

METHODS OF TREATING PANCREATIC DUCTAL ADENOCARCINOMA WITH A TOLL-LIKE RECEPTOR 9 AGONIST

PRIORITY CLAIM

[0001] This application claims priority to U.S. Provisional Application Serial No. 63/546,445 filed October 30, 2023, the entire contents of which is hereby incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The present disclosure relates generally to methods of treating cancer and methods of delivering toll-like receptor (TLR) agonists to solid tumors in the pancreas using a locoregional therapy through the vasculature.

BACKGROUND OF THE INVENTION

[0003] Cancer is a devastating disease that involves the unchecked growth of cells, which may result in the growth of solid tumors in a variety of organs such as the skin, liver, and pancreas. Tumors may first present in any number of organs or may be the result of metastases or spread from other locations.

[0004] Pancreatic cancer is the third leading cause of cancer deaths in the United States, responsible for an estimated 55,000 deaths in 2018. The 5-year survival rate of this type of cancer is only 7-8%, which is attributed to various factors including the advanced stage of the disease at which the initial diagnosis often occurs, the propensity of this type of cancer to metastasize, the resistance of the disease to chemotherapy and radiation therapy, and the complex microenvironment of pancreatic cancer tumors. Only 15-20% of patients are eligible at diagnosis for surgical resection of the primary tumor, as most patients are initially diagnosed with unresectable (metastatic or locally advanced) disease. The current standard of care for unresectable or metastatic pancreatic cancer is palliative systemic chemotherapy with either gemcitabine (Gem) monotherapy, gemcitabine/nab-paclitaxel, or folinic acid/fluorouracil /irinotecan/oxaliplatin (FOLFIRINOX). For patients with borderline resectable or locally advanced disease, combination

regimens have been used to potentially convert some borderline resectable and even some locally advanced tumors to resectability. In addition, the relatively hypovascular tumor microenvironment seen in most pancreatic adenocarcinomas makes targeted and comprehensive arterial delivery of chemotherapeutic agents challenging using conventional techniques.

[0005] Further, locally advanced pancreatic ductal adenocarcinoma (LA-PDAC) is associated with rapid progression, resistance to conventional therapies, deterioration in quality of life, significant morbidity, and a high mortality rate. PDAC tumors are characterized by dense desmoplastic stroma with a paucity of effector immune cells, rendering both drug delivery and stimulation of immune responses very challenging. Immune checkpoint inhibitors (ICI) have not demonstrated clinical benefit in the majority of pancreatic ductal adenocarcinomas (PDAC). Drug delivery challenges due to a high-pressure desmoplastic stroma and myeloid driven immunosuppression are therapeutic barriers. Therefore, there remains a need for a safe and effective treatment for treating pancreatic ductal adenocarcinomas (PDAC).

SUMMARY OF THE INVENTION

[0006] The present application relates to a method of treating pancreatic ductal adenocarcinoma (PDAC), in particular, locally advanced pancreatic ductal adenocarcinoma (LA-PDAC). The method comprises locally administering a toll-like receptor 9 (TLR9) agonist in combination with the checkpoint inhibitor. The TLR9 agonist is an oligonucleotide having the sequence:

5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 1),

or a pharmaceutically acceptable salt thereof. Specifically, the TLR9 agonist has the sequence:

5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 2), wherein the entire sequence is linked by phosphorothioate bonds. In some examples, the TLR9 agonist is a sodium salt of the oligonucleotide of SEQ ID NO: 2.

[0007] In one aspect of the present application, the plasma concentration of the TLR9 wherein the plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject. The TLR9 agonist may be locally administered via pancreatic retrograde venous infusion (PRVI). In one example, the TLR9 agonist is administered by a Pressure-Enabled Drug Delivery

(PEDD) device. The TLR9 is administered at a dose of at least about 0.5 mg or from about 0.5 mg to about 4 mg. The TLR9 may be administered in cycles with rest period between cycles.

[0008] In another aspect of the present application a method for increasing response rate to treatment with a checkpoint inhibitor in a human subject with pancreatic ductal adenocarcinoma (PDAC), in particular, locally advanced pancreatic ductal adenocarcinoma (LA-PDAC) is provided. The method comprises locally administering a toll-like receptor 9 (TLR9) agonist in combination with the checkpoint inhibitor. The TLR9 agonist is an oligonucleotide having the sequence:

5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 1),

or a pharmaceutically acceptable salt thereof. Specifically, the TLR9 agonist has the sequence:

5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 2), wherein the entire sequence is linked by phosphorothioate bonds. In some examples, the TLR9 agonist is a sodium salt of the oligonucleotide of SEQ ID NO: 2.

[0009] These and other objects, features, and advantages of the exemplary embodiments of the present disclosure will become apparent upon reading the following detailed description of the exemplary embodiments of the present disclosure, when taken in conjunction with the entire specification.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Further objects, features and advantages of the present disclosure will become apparent from the following detailed description taken in conjunction with the accompanying Figures showing illustrative embodiments of the present disclosure.

[0011] FIG. 1 shows the chemical structure of a sodium salt of nelitolimod.

[0012] FIG. 2 provides sums of longest diameters (SLD) for primary pancreatic lesions at baseline of the patients of Example 1.

[0013] FIG. 3A illustrates a Swimmers Plot of the patients of Example 1.

[0014] FIG. 3B illustrates a Spider Plot of the patients of Example 1.

- [0015] FIG. 4 illustrates gene expression levels within tumor biopsies of the patients of Example 1 collected at baseline and on Day 57.
- [0016] FIG. 5A shows changes in cytokine signaling for peripheral white blood cell counts (WBCs) of the patients of Example 1.
- [0017] FIG. 5B shows changes in chemokine signaling for peripheral white blood cell counts (WBCs) of the patients of Example 1.
- [0018] FIG. 5C shows changes in Th1 activation for peripheral white blood cell counts (WBCs) of the patients of Example 1.
- [0019] FIG. 5D shows changes in Th2 activation for peripheral white blood cell counts (WBCs) of the patients of Example 1.
- [0020] FIG. 5E shows changes in TLR signaling for peripheral white blood cell counts (WBCs) of the patients of Example 1.
- [0021] FIG. 5F shows changes in lymphocyte activation for peripheral white blood cell counts (WBCs) of the patients of Example 1.
- [0022] FIG. 5G shows changes in T cell activation and checkpoint signaling for peripheral white blood cell counts (WBCs) of the patients of Example 1.
- [0023] FIG. 5H shows changes in interferon signaling for peripheral white blood cell counts (WBCs) of the patients of Example 1.
- [0024] FIG. 6A shows changes to circulating MCP-1 (CCL2) of the patients of Example 1.
- [0025] FIG. 6B shows changes to circulating IL-18 of the patients of Example 1.
- [0026] FIG. 6C shows changes to circulating CXCL10 of the patients of Example 1.
- [0027] FIG. 6D shows changes to circulating IL-2R of the patients of Example 1.
- [0028] FIG. 6E shows changes to circulating Eotaxin of the patients of Example 1.
- [0029] FIG. 6F shows changes to circulating NT5E (CD73) of the patients of Example 1.
- [0030] FIG. 6G shows changes to circulating TIM-3 of the patients of Example 1.
- [0031] FIG. 6H shows changes to circulating IFN γ of the patients of Example 1.

[0032] FIG. 7A shows changes to circulating proliferating T cells protein expression patterns of the patients of Example 1 as determined by flow cytometry.

[0033] FIG. 7B shows changes to circulating total and proliferating NK cells protein expression patterns of the patients of Example 1 as determined by flow cytometry.

[0034] FIG. 7C shows changes to circulating activated CD8 T cells protein expression patterns of the patients of Example 1 as determined by flow cytometry.

[0035] FIG. 7D shows changes to circulating TLR9 expression protein expression patterns of the patients of Example 1 as determined by flow cytometry.

[0036] FIG. 8 shows pancreatic tumor volume change for 5 patients from Example 2.

[0037] FIG. 9 shows change in proinflammatory gene expression patterns within tumors of the patients of Example 2.

[0038] FIG. 10A shows changes to circulating IFN γ markers of the patients of Example 2.

[0039] FIG. 10B shows changes to circulating Granzyme B markers of the patients of Example 2.

[0040] FIG. 10C shows changes to circulating TNF α markers of the patients of Example 2.

[0041] FIG. 10D shows changes to circulating IL-18 markers of the patients of Example 2.

[0042] FIG. 10E shows changes to circulating MCP-1 (CCL2) markers of the patients of Example 2.

[0043] FIG. 10F shows changes to circulating VEGF-A markers of the patients of Example 2.

[0044] Throughout the drawings, the same reference numerals and characters, unless otherwise stated, are used to denote like features, elements, components or portions of the illustrated embodiments. Moreover, while the present disclosure will now be described in detail with reference to the figures, it is done so in connection with the illustrative embodiments and is not limited by the particular embodiments illustrated in the figures and the appended paragraphs.

DETAILED DESCRIPTION

[0045] The following description of embodiments provides non-limiting representative examples referencing numerals to particularly describe features and teachings of different aspects of the invention. The embodiments described should be recognized as capable of implementation separately, or in combination, with other embodiments from the description of the embodiments. A person of ordinary skill in the art reviewing the description of embodiments should be able to understand the different described aspects of the invention. The description of embodiments should facilitate understanding of the invention to such an extent that other implementations, not specifically covered but within the knowledge of a person of skill in the art having read the description of embodiments, would be understood to be consistent with application of the invention.

[0046] Unless defined otherwise, all technical and scientific terms used herein have the same meaning commonly understood to one of ordinary skill in the art to which this application pertains. Otherwise, certain terms used herein have the meanings as set in the specification. All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein.

[0047] The singular forms “a,” “an,” and, “the” include plural references unless the context clearly dictates otherwise.

[0048] Unless otherwise stated, any numerical value, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term “about.” The numerical value may include $\pm 1\%$, $\pm 2\%$, $\pm 3\%$, $\pm 4\%$, or $\pm 5\%$ of the recited value. As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0049] The term “subject” or “patient” as used herein refers to an animal, and preferably a mammal, and more preferably, a human. Examples of subjects include humans, and may also include other animals such as rats, mice and pigs. In one specific aspect, the subject is a human.

[0050] As used herein, the terms “treat,” “treating,” and “treatment” are all intended to refer to an amelioration or reversal of at least one measurable physical parameter related to a

disease, disorder, or condition. The terms “treat,” “treating,” and “treatment,” can also refer to causing regression, preventing the progression, or at least slowing down the progression of the disease, disorder, or condition. In a particular example, “treat,” “treating,” and “treatment” refer to prevention of the recurrence of the disease, disorder, or condition. In another example, “treat,” “treating,” and “treatment” refer to an increase in the survival of a subject having the disease, disorder, or condition. In a particular example, “treat,” “treating,” and “treatment” refer to elimination of the disease, disorder, or condition in the subject.

[0051] The term “therapeutically effective amount” as used herein refers to that amount of active compound or pharmaceutical agent that elicits a desired biological or medicinal response in a tissue system, animal or human, which includes improvement of one or more biomarkers for the disease, disorder or condition being treated or reduction of the severity of one or more of the symptoms of the disease, disorder or condition being treated, for example, slowing of tumor development and metastasis in a patient.

[0052] The term “agonist” as used herein refers to a compound that binds to a receptor that then increases, facilitates, sensitizes, or up-regulates the receptor.

[0053] The term “antagonist” as used herein refers to a compound that binds to a receptor that blocks or attenuates the receptor’s response to an agonist.

[0054] The term “pharmaceutically acceptable salt” refers to a salt of a compound which are known to be non-toxic and are commonly used in the pharmaceutical arts. In some examples, the pharmaceutically acceptable salt of a compound retains its biological effectiveness and is not biologically or otherwise undesirable.

[0055] As used herein, a “pharmaceutically acceptable excipient” refers to a substance that is non-toxic, biologically tolerable, and otherwise biologically suitable for administration to a subject, such as an inert substance, added to a pharmacological composition or otherwise used as a vehicle, carrier, or diluent to facilitate administration of an active compound or pharmaceutical agent and that is compatible therewith.

[0056] The present application is directed to methods of treating, ameliorating or modulating pancreatic ductal adenocarcinomas (PDAC), in particular, locally advanced PDAC (LA-PDAC), in a human subject comprising administering to the subject a therapeutically effective

amount of a toll-like receptor 9 (TLR9) agonist to a human subject in need thereof. In particular, the TLR9 agonist is locally administered to an area in the pancreas of the subject. As discussed further below, the TLR9 agonist may be administered by pancreatic retrograde venous infusions (PRVI) using transhepatic access with a Pressure-Enabled Drug Delivery (PEDD) device.

[0057] In some examples, the methods of treating, ameliorating or modulating pancreatic ductal adenocarcinomas (PDAC), in particular, locally advanced PDAC (LA-PDAC), in a human subject comprising administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and a toll-like receptor 9 (TLR9) agonist to a human subject in need thereof. The therapeutically effective combination of one or more checkpoint inhibitors and a TLR9 agonist refers to an amount of the one or more checkpoint inhibitors administered with the TLR9 agonist, such that the combined effect elicits the desired physiological or biological effect, *e.g.*, treating, ameliorating or modulating PDAC or LA-PDAC in a human subject. For example, the amount of the TLR9 agonist in the therapeutically effective combination has a combined effect with the one or more checkpoint inhibitors that is therapeutically effective, but may or may not be individually effective for treating, ameliorating or modulating PDAC or LA-PDAC in a human subject. Similarly, the amount of the one or more checkpoint inhibitors in the therapeutically effective combination has a combined effect with the TLR9 agonist that is therapeutically effective, but may or may not be individually effective for treating, ameliorating or modulating PDAC or LA-PDAC in a human subject.

Toll-like Receptor Agonists

[0058] Toll-like receptors are pattern recognition receptors that can detect microbial pathogen-associated molecular patterns (PAMPs). TLR stimulation, such as TLR9 stimulation, can not only provide broad innate immune stimulation, but can also specifically address the dominant drivers of immunosuppression in the liver. TLR1-10 are expressed in humans and recognize a diverse variety of microbial PAMPs. In this regard, TLR9 can respond to unmethylated CpG-DNA, including microbial DNA. CpG refers to the motif of a cytosine and guanine dinucleotide. TLR9 is constitutively expressed in B cells, plasmacytoid dendritic cells (pDCs), activated neutrophils, monocytes/macrophages, T cells, and MDSCs. Further, human MDSCs express TLR9 on their surface. TLR-9 plays a key role in innate immunity and bridges to

adaptive immunity. TLR-9 is expressed on suppressive immune cells such as myeloid-derived suppressor cells (MDSCs) and a TLR-9 agonist could modulate the tumor microenvironment (TME) and systemic tumor immune responses in cancer patients. Further, TLR9 and related endosomal protein TLR7 are expressed in human liver metastases tissue. TLR9 is also expressed in non-immune cells, including keratinocytes and gut, cervical, and respiratory epithelial cells. TLR9 can bind to its agonists within endosomes. Signaling may be carried out through MYD88/I κ B/Nf κ B to induce pro-inflammatory cytokine gene expression. A parallel signaling pathway through IRF7 induces type 1 and 2 interferons (e.g., IFN- α , IFN- γ , etc.) which stimulate adaptive immune responses. Further, TLR9 agonists can induce cytokine and IFN production and functional maturation of antigen presenting dendritic cells.

[0059] The TLR9 agonists may include any suitable compound or biologic molecule that binds to TLR9 that then increases, facilitates, sensitizes, or up-regulates TLR9 activity. In one example, the TLR9 agonists include synthetic CpG-oligonucleotides (CPG-ONs) mimicking the immunostimulatory nature of microbial CpG-DNA. According to an example, the oligonucleotide is an oligodeoxynucleotide (ODN). There are a number of different CpG-ODN class types, e.g. Class A, Class B, Class C, Class P, and Class S, which share certain structural and functional features. In this regard, Class A type CPG-ODNs (or CPG-A ODNs) are associated with pDC maturation with little effect on B cells as well as the highest degree of IFN α induction; Class B type CPG-ODNs (or CPG-B ODNs) strongly induce B-cell proliferation, activate pDC and monocyte maturation, NK cell activation, and inflammatory cytokine production; and Class C type CPG-ODNs (or CPG-C ODNs) can induce B-cell proliferation and IFN- α production.

[0060] According to an example, any of the CPG-C ODNs discussed herein may be present in their pharmaceutically acceptable salt forms. Suitable pharmaceutically acceptable salts of any of the CPG-C ODNs may include organic and inorganic bases. Exemplary basic salts include ammonium salts, alkali metal salts such as sodium, lithium, and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, zinc salts, salts with organic bases (for example, organic amines) such as N-Me-D-glucamine, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride, choline, tromethamine, dicyclohexylamines, t-butyl amines, and salts with amino acids such as arginine, lysine and the like. In an example, the CpG-C ODNs are in the ammonium, sodium, lithium, or potassium salt forms. In one preferred example, the CpG-C ODNs are in the sodium salt form. The CpG-C ODN may be provided in a pharmaceutical solution

comprising one or more pharmaceutically acceptable excipients. Alternatively, the CpG-C ODN may be provided as a lyophilized solid, which is subsequently reconstituted in sterile water, saline or a pharmaceutically acceptable buffer before administration.

[0061] In one example, the TLR9 agonist is a CpG-C ODN comprising the sequence of of SEQ ID NO: 1:

5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 1),

or a pharmaceutically acceptable salt thereof. More specifically, the TLR9 agonist is nelitolimod or a pharmaceutically acceptable salt thereof. Nelitolimod is a 30-mer phosphorothioate oligodeoxynucleotide, having the following sequence:

5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 2),

wherein the entire 30-mer sequence is linked by phosphorothioate bonds. The TLR agonist may be nelitolimod in the form of a free acid, or a pharmaceutically acceptable salt. In another example, the TLR9 agonist is a sodium salt. FIG. 1 illustrates the structure of a sodium salt of nelitolimod.

[0062] The molecular formula of nelitolimod in a free acid form is C₂₉₃ H₃₆₉ N₁₁₂ O₁₄₉ P₂₉ S₂₉ and the molecular mass of the nelitolimod in the free acid form is 9672 Daltons. The molecular formula of the sodium salt of nelitolimod, as shown in FIG. 1, is C₂₉₃ H₃₄₀ N₁₁₂ O₁₄₉ P₂₉ S₂₉ Na₂₉ and the molecular mass of the sodium salt of nelitolimod is 10,309 Daltons. It is noted that all of the methods described herein relating to TLR9 agonists, are applicable specifically to TLR9 type C agonists and more specifically to nelitolimod.

[0063] The TLR9 agonist may be administered to the human subject in the form of a pharmaceutical composition suitable for parenteral and/or percutaneous administration, such as, for example, an aqueous or non-aqueous solution or emulsion. The pharmaceutical composition may comprise any pharmaceutically acceptable excipient. Suitable pharmaceutically acceptable excipients of the present disclosure include, for instance, solvents, bulking agents, buffering agents, tonicity adjusting agents, and preservatives. In an example, the pharmaceutical compositions may comprise an excipient that functions as one or more of a solvent, a bulking agent, a buffering agent, and a tonicity adjusting agent (e.g., sodium chloride in saline may serve as both an aqueous vehicle and a tonicity adjusting agent). In an example, the pharmaceutical compositions comprise an aqueous vehicle as a solvent. Suitable vehicles include for instance

sterile water, saline solution, phosphate buffered saline, and Ringer's solution. In an example, the composition is isotonic.

[0064] In another example, the TLR9 agonist may be formulated in a pharmaceutical composition that is a freeze dried power that is to be reconstituted with a solvent, such as, those described above, before administration. The pharmaceutical compositions may comprise a bulking agent. Bulking agents are particularly useful when the pharmaceutical composition is lyophilized, freeze dried, or spray dried for storage and subsequently reconstituted with a solvent before administration. In an example, the bulking agent is a protectant that aids in the stabilization and prevention of degradation of the active agents during freeze or spray drying and/or during storage. Suitable bulking agents are sugars (mono-, di- and polysaccharides) such as sucrose, lactose, trehalose, mannitol, sorbitol, glucose and raffinose.

[0065] The pharmaceutical compositions may comprise a buffering agent. Buffering agents control pH to inhibit degradation of the active agent during processing, storage and optionally reconstitution. Suitable buffers include for instance salts comprising acetate, citrate, phosphate or sulfate. Other suitable buffers include for instance amino acids such as arginine, glycine, histidine, and lysine. The buffering agent may further comprise hydrochloric acid or sodium hydroxide. In some examples, the buffering agent maintains the pH of the composition within a range of 4 to 9. In an example, the pH is greater than (lower limit) 4, 5, 6, 7 or 8. In some examples, the pH is less than (upper limit) 9, 8, 7, 6 or 5. That is, the pH is in the range of from about 4 to 9 in which the lower limit is less than the upper limit.

[0066] The pharmaceutical compositions may comprise a tonicity adjusting agent. Suitable tonicity adjusting agents include for instance dextrose, glycerol, sodium chloride, glycerin, and mannitol.

[0067] The pharmaceutical compositions may comprise a preservative. Suitable preservatives include for instance antioxidants and antimicrobial agents. However, in an example, the pharmaceutical composition is prepared under sterile conditions and is in a single use container, and thus does not necessitate inclusion of a preservative.

[0068] Table 1 describes an exemplary pharmaceutical composition comprising nelitolimod, wherein nelitolimod is at a concentration of 16 g/L in the pharmaceutical composition:

Table 1

Ingredient	Grade	Concentration	Amount per batch	Exemplary Lot
				Batch Size 2.224 L
Nelitolimod Drug Substance*	GMP	1.6%	16.00 g	35.584 g ¹
Sodium phosphate, dibasic anhydrous	USP/NF, EP	1.02%	1.02 g	2.268 g
Sodium phosphate, monobasic anhydrous	USP	0.34%	0.34 g	0.747 g
Sodium chloride	USP/NF, EP	7.31%	7.31 g	16.257 g
Sterile Water for Injection	USP/NF, EP	QS	QS	2.224 L (kg) (QS)

¹Quantity based upon measured content in solution (to exclude moisture present in lyophilized powder)

* Nelitolimod Drug Substance in Table 1 reflects the totality of all oligonucleotide content, including nelitolimod.

[0069] In some examples, nelitolimod may be present in a pharmaceutical composition at a concentration from about 0.1 mg/mL to about 20 mg/mL. In particular, nelitolimod may be present in a pharmaceutical composition at a concentration of 13.4 mg/mL.

[0070] In some embodiments, the TLR9 agonist, such as nelitolimod, may be administered weekly at a dose of at least 0.5 mg. In some examples, nelitolimod may be administered weekly at a dose from about 0.5 mg to about 4 mg, from about 0.5 to about 2 mg, about 0.5 mg, about 2 mg, or about 4 mg. In some embodiments, the methods of the present invention may comprise administering a dosing regimen comprising cycles, in which one or more of the cycles comprise administering nelitolimod via PRVI using PEDD. As used herein, a “cycle” is a repeat of a dosing sequence. In one embodiment, one cycle comprises one dose per cycle. In one embodiment, a cycle of treatment according to the present invention may comprise periods of nelitolimod administration followed by “off” periods or rest periods. In another embodiment, treatment comprises administration over two cycles, with one dose per cycle and each cycle being one month apart.

Locally Administering TLR9 Agonist to the Pancreas

[0071] As discussed above, the TLR9 agonist, in particular, nelitolimod or a pharmaceutically acceptable salt thereof (e.g., the sodium salt shown in FIG. 1) is locally administered to the pancreas of the human subject by pancreatic retrograde venous infusions (PRVI) using transhepatic access with a Pressure-Enabled Drug Delivery (PEDD) device. PRVI refers to the infusion of a treatment to a solid tumor in the pancreas via a branch or branches of the pancreatic venous drainage system. In one example, the TLR9 agonists are introduced through percutaneous transhepatic introduction of a device, such as a catheter and/or a device that facilitates pressure-enabled delivery, into the branch(es) of the pancreatic venous drainage system. The device may be any device useful to achieve locoregional delivery to or near a tumor, including for example, a catheter or a catheter along with other components (e.g., filter valve, balloon, pressure sensor system, pump system, syringe, outer delivery catheter, implantable port, etc.) that may be used in combination with the catheter to administer nelitolimod via PEDD. In certain examples, the catheter is a microcatheter.

[0072] For example, delivery of the treatment by PRVI can be a more effective route of providing the TLR9 agonists to pancreatic tumors. In particular, in contrast to systemic intravenous and locoregional intra-arterial therapies, PRVI can be used to provide treatment to the tumor without relying on the arterial supply to the tumor, and, therefore may be a more effective means of delivering the TLR9 agonists and treating PDAC, specifically, LA-PDAC. For example, with PRVI, the TLR9 agonists can be delivered to the tumor via a sub-selective, catheter-directed approach utilizing the draining veins of the targeted pancreatic tumor. For example, the TLR9 agonist can be delivered to the tumor in a branch or branches of the pancreatic venous drainage system. In this regard, a digital subtraction angiography with computed tomography (CT) can be used to catheterize the veins draining the pancreatic tumor with a delivery device (e.g., catheter and/or a device that facilitates pressure-enabled delivery) in order to deliver the TLR9 agonists in a retrograde fashion.

[0073] In particular, the TLR9 agonist is infused by PRVI using transhepatic access with a PEDD device. The PEDD device may be inserted into the portal venous system of the patient, and then tracked into the target pancreatic vein. More particularly, the TLR9 agonist is infused by PRVI using a PEDD device having an expandable fluid pressure modulating structure that modulates flow and/or pressure of the vessel in which it is inserted. For example, the fluid pressure modulating structure may modulate flow and/or pressure of the vessel in which it is inserted in

sync with the cardiac cycle of the human subject. The fluid pressure modulating structure may intermittently increase pressure in the target pancreatic vein. The fluid pressure modulating structure may intermittently occlude the target pancreatic vein as it modulates the pressure of the target pancreatic vein in which it is inserted. The fluid pressure modulating structure is configured to intermittently increase pressure within the target pancreatic vein by an amount sufficient to overcoming interstitial fluid pressure and solid stress of the PDAC tumors in the pancreas of the human subject.

[0074] In some embodiments, the device for PRVI may have one or more attributes that include, but are not limited to, self-centering capability that can provide homogeneous distribution of therapy in downstream branching network of vessels; anti-reflux capability that can block or inhibit the retrograde flow of a pharmaceutical composition for infusion, e.g., a pharmaceutical fluid for infusion comprising a TLR9 agonist (for example, with the use of a valve and filter, and/or balloon); a system to measure the pressure inside the vessel; and a means to modulate the pressure inside the vessel (e.g., a one-way valve that responds dynamically to local pressure changes, an intermittently occlusive valve and/or a porous balloon) to modulate the pressure inside the vessel. In retrograde venous infusion, pressure in the vessel increases after device deployment which prevents retrograde flow. Infusion further increases vascular pressure in direct proportion to the rate of infusion. In arterial infusion, the deployment of the device reduces vascular pressure and flow. Infusion then increases vascular pressure in direct proportion to the rate of infusion. In some embodiments, the system is designed to continuously monitor real-time pressure throughout the procedure.

[0075] In one example, the mechanism for modulating the pressure generates, causes, and/or contributes to a net increase in fluid pressure within the vessel and/or target tissue or tumor. In one particular example, the mechanism for modulating the pressure may increase local vascular pressure at the target location, in particular, the increased pressure is greater than a base line arterial pressure. The mechanism for modulating the pressure may operate in sync with the cardiac cycle and/or facilitate antegrade flow. In some examples, the mechanism for modulating the pressure generates, causes, and/or contributes to a net decrease in fluid pressure within the vessel and/or target tissue or tumor. The mechanism for modulating the pressure may also redirect flow direction to improve concentration of the TLR9 agonist in tumor tissue. In some examples, the mechanism for modulating the pressure generates, causes, and/or contributes to first a decrease, then an

increase in fluid pressure within the vessel and/or target tissue or tumor. In some examples, the device supports the measurement of intravascular pressure during use.

[0076] In some examples, the device that may be used for PRVI is a device as disclosed in U.S. Patent No. 8,500,775, U.S. Patent No. 8,696,698, U.S. Patent No. 8,696,699, U.S. Patent No. 9,539,081, U.S. Patent No. 9,808,332, U.S. Patent No. 9,770,319, U.S. Patent No. 9,968,740, U.S. Patent No. 10,813,739, U.S. Patent No. 10,588,636, U.S. Patent No. 11,090,460, U.S. Patent Publication No. 2018/0193591, U.S. Patent Publication No. 2018/0250469, U.S. Patent Publication No. 2019/0298983, U.S. Patent Publication No. 2020/0038586, and U.S. Patent Publication No. 2020/0383688, which are all incorporated by reference herein in their entireties. In some examples, the device is a device as disclosed in U.S. Patent No. 9,770,319.

[0077] In some examples, the device supports the measurement of intravascular pressure during use. In some embodiments, the device is a device as disclosed in U.S. Patent Application No. 16/431,547. In certain embodiments, the device may be a device known as the TriSalus Infusion System (TIS) (sometimes also known as the SEAL device). In certain embodiments, the device may be a device known as the TriNav[®] Infusion System. In certain embodiments, the catheter device may be described as an anti-reflux microcatheter (TIS-21120-60) manufactured by TriSalus Life Sciences. In certain embodiment, the device may be a temporary occlusion device, such as, the TIS Device.

[0078] In some embodiments, the TIS Device can be a dual catheter mechanically actuated infusion system equipped with a structure at the distal end of the device that acts to reversibly occlude blood flow in a PRVI procedure. According to an embodiment, the structure at the distal end of the device can be a braided filament construct with a fluid impermeable membrane provided over a proximal portion of the braided construct and a fluid permeable coating (or covering) over a distal portion of the braided construct. The device geometry may further allow for direct continuous pressure measurements of the vasculature distal to the device infusion lumen during therapeutic delivery. Device deployment and the infusion of therapeutic may modulate distal vascular pressure during PRVI procedures.

[0079] The TIS Device is a 5.0F to 3.1F tapered coaxial infusion catheter having a 0.021" inner lumen with an expandable valve at the distal end that serves as the conduit for physician-specified agents. The valve is designed to variably expand within vessels ranging from 2 to 6mm

in diameter and forms a fluid impermeable barrier in the presence of retrograde flow. The device is further adapted to interface with standard invasive blood pressure (IBP) transducers in a manner that allows for continuous pressure monitoring in vasculature distal to the valve throughout infusion of therapeutic. During infusion, the device blocks all retrograde flow and generates pressure in the vessel, resulting in the perfusion of the venous and capillary network isolated by the device.

[0080] In some examples, the TLR9 agonist may be administered through a device via PEDD. In some examples, the TLR9 agonist may be administered while monitoring the pressure in the vessel, which can be used to adjust and correct the positioning of the device at the infusion site and/or to adjust the rate of infusion. Pressure may be monitored by, for example, a pressure sensor system comprising one or more pressure sensors. The rate of infusion may be adjusted to alter vascular pressure or flow, which may promote the penetration and/or binding of the TLR9 agonist into the target tissue or tumor or at its surface. In some examples, the rate of infusion may be adjusted and/or controlled using a syringe pump as part of the delivery system or by any other method (e.g., an infusion flow rate regulating device). In some examples, the rate of infusion may be adjusted and/or controlled using a pump system.

[0081] The rate of infusion may be adjusted to alter vascular pressure, which may promote the penetration of the TLR agonist into the target tissue or tumor. In some embodiments, the rate of infusion may be adjusted and/or controlled using a syringe pump as part of the delivery system. In some embodiments, the rate of infusion may be adjusted and/or controlled using a pump system. In some embodiments, the rate of infusion may be about 0.1 cc/min to about 40 cc/min, or about 0.1 cc/min to about 30 cc/min, or about 0.5 cc/min to about 25 cc/min, or about 0.5 cc/min to about 20 cc/min, or about 1 cc/min to about 15 cc/min, or about 1 cc/min to about 10 cc/min, or about 1 cc/min to about 8 cc/min, or about 1 cc/min to about 5 cc/min.

Checkpoint Inhibitors

[0082] As discussed above, the present application describes administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors (CPIs) and a toll-like receptor 9 (TLR9) agonist. The CPI may be a Programmed Death 1 receptor (PD-1) antagonist. A PD-1 antagonist can be any chemical compound or biological molecule that blocks binding of

Programmed Cell Death 1 Ligand 1 (PD-L1) expressed on a cancer cell to PD-1 expressed on an immune cell (T cell, B cell or NKT cell) and preferably also blocks binding of Programmed Cell Death 1 Ligand 2 (PD-L2) expressed on a cancer cell to the immune-cell expressed PD-1.

[0083] According to an example, the PD-1 antagonist can include a monoclonal antibody (mAb), or antigen binding fragment thereof, which specifically binds to PD-1 or PD-L1, and preferably specifically binds to human PD-1 or human PD-L1. The mAb may be a human antibody, a humanized antibody or a chimeric antibody, and may include a human constant region. In some examples the human constant region is selected from the group consisting of IgG1, IgG2, IgG3 and IgG4 constant regions, and in preferred examples, the human constant region is an IgG1 or IgG4 constant region. In some examples, the antigen binding fragment is selected from the group consisting of Fab, Fab'-SH, F(ab')₂, scFv and Fv fragments. For example, the PD-1 antagonist is a monoclonal antibody, or an antigen binding fragment thereof, which specifically binds to PD-1 or to PD-L1 and blocks the binding of PD-L1 to PD-1. In one example, the PD-1 antagonist is an anti-PD-1 antibody which comprises a heavy chain and a light chain, such as pembrolizumab. According to an example, pembrolizumab is administered intravenously (IV) via a peripheral vein at a dose of 200 mg every three weeks ("Q3W"). In yet another embodiment, pembrolizumab is administered concomitantly, at the same time, at about the same time, or on the same day with nelitolimod. In another embodiment, pembrolizumab is administered one a weekly, every other week, every three weeks, every four weeks, or on a monthly basis following the administration of one or more cycles of nelitolimod. In another embodiment, pembrolizumab is administered for a period of up to six months.

[0084] The present application will be further illustrated and/or demonstrated in the following Example, which is given for illustration/demonstration purposes only and is not intended to limit the invention in anyway.

EXAMPLES

Example 1

[0085] Example 1 is an open-label, phase 1/1b study (NCT05607953) of the pressure-enabled intrapancreatic infusion of nelitolimod, a TLR 9 agonist, alone or in combination with intravenous checkpoint blockade in adults with locally advanced pancreatic cancer. In Phase 1,

escalating doses of nelitolimod are administered alone via PRVI into the regional vessels of the pancreas containing the locally advanced tumor.

[0086] Patients with LA-PDAC are eligible for enrollment. Oligometastatic disease is permitted.

[0087] The inclusion criteria for the phase 1/1b study of Example 1 include:

- Patients ≥ 18 years of age with histologically or cytologically confirmed evaluable or measurable locally advanced unresectable PDAC, or previously confirmed disease in the absence of a documented complete pathologic response.
- Performance status score of 0 or 1 on the ECOG PS scale (scores range from 0 to 5, with higher numbers reflecting greater disability)
- Suitable venous anatomy on a standard portal venous phase imaging as defined by absence of portal, splenic, or superior mesenteric vein complete occlusion. As long as there is not complete occlusion and the Interventional Radiologist confirms that the target vein can be accessed, patients may be suitable for enrollment. All 3 veins do not have to be patent for eligibility.
- Having received standard of care chemoradiation therapy or a systemic chemotherapy regimen without a complete radiographic response. Standard of care chemotherapy includes gemcitabine + nab-paclitaxel, or FOLFIRINOX; for others discuss with medical monitor. Radiation with or without concurrent chemotherapy is also acceptable as a standard of care regimen.
- Able to understand the study and provide written informed consent prior to any study procedures.
- Has not received prior cytotoxic chemotherapy or targeted therapy within 14 days, or external radiation therapy within 4 weeks prior to screening.
- Low-burden, asymptomatic metastatic disease permitted if:
 - Metastatic disease poses no imminent threat to the patient
 - Patient is otherwise asymptomatic with respect to metastases
 - Metastases are limited to liver, lung, and/or bone
 - No single lesion greater than 5 cm
 - Less than 5 metastatic lesions total

- No brain or peritoneal metastases Pancreatic disease must be the dominant determinant of the patient's prognosis and clinical course
- Has no prior history of or other concurrent malignancy unless the malignancy is clinically insignificant, no ongoing treatment is required, and the patient is clinically stable
- Has a life expectancy of >3 months at screening as estimated by the Investigator
- Has a QTc interval ≤ 480 msec
- All associated clinically significant (in the judgment of the Investigator) drug-related toxicity from previous cancer therapy must be resolved (to Grade ≤ 1 or the patient's pretreatment level) prior to study treatment administration (Grade 2 alopecia, grade 2 peripheral neuropathy from prior chemotherapy, and endocrinopathies controlled on replacement therapy are allowed).
- Has adequate organ function at screening as evidence by:
 - Platelet count $> 80,000/\mu\text{L}$
 - Hemoglobin ≥ 8.0 g/dL
 - White blood cell (WBC) count $> 2,000/\mu\text{L}$
 - Serum creatinine ≤ 2.0 mg/dL unless the measured creatinine clearance is ≥ 30 mL/min calculated by Cockcroft-Gault formula.
 - Total and direct bilirubin $\leq 2.0 \times$ the upper limit of normal (ULN) and alkaline phosphatase $\leq 5 \times$ ULN. For patients with documented Gilbert's disease, total bilirubin up to 3.0 mg/dL is allowed.
 - Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq 5 \times$ ULN
 - Amylase and lipase $\leq 3 \times$ ULN
 - Prothrombin time/International Normalized Ratio (INR) or activated partial thromboplastin time (aPTT) test results at screening $\leq 1.5 \times$ ULN (this applies only to patients who do not receive therapeutic anticoagulation; patients receiving therapeutic anticoagulation should be on a stable dose for at least 4 weeks prior to the first dose of study intervention). Laboratory tests with exclusionary results judged by the Investigator as not compatible with the patient's clinical status may be repeated once for eligibility purposes.

[0088] The exclusion criteria for the phase 1/1b study of Example 1 include:

- Main portal, superior mesenteric vein, or splenic vein thrombosis with complete occlusion

- Severe portal hypertension, as evidenced by gastrointestinal (GI) bleeding, thrombocytopenia with splenomegaly
- Chronic pancreatitis
- Active autoimmune disease or history of IgG4 related pancreatitis
- Conversion to local resectability following prior treatment
- Has received chemotherapy or an investigational agent within 14 days (or 5 half-lives, whichever is shorter) before screening
- Has active, untreated brain metastasis
- Active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection
- Has main portal vein thrombosis, or severe portal hypertension as defined by a history of variceal hemorrhage or active ascites accumulation refractory to medical management
- Has Child-Pugh Class B or C cirrhosis.
- Has experienced a Grade 3 or higher immune-related AE from prior CPI therapy
- Is unable to be temporarily removed from chronic anticoagulation therapy
- Has a history of bleeding disorder
- Has active coronavirus disease 2019 (COVID-19), other severe infection, including a liver infection or acute pancreatitis, within 2 weeks before the first dose of study drug, or uncontrolled human immunodeficiency virus (HIV) infection at screening
- Has had bacterial pneumonia within 8 weeks of first dose of study drug
- Has active, known, or suspected autoimmune disease or immune-mediated disease. Type I diabetes mellitus, hypothyroidism only requiring hormone replacement, skin disorders (such as vitiligo, psoriasis or alopecia) not requiring systemic treatment or conditions not expected to recur in the absences of an external trigger are not exclusionary
- Is receiving systemic steroid therapy >10 mg of prednisone daily or equivalent or any other immunosuppressive medication at any dose level. Local steroid therapies (e.g., otic, ophthalmic, intra-articular or inhaled medications) are acceptable
- Has significant concurrent or intercurrent illness, psychiatric disorder, or alcohol or chemical dependence that would, in the opinion of the Investigator and/or Medical Monitor, compromise their safety or compliance or interfere with interpretation of the study
- Lactating women are excluded from study participation

- Has previously received SD-101
- Medical history of significant hypersensitivity, severe and unresolved immune-mediated reactions, severe infusion-related reactions, or allergic reaction to TLR9 agonists or CPI agents in the judgment of the Investigator

[0089] In Example 1, infusions of a class C TLR9 agonist, nelitolimod, are administered to a human patient in a dose-escalation clinical study via PRVI using a PEDD with the TriSalus Infusion System. Nelitolimod is delivered over 2 cycles (1 dose/cycle), during outpatient PRVI procedures using transhepatic access with the TriSalus Infusion System PEDD device. The PEDD infusion system is inserted into the portal venous system, and then tracked into the target pancreatic vein.

[0090] The first three (3) patients were enrolled at the lowest nelitolimod dose of 0.5 mg. Nelitolimod was administered to 3 patients on Day 1 and Day 57. Table 2 below provides patient characteristics for the first 3 patients with locally advanced PDAC. These patients were administered nelitolimod at a dose of 0.5 mg on Day 1 and Day 57 via pancreatic retrograde venous infusions (PRVI) using a PEDD.

Table 2.

Patient Characteristics	n = 3 (%)
Gender	
Female	0
Male	3 (100)
Age (years, range)	57-66
Prior Lines of Therapy	
0	0
1	0
2	0
≥3	3 (100)
N/A	
Number of Lesions at Baseline	
≤10	3 (100)
> 10	0
N/A	0
Largest Lesion at BL	
≤ 50mm	3 (100)
> 50mm	0
N/A	0

[0091] FIG. 2 provides sums of longest diameters (SLD) for primary pancreatic lesions at baseline of the patients of Table 2.

[0092] The adverse events related to the administration of nelitolimod is shown below in Table 3. All PRVI procedures were successfully completed, with no safety events related to the infusions.

Table 3.

Phase 1 – 0.5 mg Nelitolimod Monotherapy: Adverse Events related to nelitolimod	
Preferred Term (MedDRA v24.0)	Nelitolimod 0.5 mg n=3
All Grades (events in $\geq 5\%$ of patients), n (%)	
AT LEAST ONE EVENT	3 (100)
Abdominal Pain	1 (33)
Fatigue	2 (67)
Nausea	1 (33)
Vomiting	1 (33)
Platelet Count Decreased	2 (67)
Alanine Aminotransferase Increased	2 (67)
Aspartate Aminotransferase Increased	2 (67)
Anemia	1 (33)
Fever	1 (33)
Chills	1 (33)
Alkaline Phosphatase Increased	1 (33)
Hyperthyroidism	1 (33)
Grade ≥ 3 (Events in ≥ 1 patient), n (%)	
AT LEAST ONE EVENT	1 (33)
Alanine Aminotransferase Increased	1 (33)

[0093] FIG. 3A illustrates a Swimmers Plot illustrating survival of the patients of Example 1. FIG. 3A shows the survival rates of the patients exceeding 200 days. FIG. 3B illustrates a Spider Plot of response to nelitolimod of the patients of Example 1. A minor response is about 10-29% decrease. A partial response is about $\geq 30\%$ decrease. Progressive disease refers to an increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since treatment started. Stable disease refers to any cases that do not qualify for either partial response, minor response or progressive disease. FIG. 3B demonstrates that after 100 days on 0.5 mg nelitolimod, two patients having stable disease, and one patient has progressive disease.

[0094] FIG. 4 shows myeloid derived suppressor cell (MDSC)-associated gene expression levels within tumor biopsies of the patients of Example 1 collected at baseline and on Day 57 quantified by NanoString (n=3). Comparison of pre- and post- nelitolimod infusion PDAC tumor specimens revealed decreases in expression of MDSC associated genes TGF β , NT5E, ARG1, ROS1, and NOS2.

[0095] FIGS. 5A to 5H shows changes in pathway scores determined by advanced analysis of NanoString gene expression data of peripheral WBCs of the patients of Example 1 (n=3). FIG. 5A shows changes to cytokine signaling pathway scores. FIG. 5B shows changes to chemokine signaling pathway scores. FIG. 5C shows changes to Th1 activation pathway scores. FIG. 5D shows changes Th2 activation pathway scores. FIG. 5E shows changes to TLR signaling pathway scores. FIG. 5F shows changes to lymphocyte activation pathway scores. FIG. 5G shows changes to T cell activation and checkpoint signaling pathway scores. FIG. 5H shows changes to interferon signaling pathway scores.

[0096] The NanoString analysis of peripheral WBCs shown in FIGS. 5A to 5H demonstrate increase in pathway scores for lymphocyte activation, cytokine signaling, and chemokine signaling.

[0097] FIGS. 6A to 6H show changes in circulating immune markers of the patients of Example 1 determined by Luminex. FIG. 6A shows changes to circulating MCP-1 (CCL2). FIG. 6B shows changes to circulating IL-18. FIG. 6C shows changes to circulating CXCL10. FIG. 6D shows changes to circulating IL-2R. FIG. 6E shows changes to circulating Eotaxin. FIG. 6F shows changes to circulating NT5E (CD73). FIG. 6G shows changes to circulating TIM-3. FIG. 6H shows changes to circulating IFN γ .

[0098] FIGS. 7A to 7D shows changes in circulating WBC protein expression patterns of the patients of Example 1 as determined by flow cytometry. FIG. 7A shows changes to proliferating T cells protein expression patterns. FIG. 7B shows changes to total and proliferating NK cells protein expression patterns. FIG. 7C shows changes to activated CD8 T cells protein expression patterns. FIG. 7D shows changes to TLR9 expression protein expression patterns. Flow cytometry of peripheral WBCs revealed increases in Ki-67+ CD8 T cells, CD4 T cells, and NK Cells as well as ICOS+ and CD69+ CD8 T cells.

[0099] Additionally, in one patient (patient 101-002) changes to tumor size (as determined by sequential CT scans with intravenous contrast were measured as indicated below in Table 4.

Table 4

Patient 101-002	
Time	Tumor measurements
Baseline (Day 1)	1.87 cm
Day 53	2.26 cm
Day 100	1.86 cm

[00100] The data of Example 1 show that nelitolimod PRVI infusions with PEDD were well tolerated in the initial 3 patients. Infusions were associated with potentially favorable immune changes in the periphery and tumors.

Example 2

[00101] Example 2 provides additional data from the Phase 1 clinical study of Example 1 described above. In Example 2, escalating doses of nelitolimod are administered alone via PRVI into the regional vessels of the pancreas containing the locally advanced tumor. For Example 2, patients with locally advanced (LA) and metastatic PDAC with limited disease burden who progressed on at least one line of therapy are eligible.

[00102] Nelitolimod is delivered over 2 cycles (1 dose per cycle over 2 months), during outpatient interventional radiology procedures via percutaneous hepatic or splenic access. The infusion system is tracked to the pancreatic vein(s) draining the primary tumor. These patients were administered nelitolimod at a dose of 0.5 mg, 2 mg or 4 mg on Day 1 and Day 57 via pancreatic retrograde venous infusions (PRVI) using a PEDD.

[00103] Tumor response was assessed by RECIST 1.1 and volumetric assessments. Overall survival (OS) was calculated from beginning of the trial for the Intent to Treat population. Exploratory correlative studies using pre- and post-treatment blood and endoscopic ultrasound tumor core needle biopsy specimens are included and analyzed for cytokines (Luminex), immune cells (flow cytometry) and gene expression profile (NanoString).

[00104] In Example 2, 12 patients were enrolled at three dose levels (0.5, 2, and 4 mg). The patient of Example 2 include the initial 3 patients discussed above in Example 1. Among 11 evaluable patients, 9 were male and 2 are female. Eight patients had 3 or more lines of prior treatment, 2 patients received 2 prior lines, and 1 patient having received 1 line of therapy. 1 patient withdrew consent before treatment. There have been no serious safety events related to the PEDD devices or PRVI procedures. Two patients experienced grade 3 SAEs, but none related to study drug or procedure. The follow-up duration is 2.27-17.87 months.

[00105] FIG. 8 shows pancreatic tumor volume change for 5 patients from Example 2. The patients identified with numerals 1, 2 and 3 were administered at a dose of 0.5 mg, and patients identified with numerals 4 and 5 were administered at a dose of 2 mg.

[00106] FIG. 9 shows change in proinflammatory gene expression patterns within tumors of the patients of Example 2.

[00107] FIGS. 10A to 10F show changes in circulating immune markers of the patients of Example 2 determined by Luminex. FIG. 10A shows changes to circulating IFN γ . FIG. 10B shows changes to circulating Granzyme B. FIG. 10C shows changes to circulating TNF α . FIG. 10D shows changes to circulating IL-18. FIG. 10E shows changes to circulating MCP-1 (CCL2). FIG. 10F shows changes to circulating VEGF-A.

[00108] Disease control (SD) was noted in all with available imaging assessments (n=8), and volumetric assessments in patients at the first two dose levels revealed an average 27% decrease following treatment (p=0.056). For patients with a minimum of 6 months follow-up (n=8), the median OS is 11.6 months (range 6.2-16.6) and the median PFS is 6.7 months (range 6.2-12.5). Systemic immune activation has been noted by elevation in serum soluble IL2R, increases in peripheral T cell proliferation, decreased MDSCs and expansion of peripheral M1 macrophages. Transcriptomic analysis in tumor biopsy specimens have revealed increased TLR signaling, cytokine signaling, Th1 activation, and interferon signaling at the 2 and 4 mg doses. Increased expression of IFN α 2, IFN γ , IL15, IL18, granzyme A, ICOS, and TLR9 have been noted as well.

[00109] Nelitolimod PRVI infusions with PEDD have been well tolerated with potentially favorable intra-tumoral and systemic immune signals.

[00110] The foregoing merely illustrates the principles of the disclosure. Various modifications and alterations to the described embodiments will be apparent to those skilled in the art in view of the teachings herein. It will thus be appreciated that those skilled in the art will be able to devise numerous systems, arrangements, and procedures which, although not explicitly shown or described herein, embody the principles of the disclosure and can be thus within the spirit and scope of the disclosure. Various different exemplary embodiments can be used together with one another, as well as interchangeably therewith, as should be understood by those having ordinary skill in the art. In addition, certain terms used in the present disclosure, including the specification, can be used synonymously in certain instances, including, but not limited to, for example, data and information. It should be understood that, while these words, and/or other words that can be synonymous to one another, can be used synonymously herein, that there can be instances when such words can be intended to not be used synonymously. Further, to the extent that the prior art knowledge has not been explicitly incorporated by reference herein above, it is explicitly incorporated herein in its entirety. All publications referenced are incorporated herein by reference in their entireties.

CLAIMS

1. A method of treating pancreatic ductal adenocarcinoma (PDAC), the method comprising:
locally administering a therapeutically effective amount of a toll-like receptor 9 (TLR9) agonist to a human subject in need thereof, wherein the TLR9 agonist is an oligonucleotide having the sequence:
5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 1),
or a pharmaceutically acceptable salt thereof.
2. The method of claim 1, wherein the TLR9 agonist has the sequence:
5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 2),
wherein the entire sequence is linked by phosphorothioate bonds.
3. The method of claim 2, wherein the TLR9 agonist is a sodium salt of the oligonucleotide of SEQ ID NO: 2.
4. The method of any one of claims 1 to 3, wherein a plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject.
5. The method of any one of claims 1 to 3, wherein the PDAC is locally advanced pancreatic ductal adenocarcinoma (LA-PDAC).
6. The method of any one of claims 1 to 3, wherein the TLR9 agonist is locally administered via pancreatic retrograde venous infusion (PRVI).
7. The method of claim 6, wherein the TLR9 agonist is administered by a Pressure-Enabled Drug Delivery (PEDD) device.

8. The method of claim 6, wherein the TLR9 agonist is administered at a dose of at least about 0.5 mg.
9. The method of claim 8, wherein the TLR9 agonist is administered at a dose from about 0.5 mg to about 4 mg.
10. The method of claim 8, wherein the TLR9 agonist is administered in cycles with rest period between cycles.
11. The method of any one of claims 1 to 3, wherein the administration of the TLR9 agonist increases peripheral lymphocyte activation, peripheral cytokine signaling, or peripheral chemokine signaling.
12. A method for increasing response rate to treatment with a checkpoint inhibitor in a human subject with pancreatic ductal adenocarcinoma (PDAC), the method comprising:
locally administering a toll-like receptor 9 (TLR9) agonist in combination with the checkpoint inhibitor, wherein the TLR9 agonist is an oligonucleotide having the sequence:
5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 1),
or a pharmaceutically acceptable salt thereof.
13. The method of claim 12, wherein the TLR9 agonist has the sequence:
5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 2),
wherein the entire sequence is linked by phosphorothioate bonds.

14. The method of claim 13, wherein the TLR9 agonist is a sodium salt of the oligonucleotide of SEQ ID NO: 2.
15. The method of any one of claims 12 to 14, wherein a plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject.
16. The method of any one of claims 12 to 14, wherein the subject has locally advanced pancreatic ductal adenocarcinoma (LA-PDAC).
17. The method of any one of claims 12 to 14, wherein the TLR9 agonist is locally administered via pancreatic retrograde venous infusion (PRVI).
18. The method of claim 17, wherein the TLR9 agonist is administered by a Pressure-Enabled Drug Delivery (PEDD) device.
19. The method of claim 17, wherein the TLR9 agonist is administered at a dose of at least about 0.5 mg.
20. The method of claim 19, wherein the TLR9 agonist is administered at a dose from about 0.5 mg to about 4 mg.
21. The method of claim 20, wherein the TLR9 agonist is administered in cycles with rest period between cycles.
22. The method of claim 17, wherein the one or more checkpoint inhibitors is administered intravenously.

23. The method of 22, wherein the one or more checkpoint inhibitors comprises pembrolizumab.
24. The method of any one of claims 12 to 14, wherein the administration of the TLR9 agonist in combination with the checkpoint inhibitor increases peripheral lymphocyte activation, peripheral cytokine signaling, or peripheral chemokine signaling.
25. A toll-like receptor 9 (TLR9) agonist, wherein the TLR9 agonist is an oligonucleotide having the sequence:
5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 1),
or a pharmaceutically acceptable salt thereof for use in the treatment of pancreatic ductal adenocarcinoma (PDAC), wherein the TLR9 agonist is locally administered.
26. The TLR9 agonist of claim 25, wherein the TLR9 agonist has the sequence:
5'-TCG AAC GTT CGA ACG TTC GAA CGT TCG AAT-3' (SEQ ID NO: 2),
wherein the entire sequence is linked by phosphorothioate bonds.
27. The TLR9 agonist of claim 26, wherein the TLR9 agonist is a sodium salt of the oligonucleotide of SEQ ID NO: 2.
28. The TLR9 agonist of any one of claims 25 to 27, wherein the PDAC is locally advanced pancreatic ductal adenocarcinoma (LA-PDAC).
29. The TLR9 agonist of any one of claims 25 to 27, wherein the TLR9 agonist is locally administered via pancreatic retrograde venous infusion (PRVI).

30. The TLR9 agonist of claim 29, wherein the TLR9 agonist is administered by a Pressure-Enabled Drug Delivery (PEDD) device.
31. The TLR9 agonist of claim 29, wherein the TLR9 agonist is administered at a dose of at least about 0.5 mg.
32. The TLR9 agonist of claim 31, wherein the TLR9 agonist is administered at a dose from about 0.5 mg to about 4 mg.
33. The TLR9 agonist of claim 29, wherein the TLR9 agonist is administered in cycles with rest period between cycles.