

**METHODS OF TREATING UVEAL MELANOMA LIVER METASTASES WITH A
THERAPEUTICALLY EFFECTIVE COMBINATION OF ONE OR MORE
CHECKPOINT INHIBITORS AND A TOLL-LIKE RECEPTOR 9 AGONIST**

PRIORITY CLAIM

[0001] This application claims priority to U.S. Provisional Application Serial No. 63/470,653 filed June 2, 2023, and U.S. Provisional Application Serial No. 63/546,424 filed October 30, 2023, the entire contents of each of these applications identified above are hereby incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

[0002] The present disclosure relates generally to methods of treating cancer, in particular, liver cancer, and methods of delivering toll-like receptor (TLR) agonists to solid tumors in the liver 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] Melanoma is a clinically and molecularly diverse disease encompassing a wide range of subtypes and presentation features. Within melanoma is a constellation of rare subtypes and presentations, including melanoma with brain metastases and leptomeningeal disease, acral melanoma, pediatric melanoma, and melanoma of unknown primary, as well as uncommon sites of presentation like extracutaneous melanoma. Melanoma research, therefore, has had to remain at the forefront of development with inclusion of special populations that would otherwise be excluded from most therapeutic clinical trials. Uveal melanoma is a biologically unique disease, demonstrating stark cellular, molecular, and clinical differences from cutaneous melanoma (CM). Furthermore, UM and CM are distinct malignancies that require distinct approaches.

[0005] Liver metastases (LM) are a dominant cause of morbidity and mortality for a number of solid tumors. Uveal melanoma (UM) is among the solid organ malignancies in which

metastatic spread to the liver results in a rapidly progressive and often lethal condition, for which there are limited therapeutic options. UM is a very rare condition, with fewer than 3000 new diagnoses per year in the United States (US). Primary UM tumors can be effectively managed with surgical or radiation therapy, but metastatic disease occurs in more than 50% of patients. These poor outcome data are compounded by the high predilection for UM to spread to the liver hematogenously, an event which occurs in greater than 90% of cases.

[0006] The liver is a unique organ which is intrinsically immunosuppressive and drives the programming and expansion of suppressive cells such as myeloid derived suppressor cells (MDSCs). In this regard, MDSCs expand in response to malignancy. MDSCs also drive expansion of other suppressor cell types such as T regulatory cells (Tregs), tumor-associated macrophages (TAMs), and cancer-associated fibroblasts (CAFs). MDSCs may downregulate immune cells and interfere with the effectiveness of immunotherapeutics. Further, high MDSC levels generally predict poor outcomes in cancer patients. In the setting of LM, MDSC are critical drivers of intrahepatic immunosuppression, enabling growth and progression of malignant tumors. MDSC have specifically been implicated in the pathogenesis of UM LM. Suppressive immune cells in the liver not only dampen endogenous antitumor immunity but limit the effectiveness of immunology therapies for LM, as described below.

[0007] UM has been shown to be highly refractory to immunotherapy. Metastatic disease has very poor outcomes, with a 1-year overall survival (OS) rate of 43% from the time of the original diagnosis and response rates below 20%. Furthermore, the presence of LM have also been shown to limit effectiveness of checkpoint inhibitor therapies. Therefore, there remains a need for a safe and effective treatment for UM LM.

SUMMARY OF THE INVENTION

[0008] The present application relates to a method for treating uveal melanoma liver metastases in a human subject. The subject may have Stage IV uveal melanoma. The method comprises administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and a toll-like receptor 9 (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. In one example, the TLR9 agonist has the sequence:

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

the entire sequence is linked by phosphorothioate bonds. Specifically, the TLR9 agonist is SD-101 (also referred to herein as nelitolimod) or a pharmaceutically acceptable salt thereof. More specifically, the TLR9 agonist is a sodium salt of SD-101, such as that shown in FIG. 1. In particular, the TLR9 agonist is locally administered to an area in the liver of the subject external to the uveal melanoma liver metastases.

[0009] In another aspect of the present application, a method for increasing survival rate in a human subject with uveal melanoma liver metastases is provided. The method comprises administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and a toll-like receptor 9 (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. In one example, the TLR9 agonist has the sequence:

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the entire sequence is linked by phosphorothioate bonds. Specifically, the TLR9 agonist is SD-101 or a pharmaceutically acceptable salt thereof. More specifically, the TLR9 agonist is a sodium salt of SD-101, such as that shown in FIG. 1. In particular, the TLR9 agonist is locally administered to an area in the liver of the subject external to the uveal melanoma liver metastases. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases progression-free survival (PFS) of the subject.

[0010] In a further aspect of the present application, a method for treating uveal melanoma liver metastases in a human subject is provided. The method comprises administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and a toll-like receptor 9 (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. In one example, the TLR9 agonist has the sequence:

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the entire sequence is linked by phosphorothioate bonds. Specifically, the TLR9 agonist is SD-101 or a pharmaceutically acceptable salt thereof. More specifically, the TLR9 agonist is a sodium salt of SD-101, such as that shown in FIG. 1. In particular, the TLR9 agonist is locally administered to an area in the liver of the subject external to the uveal melanoma liver metastases. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases progression-free survival (PFS) of the subject. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist reduces circulating tumor DNA (ctDNA) in the subject.

[0011] In a further aspect of the present application, a method for reducing circulating tumor DNA (ctDNA) in a human subject with uveal melanoma liver metastases is provided. The method comprises administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and a toll-like receptor 9 (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. In one example, the TLR9 agonist has the sequence:

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

the entire sequence is linked by phosphorothioate bonds. Specifically, the TLR9 agonist is SD-101 or a pharmaceutically acceptable salt thereof. More specifically, the TLR9 agonist is a sodium salt of SD-101, such as that shown in FIG. 1. In particular, the TLR9 agonist is locally administered to an area in the liver of the subject external to the uveal melanoma liver metastases.

[0012] In another aspect of the present application, a method for increasing response rate to treatment with a checkpoint inhibitor in a human subject with uveal melanoma liver metastases is provided. The method comprises administering to the subject a therapeutically

effective combination of one or more checkpoint inhibitors and a toll-like receptor 9 (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. In one example, the TLR9 agonist has the sequence:

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

the entire sequence is linked by phosphorothioate bonds. Specifically, the TLR9 agonist is SD-101 or a pharmaceutically acceptable salt thereof. More specifically, the TLR9 agonist is a sodium salt of SD-101, such as that shown in FIG. 1. In particular, the TLR9 agonist is locally administered to an area in the liver of the subject external to the uveal melanoma liver metastases. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases survival rate of the subject. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases progression-free survival (PFS) of the subject. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist reduces circulating tumor DNA (ctDNA) in the subject. In some examples, the administration of the TLR9 agonist in combination with the checkpoint inhibitor increases immune cell activation within the uveal melanoma liver metastases of the subject. In some examples, the administration of the TLR9 agonist in combination with the checkpoint inhibitor increases systemic cytokine signaling in the blood of the subject.

[0013] For each of the above aspects of the present application, the one or more checkpoint inhibitors are systematically administered to the subject. The one or more checkpoint inhibitors may be administered intravenously, intraperitoneally or subcutaneously. The one or more checkpoint inhibitor may be administered concurrently with the TLR9 agonist, before the TLR9 agonist, or after the TLR9 agonist.

[0014] In some examples, the one or more checkpoint inhibitor may be selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab. In one example, the one or more checkpoint inhibitor is nivolumab. In another example, the one or more checkpoint inhibitor is pembrolizumab. In another example, the one or more checkpoint

inhibitor is ipilimumab. In a further example, the one or more checkpoint inhibitor is crefmirlimab. In some examples, two checkpoint inhibitors are administered. For example, the two checkpoint inhibitors are nivolumab and ipilimumab. In another example, the two checkpoint inhibitors are nivolumab and relatlimab.

[0015] The TLR9 agonist is locally administered to the liver of the subject via hepatic artery infusion at a dose sufficient to provide a concentration in the liver that is therapeutically effective in combination with the checkpoint inhibitor and to provide a plasma concentration of the TLR9 agonist that is below the concentration in the liver. In some examples, the plasma concentration of the TLR9 agonist is at a dose that is subtherapeutic when the TLR9 agonist is administered without the one or more checkpoint inhibitors. In other examples, the plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject. For example, the concentration of the TLR9 agonist in the liver is from about 750 ng/g to about 3000 ng/g. In some examples, the plasma concentration of the TLR9 agonist is less than about 750 ng/mL, or less than about 700 ng/mL, or less than about 600 ng/mL. In some examples, a ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:2 to about 1:10. In another example, the ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:3 to about 1:7.

[0016] In some examples, the TLR9 agonist is administered through a catheter device. The catheter device may comprise a one-way valve that responds dynamically to local pressure and/or flow changes. In some examples, the TLR9 agonist is administered through the catheter device via pressure-enabled drug delivery (PEDD).

[0017] In some examples, the TLR9 agonist is administered weekly at a dose from about 0.5 mg to about 20 mg. In other examples, the TLR9 agonist is administered weekly at a dose from about 1 mg to about 10 mg. In another example, the TLR9 agonist is administered weekly at a dose from about 2 mg to about 8 mg. In a further example, the TLR9 agonist is administered weekly at a dose from about 4 mg to about 8 mg. In some examples, the TLR9 agonist is administered weekly at a dose of about 2 mg.

[0018] In one particular example of method of treating uveal melanoma (e.g., Stage IV uveal melanoma) comprising locally administering the TLR9 agonist having the sequence of

SEQ ID NO: 2 at a dose of about 2 mg or 2 mg, preferably via hepatic arterial infusion (HAI) and more preferably with PEDD. The method also comprises systemically administering a checkpoint inhibitor, in particular, an anti-PD1 or anti-PDL1 antibody, and specifically nivolumab. The method may further comprise administering an anti-CTLA4 antibody (e.g., ipilimumab) in addition to the anti-PD1 or anti-PDL1 antibody.

[0019] In some examples, the TLR9 agonist is administered weekly. In certain examples, the TLR9 agonist may be administered weekly in 3 week cycles with a rest period between cycles. The TLR9 agonist may be administered each week for a period of time of about 10 to about 200 minutes. The TLR9 agonist may be administered through a catheter device. The catheter device may comprise a one-way valve that responds dynamically to local pressure and/or flow changes. For example, the TLR9 agonist may be administered through the catheter device via pressure-enabled drug delivery (PEDD). In other examples, the TLR9 agonist is administered weekly for a period of time of about 10 to about 60 minutes. In a further example, the TLR9 agonist is administered for a period of time of about 25 minutes.

[0020] 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

[0021] 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 examples of the present disclosure.

[0022] FIG. 1 shows the chemical structure of a sodium salt of SD-101.

[0023] FIG. 2A illustrates a Phase 1/1B clinical study protocol described in Example 1.

[0024] FIG. 2B provides characteristics of patients that underwent the Phase 1/1B clinical trial of Fig. 2A, wherein LDH is the abbreviation for lactic dehydrogenase levels, and ULN is the abbreviation for upper limit of normal.

- [0025] FIG. 2C provides the safety summary and adverse events for Cohorts A, B, and C administered SD-101 and/or CPI, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0026] FIG. 3 provides serum pharmacokinetic profiles of SD-101 following administration at 2 mg, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0027] FIG. 4 provides serum pharmacokinetic profiles of SD-101 following administration at 4 mg, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0028] FIG. 5 provides serum pharmacokinetic profiles of SD-101 following administration at 8 mg, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0029] FIG. 6 provides liver tissue pharmacokinetic profiles of SD-101 following administration at 8 mg, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0030] FIG. 7A provides serum cytokine analysis for CXCL10 (IP-10) following TLR9 agonist administration, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0031] FIG. 7B provides serum cytokine analysis for IL-8 following TLR9 agonist administration, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0032] FIG. 8A provides serum cytokine analysis for IL-2R following TLR9 agonist administration, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0033] FIG. 8B provides serum cytokine analysis for IFN γ following TLR9 agonist administration, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0034] FIG. 9A illustrates uveal melanoma liver metastasis TME modulation at Day 57 with MDSC related genes, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0035] FIG. 9B illustrates uveal melanoma liver metastasis TME modulation at Day 57 with T cell related genes, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0036] FIG. 10 illustrates changes in M-MDSCs within tumors after 57 days following administration of SD-101, according to the Phase 1/1B clinical study protocol of FIG. 2A.
- [0037] FIG. 11A illustrates circulating immune cell analysis following administration of SD-101 for changes in circulating NK cell protein expression patterns (Day 1 – Day 36), according to the Phase 1/1B clinical study protocol of FIG. 2A. Data represented as Mean + SEM (n=19).

[0038] FIG. 11B illustrates circulating immune cell analysis following administration of SD-101 for changes in circulating CD8 T cell protein expression patterns (Day 1 – Day 36), according to the Phase 1/1B clinical study protocol of FIG. 2A. Data represented as Mean + SEM (n=23).

[0039] FIG. 12A illustrates circulating immune cell analysis following administration of SD-101 for changes in circulating CD8 T cell protein expression patterns (Day 1 – Day 36), according to the Phase 1/1B clinical study protocol of FIG. 2A. Data represented as Mean + SEM (n=23).

[0040] FIG. 12B illustrates circulating immune cell analysis following administration of SD-101 for changes in protein expression on circulating CD8+ T cells (Day 1- Day 36) for Cohorts A, B and C, according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0041] FIG. 12C provides the data of FIG. 12B separated by based on the amount of SD-101 (also known as nelitolimod) administered.

[0042] FIG. 12D illustrates circulating immune cell analysis following administration of SD-101 for changes in protein expression on circulating NK cells (Day 1- Day 36) for Cohorts A, B and C, according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0043] FIG. 12E provides the data of FIG. 12D separated by based on the amount of SD-101 (also known as nelitolimod) administered.

[0044] FIG. 13A provides response and survival times for cohorts A, B, and C for heavily pre-treated patients, according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0045] FIG. 13B provides response and survival times for cohort B for heavily pre-treated patients, according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0046] FIG. 14A shows changes in ctDNA levels in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0047] FIG. 14B provides additional data illustrating changes in ctDNA levels in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0048] FIG. 14C provides additional data illustrating changes in ctDNA levels in Cohort B patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0049] FIG. 14D provides data illustrating changes in ctDNA levels in Cohort B and C patients for Post-Cycle 1 time points (including Day 36 and Day 57 unless otherwise noted) treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0050] FIG. 14E provides the data of FIG. 14D separated by Cohorts B and C.

[0051] FIG. 14F provides data illustrating reductions in ctDNA mutant allele fractions (MAF) from baseline in Cohort B and C patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0052] FIG. 14G provides the data of FIG. 14F separated by Cohorts B and C.

[0053] FIG. 15A shows changes in CD8⁺ T cells for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0054] FIG. 15B shows changes in CD4⁺ T cells for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0055] FIG. 15C shows changes in NK cells for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0056] FIG. 15D shows changes in M1 Macrophages for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0057] FIG. 16A shows changes in in CD8⁺ T cells for patients in cohort B in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0058] FIG. 16B shows changes in in CD4⁺ T cells for patients in cohort B in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0059] FIG. 16C shows changes in in NK cells for patients in cohort B in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0060] FIG. 16D shows changes in in M1 Macrophages for patients in cohort B in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0061] FIG. 17A shows changes in tumor pathway scores from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0062] FIG. 17B shows changes in PBMC pathway scores from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0063] FIG. 17C shows changes in tumor pathway scores split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0064] FIG. 17D shows the changes in tumor pathway scores of FIG. 17C split by dose of SD-101 administered.

[0065] FIG. 17E shows changes in PBMC pathway scores split by Cohort from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0066] FIG. 17F shows the changes in PBMC pathway scores of FIG. 17E split by dose of SD-101 administered.

[0067] FIG. 17G shows changes in tumor gene expression levels split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0068] FIG. 17H shows the changes in tumor gene expression levels of FIG. 17G split by dose of SD-101 administered.

[0069] FIG. 17I shows additional data demonstrating changes in tumor gene expression levels split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0070] FIG. 17J shows the changes in tumor gene expression levels of FIG. 17I split by dose of SD-101 administered.

[0071] FIG. 17K shows changes in PBMC gene expression levels split by Cohort from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0072] FIG. 17L shows the changes in PBMC gene expression levels of FIG. 17K split by dose of SD-101 administered.

[0073] FIG. 17M shows additional data demonstrating changes in PBMC gene expression levels split by Cohort from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0074] FIG. 17N shows the changes in PBMC gene expression levels of FIG. 17M split by dose of SD-101 administered.

[0075] FIG. 18A shows changes in Treg with increasing doses of SD-101 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0076] FIG. 18B shows changes in M-MDSC with increasing doses of SD-101 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0077] FIG. 18C shows changes in Granzyme B with increasing doses of SD-101 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0078] FIG. 18D shows changes in IL-15 with increasing doses of SD-101 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0079] FIG. 19A illustrates the change in cell concentration from baseline data for Treg cells for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0080] FIG. 19B illustrates the change in cell concentration from baseline data for M-MDSC cells for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0081] FIG. 19C illustrates the change in cell concentration from baseline data for total MSDC cells for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0082] FIG. 19D illustrates the change in cell concentration from baseline data for M2 macrophage cells for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0083] FIG. 19E illustrates the change in cell concentration from baseline data for Granzyme B for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0084] FIG. 19F illustrates the change in cell concentration from baseline data for IL-15 for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0085] FIG. 20A shows changes in peripheral immune signature CXCL10 (IP-10) in patients treated with different doses of SD-101 delivered via PEDD according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0086] FIG. 20B shows changes in peripheral immune signature IFN γ in patients treated with different doses of SD-101 delivered via PEDD according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0087] FIG. 20C shows changes in peripheral immune signature TNF α in patients treated with different doses of SD-101 delivered via PEDD according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0088] FIG. 20D shows changes in peripheral immune signature IL-2R induced by SD-101 in patients treated with different doses of SD-101 delivered via PEDD according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0089] FIG. 20E shows changes in peripheral immune signature IL-15 in patients treated with different doses of SD-101 delivered via PEDD according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0090] FIG. 20F shows changes in peripheral immune signature IL-18 in patients treated with different doses of SD-101 delivered via PEDD according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0091] FIG. 21A shows changes in immune signature in circulation of CXCL10 (IP-10) for Cohorts A, B and C according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0092] FIG. 21B shows changes in immune signature in circulation of IFN γ for Cohorts A, B and C according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0093] FIG. 21C shows changes in immune signature in circulation of TNF α for Cohorts A, B and C according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0094] FIG. 21D shows changes in immune signature in circulation of IL-2R induced by SD-101 for Cohorts A, B and C according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0095] FIG. 21E shows changes in immune signature in circulation of IL-15 for Cohorts A, B and C according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0096] FIG. 21F shows changes in immune signature in circulation of IL-18 for Cohorts A, B and C according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0097] FIG. 22A shows changes in immune signature in circulation of CXCL10 (IP-10) for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0098] FIG. 22B shows changes in immune signature in circulation of IFN γ for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[0099] FIG. 22C shows changes in immune signature in circulation of TNF α for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00100] FIG. 22D shows changes in immune signature in circulation of IL-2R induced by SD-101 for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00101] FIG. 22E shows changes in immune signature in circulation of IL-15 for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00102] FIG. 22F shows changes in immune signature in circulation of IL-18 for cohort B at 2 mg, 4 mg, and 8 mg according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00103] FIG. 23A shows changes in circulating CD8 $^{+}$ T cells from Day 1 to Day 36 in all cohorts A, B and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00104] FIG. 23B shows changes in protein expression patterns on circulating NK $^{+}$ cells from Day 1 to Day 36 in cohort B at 2 mg, 4 mg, and 8 mg in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00105] FIG. 23C shows systemic increase in proliferation of NK cells in all cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00106] FIG. 23D shows systemic increase in proliferation of NK cells in cohort B at 2 mg, 4 mg, and 8 mg in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00107] FIG. 24A shows objective RECIST 1.1 response rates for patients in cohorts B at 2 mg, 4 mg, and 8 mg, and cohort C at 2 mg in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00108] FIG. 24B shows objective RECIST 1.1 response rates for patients from FIG 24A and additional patients in cohorts B at 2 mg, 4 mg, and 8 mg, and cohort C at 2 mg and 4 mg treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00109] FIG. 25A illustrates the overall survival proportions of cohorts A, B, and C, and the probability of survival over weeks for 2 mg SD-101 via PEDD and IV anti-PD-1 in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00110] FIG. 25B illustrates the overall survival proportions of cohort B, and the probability of survival over weeks for 2 mg, 4 mg, and 8 mg SD-101 in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00111] FIG. 25C illustrates the overall survival proportions of cohorts A, B, and C, and the probability of survival over weeks for patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. The data show in FIG. 25 includes the data provided in FIG. 25A and additional data.

[00112] FIG. 25D illustrates overall survival proportions for Cohort B at 2 mg, 4 mg, and 8 mg dose of SD-101 from the data of FIG. 25C.

[00113] FIG. 25E illustrates overall survival proportions for Cohort B and C at 2 mg and 4 mg dose of SD-101 from the data of FIG. 25C.

[00114] FIG. 26A illustrates changes in cell densities within tumors split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00115] FIG. 26B shows the changes in cell densities within tumors of FIG. 26A split by dose of SD-101 administered.

[00116] FIG. 26C provides additional data demonstrating changes in cell densities within tumors split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00117] FIG. 26D shows the additional changes in cell densities within tumors of FIG. 26C split by dose of SD-101 administered.

[00118] FIG. 26E provides further demonstrating changes in cell densities within tumors split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00119] FIG. 26F shows the further changes in cell densities within tumors of FIG. 26E split by dose of SD-101 administered.

[00120] FIG. 26G provides further demonstrating changes in cell densities within tumors split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00121] FIG. 26H shows the further changes in cell densities within tumors of FIG. 26G split by dose of SD-101 administered.

[00122] FIG. 26I provides further demonstrating changes in cell densities within tumors split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00123] FIG. 26J shows the further changes in cell densities within tumors of FIG. 26I split by dose of SD-101 administered.

[00124] FIG. 26K provides further demonstrating changes in cell densities within tumors split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00125] FIG. 26L shows the further changes in cell densities within tumors of FIG. 26K split by dose of SD-101 administered.

[00126] FIG. 26M provides further demonstrating changes in cell densities within tumors split by Cohort from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00127] FIG. 26N shows the further changes in cell densities within tumors of FIG. 26M split by dose of SD-101 administered.

[00128] FIG. 27A shows changes in plasma immune markers split by Cohort from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00129] FIG. 27B shows the changes in plasma immune markers of FIG. 27A split by dose of SD-101 administered.

[00130] FIG. 27C provides additional data showing changes in plasma immune markers split by Cohort from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00131] FIG. 27D shows the additional changes in plasma immune markers of FIG. 27C split by dose of SD-101 administered.

[00132] FIG. 27E provides further data showing changes in plasma immune markers split by Cohort from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00133] FIG. 27F shows the further changes in plasma immune markers of FIG. 27E split by dose of SD-101 administered.

[00134] FIG. 27G provides further data showing changes in plasma immune markers split by Cohort from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A.

[00135] FIG. 27H shows the further changes in plasma immune markers of FIG. 27G split by dose of SD-101 administered.

[00136] 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

[00137] 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.

[00138] 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.

[00139] The singular forms “a,” “an,” and, “the” include plural references unless the context clearly dictates otherwise.

[00140] 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.

[00141] 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.

[00142] 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.

[00143] 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.

[00144] 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.

[00145] 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.

[00146] 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.

[00147] 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.

[00148] The present application is directed to methods of treating, ameliorating or modulating uveal melanoma liver metastases 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 subject may be any human in need of treatment for uveal melanoma liver metastases. In particular, the human subject may have Stage IV uveal melanoma. In some examples, the human subject may have UM LM with liver-only or liver-dominant disease. According to another example, the human subject is male or female, and is eighteen years of age or older. The therapeutically effective combination of one or more checkpoint inhibitors and a toll-like receptor 9 (TLR9) agonist may provide improved disease control as compared administration of the one or more checkpoint inhibitors without the TLR9 agonist or administration of the TLR9 agonist without the one or more checkpoint inhibitors.

[00149] 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 UM LM 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 UM LM 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 UM LM in a human subject.

[00150] The therapeutically effective combination of one or more checkpoint inhibitors and a TLR9 agonist may be administered to the human subject concurrently with (*e.g.*, at the same time), at separate times, or administered intermittently with each other. For example, the therapeutically effective combination may include administering the TLR9 agonist over the same period of time during which the one or more checkpoint inhibitors is administered to the human

subject. In particular, the human subject may be undergoing therapy where the TLR9 agonist and the one or more checkpoint inhibitors are administered within the same day. In another example, the human subject may be undergoing therapy where the TLR9 agonist and the one or more checkpoint inhibitors are administered separately. The length of time between administration of the TLR9 agonist and the one or more checkpoint inhibitors may be adjusted to achieve the desired therapeutic effect. The one or more checkpoint inhibitor may be administered before the TLR9 agonist, or after the TLR9 agonist.

[00151] Direct needle injection is often not feasible for patients with multiple liver metastases (LM) and does not permit uniform drug delivery within individual tumors. In some examples, the TLR9 agonist is locally administered to an area in the liver of the subject external to the uveal melanoma liver metastases. As discussed further below Pressure-Enabled Drug Delivery (PEDD) via hepatic artery infusion (PEDD/HAI) may be used to administer TLR9 agonists to the liver of the human subject to enable effective delivery of the TLR9 agonist to UM LM and penetration into high-pressure tumors, while limiting system exposure. The intravascular route also permits treatment of dysfunctional immune cells throughout the organ.

Toll-like Receptor Agonists

[00152] 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. 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.

[00153] 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.

[00154] 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.

[00155] In one example, the TLR9 agonist is a CpG-C ODN comprising the sequence 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 SD-101 (also referred to herein as nelitolimod) or a pharmaceutically acceptable salt thereof. SD-101 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 SD-101 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 SD-101.

[00156] The molecular formula of SD-101 in a free acid form is C₂₉₃ H₃₆₉ N₁₁₂ O₁₄₉ P₂₉ S₂₉ and the molecular mass of the SD-101 in the free acid form is 9672 Daltons. The molecular formula of the sodium salt of SD-101, as shown in FIG. 1, is C₂₉₃ H₃₄₀ N₁₁₂ O₁₄₉ P₂₉ S₂₉ Na₂₉ and the molecular mass of the sodium salt of SD-101 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 SD-101.

[00157] The TLR9 agonist, specifically SD-101, may impact multiple cell types to prime the tumor microenvironment (TME) for checkpoint inhibitor treatment. Administration of SD-101 may lead to MDSC depletion, T-cell activation and recruitment.

[00158] The TLR9 agonist may be a CpG-C ODN containing naturally-occurring or modified, non-naturally occurring bases, and may contain modified sugar, phosphate, and/or termini as compared to SD-101. For example, in addition to phosphodiester linkages, phosphate modifications include, but are not limited to, methyl phosphonate, phosphorothioate, phosphoramidate (bridging or non-bridging), phosphotriester and phosphorodithioate and may be used in any combination. In an example, CpG-C ODNs have only phosphorothioate linkages, only phosphodiester linkages, or a combination of phosphodiester and phosphorothioate linkages.

[00159] Sugar modifications known in the field, such as 2'-alkoxy-RNA analogs, 2'-amino-RNA analogs, 2'-fluoro-DNA, and 2'-alkoxy- or amino-RNA/DNA chimeras and others described herein, may also be made and combined with any phosphate modification. Examples of base modifications include but are not limited to addition of an electron-withdrawing moiety

to C-5 and/or C-6 of a cytosine of the CpG-C ODN (e.g., 5-bromocytosine, 5-chlorocytosine, 5-fluorocytosine, 5-iodocytosine) and C-5 and/or C-6 of a uracil of the CpG-C ODN (e.g., 5-bromouracil, 5-chlorouracil, 5-fluorouracil, 5-iodouracil). As noted above, use of a base modification in a palindromic sequence of a CpG-C ODN should not interfere with the self-complementarity of the bases involved for Watson-Crick base pairing. However, outside of a palindromic sequence, modified bases may be used without this restriction. For instance, 2'-O-methyl-uridine and 2'-O-methyl-cytidine may be used outside of the palindromic sequence, whereas, 5-bromo-2'-deoxycytidine may be used both inside and outside the palindromic sequence. Other modified nucleotides, which may be employed both inside and outside of the palindromic sequence include 7-deaza-8-aza-dG, 2-amino-dA, and 2-thio-dT.

[00160] Duplex (i.e., double stranded) and hairpin forms of most ODNs are often in dynamic equilibrium, with the hairpin form generally favored at low oligonucleotide concentration and higher temperatures. Covalent interstrand or intrastrand cross-links increase duplex or hairpin stability, respectively, towards thermal-, ionic-, pH-, and concentration-induced conformational changes. Chemical cross-links can be used to lock the polynucleotide into either the duplex or the hairpin form for physicochemical and biological characterization. Cross-linked ODNs that are conformationally homogeneous and are “locked” in their most active form (either duplex or hairpin form) could potentially be more active than their uncross-linked counterparts. Accordingly, some CpG-C ODNs of the present disclosure can contain covalent interstrand and/or intrastrand cross-links.

[00161] The TLR9 agonist may be a Class type C CPG -ODN (CpG-C) oligonucleotide (ODN) modified from SD-101. In the CpG-C oligonucleotide, the sugar moiety is preferably the furanoside of ribose, deoxyribose, arabinose or 2'-O-alkylribose, and the sugar can be attached to the respective heterocyclic bases in either anomeric configuration. Sugar modifications may also be made and combined with any phosphate modification in the preparation of a CpG-C ODN. The heterocyclic bases, or nucleic acid bases, which are incorporated in the CpG-C ODN can be the naturally-occurring principal purine and pyrimidine bases, (namely uracil, thymine, cytosine, adenine and guanine, as mentioned above), as well as naturally-occurring and synthetic modifications of said principal bases. Thus, a CpG-C ODN may include one or more of inosine, 2'-deoxyuridine, and 2-amino-2'-deoxyadenosine.

[00162] 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).

[00163] 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.

[00164] 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.

[00165] 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.

[00166] The pharmaceutical compositions may comprise a tonicity adjusting agent. Suitable tonicity adjusting agents include for instance dextrose, glycerol, sodium chloride, glycerin, and mannitol.

[00167] 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.

[00168] Table 1 describes an exemplary pharmaceutical composition comprising SD-101, wherein SD-101 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
SD-101 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)

* SD-101 Drug Substance in Table 1 reflects the totality of all oligonucleotide content, including SD-101.

[00169] In some examples, SD-101 may be present in a pharmaceutical composition at a concentration from about 0.1 mg/mL to about 20 mg/mL. In particular, SD-101 may be present in a pharmaceutical composition at a concentration of 13.4 mg/mL.

Locally Administering TLR9 Agonist to the Liver

[00170] As discussed above, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof (e.g., the sodium salt shown in FIG. 1) is locally

administered to the liver of the human subject via hepatic artery infusion (HAI). HAI refers to the infusion of a treatment into the hepatic artery of the liver or branches of the hepatic artery. For example, the TLR9 agonist is introduced through percutaneous introduction of a device into a branch of a hepatic artery. 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. In certain examples, the catheter is a microcatheter. In one example, the local administration of the TLR9 agonist provides penetration of the TLR9 agonist, more particularly, SD-101, throughout the UM LM tumors in the liver, substantially throughout the entirety of the UM LM tumors in the liver, or throughout the entire liver of the human subject.

[00171] In particular, the TLR9 agonist is infused via HAI into the hepatic artery or a branch of the hepatic artery under pressure (*i.e.*, at an increased pressure relative to the unaltered intravascular pressure of the hepatic artery). More particularly, the TLR9 agonist is infused via HAI using a 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 hepatic artery or a branch of the hepatic artery relative to the unaltered intravascular pressure of the hepatic artery. The fluid pressure modulating structure may intermittently occlude the hepatic artery or a branch of the hepatic artery as it modulates the pressure of the hepatic artery or the branch of the hepatic artery in which it is inserted. The fluid pressure modulating structure is configured to intermittently increase pressure within the hepatic artery or a branch of the hepatic artery by an amount sufficient to overcoming interstitial fluid pressure and solid stress of the UM LM tumors in the liver of the human subject.

[00172] In some examples, the device for hepatic artery infusion (HAI) 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 or mechanism (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, such as by causing a decrease in pressure at placement and during the TLR9 agonist infusion, and an increase of pressure during saline bolus or during bolus infusion of the TLR9 agonist. In some examples, the system is designed to continuously monitor real-time pressure or flow throughout the procedure. 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 while allowing for whole liver treatment. 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.

[00173] In some examples, the device that may be used for HAI 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. In certain examples, the device may be a device known as the Surefire Infusion System.

[00174] More specifically, the TLR9 agonist is infused via HAI into the hepatic artery or a branch of the hepatic artery using a Pressure-Enabled Drug-Delivery (PEDD) device. PEDD devices are configured to overcome intratumoral pressure of the UM LM tumors through

creation of a favorable pressure gradient, while at the same time protecting off-target organs through anti-reflux properties.

[00175] In some examples, the PEDD device is a device as disclosed in U.S. Patent Publication No. 2020-0383688. In certain examples, the PEDD device may be a device known as the TriSalus Infusion System. In certain examples, the PEDD device may be a device known as the TriNav[®] Infusion System. The TriNav[®] Infusion System is a single lumen catheter equipped with a one-way valve that responds dynamically to local pressure and flow changes, such as those arising from the cardiac cycle or generated by infusion. The valve structure modulates distal vascular pressures and blood flow. This in turn may alter therapeutic distribution and first-pass absorption due to increased contact time within the vasculature.

[00176] 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.

[00177] In one example, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof, is infused on a weekly basis using an infusion procedure that lasts approximately 10-200 minutes. In another example, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof, is infused on a weekly basis using an infusion procedure that lasts approximately 10-60 minutes. In another example, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof, is infused on a weekly basis using an infusion procedure that lasts approximately 25 minutes.

[00178] In some examples, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof, is locally administered to the liver of the subject via hepatic artery infusion at a dose sufficient to provide a concentration in the liver that is therapeutically effective in combination with the checkpoint inhibitor while providing a plasma concentration of the

TLR9 agonist that is below the concentration in the liver. In particular, the plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject. In one example, the plasma concentration of the TLR9 agonist is at a level that is a subtherapeutic level, *i.e.*, not therapeutic for the treatment of UM LM alone or in combination with one or more checkpoint inhibitors.

[00179] The amount of TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof, may be administered at a dose that is lower than the maximally tolerated dose, specifically, when it is administered locally via HAI, and more specifically when it is administered via HAI with PEDD. In some examples, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof, is locally administered to the liver of the subject via HAI at a dose sufficient to provide a concentration in the liver from about 750 ng/g to about 3000 ng/g, while providing a plasma concentration of the TLR9 agonist that is below the concentration in the liver, such as, for example, at a plasma concentration of less than about 750 ng/mL, less than about 700 ng/mL, or less than about 600 ng/mL. In other examples, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof, is locally administered to the liver of the subject via HAI at a dose that provides a ratio of a plasma concentration of the TLR9 agonist to a concentration of the TLR9 agonist is from about 1:2 to about 1:10, or from about 1:3 to about 1:7.

[00180] In some examples, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof may be administered weekly at a dose from about 0.5 mg to about 20 mg, from about 1 mg to about 10 mg, from about 2 mg to about 8 mg, or from about 4 mg to about 8 mg. In some examples, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof may be administered weekly at a dose of about 1.5 mg, about 2 mg, about 2.5 mg, about 3 mg, about 3.5 mg, about 4 mg, about 4.5 mg, about 5 mg, about 5.5 mg, about 6 mg, about 6.5 mg, about 7 mg, about 7.5 mg, or about 8 mg. The milligram amounts (e.g. about 2 mg) of SD-101 described in the present application refers to an amount (about 2 mg) of the sodium salt of SD-101 illustrated in FIG. 1, unless otherwise noted. Equivalent molar amounts of a free acid or other pharmaceutically acceptable salts are also contemplated.

[00181] In some examples, SD-101 is administered for the treatment of UM LM in a human subject a weekly dose of 2 mg through HAI, and in some examples, the SD-101 is further

administered through a device that modulates pressure (i.e. PEDD). The pressure may be modulated in sync with the cardiac cycle of the human subject, such as, for example, with the TriNav® Infusion System as described above. In some examples, SD-101 is administered for the treatment of UM LM in a human subject a weekly dose of 4 mg through HAI, and in some examples, the SD-101 is further administered through a device that modulates pressure (i.e. PEDD), wherein the pressure may be modulated in sync with the cardiac cycle of the human subject, such as, for example, with the TriNav® Infusion System as described above. In some examples, SD-101 is administered for the treatment of UM LM in a human subject a weekly dose of 8 mg through HAI, and in some examples, the SD-101 is further administered through a device that modulates pressure (i.e. PEDD), wherein the pressure may be modulated in sync with the cardiac cycle of the human subject, such as by using the TriNav® Infusion System as described above.

[00182] In some examples, the TLR9 agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof may be administered in a dosing regimen comprising cycles, in which one or more of the cycles comprise administering SD-101 via HAI and/or PEDD. As used herein, a “cycle” is a repeat of a dosing sequence. In one example, one cycle comprises three weekly doses per cycle (i.e. administration of SD-101 once per week over three consecutive weeks). In one example, a cycle of treatment according to the present application may comprise periods of SD-101 administration followed by “off” periods or rest periods. In another example, in addition to three weekly doses per cycle, the cycle further comprises one week, two weeks, three weeks, or four weeks as a rest period following the weekly administration of SD-101. In yet another example, in addition to three weekly doses per cycle, the cycle further comprises about thirty-eight days as a rest period following the weekly administration of SD-101. In another example, the entire cycle comprises about fifty-two days. In another example, the dosing regimen comprises at least one, at least two, or at least three cycles, or longer. The weekly doses as described herein may be modified to be administered weekly within a cycle.

Checkpoint Inhibitors

[00183] 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. Alternative names or synonyms for PD-1 and its ligands include: PDCD1, PD1, CD279 and SLEB2 for PD-1; PDCD1L1, PDL1, B7H1, B7-4, CD274 and B7-H for PD-L1; and PDCD1L2, PDL2, B7-DC, Btde and CD273 for PD-L2. Specifically, in the treatment methods, medicaments and uses of the present application in which a human subject is being treated, the PD-1 antagonist blocks binding of human PD-L1 to human PD-1, and preferably blocks binding of both human PD-L1 and PD-L2 to human PD-1.

[00184] 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.

[00185] According to an examples, the PD-1 antagonist can include an immunoadhesin that specifically binds to PD-1 or PD-L1, and preferably specifically binds to human PD-1 or human PD-L1, e.g., a fusion protein containing the extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region such as an Fc region of an immunoglobulin molecule.

[00186] According to an examples, the PD-1 antagonist can block PD-L1 expressed by tumor cells and MDSC, and other suppressive immune cells.

[00187] According to an examples, the PD-1 antagonist can inhibit the binding of PD-L1 to PD-1, and preferably also inhibits the binding of PD-L2 to PD-1. In some examples of the above treatment method, medicaments and uses, 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.

[00188] According to an example, the PD-1 antagonist can be one of nivolumab, pembrolizumab, and cemiplimab. According to another example, nivolumab is administered intravenously (IV) via a peripheral vein at a dose of 480 mg every four weeks (“Q4W”) or 240 mg every two weeks (“Q2W”). According to another example, nivolumab is administered intravenously (IV) via a peripheral vein at a dose of nivolumab 360 mg every three weeks (“Q3W”). In another example, nivolumab dosing is weight-based, at nivolumab 3 mg/kg Q2W or 10 mg/kg Q2W. In another example, nivolumab dosing is weight-based at nivolumab 1 mg/kg Q3W. In yet another example, nivolumab is administered concomitantly, at the same time, at about the same time, or on the same day with SD-101. In another example, nivolumab 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 SD-101. A “cycle” of administration of SD-101 is as described above.

[00189] According to another example, pembrolizumab is administered intravenously (IV) via a peripheral vein at a dose of 200 mg Q3W or 400 mg every 6 weeks (“Q6W”). In another example, pembrolizumab is administered concomitantly, at the same time, at about the same time, or on the same day with SD-101.

[00190] According to another example, the CPI can include a PD-L1 antagonist. In this regard, the PD-L1 antagonist can be one of atezolizumab, avelumab, and durvalumab.

[00191] According to another example, the CPI can include a CTLA-4 antagonist. In this regard, the CTLA-4 antagonist can be ipilimumab. According to another example, ipilimumab is administered intravenously (IV) via a peripheral vein at a dose of 3 mg/kg every three weeks. In yet another example, ipilimumab is administered concomitantly, at the same time, at about the same time, or on the same day with SD-101 and/or nivolumab. In another example, ipilimumab is administered once a week, every other week, every three weeks, every four weeks, or on a monthly basis following the administration of one or more cycles of SD-101 and/or nivolumab.

[00192] According to another example, the CPI can include a lymphocyte activation gene-3 (LAG-3) inhibitor. In this regard, the LAG-3 inhibitor can be relatlimab.

[00193] According to another example, the combination of one or more checkpoint inhibitors and a TLR9 agonist may be administer with other cancer therapeutics such as 33ressu-modulators, tumor-killing agents, and/or other targeted therapeutics.

[00194] According to an example, the combination of one or more checkpoint inhibitors and a TLR9 agonist may be administered in combination with cell therapy (thereby enabling cell therapy by modulation of the immune system), chemoembolic treatment, or radioembolic treatment.

[00195] In some examples, the human subject is administered a toll-like receptor 9 (TLR9) agonist, in particular, SD-101 or a pharmaceutically acceptable salt thereof, and one or more checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab. The human subject may be administered SD-101 in combination with nivolumab. The human subject may be administered SD-101 in combination with pembrolizumab. The human subject may be administered SD-101 in combination with ipilimumab. The human subject may be administered SD-101 in combination with crefmirlimab. The human subject may be administered SD-101 in combination with two checkpoint inhibitors. For example, the human subject may be administered SD-101 in combination with nivolumab and ipilimumab. In another example, the human subject may be administered SD-101 in combination with nivolumab and relatlimab.

[00196] In some examples, SD-101 is administered for the treatment of UM LM in a human subject a weekly dose of 2 mg through HAI, and in some examples, the SD-101 is further administered through a device that modulates pressure (e.g., dynamically responding to local pressure changes, more specifically, PEDD). The pressure, as described herein in this paragraph, may be modulated in sync with the cardiac cycle of the human subject, such as, for example, with the TriNav® Infusion System as described above. In some examples, SD-101 is administered at a dose of 2 mg through HAI through a device that modulates vascular pressure (e.g., dynamically responding to local pressure changes, more specifically, PEDD) in combination with a CPI. For example, the CPI may be administered intravenously (IV) or subcutaneously (SQ). In some examples, SD-101 is administered at a weekly dose of 2 mg through HAI through a device that modulates vascular pressure in combination with a CPI, wherein the CPI is nivolumab. In other examples, SD-101 is administered at a weekly dose of 2 mg through HAI and through a device that modulates pressure in combination with pembrolizumab. In another examples, SD-101 is administered at a weekly dose of 2 mg through HAI and through a device that modulates pressure in combination with ipilimumab. In another examples, SD-101 is administered at a weekly dose of 2 mg through HAI and through a device

that modulates pressure in combination with crefmirlimab. In another examples, SD-101 is administered at a weekly dose of 2 mg through HAI and through a device that modulates pressure in combination with nivolumab and ipilimumab. In another examples, SD-101 is administered at a dose of 2 mg through HAI and through a device that modulates pressure in combination with nivolumab and relatlimab.

[00197] In some examples, SD-101 is administered for the treatment of UM LM in a human subject a weekly dose of 4 mg through HAI, and in some examples, the SD-101 is further administered through a device that modulates pressure (e.g., dynamically responding to local pressure changes, more specifically, PEDD). The pressure, as described herein in this paragraph, may be modulated in sync with the cardiac cycle of the human subject, such as, for example, with the TriNav® Infusion System as described above. In some examples, SD-101 is administered at a dose of 4 mg through HAI through a device that modulates vascular pressure (e.g., dynamically responding to local pressure changes, more specifically, PEDD) in combination with a CPI. For example, the CPI may be administered intravenously (IV) or subcutaneously (SQ). In some examples, SD-101 is administered at a weekly dose of 4 mg through HAI through a device that modulates vascular pressure in combination with a CPI, wherein the CPI is nivolumab. In other examples, SD-101 is administered at a weekly dose of 4 mg through HAI and through a device that modulates pressure in combination with pembrolizumab. In another examples, SD-101 is administered at a weekly dose of 4 mg through HAI and through a device that modulates pressure in combination with ipilimumab. In another examples, SD-101 is administered at a weekly dose of 4 mg through HAI and through a device that modulates pressure in combination with crefmirlimab. In another examples, SD-101 is administered at a weekly dose of 4 mg through HAI and through a device that modulates pressure in combination with nivolumab and ipilimumab. In another examples, SD-101 is administered at a dose of 4 mg through HAI and through a device that modulates pressure in combination with nivolumab and relatlimab.

[00198] In some examples, SD-101 is administered for the treatment of UM LM in a human subject a weekly dose of 8 mg through HAI, and in some examples, the SD-101 is further administered through a device that modulates pressure (e.g., dynamically responding to local pressure changes, more specifically, PEDD). The pressure, as described herein in this paragraph, may be modulated in sync with the cardiac cycle of the human subject, such as, for example,

with the TriNav® Infusion System as described above. In some examples, SD-101 is administered at a dose of 8 mg through HAI through a device that modulates vascular pressure (e.g., dynamically responding to local pressure changes, more specifically, PEDD) in combination with a CPI. For example, the CPI may be administered intravenously (IV) or subcutaneously (SQ). In some examples, SD-101 is administered at a weekly dose of 8 mg through HAI through a device that modulates vascular pressure in combination with a CPI, wherein the CPI is nivolumab. In other examples, SD-101 is administered at a weekly dose of 8 mg through HAI and through a device that modulates pressure in combination with pembrolizumab. In another examples, SD-101 is administered at a weekly dose of 8 mg through HAI and through a device that modulates pressure in combination with ipilimumab. In another examples, SD-101 is administered at a weekly dose of 8 mg through HAI and through a device that modulates pressure in combination with cefmirlimab. In another examples, SD-101 is administered at a weekly dose of 8 mg through HAI and through a device that modulates pressure in combination with nivolumab and ipilimumab. In another examples, SD-101 is administered at a dose of 4 mg through HAI and through a device that modulates pressure in combination with nivolumab and relatlimab.

[00199] In one aspect of the present application, a method for treating uveal melanoma liver metastases in a human subject is provided. The method comprises administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and a TLR9 agonist. For example, the TLR9 agonist is a TLR9 type C agonist, specifically SD-101 or a hydrochloride salt of SD-101 as shown in FIG. 1. In one example, the TLR9 agonist is an oligonucleotide having the sequence of SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, and more specifically, the TLR9 agonist is an oligonucleotide having the sequence of SEQ ID NO: 2, wherein the entire sequence is linked by phosphorothioate bonds, or a pharmaceutically acceptable salt thereof. The TLR 9 agonist may be locally administered to the liver of the subject, such as via HAI (specifically HAI with PEDD) at a dose sufficient to provide a concentration in the liver that is therapeutically effective in combination with the checkpoint inhibitor and to provide a plasma concentration of the TLR9 agonist that is below the concentration in the liver. In particular, the TLR9 agonist is locally administered to an area in the liver of the subject external to the uveal melanoma liver metastases. In some examples, the TLR9 agonist is infused while modulating distal vascular pressures and blood flow in the

subject. The one or more checkpoint inhibitors may comprise any of the checkpoint inhibitors described above, such as, for example, anti-PD-1 antibodies, anti-PD-L1 antibodies. Specifically, the one or more checkpoint inhibitors comprises nivolumab. The one or more checkpoint inhibitor may be administered in any suitable manner to the human subject. In one example, the one or more checkpoint inhibitors are systematically administered to the subject. The one or more checkpoint inhibitor may be selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab. In a particular example, the TLR9 agonist is SD-101 administered weekly at a dose of about 2 mg in 3 week cycles with a rest period between cycles in combination with nivolumab. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases progression-free survival (PFS) of the subject. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist reduces circulating tumor DNA (ctDNA) in the subject.

[00200] In another one aspect of the present application, a method for increasing survival rate in a human subject with uveal melanoma liver metastases is provided. The method comprises administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and a toll-like receptor 9 (TLR9) agonist. The administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist may increase progression-free survival (PFS) of the subject. The TLR9 agonist is, for example, a TLR9 type C agonist, specifically SD-101 or a hydrochloride salt of SD-101 as shown in FIG. 1. In one example, the TLR9 agonist is an oligonucleotide having the sequence of SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, and more specifically, the TLR9 agonist is an oligonucleotide having the sequence of SEQ ID NO: 2, wherein the entire sequence is linked by phosphorothioate bonds, or a pharmaceutically acceptable salt thereof. The TLR 9 agonist may be locally administered to the liver of the subject, such as via HAI (specifically HAI with PEDD). In particular, the TLR9 agonist is locally administered to an area in the liver of the subject external to the uveal melanoma liver metastases. In some examples, the TLR9 agonist is infused while modulating distal vascular pressures and blood flow in the subject. The one or more checkpoint inhibitors may comprise any of the checkpoint inhibitors described above, such as, for example, anti-PD-1 antibodies, anti-PD-L1 antibodies. Specifically, the one or more checkpoint inhibitors comprises nivolumab. The one or more checkpoint inhibitor may be

administered in any suitable manner to the human subject. In one example, the one or more checkpoint inhibitors are systematically administered to the subject. The one or more checkpoint inhibitor may be selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab. In a particular example, the TLR9 agonist is SD-101 administered weekly at a dose of about 2 mg in 3 week cycles with a rest period between cycles in combination with nivolumab.

[00201] In another aspect of the present application, a method for treating uveal melanoma liver metastases in a human subject is provided. The method comprise administering to the subject a therapeutically effective amount of one or more checkpoint inhibitors; and locally administering, such as via HAI (specifically HAI with PEDD), a toll-like receptor 9 (TLR9) agonist. In some examples, the TLR9 agonist is infused while modulating distal vascular pressures and blood flow in the subject. The one or more checkpoint inhibitors and the TLR9 agonist are administered in a therapeutically effective combination for reducing tumor burden on the subject. For example, the TLR9 agonist is a TLR9 type C agonist, specifically SD-101 or a hydrochloride salt of SD-101 as shown in FIG. 1. In one example, the TLR9 agonist is an oligonucleotide having the sequence of SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, and more specifically, the TLR9 agonist is an oligonucleotide having the sequence of SEQ ID NO: 2, wherein the entire sequence is linked by phosphorothioate bonds, or a pharmaceutically acceptable salt thereof. The one or more checkpoint inhibitors may comprise any of the checkpoint inhibitors described above, such as, for example, anti-PD-1 antibodies, anti-PD-L1 antibodies. Specifically, the one or more checkpoint inhibitors comprises nivolumab. The one or more checkpoint inhibitor may be administered in any suitable manner to the human subject. In one example, the one or more checkpoint inhibitors are systematically administered to the subject. The one or more checkpoint inhibitor may be selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab. In a particular example, the TLR9 agonist is SD-101 administered weekly at a dose of about 2 mg in 3 week cycles with a rest period between cycles in combination with nivolumab. In one example, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases survival rate of the subject. In another example, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases progression-free survival (PFS) of the subject. In a further example, administration of the

combination of the one or more checkpoint inhibitors and the TLR9 agonist reduces circulating tumor DNA (ctDNA) in the subject.

[00202] In another aspect of the present application, a method for reducing circulating tumor DNA (ctDNA) in a human subject with uveal melanoma liver metastases is provided. The method comprising administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and a toll-like receptor 9 (TLR9) agonist. For example, the TLR9 agonist is a TLR9 type C agonist, specifically SD-101 or a hydrochloride salt of SD-101 as shown in FIG. 1. In one example, the TLR9 agonist is an oligonucleotide having the sequence of SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, and more specifically, the TLR9 agonist is an oligonucleotide having the sequence of SEQ ID NO: 2, wherein the entire sequence is linked by phosphorothioate bonds, or a pharmaceutically acceptable salt thereof. The TLR 9 agonist may be locally administered to the liver of the subject, such as via HAI (specifically HAI with PEDD) at a dose sufficient to provide a concentration in the liver that is therapeutically effective in combination with the checkpoint inhibitor. In some examples, the TLR9 agonist is infused while modulating distal vascular pressures and blood flow in the subject. The one or more checkpoint inhibitors may comprise any of the checkpoint inhibitors described above, such as, for example, anti-PD-1 antibodies, anti-PD-L1 antibodies. Specifically, the one or more checkpoint inhibitors comprises nivolumab. The one or more checkpoint inhibitor may be administered in any suitable manner to the human subject. In one example, the one or more checkpoint inhibitors are systematically administered to the subject. The one or more checkpoint inhibitor may be selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab. In a particular example, the TLR9 agonist is SD-101 administered weekly at a dose of about 2 mg in 3 week cycles with a rest period between cycles in combination with nivolumab.

[00203] In another aspect of the present application, a method for increasing response rate to treatment with a checkpoint inhibitor in a human subject with uveal melanoma liver metastases is provided. The method comprising locally administering a toll-like receptor 9 (TLR9) agonist in combination with the checkpoint inhibitor. For example, the TLR9 agonist is a TLR9 type C agonist, specifically SD-101 or a hydrochloride salt of SD-101 as shown in FIG. 1. In one example, the TLR9 agonist is an oligonucleotide having the sequence of SEQ ID NO: 1, or a pharmaceutically acceptable salt thereof, and more specifically, the TLR9 agonist is an

oligonucleotide having the sequence of SEQ ID NO: 2, wherein the entire sequence is linked by phosphorothioate bonds, or a pharmaceutically acceptable salt thereof. The TLR 9 agonist may be locally administered to the liver of the subject, such as via HAI (specifically HAI with PEDD) at a dose sufficient to provide a concentration in the liver that is therapeutically effective in combination with the checkpoint inhibitor. In some examples, the TLR9 agonist is infused while modulating distal vascular pressures and blood flow in the subject. The one or more checkpoint inhibitors may comprise any of the checkpoint inhibitors described above, such as, for example, anti-PD-1 antibodies, anti-PD-L1 antibodies. Specifically, the one or more checkpoint inhibitors comprises nivolumab. The one or more checkpoint inhibitor may be administered in any suitable manner to the human subject. In one example, the one or more checkpoint inhibitors are systematically administered to the subject. The one or more checkpoint inhibitor may be selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab. In a particular example, the TLR9 agonist is SD-101 administered weekly at a dose of about 2 mg in 3 week cycles with a rest period between cycles in combination with nivolumab. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases survival rate of the subject. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases progression-free survival (PFS) of the subject. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist reduces circulating tumor DNA (ctDNA) in the subject. In some examples, the administration of the TLR9 agonist in combination with the checkpoint inhibitor increases immune cell activation within the uveal melanoma liver metastases of the subject. In some examples, administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases immune cell densities (*e.g.*, CD4 T cells, CD8 T cells and/or MDSCs) in the subject. In some examples, the administration of the TLR9 agonist in combination with the checkpoint inhibitor increases systemic cytokine signaling in the blood of the subject.

[00204] For the methods described above, in some examples, the plasma concentration of the TLR9 agonist is at a dose that is subtherapeutic when the TLR9 agonist is administered without the one or more checkpoint inhibitors. In certain examples, the plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject. In certain examples, the subject has Stage IV uveal melanoma. In certain examples, the TLR9 agonist is locally

administered to an area in the liver of the subject that is external to the uveal melanoma liver metastases. In certain examples, the one or more checkpoint inhibitors is administered intravenously, intraperitoneally or subcutaneously. In certain examples, a ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:2 to about 1:10. In certain examples, the ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:3 to about 1:7. In certain examples, the concentration of the TLR9 agonist in the liver is from about 750 ng/g to about 3000 ng/g. In certain examples, the plasma concentration of the TLR9 agonist is less than about 750 ng/mL. In certain examples, the plasma concentration of the TLR9 agonist is less than about 700 ng/mL. In certain examples, the plasma concentration of the TLR9 agonist is less than about 600 ng/mL. In certain examples, the TLR9 agonist is administered weekly at a dose from about 0.5 mg to about 20 mg. In certain examples, the TLR9 agonist is administered weekly at a dose from about 1 mg to about 10 mg. In certain examples, the TLR9 agonist is administered weekly at a dose from about 2 mg to about 8 mg. In certain examples, the TLR9 agonist is administered weekly at a dose from about 4 mg to about 8 mg. In certain examples, the TLR9 agonist is administered weekly at a dose of about 2 mg. In certain examples, the TLR9 agonist is administered weekly for a period of time of about 10 to about 200 minutes. In certain examples, the TLR9 agonist is administered weekly for a period of time of about 10 to about 60 minutes. In certain examples, the TLR9 agonist is administered for a period of time of about 25 minutes. In certain examples, the one or more checkpoint inhibitor is administered concurrently with the TLR9 agonist. In certain examples, the one or more checkpoint inhibitor is administered before the TLR9 agonist. In certain examples, the one or more checkpoint inhibitor is administered after the TLR9 agonist. In certain examples, the one or more checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab. In certain examples, the one or more checkpoint inhibitor is nivolumab. In certain examples, the one or more checkpoint inhibitor is pembrolizumab. In certain examples, the one or more checkpoint inhibitor is ipilimumab. In certain examples, the one or more checkpoint inhibitor is crefmirlimab. In certain examples, two checkpoint inhibitors are administered.

[00205] 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.

EXAMPLE**Example 1:**

[00206] Metastatic uveal melanoma liver metastasis (MUM-LM) are resistant to immune checkpoint inhibitors (ICIs) for several reasons including the prevalence of myeloid-derived suppressor cells (MDSCs). PFS has been limited, even with approved therapies such as tebentafusp (median 3.3 months) with grade 3/4 AE rates typically >30%. In Example 1, infusions of a class C TLR9 agonist, SD-101, are administered to a human patient in a dose-escalation clinical study (NCT04935229) via pressure-enabled hepatic artery infusion (PEDD/HAI) using the TriNav® Infusion System, with the aim of enhancing response rates to checkpoint inhibitor (CPI) therapy, such as ipilimumab and nivolumab, in human patients with uveal melanoma metastatic to the liver (MUM). SD-101 is delivered over 2 outpatient cycles, with 3 weekly doses/cycle.

[00207] The study included Cohorts A, B, and C, whereby Cohort A is administered SD-101 as a single agent (where n = 3-12), Cohort B is administered SD-101 in combination with an anti-PD-1 CPI, nivolumab (where n = 3-12), and Cohort C is administered SD-101 in combination with nivolumab and an anti-CTLA-4 CPI, ipilimumab (where n = 3-12). The amount of SD-101 administered to human patients in Cohorts A, B, and C is across a dose escalation range from about 2 mg (minimum anticipated biological effect level, MABEL) to about 8 mg per dose (i.e., 2 mg, 4 mg and 8 mg).

[00208] The dosing regimen includes administering SD-101 via PEDD over two cycles, with each cycle comprising three weekly doses of SD-101 (i.e., administration of SD-101 once per week over three consecutive weeks) and a rest period of five weeks following the first cycle of weekly administration of SD-101. Plasma was analyzed for SD-101 levels by LC-MS and cytokine levels by Luminex. NanoString was used to analyze gene expression levels within PBMCs and LM. The nSolver advanced analysis was performed for pathway scoring. FFPE tissue sections were analyzed by multiplex IF using the Akoya Bioscience PhenoCycler-Fusion system staining for DAPI, CD14, HLA-DR, CD79a, CD163, CD68, CD11b, CD8, CD15, CD56, CD3e, CD4, CD20, FOXP3, gp100, & CD45. Quantitative cell density data was generated using Qupath 0.4.3. Flow cytometry was performed to evaluate CD8+ T cell proliferation

(CD45+CD3+CD8+Ki67+), activation (CD45+CD3+CD8+ICOS+) and NK cell proliferation (CD45+CD3-CD56+Ki67+), using Cytoflex.

[00209] FIG. 2A illustrates a Phase 1/1B clinical study protocol including Cohorts A, B, and C and optionally Cohort D. This study is conducted in 2 phases. In Phase 1, a Sentinel Cohort is enrolled to determine the safety of SD-101 delivered via PEDD/HAI with a 2-dose intra-patient dose escalation. The Sentinel Cohort patients receives 2 infusions (2 weeks apart) with assessments for toxicity prior to escalating from the first dose level (0.5 mg) to the second dose level (2 mg). Patient enrollment in the Sentinel Cohort are staggered by 7 days. In the absence of dose-limiting toxicities (DLTs), each patient is eligible to transition into Cohort A at the second infusion time point for dose level 1 (i.e., Cohort A, Day 8 dose). Following completion of the Sentinel Cohort, escalating doses of SD-101 is administered alone (Cohort A), together with nivolumab (Cohort B), and together with combined ipilimumab and nivolumab (Cohort C).

[00210] Cohort A is a dose escalating 3 + 3 design and dose expansion study that initiates with a 2 mg (minimum anticipated biological effect level, MABEL) dose of SD-101, is followed by a 4 mg dose, and is then followed by an 8 mg dose. After the maximum tolerated dose (MTD) for once daily (OD) was determined, an optional dose expansion study is performed. Cohort B is a dose re-escalating 3 + 3 study that is initiated with a 2 mg MABEL dose of SD-101 and 1 mg/kg dose of nivolumab, which is followed by a 4 mg dose of SD-101 and 1 mg/kg dose of nivolumab. After the MTD/OD is determined, an 8 mg dose of SD-101 and 1 mg/kg dose of nivolumab is administered, and an optional Cohort B1 that included SD-101, 2 mg (MABEL), 1 mg/kg dose of nivolumab, and 3 mg/kg dose of ipilimumab may be initiated. Cohort C is a dose re-escalating 3 + 3 study of 2 mg MABEL dose of SD-101 combined with a 1 mg/kg dose of nivolumab and a 3 mg/kg dose of ipilimumab, which is followed by 4 mg SD-101 combined with a 1 mg/kg dose of nivolumab and a 3 mg/kg dose of ipilimumab. After the MTD/OD is determined, an optional dose expansion study of SD-101, nivolumab, and ipilimumab may be performed. In addition, an optional Cohort C1 may be performed that includes SD-101, nivolumab, and relatimab. Cohort D is an optional dose re-escalating 3 + 3 study that determines the MTD/OD of PEDD/HAI of SD-101 for a total of four doses over two cycles + CPI.

[00211] Briefly, in Cohorts A, B and C, SD-101 is administered for two cycles at 1 dose per week for 3 weeks per cycle. The first cycle is administered weekly for three weeks, followed by an off period for five weeks, which is followed by a second cycle of weekly administration of SD-101 for three weeks. In Cohorts B and C, CPI as indicated above is also administered to the human subject. Specifically, in Cohort B, nivolumab 480 mg is administered IV every 4 weeks (Q4W). For Cohort C, dual CPI as administered in the following manner: IV ipilimumab 3 mg/kg and IV nivolumab 1 mg/kg Q3W for 4 doses each followed thereafter by nivolumab 480 mg IV Q4W. In Cohort D (to be conducted), SD-101 may be administered with a dosing schedule with only 2 weekly SD-101 infusions per cycle for 2 cycles in combination with one or more of the following three CPI regimens: (1) Single-agent nivolumab IV at 480 mg every 4 weeks; (2) The CPI doublet of either IV ipilimumab 3 mg/kg and IV nivolumab 1 mg/kg every 3 weeks (Q3W) for 4 doses each followed thereafter by nivolumab 480 mg IV Q4W; or (3) nivolumab 480 mg and relatlimab 160 mg IV Q4W. The checkpoint inhibitors, as described above for Cohorts B, C and D, are concomitantly administered to the human subjects while the subjects are treated with SD-101 over the two cycles and human subjects may be continued to be administered the checkpoint inhibitors for up to 12 months.

[00212] The SD-101 solution is infused via the hepatic arterial system, optionally using TriNav[®] Infusion System. Femoral or brachial/radial access may be used. For the SD-101 infusion procedures, the drug is prepared and delivered in a 50-mL syringe (therapeutic dose) and 100-mL vial containing the volume necessary for the therapeutic flush (10 mL), both at the therapeutic concentration. The 50 mL volume to be administered is allocated by per segment or sector of the liver.

[00213] FIG. 2B illustrates the patient characteristics for patients that underwent testing during Phase 1 clinical trials. Overall, this was a heavily pre-treated population with heavy disease burden in many cases.

[00214] FIG. 2C illustrates the safety summary and adverse events (Aes) for Cohorts A, B, and C, including dose-limiting toxicities (DLTs) and serious adverse events (SAEs). The most common adverse events for all cohorts was gastrointestinal disorders (41%), fatigue (30%), and skin related events (27%). The following are considered DLTs when observed during either

SD-101 cycle or within 4 weeks after the last SD-101 dose in Cycle 1 and are considered attributable to study intervention (SD-101 or CPI therapy) and/or the PEDD device:

- $\geq$ Grade 4 cytokine release syndrome (CRS) that does not recover to $\leq$ Grade 2 within 7 days per National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE)
- Grade 3 CRS per NCI CTCAE that does not recover to $\leq$ Grade 2 within 7 days
- autoimmune AE $\geq$ Grade 3 per NCI CTCAE
- allergic reaction Aes $\geq$ Grade 3 per NCI CTCAE
- Grade 4 hematologic Aes that do not recover to $\leq$ Grade 2 within 7 days
- Any Grade 4 AE per NCI CTCAE in any organ system

[00215] The data of FIG. 2C shows that the addition of SD-101 delivered into the liver via HAI with PEDD to a treatment regimen with CPI is well tolerated and has similar safety profile to SPI in the absence of SD-101.

[00216] FIGS. 3-5 illustrate the serum pharmacokinetic profile of SD-101 following each liver infusion at 2, 4, and 8 mg, respectively. Plasma was collected on infusion days at the indicated time points and analyzed for SD-101 levels by LC-MS. Notably, the TriNav[®] Infusion System, which is a PEDD device, can achieve high liver SD-101 levels with limited and safe systemic exposure. SD-101 was transiently detected (< 2 hr) in serum following SD-101 at all doses shown in FIGS. 3-5. Also, the SD-101 pharmacokinetic profile was not impacted by CPI administration. FIG. 6 illustrates the SD-101 concentrations within liver tissue post-infusion. Liver tissue levels were observed up to 2,720 ng/g at a dose of 8 mg. FIGS. 3-6 demonstrate that plasma levels of SD-101 are transient and remain low following PEDD while high drug levels are observed in the liver. This data demonstrates that SD-101 delivered via HAI with PEDD concentrates the drug in the target organ while minimizing systemic exposure.

[00217] FIG. 7A provides serum cytokine analysis for CXCL10 (IP-10) following TLR9 agonist administration, according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 7B provides serum cytokine analysis for IL-8 following TLR9 agonist administration, according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 8A provides serum cytokine analysis for

IL-2R following TLR9 agonist administration, according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 8B provides serum cytokine analysis for IFN γ following TLR9 agonist administration, according to the Phase 1/1B clinical study protocol of FIG. 2A. The data of FIGS. 7A, 7B, 8A and 8B illustrate an increase in serum cytokine levels despite a low systemic exposure to SD-101.

[00218] FIG. 9A illustrates uveal melanoma liver metastasis TME modulation at Day 57 with MDSC related genes, according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 9B illustrates uveal melanoma liver metastasis TME modulation at Day 57 with T cell related genes, according to the Phase 1/1B clinical study protocol of FIG. 2A. FIGS. 9A and 9B illustrate that SD-101 induces broad and favorable gene expression changes after 57 days. This data demonstrates an improvement in uveal melanoma liver metastasis TME modulation. FIG. 10 show a change in M-MDSCs within tumors after 57 days.

[00219] FIG. 11A illustrates circulating immune cell analysis following administration of SD-101 for changes in circulating NK cell protein expression patterns (Day 1 – Day 36), according to the Phase 1/1B clinical study protocol of FIG. 2A. Data represented as Mean + SEM (n=19). FIG. 11B illustrates circulating immune cell analysis following administration of SD-101 for changes in circulating CD8 T cell protein expression patterns (Day 1 – Day 36), according to the Phase 1/1B clinical study protocol of FIG. 2A. Data represented as Mean + SEM (n=23). FIGS. 11A and 11B illustrate changes in flow cytometry despite low systemic exposure to SD-101. For the data shown in FIGS. 11A and 11B, at Day 36, NK cells increased from 13 of 16 subjects.

[00220] FIGS. 11C-11F provide data for immune signatures in circulation associated with tumor regression. FIG. 11C illustrates circulating immune cell analysis following administration of SD-101 for changes in protein expression on circulating CD8⁺ T cells (Day 1- Day 36) for Cohorts A, B and C, according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 11D provides the data of FIG. 11C separated by based on the amount of SD-101 (also known as nelitolimod) administered. FIGS. 11C and 11D show systemic increase in proliferation and activation of CD8⁺ T cells in all cohorts. FIG. 11E illustrates circulating immune cell analysis following administration of SD-101 for changes in protein expression on circulating NK cells (Day 1- Day 36) for Cohorts A, B and C, according to the Phase 1/1B clinical study protocol of

FIG. 2A. FIG. 11F provides the data of FIG. 11E separated by based on the amount of SD-101 (also known as nelitolimod) administered. FIGS. 11E and 11F show systemic increase in proliferation of NK cells.

[00221] FIG. 12A illustrates changes in flow cytometry despite low systemic exposure to SD-101. FIGS. 12B-12E provide data for immune signatures in circulation associated with tumor regression. FIG. 12B illustrates circulating immune cell analysis following administration of SD-101 for changes in protein expression on circulating CD8+ T cells (Day 1- Day 36) for Cohorts A, B and C, according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 12C provides the data of FIG. 12B separated by based on the amount of SD-101 (also known as nelitolimod) administered. FIGS. 12B and 12C show systemic increase in proliferation and activation of CD8+ T cells in all cohorts. FIG. 12D illustrates circulating immune cell analysis following administration of SD-101 for changes in protein expression on circulating NK cells (Day 1- Day 36) for Cohorts A, B and C, according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 12E provides the data of FIG. 12D separated by based on the amount of SD-101 (also known as nelitolimod) administered. The data provided in FIGS. 12D and 12E show systemic increase in proliferation of NK cells.

[00222] FIG. 13A illustrates a swimmer plot of patients showing superior results for 16 of 25 patients with SD-101, 1 of 25 patients with a minor response (MR), and a potential for prolonged survival with progressive disease (PD). FIG. 13B is a continuation of FIG. 13A and shows additional study days for Cohort B alone. The data shows superior progression-free survival (PFS) rates with 2 mg SD-101 in combination with nivolumab (Nivo), as opposed to patients receiving 4 mg, or 8 mg SD-101 combinations with Nivo. The median PFS for the 2 mg SD-101 group was 11.7 months (~356 days), and overall survival (OS) rate at 1 year was 86%. The data of FIG. 13B shows that 70% of the patients in Cohort B are 2L and beyond, including 4L and 6L patients. The data also shows 27% ctDNA clearance and that 5 of 7 of 2mg + nivo patients demonstrated >50% decrease in ctDNA including 2 complete ctDNA responders. This data demonstrates that even progressive disease patients with reduced ctDNA may demonstrate improved survival rate.

[00223] FIGS. 14A, 14B and 14C illustrate changes in ctDNA levels in heavily treated patients stratified by dose levels. FIG. 14A shows changes in ctDNA levels in patients treated

according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 14A illustrates ctDNA levels decreased for 8 of 13 patients, with 3 patients having cleared ctDNA. Also, 61% of patients with measurable ctDNA at baseline showed a favorable decrease in ctDNA levels. FIG. 14B provides additional data illustrating changes in ctDNA levels in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 14C provides additional data illustrating changes in ctDNA levels in Cohort B patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIGS. 14B and 14C illustrate changes in ctDNA levels for additional patients (and specifically for patients in Cohort B), and shows a decrease in ctDNA levels for 16 of 26 patients, with 7 patients having cleared ctDNA. In FIGS. 14B and 14C ctDNA was quantified during Cycle 1 (on-treatment, weeks 1-3) and at the latest available time point following Cycle 1 (post-treatment, week 6 or 9). Change in ctDNA levels in Cohort B (FIG. 14C) and in Cohorts B and C combined by dose (FIG. 14B) were determined by Next Generation Sequencing.

[00224] The data provided in FIGS. 14D to 14G show ctDNA decreases in heavily pre-treated patients. FIG. 14D provides data illustrating changes in ctDNA levels in Cohort B and C patients for Post-Cycle 1 time points (including Day 36 and Day 57 unless otherwise noted) treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 14E provides the data of FIG. 14D separated by Cohorts B and C. The data of FIGS. 14D and 14E shows 69.6% (16/23) had a decrease in ctDNA and 21.7% (5/23) cleared ctDNA. At the 2 mg dose level for SD-101, 81.8% (9/11) had a decrease and 36% (4/11) cleared ctDNA. In FIGS. 14D and 14E, the Post-Cycle 1 time points include Day 36 and Day 57. 27 patients were not included in FIGS. 14D and 14E because they were unevaluable due to undetectable ctDNA at baseline or unavailable baseline data. FIG. 14F provides data illustrating reductions in ctDNA mutant allele fractions (MAF) from baseline in Cohort B and C patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 14G provides the data of FIG. 14F separated by Cohorts B and C. The data of FIGS. 14F and 14G shows 76.9% (20/26) molecular response rate ($\geq 50\%$ reduction in MAF from baseline) and 50% (13/26) cleared ctDNA. At the 2 mg dose level for SD-101, 90.9% (10/11) molecular response rate ($\geq 50\%$ reduction in MAF from baseline) and 54.5% (6/11) cleared ctDNA.

[00225] FIG. 15A illustrates the change in cell concentration for CD8+ T cells for cohorts A, B, and C. FIG. 15B illustrates the change in cell concentration for CD4+ T cells for cohorts

A, B, and C. FIG. 15C illustrates the change in cell concentration for NK cells for cohorts A, B, and C. FIG. 15D illustrates the change in cell concentration for M1 macrophage cells for cohorts A, B, and C. These figures show the consistent increase in CD8⁺ T cells, CD4⁺ T cells, NK cells, and M1 macrophage cells in tumors for cohort B at Day 57 compared to baseline. Data of FIGS. 15A-D represented as Mean $\pm$ SEM.

[00226] FIG. 16A illustrates the change in cell concentration for CD8⁺ T cells for cohort B at 2 mg, 4 mg, and 8 mg. FIG. 16B illustrates the change in cell concentration for CD4⁺ T cells for cohort B at 2 mg, 4 mg, and 8 mg. FIG. 16C illustrates the change in cell concentration for CD8⁺ T cells for cohort B at 2 mg, 4 mg, and 8 mg. FIG. 16D illustrates the change in cell concentration for M1 macrophage cells for cohort B at 2 mg, 4 mg, and 8 mg. Data represented as Mean $\pm$ SEM.

[00227] FIG. 17A shows changes in tumor pathway scores determined by advanced analysis of NanoString gene expression data from baseline to Day 57 for cohorts A, B, and C (Cohort A (2mg n=3, 4mg n=2, 8mg n=2); Cohort B (2mg n=2, 4mg n=5, 8mg n=6); Cohort C (2mg n=4)). FIG. 17A illustrates hepatic artery infusion of SD-101 via PEDD associated with induction of T cell activation and cytokine signaling in liver metastases for cohorts A, B, and C at baseline and Day 57. The data of FIG. 17A show cohort B has a consistent positive change in tumor pathway scores and an increase immune cell activation (e.g., T cell activation and cytokine signaling) within liver metastases. Data represented as Mean $\pm$ SEM.

[00228] FIG. 17B shows changes in PBMC pathway scores determined by advanced analysis of NanoString gene expression data from baseline to Day 36 for cohorts A, B, and C (Cohort A (2mg n=4, 4mg n=3, 8mg n=3); Cohort B (2mg n=1, 4mg n=7, 8mg n=7); Cohort C (2mg n=7)). FIG. 17B illustrates hepatic artery infusion of SD-101 via PEDD associated with induction of T cell activation and cytokine signaling in the blood for cohorts A, B, and C at baseline and Day 36, despite low levels of SD-101 outside the liver. Data represented as Mean $\pm$ SEM.

[00229] FIG. 17C shows changes in tumor pathway scores split by Cohort determined by advanced analysis of NanoString gene expression data from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 17D shows the changes in tumor pathway scores of FIG. 17C split by dose of SD-101

administered. In the data of FIGS. 17C and 17D, a dose response was observed for pathway score changes within tumors, with 8 mg nelitolimod producing the greatest increase in immune signaling and activation pathway scores following Cycle 1.

[00230] FIG. 17E shows changes in PBMC pathway scores split by Cohort determined by advanced analysis of NanoString gene expression data from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 17F shows the changes in tumor pathway scores of FIG. 17E split by dose of SD-101 administered. The data of FIGS. 17E and 17F shows that 4 mg nelitolimod produced the greatest increase in immune signaling and activation pathway scores within PBMCs following Cycle 1.

[00231] FIG. 17G shows changes in tumor gene expression levels split by Cohort determined by advanced analysis of NanoString gene expression data from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 17H shows the changes in tumor gene expression levels of FIG. 17G split by dose of SD-101 administered. The data of FIGS. 17G and 17H shows that across all Cohorts, there was an increase in gene expression levels for proinflammatory genes within tumors following Cycle 1.

[00232] FIG. 17I shows additional data demonstrating changes in tumor gene expression levels split by Cohort determined by advanced analysis of NanoString gene expression data from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 17J shows the changes in tumor gene expression levels of FIG. 17I split by dose of SD-101 administered. The data of FIGS. 17I and 17J shows that within tumors, the 8 mg dose levels produced an increase in the expression of most MDSC-associated genes while 2 mg produced a decrease in the expression of most MDSC-associated genes.

[00233] FIG. 17K shows changes in PBMC gene expression levels split by Cohort determined by advanced analysis of NanoString gene expression data from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 17L shows the changes in PBMC gene expression levels of FIG. 17K split by dose of SD-101 administered. The data of FIGS. 17K and 17L shows that across all Cohorts,

IFNB1 expression increased with PBMCs following Cycle 1 and that Cohorts B and C had an increase in CXCL10, granzyme A, and ICOS within PBMCs following Cycle 1.

[00234] FIG. 17M shows additional data demonstrating changes in PBMC gene expression levels split by Cohort determined by advanced analysis of NanoString gene expression data from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. FIG. 17N shows the changes in PBMC gene expression levels of FIG. 17M split by dose of SD-101 administered. The data of FIGS. 17M and 17N shows that across all doses, IFNA2 and IFNB1 expression increased within PBMCs following Cycle 1 and that the 2 mg and 4 mg nelitolimod dose levels had an increase in granzyme A and ICOS within PBMCs following Cycle 1.

[00235] FIG. 18A illustrates the change in cell concentration from baseline data for Regulatory T cells (Treg) for cohorts A, B, and C. FIG. 18B illustrates the change in cell concentration from baseline data for Monocytic Myeloid-derived Suppressor Cells (M-MDSC) for cohorts A, B, and C. FIG. 18C illustrates the change in cell concentration from baseline data for total MDSC cells for cohorts A, B, and C. FIG. 18D illustrates the change in cell concentration from baseline data for M2 macrophage cells for cohorts A, B, and C. A reduction in Treg and MDSC cells in liver tumors results in fewer cells that drives CPI failure. Data of FIGS. 18A-D represented as Mean $\pm$ SEM.

[00236] FIG. 19A illustrates the change in cell concentration from baseline data for Treg cells for cohort B at 2 mg, 4 mg, and 6 mg. FIG. 19B illustrates the change in cell concentration from baseline data for M-MDSC cells for cohort B at 2 mg, 4 mg, and 6 mg. FIG. 19C illustrates the change in cell concentration from baseline data for total MSDC cells for cohort B at 2 mg, 4 mg, and 6 mg. FIG. 19D illustrates the change in cell concentration from baseline data for M2 macrophage cells for cohort B at 2 mg, 4 mg, and 6 mg. FIG. 19E illustrates the change in cell concentration from baseline data for Granzyme B for cohort B at 2 mg, 4 mg, and 6 mg. FIG. 19F illustrates the change in cell concentration from baseline data for IL-15 for cohort B at 2 mg, 4 mg, and 6 mg. Increase in Granzyme B helps T cells to kill tumor cells. Increase in IL-15 increases the activation of T cells and NK cells and has a role in persistence of CD8+ memory T cells. A decrease in MDSC and Treg in liver tumor corresponds to fewer cells that drive CPI failure. Data of FIGS. 19A-F represented as Mean $\pm$ SEM.

[00237] The clinical data described herein for Example 1 shows that at 2 mg SD-101 via PEDD + nivolumab provides >80% ctDNA response rate, >80% disease control rate, and 11.7-month progression free survival (PFS).

[00238] FIGS. 20A-F illustrate peripheral immune signatures induced by SD-101 delivered via PEDD. Data represented as Mean $\pm$ SEM. In particular, FIGS. 20A-F show systemic immune activation with an increase in pro-inflammation cytokines (peripheral immune signatures) induced by SD-101 on Day 36 delivered by PEDD. FIG. 20A illustrates activation by pro-inflammatory cytokine CXCL10 (IP-10). FIG. 20B illustrates activation by pro-inflammatory cytokine IFN γ . FIG. 20C illustrates activation by pro-inflammatory cytokine TNF α . FIG. 20D illustrates activation by pro-inflammatory cytokine IL-2R. FIG. 20E illustrates activation by pro-inflammatory cytokine IL-15. FIG. 20F illustrates activation of pro-inflammatory cytokine IL-18.

[00239] FIGS. 21A-F illustrate immune signatures in circulation associated with tumor regression in Cohort A, B and C. Data represented as Mean $\pm$ SEM. FIG. 21A illustrates activation by pro-inflammatory cytokine CXCL10 (IP-10). FIG. 21B illustrates activation by pro-inflammatory cytokine IFN γ . FIG. 21C illustrates activation by pro-inflammatory cytokine TNF α . FIG. 21D illustrates activation by pro-inflammatory cytokine IL-2R. FIG. 21E illustrates activation by pro-inflammatory cytokine IL-15. FIG. 21F illustrates activation of pro-inflammatory cytokine IL-18.

[00240] FIGS. 22A-F illustrate immune signatures in circulation associated with tumor regression in Cohort B. Data represented as Mean $\pm$ SEM. FIG. 22A illustrates activation by pro-inflammatory cytokine CXCL10 (IP-10). FIG. 22B illustrates activation by pro-inflammatory cytokine IFN γ . FIG. 22C illustrates activation by pro-inflammatory cytokine TNF α . FIG. 22D illustrates activation by pro-inflammatory cytokine IL-2R. FIG. 22E illustrates activation by pro-inflammatory cytokine IL-15. FIG. 22F illustrates activation of pro-inflammatory cytokine IL-18.

[00241] FIG. 22A-D illustrates the changes in protein expression patterns on circulating cells. Data represented as Mean $\pm$ SEM. FIG. 22A illustrates systemic increase in proliferation and activation of CD8 $^{+}$ T cells in all cohorts A, B and C. FIG. 22B illustrates systemic increase in proliferation and activation of CD8 $^{+}$ T cells in cohort B at 2 mg, 4 mg, and 8 mg. FIG. 22C

illustrates systemic increase in proliferation of NK cells in all cohorts A, B, and C. FIG. 22D illustrates systemic increase in proliferation of NK cells in cohort B at 2 mg, 4 mg, and 8 mg.

[00242] The data of FIGS. 20A-F, 21A-F, 22A-F and 23A-D show systemic increase in proliferation and activation of CD8⁺ T cells in all cohorts, and systemic increase in proliferation of NK cells.

[00243] The Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 overall response rate (ORR) and 12 month overall success (OS) to PEDD/HAI SD 101 in combination with systemic, i.e. intravenous (IV), immunological checkpoint blockade of the patients are assessed are also assessed.

[00244] FIG. 24A illustrates objective RECIST 1.1 response rates for patients in cohorts B at 2 mg, 4 mg, and 8 mg, and cohort C at 2 mg. 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. Results demonstrate 81% disease control rate (DCR) at an optimal biological dose of SD-101 at 2 mg, and 58% DCR across all SD-101 doses in combination with IV CPI. Delayed response noted in some subjects, including conversion of PD to SD or MR. FIG. 24B shows objective RECIST 1.1 response rates for patients from FIG 24A and additional patients in Cohorts B at 2 mg, 4 mg, and 8 mg, and Cohort C at 2 mg and 4 mg treated according to the Phase 1/1B clinical study protocol of FIG. 2A. The data shown in FIGS. 24A and 24B highlight the importance of ctDNA and PFS for the measurement of disease control.

[00245] FIG. 25A illustrates Kaplan-Meier curves showing overall survival (OS) proportions for cohorts A, B, and C. FIG. 25B illustrates overall survival proportions for cohort B at 2 mg, 4 mg, and 8 mg. The optimal biological dose for OS is 2 mg in cohort B. As shown in Fig. 25B, the overall survival (OS) rate at 1 year of patients in Cohort B administered with a 2 mg weekly dose of SD-101 (in 3 week cycles, with 5 weeks in between each cycle) and nivolumab is 86%.

[00246] In three (3) patients administered with a 2 mg or 4 mg weekly dose of SD-101 (in 3 week cycles, with 5 weeks in between each cycle) and nivolumab according to the protocol for Cohort B. Sequential MRI/CT scans were obtained and tumor dimension are provided below in Table 2. Two of the patients show a response to treatment in reduction of tumor size.

Table 2

Patient 101-016: Cohort B 2mg SD-101 + Nivo		Patient 111-014: Cohort B 2mg SD-101 + Nivo		Patient 111-025: Cohort B 4mg SD-101 + Nivo	
Time	Tumor measurements	Time	Tumor measurements	Time	Tumor measurements
Baseline (Day 1)	5.9 x 4.0 cm	Baseline (Day 1)	2.4 x 2.5 cm	Baseline (Day 1)	1.8 x 2.0 cm
Day 62	6.4 x 3.9 cm	Day 70	2.3 x 2.3 cm	Day 59	1.6 x 1.3 cm
Day 92	6.3 x 3.9 cm	Day 105	2.3 x 2.3 cm	Day 92	1.0 x 0.8 cm
Day 182	4.7 x 3.5 cm	Day 185	2.4 x 2.6 cm	Day 177	1.2 x 0.8 cm
Day 266	4.5 x 3.2 cm	Day 273	3.1 x 2.8 cm	Day 264	1.1 x 0.9 cm
Day 350	4.3 x 3.2 cm	Day 368	3.7 x 3.3 cm		

[00247] Treatment as described in Example 1 resulted in high liver drug levels within the liver and low, transient levels within the peripheral blood. Broad immunostimulatory gene expression changes were noted in tumor and normal liver following SD-101. Increases in serum IL-18 and IFN γ were observed. An increase in CD8 $^{+}$ T cells, NK cells, and M1 macrophage infiltration in tumors was observed. A decrease in immunosuppressive Tregs, M-MDSCs, and M2 macrophages in tumors was observed. Gene expression levels in LM and in PBMCs demonstrated increased expression levels of genes related to CD8 $^{+}$ cytotoxic T lymphocytes activity, Th1 activation, cytokine, and chemokine signaling. Systemic immune activation was observed by an increase in pro-inflammatory cytokines. Systemic increase in NK cell proliferation and CD8 $^{+}$ T cell proliferation and activation was observed. A median PFS of 11.7 months was observed for SD-101 at 2 mg + nivolumab.

[00248] Based on the data provided above, the TriNav PEDD device achieved high liver SD-101 levels with limited and safe systemic exposure. PEDD of SD-101 via hepatic artery infusion (HAI) was well tolerated, with or without systemic CPIs, and associated with encouraging immunologic activity. Evidence of biologic effects at the lower doses of SD-101 in combination with nivolumab was observed. The results also showed MDSC depletion and reduction in MDSC associated genes in liver metastases, while broad immune effects were noted

in liver metastases and periphery. Likewise, ctDNA decreases were noted in heavily pre-treated patient groups. Delivery of SD-101 by PEDD plus systemic ICI in MUM-LM patients results in clinical activity with median PFS of 11.7 months, MDSC re-programming, and evidence of peripheral and intra-tumoral immune activation.

[00249] FIG. 25C illustrates Kaplan-Meier curves showing overall survival (OS) proportions for cohorts A, B, and C. The data show in FIG. 25 includes the data provided in FIG. 25A and additional data. FIG. 25D illustrates overall survival proportions for Cohort B at 2 mg, 4 mg, and 8 mg dose of SD-101 from the data of FIG. 25C. FIG. 25E illustrates overall survival proportions for Cohort B and C at 2 mg and 4 mg dose of SD-101 from the data of FIG. 25C.

[00250] FIGS. 26A through 26N illustrate changes in cell densities within tumors obtained using multiplex immunofluorescence (IF) from baseline to Day 57 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. The data of FIGS. 26A through 26N show that on average, all cell types increased following treatment, but CD4 and CD8 T cells increased the greatest amount by far. In addition, the data show that on average, all cell types increased following treatment, but CD4 and CD8 T cells increased the greatest amount by far. Although the data shows that there was an increase in total MDSCs within tumors at the 2 mg dose level, MDSC-associated gene expression levels decreased within tumors at this dose level, indicating that MDSCs may have reduced activity post-cycle 1.

[00251] FIGS. 27A through FIG. 27H illustrate changes in plasma cytokine immune markers, as analyzed by Luminex, from baseline to Day 36 for cohorts A, B, and C in patients treated according to the Phase 1/1B clinical study protocol of FIG. 2A. The data of FIGS. 27A through 27N show that across all doses and cohorts, there was an increase in plasma levels of IL-2R, CXCL10, and granzyme B following Cycle 1.

[00252] 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 for treating uveal melanoma liver metastases 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 that 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,
wherein the one or more checkpoint inhibitors are systematically administered to the
subject, and
wherein the TLR9 agonist is locally administered to the liver of the subject via hepatic
artery infusion at a dose sufficient to provide a concentration in the liver that is therapeutically
effective in combination with the checkpoint inhibitor and to provide a plasma concentration of
the TLR9 agonist that is below the concentration in the liver.
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 the plasma concentration of the TLR9
agonist is administered at a dose that is subtherapeutic when the TLR9 agonist is administered
without the one or more checkpoint inhibitors.
5. The method of any one of claims 1 to 3, wherein the plasma concentration of the TLR9
agonist is at a level that is not toxic to the human subject.
6. The method of any one of claims 1 to 3, wherein the subject has Stage IV uveal
melanoma.

7. The method of any one of claims 1 to 3, wherein the TLR9 agonist is locally administered to an area in the liver of the subject that is external to the uveal melanoma liver metastases.
8. The method of any one of claims 1 to 3, wherein the one or more checkpoint inhibitors is administered intravenously, intraperitoneally or subcutaneously.
9. The method of any one of claims 1 to 3, wherein a ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:2 to about 1:10.
10. The method of claim 9, wherein the ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:3 to about 1:7.
11. The method of any one of claims 1 to 3, wherein the concentration of the TLR9 agonist in the liver is from about 750 ng/g to about 3000 ng/g.
12. The method of claim 11, wherein the plasma concentration of the TLR9 agonist is less than about 750 ng/mL.
13. The method of claim 12, wherein the plasma concentration of the TLR9 agonist is less than about 700 ng/mL.
14. The method of claim 13, wherein the plasma concentration of the TLR9 agonist is less than about 600 ng/mL.
15. The method of any one of claims 1 to 3, wherein the TLR9 agonist is administered weekly at a dose from about 0.5 mg to about 20 mg.

16. The method of claim 15, wherein the TLR9 agonist is administered weekly at a dose from about 1 mg to about 10 mg.
17. The method of claim 16, wherein the TLR9 agonist is administered weekly at a dose from about 2 mg to about 8 mg.
18. The method of claim 17, wherein the TLR9 agonist is administered weekly at a dose from about 4 mg to about 8 mg.
19. The method of claim 17, wherein the TLR9 agonist is administered weekly at a dose of about 2 mg.
20. The method of claim 19, wherein the TLR9 agonist is administered weekly in 3 week cycles with a rest period between cycles.
21. The method of any one of claims 1 to 20, wherein the TLR9 agonist is administered through a catheter device.
22. The method of claim 21, wherein the catheter device comprises a one-way valve that responds dynamically to local pressure and/or flow changes.
23. The method of claim 22, wherein the TLR9 agonist is administered through the catheter device via pressure-enabled drug delivery (PEDD).
24. The method of claim 21, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 200 minutes.
25. The method of claim 24, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 60 minutes.

26. The method of claim 25, wherein the TLR9 agonist is administered for a period of time of about 25 minutes.
27. The method of any one of claims 1 to 3, wherein the one or more checkpoint inhibitor is administered concurrently with the TLR9 agonist.
28. The method of any one of claims 1 to 3, wherein the one or more checkpoint inhibitor is administered before the TLR9 agonist.
29. The method of any one of claims 1 to 3, wherein the one or more checkpoint inhibitor is administered after the TLR9 agonist.
30. The method of any one of claims 1 to 3, wherein the one or more checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab.
31. The method of claim 30, wherein the one or more checkpoint inhibitor is nivolumab.
32. The method of claim 30, wherein the one or more checkpoint inhibitor is pembrolizumab.
33. The method of claim 30, wherein the one or more checkpoint inhibitor is ipilimumab.
34. The method of claim 30, wherein the one or more checkpoint inhibitor is crefmirlimab.
35. The method of claim 30, wherein two checkpoint inhibitors are administered.
36. The method of claim 35, wherein the two checkpoint inhibitors are nivolumab and ipilimumab.
37. The method of claim 35, wherein the two checkpoint inhibitors are nivolumab and relatlimab.

38. A method for increasing survival rate in a human subject with uveal melanoma liver metastases comprising:

administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and 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.

39. The method of claim 38, 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.

40. The method of claim 39, wherein the TLR9 agonist is a sodium salt of the oligonucleotide of SEQ ID NO: 2.

41. The method of any one of claims 38 to 40, wherein the one or more checkpoint inhibitors are systematically administered to the subject.

42. The method of any one of claims 38 to 41, wherein the plasma concentration of the TLR9 agonist is at a dose that is subtherapeutic when the TLR9 agonist is administered without the one or more checkpoint inhibitors.

43. The method of any one of claims 38 to 41, wherein the plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject.

44. The method of any one of claims 38 to 41, wherein the subject has Stage IV uveal melanoma.

45. The method of any one of claims 38 to 41, wherein the TLR9 agonist is locally administered to an area in the liver of the subject that is external to the uveal melanoma liver metastases.
46. The method of claim 41, wherein the one or more checkpoint inhibitors is administered intravenously, intraperitoneally or subcutaneously.
47. The method of any one of claims 38 to 41, wherein a ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:2 to about 1:10.
48. The method of claim 47, wherein the ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:3 to about 1:7.
49. The method of any one of claims 38 to 41, wherein the concentration of the TLR9 agonist in the liver is from about 750 ng/g to about 3000 ng/g.
50. The method of claim 49, wherein the plasma concentration of the TLR9 agonist is less than about 750 ng/mL.
51. The method of claim 50, wherein the plasma concentration of the TLR9 agonist is less than about 700 ng/mL.
52. The method of claim 51, wherein the plasma concentration of the TLR9 agonist is less than about 600 ng/mL.
53. The method of any one of claims 38 to 41, wherein the TLR9 agonist is administered weekly at a dose from about 0.5 mg to about 20 mg.
54. The method of claim 53, wherein the TLR9 agonist is administered weekly at a dose from about 1 mg to about 10 mg.

55. The method of claim 54, wherein the TLR9 agonist is administered weekly at a dose from about 2 mg to about 8 mg.
56. The method of claim 55, wherein the TLR9 agonist is administered weekly at a dose from about 4 mg to about 8 mg.
57. The method of claim 55, wherein the TLR9 agonist is administered weekly at a dose of about 2 mg.
58. The method of claim 57, wherein the TLR9 agonist is administered weekly in 3 week cycles with a rest period between cycles.
59. The method of claims 38 to 58, wherein the TLR9 agonist is locally administered to the liver of the subject via hepatic artery infusion.
60. The method of claim 59, wherein the TLR9 agonist is administered through a catheter device.
61. The method of claim 59, wherein the TLR9 agonist is infused while modulating distal vascular pressures and blood flow in the subject.
62. The method of claim 59, wherein the catheter device comprises a one-way valve that responds dynamically to local pressure and/or flow changes.
63. The method of claim 60, wherein the TLR9 agonist is locally administered to the liver of the subject via pressure-enabled drug delivery (PEDD).
64. The method of claim 59, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 200 minutes.

65. The method of claim 64, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 60 minutes.
66. The method of claim 65, wherein the TLR9 agonist is administered for a period of time of about 25 minutes.
67. The method of any one of claims 38 to 41, wherein the one or more checkpoint inhibitor is administered concurrently with the TLR9 agonist.
68. The method of any one of claims 38 to 41, wherein the one or more checkpoint inhibitor is administered before the TLR9 agonist.
69. The method of any one of claims 38 to 41, wherein the one or more checkpoint inhibitor is administered after the TLR9 agonist.
70. The method of any one of claims 38 to 41, wherein the one or more checkpoint inhibitor comprises an anti-PD1 or anti-PD-L1 antibody or an antigen binding fragment thereof.
71. The method of claim 70, wherein the one or more checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab.
72. The method of claim 71, wherein the one or more checkpoint inhibitor is nivolumab.
73. The method of claim 71, wherein the one or more checkpoint inhibitor is pembrolizumab.
74. The method of claim 71, wherein the one or more checkpoint inhibitor is ipilimumab.
75. The method of claim 71, wherein the one or more checkpoint inhibitor is crefmirlimab.
76. The method of any one of claims 38 to 41, wherein two checkpoint inhibitors are administered.

77. The method of claim 76, wherein the two checkpoint inhibitors are nivolumab and ipilimumab.
78. The method of claim 76, wherein the two checkpoint inhibitors are nivolumab and relatlimab.
79. The method of any one of claims 38 to 41, wherein administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases progression-free survival (PFS) of the subject.
80. A method for treating uveal melanoma liver metastases in a human subject comprising:
administering to the subject a therapeutically effective amount of one or more checkpoint inhibitors; and
locally administering 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,
wherein the one or more checkpoint inhibitors and the TLR9 agonist are administered in a therapeutically effective combination for reducing tumor burden on the subject.
81. The method of claim 80, 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.
82. The method of claim 81, wherein the TLR9 agonist is a sodium salt of the oligonucleotide of SEQ ID NO: 2.
83. The method of any one of claims 80 to 82, wherein the plasma concentration of the TLR9 agonist is at a dose that is subtherapeutic when the TLR9 agonist is administered without the one or more checkpoint inhibitors.

84. The method of any one of claims 80 to 82, wherein the plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject.
85. The method of any one of claims 80 to 82, wherein the subject has Stage IV uveal melanoma.
86. The method of any one of claims 80 to 82, wherein the TLR9 agonist is locally administered to an area in the liver of the subject that is external to the uveal melanoma liver metastases.
87. The method of any one of claims 80 to 82, wherein the one or more checkpoint inhibitors is administered intravenously, intraperitoneally or subcutaneously.
88. The method of any one of claims 80 to 82, wherein a ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:2 to about 1:10.
89. The method of claim 88, wherein the ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:3 to about 1:7.
90. The method of any one of claims 80 to 82, wherein the concentration of the TLR9 agonist in the liver is from about 750 ng/g to about 3000 ng/g.
91. The method of claim 90, wherein the plasma concentration of the TLR9 agonist is less than about 750 ng/mL.
92. The method of claim 91, wherein the plasma concentration of the TLR9 agonist is less than about 700 ng/mL.

93. The method of claim 92, wherein the plasma concentration of the TLR9 agonist is less than about 600 ng/mL.
94. The method of any one of claims 80 to 82, wherein the TLR9 agonist is administered weekly at a dose from about 0.5 mg to about 20 mg.
95. The method of claim 94, wherein the TLR9 agonist is administered weekly at a dose from about 1 mg to about 10 mg.
96. The method of claim 95, wherein the TLR9 agonist is administered weekly at a dose from about 2 mg to about 8 mg.
97. The method of claim 96, wherein the TLR9 agonist is administered weekly at a dose from about 4 mg to about 8 mg.
98. The method of claim 96, wherein the TLR9 agonist is administered weekly at a dose of about 2 mg.
99. The method of claim 96, wherein the TLR9 agonist is administered weekly in 3 week cycles with rest period between cycles.
100. The method of any one of claims 80 to 82, wherein the TLR9 agonist is locally administered to the liver of the subject via hepatic artery infusion.
101. The method of claim 100, wherein the TLR9 agonist is administered through a catheter device.
102. The method of claim 101, wherein the TLR9 agonist is infused while modulating distal vascular pressures and blood flow in the subject.

103. The method of claim 101, wherein the catheter device comprises a one-way valve that responds dynamically to local pressure and/or flow changes.
104. The method of claim 100, wherein the TLR9 agonist is locally administered to the liver of the subject via pressure-enabled drug delivery (PEDD).
105. The method of claim 100, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 200 minutes.
106. The method of claim 105, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 60 minutes.
107. The method of claim 106, wherein the TLR9 agonist is administered for a period of time of about 25 minutes.
108. The method of any one of claims 80 to 82, wherein the one or more checkpoint inhibitors are systematically administered to the subject.
109. The method of any one of claims 80 to 82, wherein the one or more checkpoint inhibitor is administered concurrently with the TLR9 agonist.
110. The method of any one of claims 80 to 82, wherein the one or more checkpoint inhibitor is administered before the TLR9 agonist.
111. The method of any one of claims 80 to 82, wherein the one or more checkpoint inhibitor is administered after the TLR9 agonist.
112. The method of any one of claims 80 to 82, wherein the one or more checkpoint inhibitor comprises an anti-PD1 or anti-PD-L1 antibody or an antigen binding fragment thereof.

113. The method of claim 112, wherein the one or more checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab.
114. The method of claim 113, wherein the one or more checkpoint inhibitor is nivolumab.
115. The method of claim 113, wherein the one or more checkpoint inhibitor is pembrolizumab.
116. The method of claim 113, wherein the one or more checkpoint inhibitor is ipilimumab.
117. The method of claim 113, wherein the one or more checkpoint inhibitor is crefmirlimab.
118. The method of any one of claims 80 to 82, wherein two checkpoint inhibitors are administered.
119. The method of claim 118, wherein the two checkpoint inhibitors are nivolumab and ipilimumab.
120. The method of claim 118, wherein the two checkpoint inhibitors are nivolumab and relatlimab.
121. The method of any one of claims 80 to 82, wherein administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases survival rate of the subject.
122. The method of any one of claims 80 to 82, wherein administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases progression-free survival (PFS) of the subject.
123. The method of any one of claims 80 to 82, wherein administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist reduces circulating tumor DNA (ctDNA) in the subject.

124. A method for reducing circulating tumor DNA (ctDNA) in a human subject with uveal melanoma liver metastases, the method comprising:

administering to the subject a therapeutically effective combination of one or more checkpoint inhibitors and 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.

125. The method of claim 124, 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.

126. The method of claim 125, wherein the TLR9 agonist is a sodium salt of the oligonucleotide of SEQ ID NO: 2.

127. The method of any one of claims 124 to 126, wherein the TLR9 agonist is present in a plasma concentration that is subtherapeutic when the TLR9 agonist is administered without the one or more checkpoint inhibitors.

128. The method of claim 127, wherein the plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject.

129. The method of any one of claims 124 to 126, wherein the subject has Stage IV uveal melanoma.

130. The method of any one of claims 124 to 126, wherein the TLR9 agonist is locally administered to an area in the liver of the subject that is external to the uveal melanoma liver metastases.

131. The method of any one of claims 124 to 126, wherein the one or more checkpoint inhibitors is administered intravenously, intraperitoneally or subcutaneously.
132. The method of any one of claims 124 to 126, wherein a ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:2 to about 1:10.
133. The method of claim 132, wherein the ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:3 to about 1:7.
134. The method of any one of claims 124 to 126, wherein the concentration of the TLR9 agonist in the liver is from about 750 ng/g to about 3000 ng/g.
135. The method of claim 134, wherein the plasma concentration of the TLR9 agonist is less than about 750 ng/mL.
136. The method of claim 135, wherein the plasma concentration of the TLR9 agonist is less than about 700 ng/mL.
137. The method of claim 136, wherein the plasma concentration of the TLR9 agonist is less than about 600 ng/mL.
138. The method of any one of claims 124 to 126, wherein the TLR9 agonist is administered weekly at a dose from about 0.5 mg to about 20 mg.
139. The method of claim 138, wherein the TLR9 agonist is administered weekly at a dose from about 1 mg to about 10 mg.
140. The method of claim 139, wherein the TLR9 agonist is administered weekly at a dose from about 2 mg to about 8 mg.

141. The method of claim 140, wherein the TLR9 agonist is administered weekly at a dose from about 4 mg to about 8 mg.
142. The method of claim 140, wherein the TLR9 agonist is administered weekly at a dose of about 2 mg.
143. The method of claim 140, wherein the TLR9 agonist is administered weekly in 3 week cycles with rest period between cycles.
144. The method of claims 124 to 126, wherein the TLR9 agonist is locally administered to the liver of the subject via hepatic artery infusion.
145. The method of claim 144, wherein the TLR9 agonist is administered through a catheter device.
146. The method of claim 144, wherein the TLR9 agonist is infused while modulating distal vascular pressures and blood flow in the subject.
147. The method of claim 145, wherein the catheter device comprises a one-way valve that responds dynamically to local pressure and/or flow changes.
148. The method of claim 147, wherein the TLR9 agonist is locally administered to the liver of the subject via pressure-enabled drug delivery (PEDD).
149. The method of claim 144, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 200 minutes.
150. The method of claim 149, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 60 minutes.

151. The method of claim 150, wherein the TLR9 agonist is administered for a period of time of about 25 minutes.

152. The method of any one of claims 124 to 126, wherein the one or more checkpoint inhibitors are systematically administered to the subject.

153. The method of any one of claims 124 to 126, wherein the one or more checkpoint inhibitor is administered concurrently with the TLR9 agonist.

154. The method of any one of claims 124 to 126, wherein the one or more checkpoint inhibitor is administered before the TLR9 agonist.

155. The method of any one of claims 124 to 126, wherein the one or more checkpoint inhibitor is administered after the TLR9 agonist.

156. The method of any one of claims 124 to 126, wherein the one or more checkpoint inhibitor comprises an anti-PD1 or anti-PD-L1 antibody or an antigen binding fragment thereof.

157. The method of claim 156, wherein the one or more checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab.

158. The method of claim 157, wherein the one or more checkpoint inhibitor is nivolumab.

159. The method of claim 157, wherein the one or more checkpoint inhibitor is pembrolizumab.

160. The method of claim 157, wherein the one or more checkpoint inhibitor is ipilimumab.

161. The method of claim 157, wherein the one or more checkpoint inhibitor is crefmirlimab.

162. The method of any one of claims 124 to 126, wherein two checkpoint inhibitors are administered.
163. The method of claim 162, wherein the two checkpoint inhibitors are nivolumab and ipilimumab.
164. The method of claim 162, wherein the two checkpoint inhibitors are nivolumab and relatlimab.
165. The method of any one of claims 124 to 126, wherein administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases response rate of the subject to the one or more checkpoint inhibitors.
166. The method of any one of claims 124 to 126, wherein administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases survival rate of the subject.
166. The method of any one of claims 124 to 126, wherein the TLR9 agonist is locally administered to the liver of the subject via hepatic artery infusion.
167. A method for increasing response rate to treatment with a checkpoint inhibitor in a human subject with uveal melanoma liver metastases, 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.
168. The method of claim 167, 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.

169. The method of claim 168, wherein the TLR9 agonist is a sodium salt of the oligonucleotide of SEQ ID NO: 2.

170. The method of any one of claims 167 to 169, wherein the TLR9 agonist is present in a plasma concentration that is subtherapeutic when the TLR9 agonist is administered without the one or more checkpoint inhibitors.

171. The method of any one of claims 167 to 169, wherein the plasma concentration of the TLR9 agonist is at a level that is not toxic to the human subject.

172. The method of any one of claims 167 to 169, wherein the subject has Stage IV uveal melanoma.

173. The method of any one of claims 167 to 169, wherein the TLR9 agonist is locally administered to an area in the liver of the subject that is external to the uveal melanoma liver metastases.

174. The method of any one of claims 167 to 169, wherein the one or more checkpoint inhibitors is administered intravenously, intraperitoneally or subcutaneously.

175. The method of any one of claims 167 to 169, wherein a ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:2 to about 1:10.

176. The method of claim 175, wherein the ratio of the plasma concentration of the TLR9 agonist to the concentration of the TLR9 agonist in the liver is from about 1:3 to about 1:7.

177. The method of any one of claims 167 to 169, wherein the concentration of the TLR9 agonist in the liver is from about 750 ng/g to about 3000 ng/g.

178. The method of claim 177, wherein the plasma concentration of the TLR9 agonist is less than about 750 ng/mL.

179. The method of claim 178, wherein the plasma concentration of the TLR9 agonist is less than about 700 ng/mL.

180. The method of claim 179, wherein the plasma concentration of the TLR9 agonist is less than about 600 ng/mL.

181. The method of any one of claims 167 to 169, wherein the TLR9 agonist is administered weekly at a dose from about 0.5 mg to about 20 mg.

182. The method of claim 181, wherein the TLR9 agonist is administered weekly at a dose from about 1 mg to about 10 mg.

183. The method of claim 182, wherein the TLR9 agonist is administered weekly at a dose from about 2 mg to about 8 mg.

184. The method of claim 183, wherein the TLR9 agonist is administered weekly at a dose from about 4 mg to about 8 mg.

185. The method of claim 183, wherein the TLR9 agonist is administered weekly at a dose of about 2 mg.

186. The method of claim 183, wherein the TLR9 agonist is administered weekly in 3 week cycles with rest period between cycles.

187. The method of any one of claims 167 to 169, wherein the TLR9 agonist is locally administered to the liver of the subject via hepatic artery infusion.

188. The method of claim 187, wherein the TLR9 agonist is administered through a catheter device.

189. The method of claim 188, wherein the TLR9 agonist is infused while modulating distal vascular pressures and blood flow in the subject.

190. The method of claim 188, wherein the catheter device comprises a one-way valve that responds dynamically to local pressure and/or flow changes.

191. The method of claim 188, wherein the TLR9 agonist is locally administered to the liver of the subject via pressure-enabled drug delivery (PEDD).

192. The method of claim 187, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 200 minutes.

193. The method of claim 192, wherein the TLR9 agonist is administered weekly for a period of time of about 10 to about 60 minutes.

194. The method of claim 193, wherein the TLR9 agonist is administered for a period of time of about 25 minutes.

195. The method of any one of claims 167 to 169, wherein the one or more checkpoint inhibitors are systematically administered to the subject.

196. The method of any one of claims 167 to 169, wherein the one or more checkpoint inhibitor is administered concurrently with the TLR9 agonist.

197. The method of any one of claims 167 to 169, wherein the one or more checkpoint inhibitor is administered before the TLR9 agonist.

198. The method of any one of claims 167 to 169, wherein the one or more checkpoint inhibitor is administered after the TLR9 agonist.
199. The method of any one of claims 167 to 169, wherein the one or more checkpoint inhibitor comprises an anti-PD1 or anti-PD-L1 antibody or an antigen binding fragment thereof.
200. The method of claim 199, wherein the one or more checkpoint inhibitor is selected from the group consisting of nivolumab, pembrolizumab, ipilimumab, relatlimab, and crefmirlimab.
201. The method of claim 200, wherein the one or more checkpoint inhibitor is nivolumab.
202. The method of claim 200, wherein the one or more checkpoint inhibitor is pembrolizumab.
203. The method of claim 200, wherein the one or more checkpoint inhibitor is ipilimumab.
204. The method of claim 200, wherein the one or more checkpoint inhibitor is crefmirlimab.
205. The method of any one of claims 80 to 82, wherein two checkpoint inhibitors are administered.
206. The method of claim 118, wherein the two checkpoint inhibitors are nivolumab and ipilimumab.
207. The method of claim 118, wherein the two checkpoint inhibitors are nivolumab and relatlimab.
208. The method of any one of claims 167 to 169, wherein administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases survival rate of the subject.

209. The method of any one of claims 167 to 169, wherein administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist increases progression-free survival (PFS) of the subject.

210. The method of any one of claims 167 to 169, wherein administration of the combination of the one or more checkpoint inhibitors and the TLR9 agonist reduces circulating tumor DNA (ctDNA) in the subject.

211. The method of any one of claims 167 to 169, wherein the administration of the TLR9 agonist in combination with the checkpoint inhibitor increases immune cell activation within the uveal melanoma liver metastases of the subject.

212. The method of any one of claims 167 to 169, wherein the administration of the TLR9 agonist in combination with the checkpoint inhibitor increases systemic cytokine signaling in the blood of the subject.

213. The method of any one of claims 167 to 169, wherein the administration of the TLR9 agonist in combination with the checkpoint inhibitor increases one or more immune cell densities within the uveal melanoma liver metastases of the subject.