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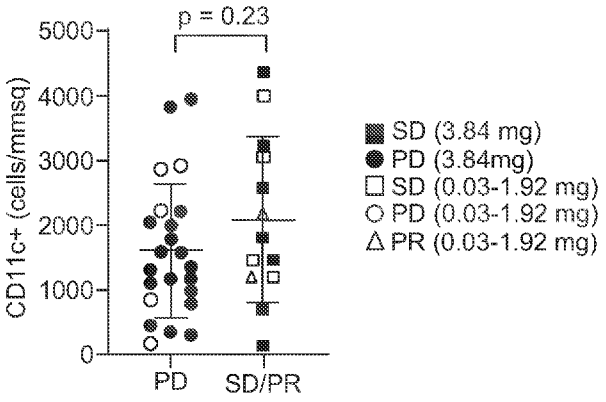

FIG. 4

(57) Abstract: Provided herein are methods related to cancer treatment and prognosis, and more specifically to treatment of cancers positive of CD11c with Toll-Like Receptor ("TLR") agonists.

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TLR AGONIST COMPOUNDS AND RELATED CANCER IMMUNOTHERAPY METHODS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application No. 63/110,897, filed on November 6, 2020, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0002] Provided herein are methods related to cancer treatment and prognosis, and more specifically to treatment of such conditions with Toll-Like Receptor ("TLR") agonist compounds.

BACKGROUND

[0003] TLRs are a class of type I transmembrane proteins and play an essential role in the innate immune system by recognizing pathogen-associated molecular patterns derived from microbes. There are several TLRs in human, including TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, and TLR10. Various combinations of TLRs are expressed on several cell types belonging to the innate and adaptive immune system, such as monocytes, macrophages, dendritic cells (DCs), neutrophils, B cells, and T cells. Activated TLR signaling triggers the NF-κB and type I interferon (IFN) pathways resulting in the production of pro-inflammatory cytokines, chemokines, and type I IFNs. TLR agonists are potential cancer immunotherapy candidates due to their immune stimulatory effects and abilities to modulate the tumor environment from a tumor-promoting to a tumor-suppressive (inflammatory) environment.

[0004] Activating the immune system has the potential to produce durable responses in human cancers (Hodi, 2010). Recent attention in immunotherapy has focused on immune activation using checkpoint inhibitor antibodies (Topalian, 2014). However, direct immune stimulation using cytokines can also drive immune-mediated cures (Hanzly, 2014). Aldesleukin directly stimulates the immune system and has been shown to lead to remission in ~10% of patients with metastatic melanoma (MEL) and renal cancer (Payne, 2014). TLR ligands are a promising emerging class of immune

response enhancers with the potential to generate an effective anti-tumor immune response. TLRs activate both the innate and adaptive immune systems, and are involved in antiviral and anti-tumor immunity. Both the innate and the adaptive arm of the immune system can contribute to eradication of tumor cells, with natural killer (NK) cells and T cells, respectively, acting as key players. Specifically, activation of TLR7 and TLR8 signaling pathways has been identified as a target for oncology indications (Toussi, 2014).

[0005] The systemic administration of TLR agonists for use in cancer therapy, however, has shown only limited activity (Chan, 2015), which may in part be due to the fact that activated T cells failed to migrate to the tumor (Lou, 2011). The activation of TLR7/8 with imiquimod as a topical local application demonstrated clinical and histological clearance of superficial basal cell carcinoma in a controlled Phase 3 study (Eigentler, 2007). Effective TLR7 and TLR8 activation have been achieved with resiquimod (a TLR7/8 agonist that can, for example, activate TLR7 and/or TLR8), which was previously studied as an oral agent in a Phase 2 study for the treatment of hepatitis C virus (HCV; Pockros, 2007).

[0006] (2,7-(bis-methoxyPEG$_{10kD}$-carboxyamide)(9H-fluorene-9-yl)methyl N-carbamate)$_{6\ avg}$interleukin-2 (wherein the interleukin-2 is aldesleukin), also referred to herein as RSLAIL-2, is a long-acting, CD122-preferential, IL-2Rβ preferential agonist that is able to activate adaptive immunity by increasing proliferation and tumor infiltration of $CD8^+$ T cells and NK cells in the tumor microenvironment. Exemplary IL-2Rβ-preferential agonists, including RSLAIL-2, are described in International Patent Publication Nos. WO 2012/065068 and WO 2015/125159, the entire disclosures of which are incorporated by reference herein.

[0007] As described in International Patent Publication No. WO 2018/132496, the combination of a TLR7/8 agonist such as pentaerythritolyl-4-arm-(PEG-1-methylene-2-oxo-vinylamino acetamide linked-resiquimod)-20kD (4-arm-PEG20k-CM-Gly-N-R848) and (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 (RSLAIL-2) may lead to synergistic activation of both the innate and adaptive anti-tumor immune response and enhanced antigen presentation with sustained T cell activation, resulting in tumor growth inhibition of treated and abscopal lesions in preclinical murine models.

[0008] A Phase 1b/2 clinical trial has been initiated to evaluate the combination therapy of pentaerythritolyl-4-arm-(PEG-1-methylene-2-oxo-vinylamino acetamide linked-resiquimod)-20kD (4-arm-PEG20k-CM-Gly-N-R848) with (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 in patients with refractory advanced or metastatic solid tumors (see Example 1 for details). Briefly, the design of the trial involves administering to patients escalating doses of 4-arm-PEG20k-CM-Gly-N-R848 (0.03 mg to 3.84 mg intratumoral (i.t.)) plus (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 (0.006 mg/kg intravenous (i.v.)) in 3-week treatment cycles. The primary endpoint is safety and tolerability, including definition of the maximum tolerated dose/recommended Phase 2 dose (RP2D). Other endpoints include antitumor activity, immune activation, cytokines, PD-L1 status, and pharmacokinetics.

[0009] Ongoing clinical trial data have demonstrated that the addition of nivolumab (Opdivo®) to (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 enhanced an immune-stimulatory response compared to (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 monotherapy (Diab, 2017). Therefore, the combination of a TLR7/8 agonist and (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 with or without nivolumab (or other suitable anti-PD-1 or anti-PDL-1 checkpoint inhibitor, or immune checkpoint blockage therapeutic) could engage the entire immune activation cascade required for systemic tumor clearance from local tumor antigen production to a sustained systemic T cell response and therefore may offer a number of possible synergies and provide new treatment options for patients with cancer.

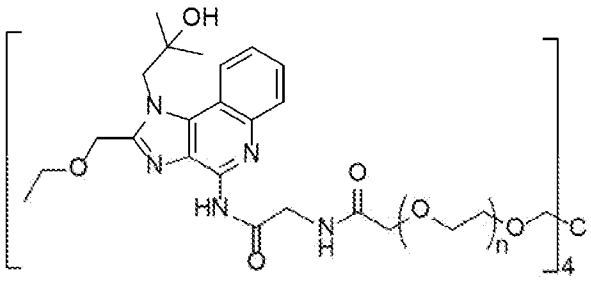
SUMMARY

[0010] The present disclosure is generally directed to methods of treating or preventing cancer, including solid tumors, that are positive for expression of cluster of differentiation (CD)11c with one or more immunotherapy drugs, including a TLR7/8 agonist and/or CD-122 preferential agonist.

[0011] In a first aspect, the disclosure is directed to a method of treating a subject having a solid cancer where the cancer has a baseline cluster of differentiation (CD)11c

expression level at least about 500 to at least about 5000 CD11c positive cells/mm^2 by administering a TLR7/8 agonist. More particularly, the subject is treated with the TLR7/8 agonist where the cancer has a CD11c+ baseline expression of at least about 500 to at least about 2000 CD11c positive cells/mm^2 or about 500 to at least about 2000 CD11c positive cells/mm^2. Preferably the cancer has a CD11c+ baseline expression of at least about 500 to at least about 2000 CD11c positive cells/mm^2, or at least about 500 to at least about 1000 CD11c positive cells/mm^2, or at least about 1000 to at least about 2000 CD11c positive cells/mm^2.

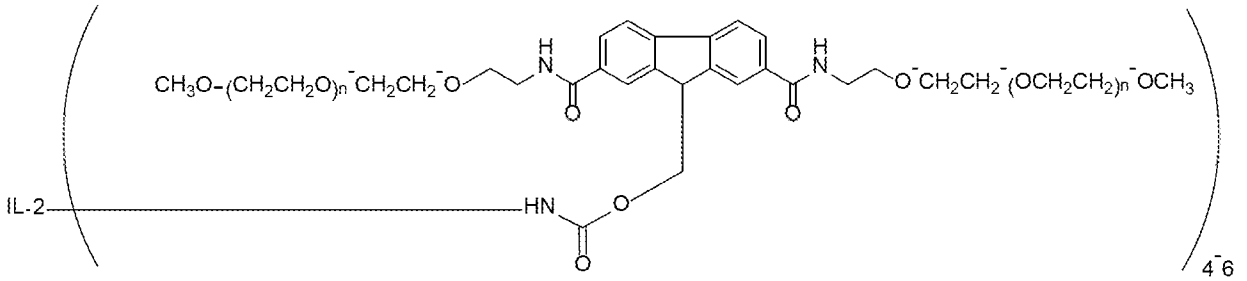
[0012] More particularly, the TLR7/8 agonist comprises a multi-arm polymer conjugated to a TLR receptor agonist. In preferred embodiments, the TLR7/8 receptor agonist has the formula:



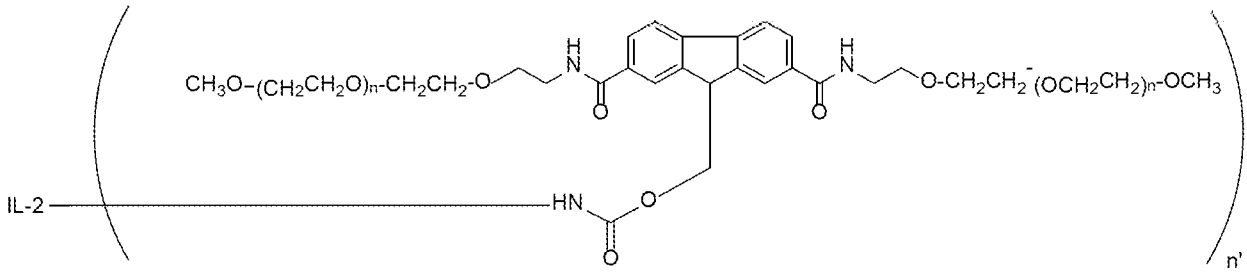
wherein each n is independently an integer from 40 to 350. In embodiments, n may be independently selected from 100 to 250. Among other things, the TLR7/8 agonists are locally administered, e.g., intratumorally to a tumor site, where the TLR7/8 agonist is effective to increase tumor antigen presentation and T-cell stimulation (i.e., to result in enhanced CD8 T cell priming), that is, to elicit an innate immune response, while accompanied by minimal toxic side effects due to localized activity. In some embodiments, the TLR7/8 agonist is administered at a therapeutically effective dose such as a dose of about 30 µg to about 4 mg. In more particular embodiments, the TLR7/8 agonist is administered at a dose of about 0.03 mg to 7.68 mg. In one specific embodiment the TLR7/8 agonist is administered at a dose of about 3.84 mg.

[0013] In some embodiments, the present methods further comprise administering a therapeutically effective amount of a CD-122 preferential agonist to the subject. In some embodiments, the CD-122 preferential agonist comprises (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2.

In certain embodiments, the combination is effective to promote activation of the immune system (for example through promotion of CD8 T cells, CD11c+ and CD8+ dendritic cells, and neutrophils), while also overcoming immune suppression (for example though suppression of T regulatory cells, macrophages, and monocytes). In particular embodiments, the CD-122 preferential agonist comprises compounds encompassed by the formula:



wherein IL-2 is an interleukin-2, where "n" is an integer from about 3 to about 4000, or pharmaceutically acceptable salts thereof. In some further embodiments, the CD-122 preferential agonist is comprised in a composition comprising no more than 10% (based on a molar amount) of compounds encompassed by the following formula:



wherein IL-2 is interleukin-2, n' is an integer selected from the group consisting of 1, 2, 3, 7 and >7, or pharmaceutically acceptable salts thereof. In one preferred embodiment, the CD-122 preferential agonist is administered at a dose of about 0.006 mg/kg.

[0014] In one or more embodiments, the TLR agonist is administered locally and the CD-122 preferential agonist is administered parenterally. In one or more related embodiments, the TLR agonist is administered directly to the site of a tumor.

[0015] By way of clarity, with regard to the sequence of administering, the TLR 7/8 agonist and the CD-122 preferential agonist may be administered concurrently or sequentially and in any order, and via the same and/or different routes of administration,

each in an immunomodulating amount. Moreover, treatment may comprise a single cycle of therapy, or may comprise multiple (i.e., two or more) cycles of therapy.

[0016] In yet one or more embodiments, one or more additional therapeutic agents to the subject. In particular embodiments, the additional therapeutic agent comprises an immunotherapy agent such as nivolumab. In a particular embodiment where the additional agent is nivolumab, the nivolumab is administered at a dose of about 360 mg.

[0017] In one or more embodiments, the cancer for treatment may be any cancer that expresses CD11c. In some particular embodiments, the methods described herein are suitable for treatment of a solid tumor is selected from the group consisting of melanoma, sarcoma, Merkel cell carcinoma, colorectal cancer, head and neck cancer, renal cell carcinoma, and breast cancer. In particular embodiments, the sarcoma is any one or more of osteosarcoma, chondrosarcoma, undifferentiated pleomorphic sarcoma, fibrous histocytoma, liposarcoma, angiosarcoma and leiomyosarcoma.

[0018] In performing the methods, a particular embodiment involves obtaining a sample by a biopsy of the solid tumor. In some further embodiments, the baseline CD11c expression level in the sample may be determined by an immunohistochemistry assay of the sample such as an assay using an anti-CD11c antibody.

[0019] In one or more embodiments, the method treats or prevents cancer by promoting anti-antitumor immune response in the tumor microenvironment in the subject. In further embodiments, treating or preventing cancer comprises reducing tumor size or tumor cell number in the subject. In even further embodiments, treating comprises reducing tumor burden or loci in the subject including reducing a metastatic tumor burden. In yet another embodiment, treating the cancer comprises increasing cancer remission or cancer survival rate in the subject.

[0020] In another aspect, i.e. a second, aspect, the disclosure provides a method of predicting the efficacy of and treatment with a TLR7/8 agonist in a subject having one or more solid tumors, comprising evaluating the subject for determining a baseline CD11c expression level a solid tumor sample obtained from the subject; and administering a therapeutically effective amount of the TLR7/8 agonist to a subject having a CD11c+ baseline expression of at least about 500 to about 2000, at least about 500 to about 1000, or at least about 1000 to about 2000 CD11c positive cells/mm^2. In some embodiments,

the improved efficacy is selected from one or more of increased survival rate, decreased tumor size, decreased tumor numbers, and reduced tumor burden or loci.

[0021] In yet another, i.e., third, aspect, disclosed is an improved method of treating a subject having a solid tumor by administering to the subject a TLR7/8 agonist, where the improvement comprises prior to the administering step, determining, from a tissue sample of the subject's solid tumor, a baseline CD11c expression level of at least about 500 to at least about 2000, at least about 500 to about 1000, or at least about 1000 to about 2000 CD11c positive cells/mm^2. In this method, the expression level is predictive of a favorable response of the subject to treatment.

[0022] In a further, i.e., fourth, aspect, disclosed is a method of treating a subject having a solid cancer, comprising administering a TLR7/8 agonist to a subject having a solid tumor that is positive for expression of cluster of differentiation (CD)11c.

[0023] In yet another, i.e. fifth, aspect, provided is a kit comprising a therapeutically effective amount of a TLR agonist and a therapeutically effective amount of a CD-122 preferential agonist, accompanied by instructions for use in treating a subject having cancer.

[0024] Additional embodiments of the present conjugates, compositions, methods, and the like will be apparent from the following description, examples, and claims. As can be appreciated from the foregoing and following description, each and every feature described herein, and each and every combination of two or more of such features, is included within the scope of the present disclosure provided that the features included in such a combination are not mutually inconsistent. In addition, any feature or combination of features may be specifically excluded from any embodiment.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] FIGs. 1A-1E show that combination treatment leads to sequential innate and adaptive immune activation. CT26 tumor bearing mice with bilateral flank tumors were treated with the TLR7/8 agonist 4-arm-PEG20k-CM-Gly-N-R848 in the right anatomical side tumor only on Day 0. (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 was administered intravenously (i.v.) on Day 4. Immune phenotyping by flow cytometry was conducted on Day 1 (TLR7/8 agonist single agent effect) and Day 7 (3 days after administration for combination activity). TLR7/8

agonist treated and untreated tumors were both evaluated to measure the local TLR7/8 agonist treatment site and abscopal immune response, respectively. Local TLR7/8 agonist injection site activation of neutrophils was observed (FIG. 1B). Dendritic cell response was observed locally in the injected tumor and also in the abscopal tumor (FIGs. 1D and 1E); the magnitude of $CD8^+$ T cell tumor infiltration after RSLAIL-2 administration was dependent on TLR7/8 agonist dose response.

[0026] FIGs. 2A-2C show tumor growth inhibition and increased survival by combination treatment. As seen from FIGs. 2A-2B, CT26 tumor bearing mice with bilateral flank tumors were treated once with the TLR7/8 agonist 4-arm-PEG20k-CM-Gly-N-R848 in the right anatomical side tumor only on Day 0, followed by (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 i.v. administration on Day 4 with q9dx3 regimen. TLR7/8 agonist treated (solid lines) and abscopal (dashed lines) tumor volumes were measured over 55 days following treatment start. As shown in FIG. 2C, administration of the TLR7/8 agonist dose-dependently increased survival in combination treatment with RSLAIL-2 (open symbols) compared to single agent treatments (filled symbols and "x").

[0027] FIG. 3 shows proposed activation of anti-tumor immune cascade by the TLR7/8 agonist 4-arm-PEG20k-CM-Gly-N-R848 in combination with (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 or in combination (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 and nivolumab.

[0028] FIG. 4 shows baseline $CD11c^+$ cell density (cells/mm^2) in melanoma patient biopsies categorized by best overall response.

[0029] FIG. 5 shows the change in target lesion sum of diameters at week 9 from baseline (% CFB) as compared to baseline $CD11c^+$ cell density (cells/mm^2) in patient biopsies.

[0030] FIGs. 6A-6C show association between TLR7/8 target genes and baseline $CD11c^+$ cell density.

[0031] FIGs. 7A-7C show that melanoma baseline biopsies are enriched in $CD11c^+$ cells compared to other tumor types.

DETAILED DESCRIPTION

[0032] CD11c is a cell surface marker expressed by several subsets of immune cells, including antigen-presenting cells (APCs). Many $CD11c^+$ cells express TLR7 and/or TLR8 and thus could potentially respond to treatment with a TLR7 agonist, a TLR8 agonist, and/or a TLR7/8 agonist (e.g., 4-arm-PEG20k-CM-Gly-N-R848). Without intending to be limited by any particular theory, activation of these cells can be essential for driving the TLR7/8 agonist-dependent innate anti-tumor response. According to the present technology, the concentration of $CD11c^+$ cells in tumor biopsies at baseline and a favorable response of these $CD11c^+$ tumors to the immune-oncology treatment suggest that CD11c could serve as a novel cancer biomarker and be used to optimize cancer immunotherapies, predict responsiveness, and select patient populations, for better treatment outcome.

[0033] To the extent any materials incorporated herein by reference conflict with the present disclosure, the present disclosure controls.

[0034] While the present disclosure is capable of being embodied in various forms, the description below of several embodiments is made with the understanding that the present disclosure is to be considered as an exemplification of the invention and is not intended to limit the invention to the specific embodiments illustrated.

[0035] Headings are provided for convenience only and are not to be construed to limit the invention in any manner. Embodiments illustrated under any heading may be combined with embodiments illustrated under any other heading.

Definitions

[0036] Unless otherwise specified, each of the following terms has the meaning set forth in this section.

[0037] The indefinite articles "a" and "an" denote at least one of the associated nouns and are used interchangeably with the terms "at least one" and "one or more." For example, the phrase "a module" means at least one module, or one or more modules.

[0038] The conjunctions "or" and "and/or" are used interchangeably.

[0039] The term "including" is used interchangeably with the term "including, but not limited to."

[0040] The term "about," as used herein when referring to a measurable value such as an amount or concentration and the like, is meant to encompass variations of 20%, 10%, 5%, 1%, 0.5%, or even 0.1% of the specified amount.

[0041] A "subject" means a human, mouse, or non-human primate. A human subject can be any age (e.g., an infant, child, young adult, or adult), and may suffer from a disease, such as a cancer. In some embodiments, a subject is suffering from a relevant disease, disorder, or condition. In some embodiments, a subject is susceptible to a disease, disorder, or condition. In some embodiments, a subject displays one or more symptoms or characteristics of a disease, disorder, or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a subject is someone with one or more features characteristic of susceptibility to or risk of a disease, disorder, or condition. In some embodiments, a subject is a patient. In some embodiments, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

[0042] The phrases "subject" and "patient" are used interchangeably herein.

[0043] The terms "treat," "treating," and "treatment" as used herein with regard to cancer refers to alleviating the cancer partially or entirely, inhibiting cancer cell growth, reducing the number of cancer cells, preventing the cancer, decreasing the likelihood of occurrence or recurrence of the cancer, slowing the progression or development of the cancer, or eliminating, reducing, or slowing the development of one or more symptoms associated with the cancer. For example, "treating" may refer to preventing or slowing the existing tumor from growing larger, preventing or slowing the formation or metastasis of cancer, and/or slowing the development of certain symptoms of the cancer. In some embodiments, the term "treat," "treating," or "treatment" means that the subject has a reduced number or size of tumor comparing to a subject without being administered with the treatment. In some embodiments, the term "treat," "treating," or "treatment" means that one or more symptoms of the cancer are alleviated in a subject receiving the pharmaceutical compositions as disclosed and described herein compared to a subject who does not receive such treatment.

[0044] The terms "prevent," "preventing," and "prevention" as used herein means the prevention of a disease (e.g., cancer) in a subject (e.g., in a human), including (a)

avoiding or precluding the disease; (b) affecting the predisposition toward the disease; and (c) preventing or delaying the onset of and/or reduction in frequency and/or severity of at least one symptom of the disease.

[0045] The term "antibody" as used herein is used to denote, in addition to natural antibodies, genetically engineered or otherwise modified forms of immunoglobulins, including chimeric antibodies, human antibodies, humanized antibodies, or synthetic antibodies. The antibodies disclosed herein may be monoclonal or polyclonal antibodies. In those embodiments wherein an antibody is an immunogenically active portion of an immunoglobulin molecule, the antibody may include, but are not limited to, a single chain Fv antibody (scFv), disulfide-linked Fv, single domain antibody (dAb), Fab, Fab', Fab fragment, F(ab')2, or diabody.

[0046] A "therapeutically effective amount" as used herein is an amount that produces a desired effect in a subject for treating cancer. In certain embodiments, the therapeutically effective amount is an amount that yields maximum therapeutic effect. In other embodiments, the therapeutically effective amount yields a therapeutic effect that is less than the maximum therapeutic effect. For example, a therapeutically effective amount may be an amount that produces a therapeutic effect while avoiding one or more side effects associated with a dosage that yields maximum therapeutic effect. A therapeutically effective amount for a particular composition will vary based on a variety of factors, including, but not limited to, the characteristics of the therapeutic composition (e.g., activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (e.g., age, body weight, sex, disease type and stage, medical history, general physical condition, responsiveness to a given dosage, and other present medications), the nature of any pharmaceutically acceptable carriers, excipients, and preservatives in the composition, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation, namely, by monitoring a subject's response to administration of the therapeutic composition and adjusting the dosage accordingly. For additional guidance, see, for example, Remington: The Science and Practice of Pharmacy, 22nd Edition, Pharmaceutical Press, London, 2012, and Goodman & Gilman's

The Pharmacological Basis of Therapeutics, 12th Edition, McGraw-Hill, New York, NY, 2011, the entire disclosures of which are incorporated by reference herein.

[0047] The following abbreviations may be used in the present disclosure: 1L, one line (of therapy); 1-2L, one to two lines (of therapy); 2L, two lines (of therapy); 2-3L, two to three lines (of therapy); 3L, three lines (of therapy); ACTH, adrenocorticotropic hormone; AE, adverse event; AESI, adverse event of special interest; AJCC, American Joint Committee on Cancer; ALP, alkaline phosphatase; ALT (SGPT), alanine aminotransferase (serum glutamic pyruvic transaminase); ANC, absolute neutrophil count; anti-CTLA-4, antibody against cytotoxic T-lymphocyte-associated antigen 4; APC, antigen presenting cell; AST (SGOT), aspartate aminotransferase (serum glutamic oxaloacetic transaminase); AUC, area under the curve; BM, biomarker; BOR, best overall response; BUN, blood urea nitrogen; β-HCG, beta-sub unit of human chorionic gonadotropin; CBR, clinical benefit rate; CFR, Code of Federal Regulations; CI, confidence interval; CK, creatinine kinase; CL/F, clearance; Cmax, maximum concentration; CR, complete response; CRC, colorectal carcinoma; CRF, case report form; CRS, cytokine-release syndrome; CT, computed tomography; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; CVA, cerebrovascular accident; CYP, cytochrome P450; CYP3A4, cytochrome P450 3A4; DCI, data collection instrument; DILI, drug-induced liver injury; DLT, dose-limiting toxicity; DOAC, direct oral anticoagulation; DOR, duration of response; dQTcF, baseline adjusted QTcF interval; DWI, diffusion-weighted imaging; EC50, half maximal effective concentration; ECG, electrocardiogram; ECOG, Eastern Cooperative Oncology Group; eCRF, electronic case report form; EDC, electronic data capture; EOI, end of infusion; EOT, end of treatment; ER-,estrogen-receptor-negative; ERK, extracellular signal–regulated kinase; FDA, Food and Drug Administration; FFPE, formalin-fixed paraffin-embedded; FIH, first-in-human; FNR, false-negative rate; FPR, false-positive rate; GCP, Good Clinical Practice; GGT, gamma-glutamyl transferase; GLP, Good Laboratory Practice; GMP, Good Manufacturing Practice; HBsAg, hepatitis B surface antigen; HCT, hematocrit; HCV, hepatitis C virus; HED, human equivalent dose; HER2-,human epidermal growth factor receptor 2 negative; Hgb, hemoglobin; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; HNSCC, head and neck

squamous cell carcinoma; HNSTD, highest non-severely toxic dose; ICF, informed consent form; ICH, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; IEC, Independent Ethics Committee; IFN, interferon; IFNα, interferon alpha; IFNγ, interferon gamma; IHC, immunohistochemistry; IL-2, interleukin-2; IL-6, interleukin-6; IL-12, interleukin-12; imAE, immune-mediated adverse event; IND, Investigational New Drug application; I-O, immune-oncology; IR, interventional radiographic; IRB, Institutional Review Board; irCR, immune-related complete response; Irf7, interferon regulatory factor 7; irPD, immune-related progressive disease; irPR, immune-related partial response; irRECIST, immune-related RECIST; irSD, immune-related stable disease; IT, intratumoral; IV, intravenous; L, line; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; LMWH, low molecular weight heparin; MAD, maximally administered dose; MCC, Merkel cell carcinoma; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCPyV, Merkel cell polyomavirus; MCV, mean corpuscular volume; MedDRA, Medical Dictionary for Regulatory Activities; MEL, melanoma; mg, milligram; min, minute(s); mL, milliliter; MOA, mechanism of action; MRI, magnetic resonance imaging; MRSD, maximum recommended starting dose; MSI, microsatellite instability; MTD, maximum tolerated dose; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; NE, inevaluable; NK, natural killer; (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2; NOAEL, no observable adverse effect level; NSCLC, non-small cell lung cancer; NTL, non-target lesion; NYHA, New York Heart Association; ORR, objective response rate; OS, overall survival; OTC, over-the-counter; PBMC, peripheral blood mononuclear cell; PD, progressive disease; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; PK, pharmacokinetic; PPK, population pharmacokinetics; PR, partial response; PR-,progesterone-receptor-negative; PT, prothrombin time; PTT, partial thromboplastin time; q2w, every 2 weeks; q21d, every 21 days; q3w,every 3 weeks; QTcF, Fridericia's corrected QT interval; RANKL, receptor activator of nuclear factor kappa-B ligand; R848, resiquimod; RBC, red blood cells; RCC, renal cell carcinoma; RECIST, Response Evaluation Criteria in Solid Tumors; RP2D, recommended Phase 2 dose; R/R, relapsed or refractory; SAE, serious adverse event;

SAP, statistical analysis plan; SC, subcutaneous(ly); SD, stable disease; SLD, sum of the longest diameters; SOP, standard operating procedure; SUSAR, suspected unexpected serious adverse reaction; t1/2, half-life; T3, triiodothyronine; T4, free thyroxine; TEAE, treatment-emergent adverse event; TIA, transient ischemic attack; TIL, tumor infiltrating lymphocyte; TLR, toll-like receptor; TNFα, tumor necrosis factor alpha; Tmax, time to maximum concentration; TNBC, triple-negative breast cancer; TP, total protein; Treg, regulatory T cell; TSH, thyroid stimulating hormone; TTR, time to response; ULN, upper limit of normal; USP, United States Pharmacopeia; Vd/F, volume of distribution; VS, vital signs; WBC, white blood cell; WCBP, women of child-bearing potential.

Methods of Treatment

[0048] In some aspects, the present technology provides methods of treating or preventing cancer in a subject. In some embodiments, the methods comprise determining a baseline CD11c expression level in the cancer and administering to the subject a therapeutically effective amount of a TLR7/8 agonist (e.g., 4-arm-PEG20k-CM-Gly-N-R848), alone or in combination with an IL-2Rβ-selective (i.e. a CD-122 preferential agonist) (e.g., (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2), based on the determined baseline CD11c expression level. In some embodiments, the methods comprise predicting the responsiveness of a patient to treatment with a TLR7/8 agonist, and/or selecting a suitable patient population for treatment with a TLR7/8 agonist, based on the CD11c expression level in the cancer cells.

[0049] Mechanistic Considerations: At the start of the treatment, a TLR7/8 agonist activates TLR7/8 receptor signaling in the tumor primarily in myeloid cell types. A number of parallel immune cell activities are rapidly, within hours, initiated after TLR7/8 agonist treatment: (1) initial transient IT increase and activation of early responding innate cell types including neutrophils lead to tumor cell death and release of tumor antigen; (2) dendritic cells are forced to differentiate and mature leading to tumor antigen uptake, and cross presentation by APCs; and (3) an IT cytokine milieu is generated that promotes immune stimulatory cell signaling and reduction of immune suppressive signals for infiltrating anti-tumor CD8+ T cells.

[0050] These immune mechanisms prepare the tumor microenvironment to be T cell attractive and immune stimulatory for subsequent CD8$^+$ cytotoxic T cell infiltration that is

initiated by the RSLAIL-2 component of the therapy. In addition to the fast-acting immune mechanisms listed above, TLR7/8 agonist also has a prolonged inhibitory effect on IT accumulation of regulatory T cells (Tregs) leading to a superior $CD8^+$/Treg ratio compared to RSLAIL-2 single agent therapy in nonclinical models.

[0051] A role of the IL-2Rβ-selective (i.e., a CD-122 preferential) agonist, e.g., RSLAIL-2, is to engage the interleukin-2 (IL-2) receptor complex to promote proliferation of T cells with a bias towards the $CD8^+$ subtype and to sustain T cell activation. T cells encounter APCs generated in response to TLR7/8 agonist stimulation and tumor antigen specific CD8+ cytotoxic T lymphocyte (CTL) clones are preferentially amplified and sustained by RSLAIL-2 activity. These activated CD8+ T cells migrate into tumors that have up-regulated T cell attracting chemokines like IP-10 in response to TLR7/8 agonist stimulation. RSLAIL-2 has additional inhibitory effects on monocyte IT recruitment reducing immune suppressive myeloid activity and macrophage numbers in tumors.

[0052] Activation of neutrophils in tumors and inhibition of IT accumulation of Tregs by TLR7/8 agonist activity is maintained in combination treatment with RSLAIL-2, which suppresses monocyte accumulation leading to further reduction in immune suppression. Beyond additive effects, the combination of TLR7/8 agonist and RSLAIL-2 treatment also generates a synergistic activity of both components that leads to IT increase in $CD103^+CD11c^+$ tumor antigen cross-presenting dendritic cells and decrease in tumor-associated macrophages. These additive and synergistic interactions between TLR7/8 agonist and RSLAIL-2, when administered in combination therapy lead to comprehensive multi-step anti-tumor immune response from initial tumor antigen processing to subsequent initiation of tumor-reactive T cells. For example, a TLR7/8 agonist and RSLAIL-2 combination treatment enables local initiation of anti-tumor immune response in one or more accessible tumors with administration of the TLR7/8 agonist, e.g., intratumorally, and subsequent systemic dissemination and abscopal effect of generated tumor killing T cell response throughout the body by virtue of RSLAIL-2 activity.

[0053] The TLR7/8 agonist, when administered in combination with RSLAIL-2, can engage non-overlapping complementary immune signaling pathways with both additive and synergistic interactions between treatment components leading to a sustained comprehensive anti-tumor immune response.

[0054] In some embodiments, a combination of the TLR7/8 agonist and RSLAIL-2, in combination with an anti-programmed cell death protein 1 (PD-1) checkpoint inhibitor (such as, for example, nivolumab) may further prolong tumor specific cytotoxic T cell activity by limiting immune suppression.

[0055] In one or more preferred embodiments, the cancer comprises a solid tumor. Non-limiting examples of a solid tumor suitable for treatment include melanoma e.g. metastatic melanoma, sarcoma, Merkel cell carcinoma, colorectal cancer, and breast cancer. In some embodiments, the breast cancer is triple-negative breast cancer. Renal cell carcinomas (RCC) for treatment include advanced or metastatic RCC. Colorectal cancer for treatment may be advanced, which includes either locally advanced unresectable cancer or metastatic cancer. Sarcomas for treatment include, but are not limited to osteosarcoma, chondrosarcoma, undifferentiated pleomorphic sarcoma/malignant fibrous histocytoma, dedifferentiated/pleomorphic liposarcoma, angiosarcoma and leiomyosarcoma. Sarcomas for treatment may be advanced, which includes either locally advanced unresectable cancer or metastatic cancer.

[0056] In some embodiments, the cancer comprises a solid tumor. Non-limiting examples of a solid tumor suitable for treatment of the present technology include melanoma, sarcoma, Merkel cell carcinoma, colorectal cancer, and breast cancer.

[0057] In some embodiments, the cancer suitable for treatment with the present technology is concentrated for $CD11c^+$ cells. Indeed, as described in Example 2, it was observed in patient biopsies taken after administration of an exemplary and preferred TLR 7/8 agonist, 4-arm-PEG20k-CM-Gly-N-R848, that several genes previously shown to be induced by TLR7/8 agonism were increased in response to the administration and expressed at higher levels compared to baseline biopsies, consistent with the proposed mechanism of action. As seen in FIGs 6A-6C, induction of multiple TLR7/8 target genes associated with the density of $CD11c^+$ cells in baseline biopsies provide a link between the presence of $CD11c^+$ cells in tumors and the ability of the TLR7/8 agonist to drive signaling through TLR7/8 to thereby result in more efficacious treatment. As described further herein, CD11c+ cells express TLR7 and/or TLR8 and are a target for TLR7/8 agonists, and $CD11c^+$ cells respond to TLR7/8 agonism. Therefore, based upon the supporting data provided herein, $CD11c^+$ cancers, or cancers with an elevated

concentration of $CD11c^+$ cells, appear to exhibit a more favorable response to treatment with immunotherapy agents of the present technology, such as TLR7/8 agonists.

[0058] In some embodiments, the methods comprise a step of obtaining a sample from the cancer of the subject and/or isolating cancer cells from the subject. Cancer samples and/or cells can be obtained and/or isolated from a subject using known techniques in the medical art, for example, by biopsies of the cancer. Depending on the cancer type and location, various biopsy procedures may be used, including skin biopsy, endoscopic biopsy, needle biopsy, and bone marrow biopsy. Once the sample is removed from the patient, the sample can be subject to subsequent processing (e.g., slicing, staining) for further laboratory analysis.

[0059] In some embodiments, the methods comprise a step of determining a baseline CD11c expression level of the subject-derived cancer or tumor sample. CD11c expression level of a tumor sample can be determined using methods and techniques known to a person of ordinary skill in the art, including, but not limited to, antibody-based assays. Non-limiting examples of such antibody-based assays include immunohistochemistry (IHC), Western blot, enzyme-linked immunosorbent assay (ELISA), protein immunoprecipitation, and flow cytometry. For example, IHC assay of a tumor biopsy involves contacting the tumor sample with antibodies specific for a protein of interest (e.g., anti-CD11c antibody) and allows imaging, visualization, and quantification of cells positive for that protein in the sample.

[0060] In some embodiments, the subject has a solid tumor exhibiting a baseline CD11c expression level of at least about 100 positive cells/mm^2, at least about 200 positive cells/mm^2, at least about 300 positive cells/mm^2, at least about 400 positive cells/mm^2, at least about 500 positive cells/mm^2, at least about 600 positive cells/mm^2, at least about 700 positive cells/mm^2, at least about 800 positive cells/mm^2, at least about 900 positive cells/mm^2, at least about 1000 positive cells/mm^2, at least about 1100 positive cells/mm^2, at least about 1200 positive cells/mm^2, at least about 1300 positive cells/mm^2, at least about 1400 positive cells/mm^2, at least about 1500 positive cells/mm^2, at least about 1600 positive cells/mm^2, at least about 1700 positive cells/mm^2, at least about 1800 positive cells/mm^2, at least about 1900 positive cells/mm^2, at least about 2000 positive cells/mm^2, at least about 2500 positive cells/mm^2, at least about 3000 positive

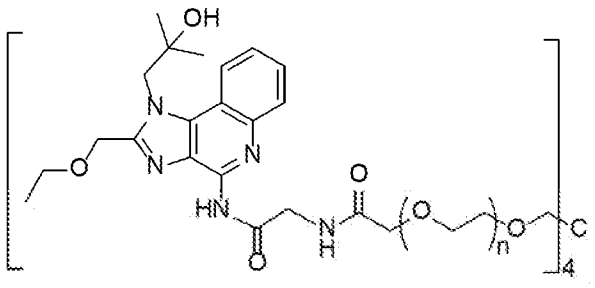
cells/mm^2, at least about 3500 positive cells/mm^2, at least about 4000 positive cells/mm^2, at least about 4500 positive cells/mm^2, at least about 5000 positive cells/mm^2, or greater than 5000 positive cells/mm^2, in the cancer sample.

[0061] In one or more embodiments, a patient diagnosed with cancer, where the baseline level of expression of CD11c on the cancer cells in a sample from the patient is at least about 500-5000 CD11c positive cells/mm^2, is treated with a TLR7/8 agonist, and optionally one or more additional immunotherapeutic agents as described herein. In further embodiments, the patient has a solid cancer determined to have a baseline level of CD11c expression of at least about 500-1000 CD11c positive cells/mm^2, at least about 500-1500 CD11c positive cells/mm^2, at least about 500-2000 CD11c positive cells/mm^2, at least about 1000-5000 CD11c positive cells/mm^2, or even at least about 1000-2000 CD11c positive cells/mm^2 threshold, wherein a baseline CD11c positive threshold may be a baseline CD11c expression level as noted above, and the patient is treated with a TLR7/8 agonist, and, optionally, one or more additional immunotherapeutic agents as described herein.

[0062] In some embodiments, the methods comprise administering a TLR7/8 agonist (e.g., 4-arm-PEG20k-CM-Gly-N-R848), alone or in combination with an IL-2Rβ-preferential or CD-122 preferential agonist (e.g., 2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2) to a subject determined to have a particular threshold baseline CD11c expression level in the cancer sample. Administration of the TLR7/8 agonist is typically via injection. Other modes of administration are also contemplated, such as pulmonary, nasal, buccal, rectal, sublingual and transdermal. As used herein, the term "parenteral" includes subcutaneous, intravenous, intra-arterial, intratumoral, intralymphatic, intraperitoneal, intracardiac, intrathecal, and intramuscular injection, as well as infusion injections. In some preferred embodiments, the TLR7/8 agonist is administered intratumorally, e.g, administered directly into a tumor, e.g., by injection. Such administration provides for a high concentration of the TLR7/8 agonist to be achieved in the tumor, with delayed release of the TLR7/8 agonist into the systemic circulation, and, in the case of a TLR7/8 compound comprising releasable linkages, into the tumor itself. An exemplary formulation for intratumoral administration of a TLR 7/8 agonist such as 4-arm-PEG20k-CM-Gly-N-R848

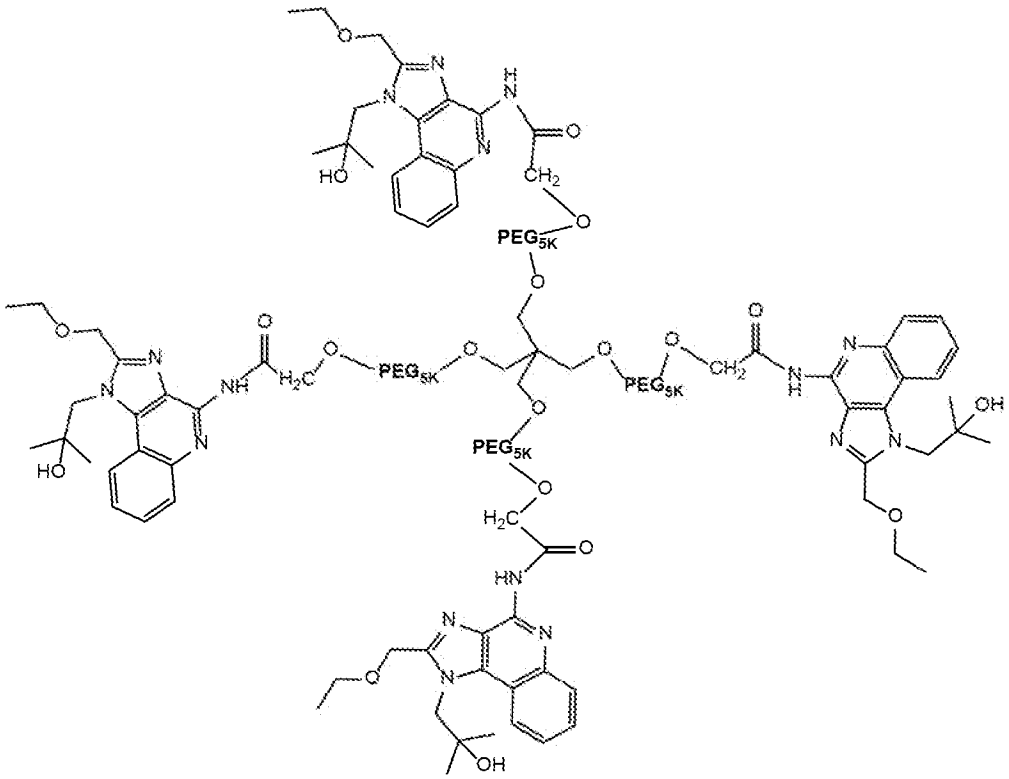
comprises Na/K phosphate buffer at pH 7.4. In some embodiments, treatment with the TLR7/8 agonist may not be commenced, or an alternative immuno-oncology treatment selected for the subject if the baseline CD11c expression level in the cancer is below the threshold level. In some embodiments, it is contemplated that for patients having a cancer wherein baseline level of expression of CD11c on the cancer cells in a sample from the patient are at least about 500-5000 CD11c positive cells/mm^2, preferably at least about 500-2000 CD11c positive cells/mm^2, at least about 500-1000 CD11c positive cells/mm^2, , at least about 500-1500 CD11c positive cells/mm^2, or at least about 1000-2000 CD11c positive cells/mm^2, a more favorable prognosis and/or outcome of treatment may result when compared to a subject having a cancer-type that exhibits baseline levels of CD11c expression below the threshold.

[0063] In one or more embodiments, the TLR7/8 agonist is a multi-arm polymer conjugate of resiquimod that optimizes the anti-tumor effect of resiquimod. In one particular embodiment, the TLR7/8 agonist is pentaerythritolyl-4-arm-(PEG-1-methylene-2 oxo-vinylamino acetamide linked –resiquimod)-20K (4-arm-PEG20k-CM-Gly-N-R848) having a 4-arm branched polyethylene glycol structure with resiquimod molecules covalently attached at the termini of the poly(ethylene glycol) "arms" via a hydrolysable linker. As a result, following administration, resiquimod is released upon hydrolysis to target TLR7 and TLR8, which are expressed in multiple cell types. 4-arm-PEG20k-CM-Gly-N-R848 is designed to be retained in the tumor micro-environment and to activate innate immunity by activating antigen presenting cells (APCs), such as dendritic cells, to create new antigen-specific cytotoxic ($CD8^+$) T cells. The structures, properties, and methods of use of exemplary TLR7/8 agonist conjugates, including 4-arm-PEG20k-CM-Gly-N-R848, are described in International Patent Publication No. WO 2018/132496, the entire disclosures of which are incorporated by reference herein. In particular, 4-arm-PEG20k-CM-Gly-N-R848 has the following structure:

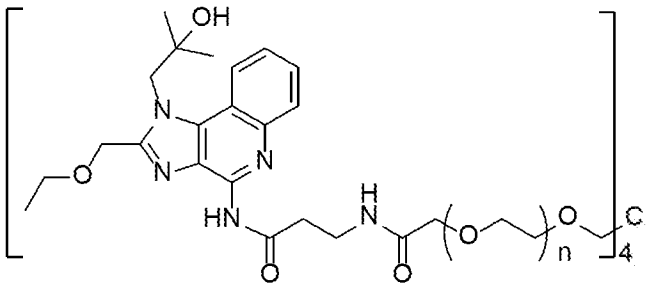


, wherein each n is independently an integer from 40 to 350. Preferably each n is independently an integer selected from 100 to 250. Further, n may be selected such that the average value of n in each of the poly(ethylene glycol) arms corresponds to a weight average molecular weight of about 5,000 daltons.

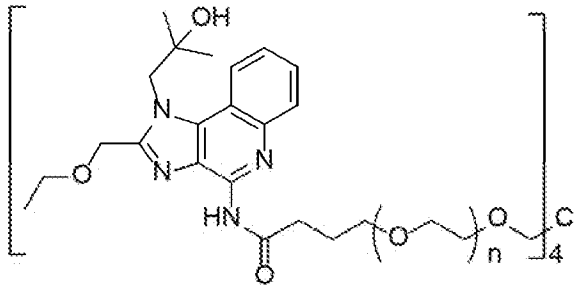
[0064] TLR7/8 agonist molecules suitable for use in the methods herein include the following, e.g., Compounds 1-10 and 12-16:



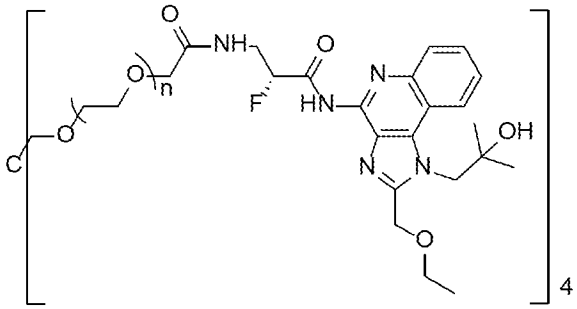
Compound 1



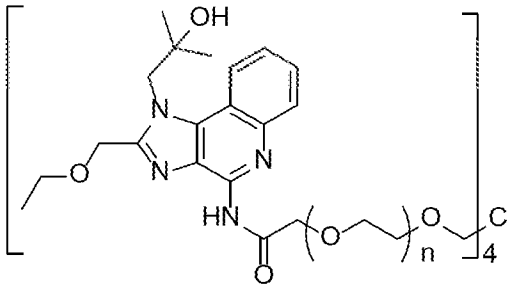
Compound 2



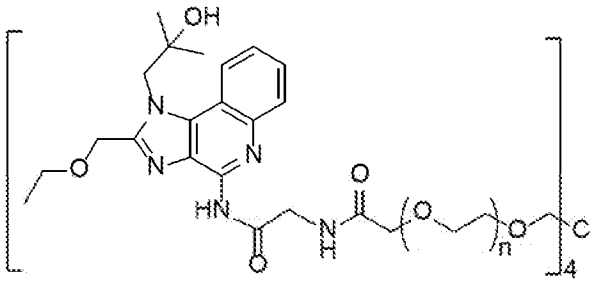
Compound 3



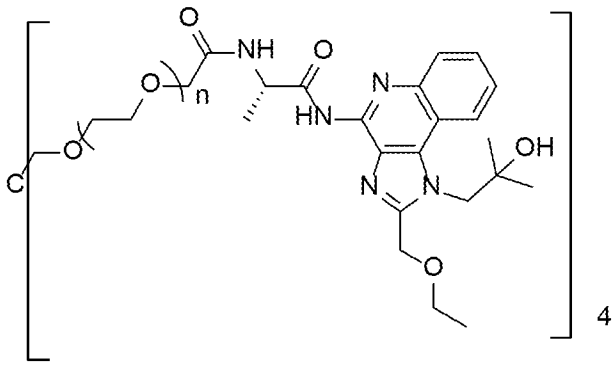
Compound 4



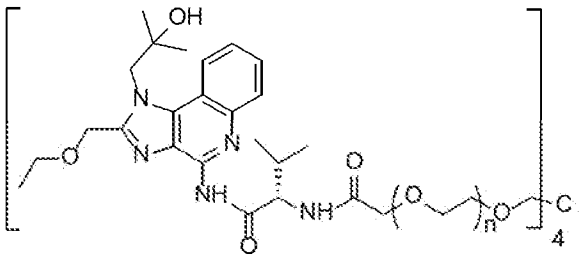
Compound 5



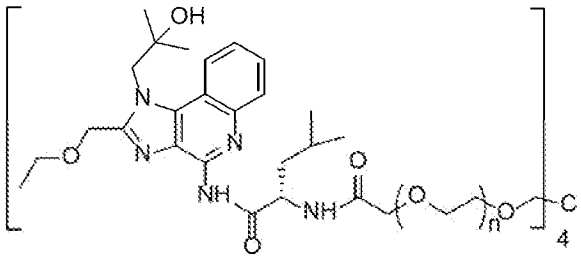
Compound 6



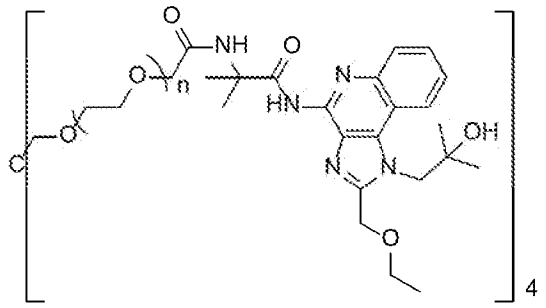
Compound 7



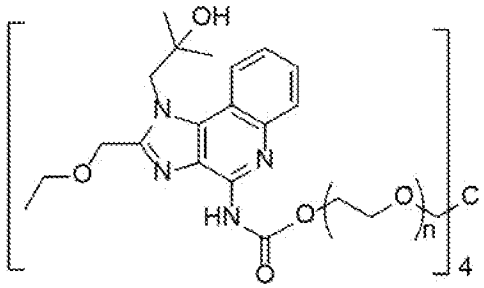
Compound 8



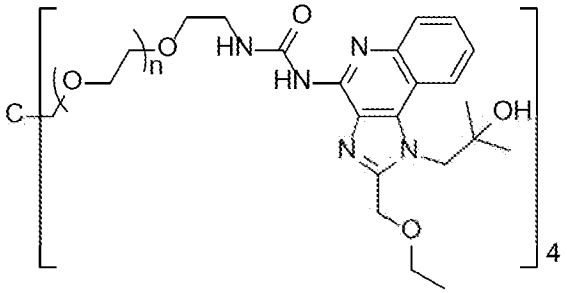
Compound 9



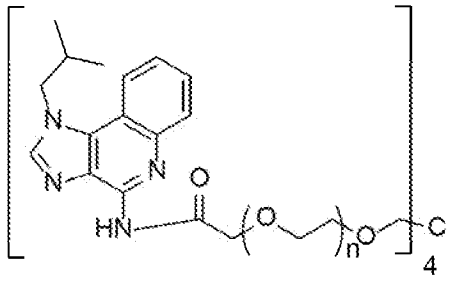
Compound 10



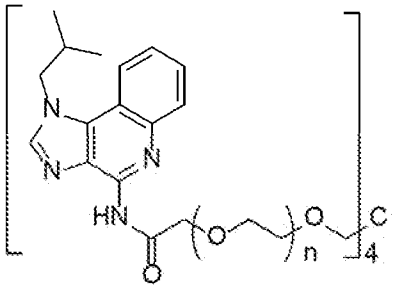
Compound 12



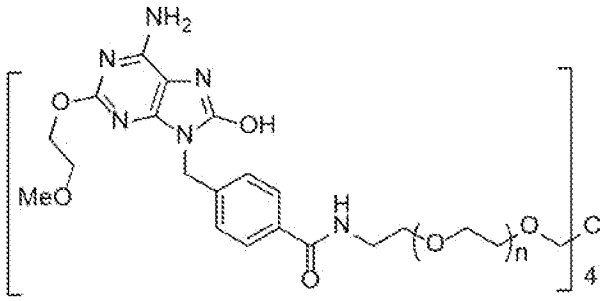
Compound 13



Compound 14



Compound 15



Compound 16

or a pharmaceutically acceptable salt or stereoisomer thereof, wherein each n is independently an integer from 40 to 350 inclusive, more preferably an integer from 100 to 250 inclusive. In some embodiments, n is independently an integer from 100-150 inclusive. In one particular embodiment, n is about 113. For any given polymer in which the molecular weight is known, it is possible to determine the number of repeating units (i.e., "n") by dividing the total weight-average molecular weight of the polymer by the molecular weight of the repeating monomer. In embodiments, n is selected such that the polymer, e.g. poly(ethylene glycol), has a molecular weight of from about 5,000 Daltons to about 40,000 Daltons, more preferably from about 5,000 Daltons to about 25,000 Daltons, or even 5,000 Daltons to about 10,000 Daltons.

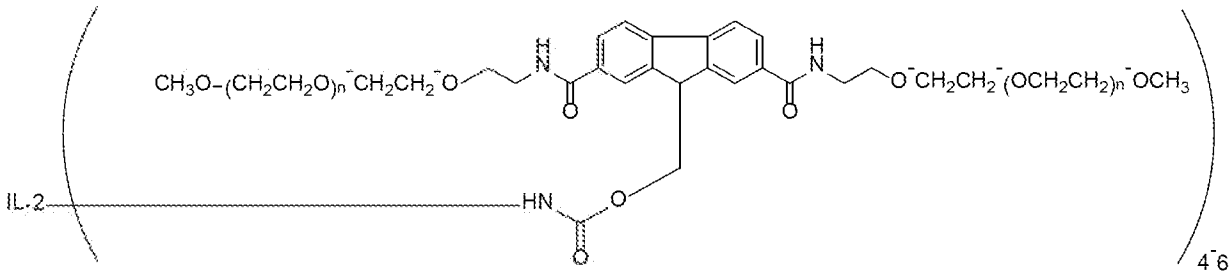
[0065] Also contemplated for use in the methods provided herein are TLR 7/8 agonists such as, e.g., MED19117 (Medimmune, LLC, MD), a lipophilic TLR7/8 agonist , DSP-0509 (a TLR7 agonist, Sumitomo Dainippon Pharma Oncology), and DN052 (TLR8 agonist, Shanghai Denovo Pharmatech Co., China).

[0066] In some embodiments, a TLR7/8 agonist (e.g., 4-arm-PEG20k-CM-Gly-N-R848) according to the present technology is administered to the subject in a dose of about 10 µg, about 20 µg, about 30 µg, about 40 µg, about 50 µg, about 60 µg, about 70 µg, about 80 µg, about 90 µg, about 100 µg, about 150 µg, about 200 µg, about 250 µg, about 300 µg, about 350 µg, about 400 µg, about 450 µg, about 500 µg, about 550 µg, about 600 µg, about 650 µg, about 700 µg, about 750 µg, about 800 µg, about 850 µg, about 900 µg, about 950 µg, about 1000 µg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.6 mg, about 1.7 mg, about 1.8 mg, about 1.9 mg, about 2.0 mg, about 2.1 mg, about 2.2 mg, about 2.3 mg, about 2.4 mg, about 2.5 mg, about 2.6 mg, about 2.7 mg, about 2.8 mg, about 2.9 mg, about 3.0 mg, about 3.1 mg, about 3.2 mg, about 3.3 mg, about 3.4 mg, about 3.5 mg, about 3.6 mg, about 3.7 mg, about 3.8 mg, about 3.9 mg, about 4.0 mg, about 4.1 mg, about 4.2 mg, about 4.3 mg, about 4.4 mg, about 4.5 mg, about 4.6 mg, about 4.7 mg, about 4.8 mg, about 4.9 mg, about 5.0 mg, or greater than 5.0 mg, or in a range between any combination of the above numbers. In some preferred embodiments, the a TLR7/8 agonist such as 4-arm-PEG20k-CM-Gly-N-R848 is administered at a dose of 0.03 mg, 0.06 mg, 0.12 mg, 0.24 mg, 0.48 mg, 0.96 mg, 1.92 mg, 3.0 mg, 3.5 mg, 3.7 mg, 3.8 mg, 3.84 mg, 3.9 mg, 4.0 mg, 4.5 mg, 5.0 mg, 5.5 mg, 6.0 mg, 6.5 mg, 7.0 mg, 7.5 mg, or 7.68 mg.

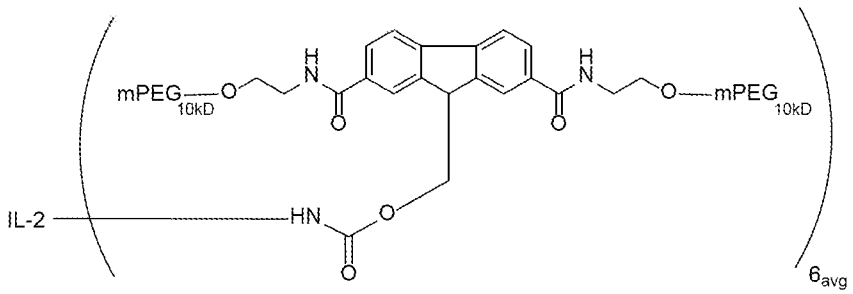
[0067] In some embodiments, administration of the TLR-7/8 agonist is in combination with an interleukin-2 receptor beta (IL-2Rβ) preferential agonist as described, for example, in U.S. Patent No. 10,101,587, and generally referred to therein as RSLAIL-2.

[0068] The releasable PEG comprised in RSLAIL-2 is based upon a 2,7,9-substituted fluorene as shown below, with poly(ethylene glycol) chains extending from the 2- and 7- positions on the fluorene ring via amide linkages (fluorene-C(O)-NH~), and having releasable covalent attachments to amino groups of interleukin-2, via a carbamate linkages. As shown below, the carbamate linkage is attached via a methylene group (-

CH_2-) to the 9-position of the fluorene ring. RSLAIL-2 comprises compounds encompassed by the following formula:



[0069] wherein IL-2 is an interleukin-2 such as aldesleukin. In certain preferred embodiments, "n" in each of the polyethylene glycol chains has a value, on average, of about 227 (i.e., where each polyethylene glycol chain extending from the central fluorenyl core has a weight average molecular weight of about 10,000 daltons, such that the weight average molecular weight of the overall branched PEG moiety is about 20,000 daltons), i.e., referred to more particularly as (2,7-(bis-methoxyPEG$_{10kD}$-carboxyamide)(9H-fluorene-9-yl)methyl N-carbamate)$_{4-6}$interleukin-2. RSLAIL-2 may also be represented by the following formula:



wherein the average number of branched PEG moieties having a structure as shown and releasably covalently attached to the IL-2 moiety is six, i.e., (2,7-(bis-methoxyPEG$_{10kD}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2.

[0070] Also suitable for use in therapeutic combinations with the TLR-7/8 agonist for treating cancer are RO7284755 (a fusion protein of an interleukin-2 variant fused with an anti-PD-1 protein moiety, PD-1-IL2v, Roche, see, for example, WO2018/184964), Thor-707 (a recombinant IL-2 with a poly(ethylene glycol) attached at an unnatural amino acid site, Sanofi, see, e.g., WO 2019/028419), ALKS-4230 (an IL-2 fusion protein comprised of a circularly-permuted IL-2 with the extracellular domain of IL-2Rα, Alkermes, see, e.g.,

U.S. Patent No. 9,359,415), APN-301 (anti-ganglioside-GD2-antibody-interleukin-2-fusion protein, Apeiron Biologics), and daromun (a combination of darleukin (L19-IL2), an immunocytokine consisting of the recombinant form of interleukin-2 (IL-2), fused to a human single-chain variable fragment (scFv) directed against the extra-domain B (ED-B) of fibronectin (L19), and fibromun (L19-TNFalpha), an immunocytokine consisting of human tumor necrosis factor alpha (TNFalpha) fused to a human scFv antibody fragment directed against the ED-B of L19, Philogen).

[0071] In some embodiments, administration of the TLR-7/8 agonist is in combination with a CD-122 preferential agonist such as, e.g., RSLAIL-2, at a dose of from about 0.001 mg/kg to about 100 mg/kg, from 0.001 mg/kg to about 10 mg/kg, or from 0.001 mg/kg to about 9 mg/kg, for example, about 0.001 mg/kg, about 0.002 mg/kg, about 0.003 mg/kg, about 0.004 mg/kg, about 0.005 mg/kg, about 0.006 mg/kg, about 0.007 mg/kg, about 0.008 mg/kg, about 0.009 mg/kg, about 0.01 mg/kg, about 0.02 mg/kg, about 0.03 mg/kg, about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3 mg/kg, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1.0 mg/kg, about 2.0 mg/kg, about 3.0 mg/kg, about 4.0 mg/kg, about 5.0 mg/kg, about 6.0 mg/kg, about 7.0 mg/kg, about 8.0 mg/kg, about 9.0 mg/kg, about 10 mg/kg, or greater than 10 mg/kg, or in a range between any combination of the above numbers. In one or more preferred embodiments, as applied to any one or more of the methods or embodiments described herein, the CD-122 preferential agonist, e.g., RSLAIL-2, is administered to the subject at a dosage of about 0.003 to about 0.009 mg/kg. In one particular embodiment, a dose of about 0.003 mg/kg of RSLAIL-2 is administered to the subject. In yet a further particular embodiment, a dose of about 0.006 mg/kg of RSLAIL-2 is administered to the subject.

[0072] It will be appreciated that the doses for the CD-122 preferential agonist as described above may refer to either of the compound or the protein equivalent. In some preferred embodiments, the doses are in protein equivalents.

[0073] In some embodiments, a single dose or multiple doses of the TLR7/8 agonist, and/or a single dose or multiple doses of the CD-122 preferential agonist, may be administered to a subject. The TLR7/8 agonist and/or the CD-122 preferential agonist

may be administered once or multiple times a day. In some embodiments, the TLR7/8 agonist and the CD-122 preferential agonist may be administered in a single formulation or in separated formulations.

[0074] As one of ordinary skill in the art would understand, the subject may be administered the TLR7/8 agonist before, concurrently with, or after administration of the CD-122 preferential agonist. In some embodiments, the TLR7/8 agonist and the CD-122 preferential agonist may be administered at the same time, on the same day, or in the same week. In some embodiments, the TLR7/8 agonist may be administered in a manner temporally separated from administration of the CD-122 preferential agonist, for example, one or more hours before or after, one or more days before or after, one or more weeks before or after, or one or more months before or after. In various embodiments, the administration frequency of the TLR7/8 agonist may be the same as, similar to, or different from the administration frequency of the CD-122 preferential agonist. One skilled in the art would be able to combine one or both of these therapies in different orders to achieve the desired therapeutic results.

[0075] For treating a subject in need thereof, the TLR7/8 agonist and/or CD-122 preferential agonist according to the present technology can be administered continuously or intermittently, for an immediate release, controlled release, or sustained release. The TLR7/8 agonist and/or CD-122 preferential agonist may be administered over a pre-determined time period. Alternatively, the TLR7/8 agonist and/or CD-122 preferential agonist may be administered until a particular therapeutic benchmark is reached. In certain embodiments, the methods provided herein include a step of evaluating one or more therapeutic benchmarks, such as, but not limited to, the level of certain biomarkers in a biological sample, such as blood circulating tumor cells, a biopsy sample, or urine to determine whether to continue administration. In certain embodiments involving cancer, the TLR7/8 agonist and/or CD-122 preferential agonist may be administered until tumor growth is arrested or reversed, until one or more tumors are eliminated, or until the number of cancer cells are reduced to a specific level.

[0076] As one of ordinary skill in the art would understand, the TLR7/8 agonist and the CD-122 preferential agonist, if used, can be administered to a subject in need thereof one or more times at the same or different doses, and/or using the same or different

routes of administration and/or formulations depending on the diagnosis and prognosis of the subject. One skilled in the art would be able to combine one or more of these therapies in different orders to achieve the desired therapeutic results. In some embodiments, the combinational therapy of the TLR7/8 agonist and the CD-122 preferential agonist achieves improved or synergistic effects in comparison to any of the treatments administered alone.

[0077] In some embodiments, the TLR7/8 agonist and/or the CD-122 preferential agonist, and/or one or more additional therapeutic agents according to the present technology may be administered in a manner appropriate to the disease, condition, or disorder to be treated, such as cancer, as determined by persons skilled in the medical art. Those of ordinary skill in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to a subject, for example, a human. For example, in some embodiments, administration may be systemic or local. In some embodiments, administration may be enteral or parenteral. In any of the embodiments of the present technology, the TLR7/8 agonist, CD-122 preferential agonist, and/or one or more additional therapeutic agents can be administered intravenously (i.v.), intraperitoneally (i.p.), subcutaneously (s.c.), intertumorally, intratumorally, into the bone marrow, into a lymph node, or into the cerebrospinal fluid so as to encounter the target tissue, organ, or cells. An appropriate dose, suitable duration, and frequency of administration of the compositions will be determined by such factors as a condition of the patient; size, type, and severity of the disease, condition, or disorder, including cancer; the undesired type or level of any side effect; the particular form of the active ingredient; and the method of administration.

[0078] In some embodiments, the TLR7/8 agonist, and/or the CD-122 preferential agonist, and/or one or more additional therapeutic agents according to the present technology administered to the subject daily (i.e., once a day), twice a day, every other day, every third day, weekly (i.e., once a week), twice a week, biweekly (i.e., every other week), every third week, monthly, every other month, or every third month, for a period of about 3 days, about 5 days, about 7 days, about 10 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9

months, about 10 months, about 11 months, about 1 year, about 1.25 years, about 1.5 years, about 1.75 years, about 2 years, about 2.25 years, about 2.5 years, about 2.75 years, about 3 years, about 3.25 years, about 3.5 years, about 3.75 years, about 4 years, about 4.25 years, about 4.5 years, about 4.75 years, about 5 years, or more than 5 years.

[0079] In some embodiments, upon administration to the subject the TLR7/8 agonist and/or the CD-122 preferential agonist according to the present technology, the subject exhibits one or more of following outcomes listed in (a)–(e):

[0080] (a) an increase in anti-tumor immune response in the tumor microenvironment compared to baseline or control (e.g., a subject that has not been administered the TLR7/8 agonist and/or the CD-122 preferential agonist);

[0081] (b) a reduction in tumor size or tumor cell number compared to baseline or control;

[0082] (c) a reduction in metastatic tumor burden or loci compared to baseline or control;

[0083] (d) an increase in cancer remission compared to baseline or control; and

[0084] (e) an increase in cancer survival rate compared to baseline or control.

[0085] In one embodiment, the methods comprise measuring baseline levels of one or more markers, risks, or conditions set forth in (a)–(e) above prior to dosing the subject or subject group. In another embodiment, the methods comprise administering a therapeutic composition as described and disclosed herein to the subject after baseline levels of one or more markers, risks, or conditions set forth in (a)–(e) are determined, and subsequently taking an additional measurement of said one or more markers, risks, or conditions.

[0086] In another embodiment, upon treatment with a therapeutic composition according to various embodiments disclosed herein, the subject exhibits one or more of:

[0087] (a) an increase in anti-antitumor immune response in the tumor microenvironment of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least

about 85%, at least about 90%, at least about 95%, or about 100% as compared to baseline or control;

[0088] (b) a reduction in tumor size or tumor cell number of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% as compared to baseline or control;

[0089] I a reduction in metastatic tumor burden or loci of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% as compared to baseline or control;

[0090] (d) an increase in cancer remission of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% as compared to baseline or control; and

[0091] (e) an increase in cancer survival rate of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% as compared to baseline or control.

[0092] In some embodiments, the method further entails administering one or more other cancer therapies, such as surgery, immunotherapy, radiotherapy, chemotherapy, and/or stem cell transplantation (SCT) to the subject sequentially or simultaneously at any desired intervals.

[0093] In some embodiments, the methods further comprise administering a pharmaceutically effective amount of one or more additional therapeutic agents to the subject to obtain improved or synergistic therapeutic effects. Such one or more additional therapeutic agents may comprise an immunotherapy agent, i.e., an agent that can modulate the immune response in the subject. In one embodiment, the additional immunotherapy agent to be used in combination with the TLR7/8 agonist and/or the CD-122 preferential agonist is nivolumab. In some further embodiments, the additional immunotherapeutic agent is a checkpoint inhibitor such as an anti-PD-1 or an anti-CTLA-4.

[0094] In some embodiments, an immunotherapy agent (e.g., nivolumab) according to the present technology is administered to the subject in a dose of about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, or greater than 1000 mg, or in a range between any combination of the above numbers. In one embodiment, nivolumab is administered to the subject at a dose of about 360 mg.

[0095] As one of ordinary skill in the art would understand, the subject may be administered the one or more additional therapeutic agents before, concurrently with, or after administration of the TLR7/8 agonist and/or the CD-122 preferential agonist. In some embodiments, the one or more additional therapeutic agents administered in combination with the TLR7/8 agonist and/or the CD-122 preferential agonist may be administered at the same time, on the same day, or in the same week. In some embodiments, the one or more additional therapeutic agents administered in combination with the TLR7/8 agonist and/or the CD-122 preferential agonist may be administered in a single formulation or in separated formulations. In certain embodiments, the one or more additional therapeutic agents may be administered in a manner temporally separated from administration of the TLR7/8 agonist and/or the CD-122 preferential agonist, for example, one or more hours before or after, one or more days before or after, one or more weeks before or after, or one or more months before or after. In various

embodiments, the administration frequency of one or more additional therapeutic agents may be the same as, similar to, or different from the administration frequency of the TLR7/8 agonist and/or the CD-122 preferential agonist. One skilled in the art would be able to combine one or more of these therapies in different orders to achieve the desired therapeutic results. In some embodiments, the combinational therapy of the TLR7/8 agonist, the CD-122 preferential agonist, and/or the one or more additional therapeutic agents achieves improved or synergistic effects in comparison to any of the treatments administered alone.

[0096] From the foregoing, it will be appreciated that specific embodiments of the invention have been described herein for purposes of illustration, but that various modifications may be made without deviating from the scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

EXAMPLES

Materials and Methods

[0097] (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2: Recombinant human IL-2 having an amino acid sequence identical to that of aldesleukin (des-alanyl-1, serine-125 human interleukin-2) was cloned and expressed and used to prepare the exemplary IL-2Rβ-preferential agonist, (2,7-(bis-methoxyPEG$_{10kD}$-carboxyamide)(9H-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 (CAS No. 1939126-74-5), also referred to as bempegaldesleukin, or more generally herein as, "RSLAIL-2". The preparation of (2,7-(bis-methoxyPEG$_{10kD}$-carboxyamide)(9H-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 is described, e.g., in WO 2018/132496 (Example 19).

[0098] 2,7-(bis-methoxyPEG$_{10kD}$-carboxyamide)(9H-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 is supplied as a sterile lyophilized powder for reconstitution in single-use glass vials for injection. The drug substance is formulated in 10 mM citrate buffer, 7% (w/v) trehalose dihydrate, pH 4.0 and provided in the form of a powder for reconstitution prior to administration: 1.0 mg/vial recombinant human interleukin 2 (rhIL-2) (1 mg/mL of rhIL-2 after reconstitution with 1.1 mL water for injection).

[0099] The drug product is administered as an IV infusion following dilution into 0.9% NaCl solution or 5% Dextrose solution, approximately 50 mL in volume.

Example 1

Combined Cancer Treatment With a TLR7/8 Agonist and a CD-122 Preferential Agonist

[0100] A phase 1/2, open-label, multicenter, dose escalation and dose expansion study of the exemplary TLR7/8 agonist, pentaerythritolyl-4-arm-(PEG-1-methylene-2-oxo-vinylamino acetamide linked-resiquimod)-20kD in combination with (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 (RSLAIL-2) or in combination with (2,7-(bis-methoxyPEG$_{10kd}$-carboxyamide)(9h-fluorene-9-yl)methyl N-carbamate)$_{6avg}$interleukin-2 (RSLAIL-2) plus nivolumab in patients with locally advanced or metastatic solid tumor malignancies was conducted.

[0101] A brief description of the Phase 1 study design follows. Cohort A involves 1-3 cycles where the TLR7/8 agonist and RSLAIL-2 are administered on the same day. Cohort B involves 1-3 cycles where the TLR7/8 agonist, RSLAIL-2, and nivolumab are administered on the same day. The exact dose of the TLR7/8 agonist is confirmed based on review of safety and PK data after a minimum of 3 patients have been enrolled in each cohort, by the Safety Review Committee. Dose escalation does not exceed double the prior dose of the TLR7/8 agonist. Dose may be rounded off to one decimal point based on study drug reconstitution calculations. No intra-patient dose escalation is conducted in any cohort. Patients may receive additional 3 cycles of TLR7/8 agonist IT injection after assessment of anti-tumor effect at each designated disease staging. Treatment with RSLAIL-2 or RSLAIL-2 with nivolumab continues every 3 weeks until progression or unacceptable toxicity.

[0102] During cycles where the TLR7/8 agonist and RSLAIL-2 are administered on the same day, TLR7/8 agonist intratumoral (IT) injection are administered prior to the RSLAIL-2 infusion. During Cohort B, TLR7/8 agonist IT injection is administered first, followed by RSLAIL-2 infusion, followed by nivolumab infusion. The dose-limiting toxicity (DLT) window for TLR7/8 agonist single agent is 21 days, the DLT window for TLR7/8 agonist in combination with RSLAIL-2 is 9 days during the dose escalation for staggered

administration (Cohorts 1 and 2). The total DLT period for the combination of TLR7/8 agonist and RSLAIL-2 (Cohorts 1-2, staggered administration) is 30 days. The total DLT period for the combination of TLR7/8 agonist and RSLAIL-2 (Numbered Cohorts 3 and greater, same-day administration) is 28 days. The DLT window of 7 days applies for same day administration of TLR7/8 agonist and RSLAIL-2, for the doublet in Cohort A and the triplet in Cohort B.

[0103] Phase 2 dose expansion initiation for both the doublet (TLR7/8 agonist and RSLAIL-2) or triplet combination (TLR7/8 agonist, RSLAIL-2, and nivolumab) cohorts is based on safety and tolerability findings and anti-tumor effect using RECIST 1.1. The TLR7/8 agonist is administered in 3-week cycles. Patients may receive an additional 3 cycles of TLR7/8 agonist intratumoral (IT) injection after assessment of anti-tumor effect at each designated disease staging. Patients treated with doublet therapy are administered TLR7/8 agonist IT injection first, followed by RSLAIL-2 infusion. Patients treated with triplet therapy are administered TLR7/8 agonist IT injection first, followed by RSLAIL-2 infusion, followed by nivolumab infusion. Treatment continues until unacceptable toxicity, death, disease progression per RECIST 1.1, Investigator's decision to discontinue treatment, the patient withdraws consent, is lost to follow-up, or a decision to terminate the trial.

TLR7/8 agonist Nonclinical Experience

[0104] The activity of the TLR7/8 agonist 4-arm-PEG20k-CM-Gly-N-R848 has been assessed in multiple in vivo and in vitro studies. Resiquimod released from 4-arm-PEG20k-CM-Gly-N-R848 was found to induce pro-inflammatory cytokines in vitro in peripheral blood mononuclear cell (PBMC) cultures in all tested species (rat, dog, human). In in vivo studies in the mouse CT26 colorectal carcinoma (CRC) model, 4-arm-PEG20k-CM-Gly-N-R848 IT delivery was found to induce immune stimulatory cytokines (interferon alpha (IFNα), interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα), interleukin-12 (IL-12)) and activate gene expression (interferon regulatory factor 7 (Irf7)) downstream of the TLR7 signaling cascade. Importantly, it was shown that immune stimulation was enhanced selectively in the tumor environment and comparatively limited systemic cytokine induction was observed.

[0105] TLR7/8 agonist pharmacodynamic response was rapid showing peak cytokine levels within one day after administration. Intratumoral 4-arm-PEG20k-CM-Gly-N-R848 and released resiquimod at the treated tumor and plasma were detectable for at least 7 days while TLR7/8 agonist induced cytokine response had largely returned to pre-treatment levels in 48 hours. Prolonged resiquimod exposure is therefore expected to have limited effect on cytokine production in plasma beyond 48 hours after treatment.

[0106] Assessed by flow cytometry analysis, a single dose of a TLR7/8 agonist that induced IT cytokine release in the CT26 tumor model correlated with rapid activation of TLR7 expressing myeloid cells including neutrophils and dendritic cells within one day of treatment. While neutrophils were locally activated in the injected tumor leading to tumor cell antigen release, a transient activation of dendritic cells was observed in both injected and abscopal tumors (FIGs. 1A-1E). Combination treatment of TLR7/8 agonist with RSLAIL-2 showed TLR7/8 agonist dose dependent enhancement of selective IT cytotoxic T cell expansion (FIGs. 1A-1E) and upregulation of checkpoint receptors cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and PD 1 indicating tumor antigen dependent $CD8^+$ T cell activity. In parallel to improving cytotoxic T cell immune response, TLR7/8 agonist and RSLAIL-2 also decreased IT immune suppression by reducing Tregs and monocytes/macrophages. $CD8^+$ T cell response was primarily concentrated to tumor sites and minimal inhibitory checkpoint induction was observed in circulating T cells.

[0107] These immune phenotyping results support a model where the TLR7/8 agonist and RSLAIL-2 combination treatment leads to sequential activation of innate and adaptive immune cell types optimizing the tumor antigen specific activity of cytotoxic T cells through synergistic non-overlapping mechanisms.

[0108] Finally, in agreement with observed pharmacodynamics, a single dose of TLR7/8 agonist combined with repeat administration of RSLAIL-2 also led to significant tumor growth inhibition in vivo in the CT26 tumor model (FIGs. 2A-2C). TLR7/8 agonist at low dose levels (0.01 µg – 1 µg) as a single agent had no effect on anti-tumor efficacy but when combined with RSLAIL-2 (0.8 mg/kg, 3 dosing cycles every 9 days) enhanced inhibition of treated and abscopal tumor growth compared to single agent treatments with minimal negative impact on treatment tolerability.

Rationale for TLR7/8 agonist in Combination with RSLAIL-2 *or in Combination with* RSLAIL-2 *and Nivolumab*

[0109] Accumulating evidence suggests that patients with low baseline CD8+ T cells within the tumor microenvironment are predicted to show poor response to checkpoint inhibitor immunotherapies (Tumeh, 2014; Daud, 2016a; Daud, 2016b); thus, agents designed to specifically activate and expand CD8+ T cells may improve clinical outcomes in patients with low TILs. Combining RSLAIL-2 with a TLR7/8 agonist will potentially enhance the expansion of tumor specific CD8+ T cells resulting in increased TIL numbers.

[0110] Combining a TLR7/8 agonist and RSLAIL-2 engages the entire immune activation cascade required for systemic tumor clearance from local tumor antigen production to a sustained systemic T cell response. Unlike treatments that stimulate downstream components of select immune pathways without eliciting systemic tumor immunity, a comprehensive anti-tumor immune activation by coordinated engagement of innate and adaptive immune cells may increase the success of immune therapy for patients. Adding checkpoint inhibition (nivolumab) to TLR7/8 agonist and RSLAIL-2 combination may further enhance efficacy by enabling sustained anti-tumor activity of cytotoxic T cells (FIG. 3).

[0111] To further support the triplet combination of TLR7/8 agonist plus RSLAIL-2 and nivolumab, recent publications (Gupta, 2017; Wölfle, 2011) indicate that usage of TLR agonists resulted in induced up-regulation of programmed cell death ligand 1 (PD-L1) in tumors in an interferon (IFN)-dependent manner. Additionally, it was shown that the RSLAIL-2 driven immune response is improved in combination with nivolumab (Diab, 2017; Bernatchez, 2017). These observations support the investigational plan to use a blocking antibody against PD 1/PD L1 axis in combination with TLR7/8 agonist and RSLAIL-2, as it is expected to contribute to an enhancement of sustained tumor specific immune surveillance by reducing immune suppressive mechanisms in tumors.

Clinical Experience with aTLR7/8 agonist as a Single Agent and in Combination with RSLAIL-2

[0112] In the phase 1/2 study, a TLR7/8 agonist is being assessed as a single agent for 21 days and subsequently combined with RSLAIL-2 (0.006 mg/kg) in Cycle 2 in the dose escalation phase. Patients have been dosed across 9 cohorts, in which TLR7/8

agonist was administered every 3 weeks (q3w) at doses ranging from 0.03 mg to 3.84 mg.

[0113] The Phase 1 portion of the study is ongoing, and the dose escalation phase is complete. Cohorts 1 through 9 were evaluated for safety and tolerability in the following tumor types: MEL (n = 24), sarcoma (n = 3), CRC (n = 3), RCC (n = 3), triple-negative breast cancer (TNBC) (n = 2), and Merkel cell carcinoma (MCC) (n = 1).

[0114] 8 patients were dosed in Cohort 9 at a TLR7/8 agonist dose level of 3.84 mg. Ongoing clinical evaluation of the safety information from the dose escalation portion of the study remains consistent with the known safety profiles of resiquimod (active moiety of the TLR7/8 agonist) and RSLAIL-2. No new safety signals or risks associated with the combination with RSLAIL-2 have been identified to date. No new risks associated with protocol-mandated procedures, including the required IT injection procedure, have been identified.

[0115] For the purpose of safety analysis, blood samples were analyzed for 4-arm-PEG20k-CM-Gly-N-R848, resiquimod (R848), and the R848 metabolite O-deethylated resiquimod (M2). Pharmacokinetic (PK) samples from 32 patients receiving 4-arm-PEG20k-CM-Gly-N-R848 IT administration have been analyzed for up to 3 cycles. There is an increase in 4-arm-PEG20k-CM-Gly-N-R848 exposure with increasing dose. 4-arm-PEG20k-CM-Gly-N-R848, R848, and M2 were quantified and analyzed in a portion of the samples. No trends were observed between the frequency or severity of AEs and 4-arm-PEG20k-CM-Gly-N-R848 or R848 PK concentrations.

[0116] In summary, a TLR7/8 agonist in combination with RSLAIL-2 has been generally well tolerated in the dose escalation phase. The safety profile of a TLR7/8 agonist in combination with RSLAIL-2 has not yet shown evidence of an increased incidence or severity of AEs over RSLAIL-2 monotherapy.

[0117] The RP2D for 4-arm-PEG20k-CM-Gly-N-R848 has been established at 3.84 mg.

Clinical Experience with RSLAIL-2 *as Monotherapy*

[0118] A Phase 1/2 open-label, multicenter, dose escalation and dose expansion monotherapy study of RSLAIL-2 in patients with locally advanced or metastatic solid tumors was previously started. The objectives of the study were to evaluate the safety

and tolerability of RSLAIL-2 to determine the MTD, as well as to assess the objective response rate (ORR) and other efficacy measures at or below the MTD, or to identify the recommended Phase 2 dose (RP2D). The RP2D for RSLAIL-2 was determined to be 0.006 mg/kg, taking in consideration the clinical safety profile associated to the robust immune system activation observed.

Rationale for Intratumoral Injection of a TLR7/8 agonist in Combination with RSLAIL-2 *and Nivolumab*

[0119] 4-arm-PEG20k-CM-Gly-N-R848, a TLR7/8 agonist, is a prodrug of resiquimod. In nonclinical studies, resiquimod release from 4-arm-PEG20k-CM-Gly-N-R848 was confirmed, and the resiquimod exposure ratio of the injected tumor to plasma was between 32.7- and 64.3-fold in both CT26 and EMT6 tumors following 4-arm-PEG20k-CM-Gly-N-R848 administration, supporting the projected goal of minimizing plasma exposure to resiquimod which is believed thereby to lead to limiting systemic cytokine activation. In the mouse tumor model, 4-arm-PEG20k-CM-Gly-N-R848 as a single agent had limited efficacy. However, the combination treatment of IT 4-arm-PEG20k-CM-Gly-N-R848 with systemically administered RSLAIL-2 has shown prominent efficacy not only in the injected tumor but also in the distant untreated tumor in mice with tumors on both flanks. Ongoing clinical trial data have demonstrated that the addition of nivolumab to RSLAIL-2 enhanced an immune-stimulatory response compared to RSLAIL-2 monotherapy. Therefore, the combination of IT TLR7/8 agonist with RSLAIL-2 or with both RSLAIL-2 and nivolumab may offer a novel therapy to benefit patients with select metastatic indications.

Rationale for TLR7/8 agonist Starting Dose

[0120] The TLR7/8 agonist starting dose of 0.03 mg IT has been established based on the rat (the most sensitive species) and the tolerated dose levels (HNSTD and NOAEL for rat and NOAEL for dog) identified from GLP toxicology studies (not shown), as well as prediction of human PK and pharmacodynamics. This starting dose includes a 30-fold safety factor over the rat NOAEL dose corrected for body surface area and assumes a 60 kg human. The 0.03 mg starting dose is expected to have pharmacological activity based on the half maximal effective concentration (EC_{50}) for activating extracellular signal–regulated kinase (ERK) phosphorylation in human PBMCs.

[0121] In addition, the 0.03 mg starting IT dose of the TLR7/8 agonist is predicted not to exceed systemic exposures to resiquimod that have been shown to be safe after SC administration to 96 patients with chronic HCV (Pockros, 2007).

Rationale for TLR7/8 agonist Recommended Phase 2 Dose

[0122] A composite of clinical data was considered for selection of the RP2D including data on safety and tolerability, confirmation of maximum target engagement, optimal biological effects without undesirable clinical effects, pharmacokinetics/pharmacodynamics, and tumor response.

[0123] The RP2D has been determined to be 3.84 mg TLR7/8 agonist in combination with RSLAIL-2 (0.006 mg/kg).

[0124] Data from Cohort 9 suggest that this dose has an acceptable safety profile and leads to significant TLR7 target engagement followed by a local biological response (see clinical experience with 4-arm-PEG20k-CM-Gly-N-R848).

[0125] Supportive biomarker data indicated the induction of Type 1 interferon genes following TLR7/8 agonist administration in a dose-dependent manner, suggesting relevant TLR7 target engagement. Of note, similar levels of TLR7 target engagement were achieved at 1.92 mg and 3.84 mg, suggesting that further dose escalation would be unlikely to lead to further TLR7 pathway activation. Data from peripheral blood in Cohorts 3 through 9 show activated type 1 interferon induced genes downstream of the TLR7 signaling pathway. Further, CXCL10/IP10, an interferon-inducible chemokine, showed a dose dependent increase in response to TLR7/8 agonist. The induction of both type 1 interferon genes and CXCL10 chemokine indicate drug-dependent target engagement.

[0126] In parallel, clear activation of the IL-2 pathway was also demonstrated by the combination of TLR7/8 agonist at 3.84 mg and RSLAIL-2 at 0.006 mg/kg leading to an increase in absolute lymphocyte count with activated and proliferating CD4, CD8, and NK cells in blood.

[0127] The composite of biological and safety elements of data suggests that TLR7/8 agonist at 3.84 mg in combination with RSLAIL-2 at 0.006 mg/kg strikes a balance between safety and pharmacodynamic effects relevant to the proposed mechanism of action (MOA).

Overall Benefit/Risk

[0128] TLR7/8 agonist IT injection is predicted to provide locally extended drug exposure in the injected tumor while limiting systemic cytokine activation. In nonclinical studies, TLR7/8 agonist as a single agent had limited anti-tumor activity. However, the combination treatment of IT TLR7/8 agonist with systemically administered RSLAIL-2 has shown prominent efficacy not only in the injected tumor but also in the distant untreated tumor in mice with tumors on both flanks. RSLAIL-2 is in clinical development in advanced cancer indications and has already shown very promising signs of anti-tumor activity in patients, with an acceptable safety profile (Bernatchez, 2017, Study 15-214-01). The safety profile of nivolumab is well characterized and manageable as a single agent and when administered in combination with other I-O products. Data from the ongoing clinical trial of RSLAIL-2 in combination with nivolumab demonstrate an adequate safety profile allowing for the proposed combination of locally delivered TLR7/8 agonist. The TLR7/8 agonist starting dose includes a 30-fold safety factor over the TLR7/8 agonist rat NOAEL dose. The starting dose is expected to have pharmacological activity based on the EC50 for activating ERK phosphorylation in human PBMCs. Therefore, the combination of TLR7/8 agonist and RSLAIL-2 with or without the addition of nivolumab may offer novel therapeutic approaches to benefit patients with select cancers including metastatic indications, outweighing the risks of these agents.

Primary Objectives

[0129] To evaluate the safety and tolerability, and define the MTD or RP2D of TLR7/8 agonist in combination with RSLAIL-2 (doublet) and the safety and tolerability of TLR7/8 agonist and RSLAIL-2 plus nivolumab (triplet).

[0130] To evaluate the anti-tumor activity of the combination of TLR7/8 agonist plus RSLAIL-2 (doublet) and the combination of TLR7/8 agonist and RSLAIL-2 plus nivolumab (triplet) by assessing the ORR by RECIST 1.1.

Secondary Objectives

[0131] To evaluate the anti-tumor activity of the combination of TLR7/8 agonist plus RSLAIL-2 (doublet) and the combination of TLR7/8 agonist and RSLAIL-2 plus nivolumab (triplet) by assessing progression-free survival (PFS) and overall survival (OS).

[0132] To evaluate the proportion of patients with an abscopal response by RECIST 1.1 after the initiation of treatment of TLR7/8 agonist plus RSLAIL-2 or TLR7/8 agonist and RSLAIL-2 plus nivolumab.

Further Objectives

[0133] To evaluate the efficacy of the combination of TLR7/8 agonist and RSLAIL-2 (doublet) and the combination of TLR7/8 agonist, RSLAIL-2 and nivolumab (triplet) by assessing ORR by immune-related RECIST (irRECIST).

[0134] To assess the effects of the combination of TLR7/8 agonist and RSLAIL-2 (doublet) and of the combination of TLR7/8 agonist and RSLAIL-2 plus nivolumab (triplet) on immune cells in blood and tumor.

[0135] To characterize the PK of TLR7/8 agonist, RSLAIL-2, and their metabolites, and nivolumab when administered in combination.

[0136] To assess the immunogenicity of RSLAIL-2 and nivolumab when given in combination with TLR7/8 agonist.

[0137] To assess the association between efficacy measures and PD-L1 expression in tumors.

[0138] To assess the association between anti-tumor activity and immune cells in tumor and blood.

[0139] To assess the association between efficacy measures and tumor mutational burden in tumors and blood.

[0140] To assess the effect of TLR7/8 agonist on QT/QTc interval using exposure response analysis.

SELECTION OF STUDY POPULATION

[0141] For the purposes of eligibility, "neoadjuvant therapy" is defined as systemic chemotherapy administered prior to definitive local surgery in a patient without distant metastases; "adjuvant therapy" is defined as systemic therapy administered following definitive local therapy (surgery or radiation) in a patient without distant metastases (with no evidence of disease).

[0142] A "line of therapy" is defined as any regimen – single-agent or combination therapy, cytotoxic therapy, I-O therapy separately or in combination – that is given for

patients with advanced disease, and that is stopped for any reason, including progression of disease, toxicity, physician decision, or patient withdrawal of consent.

Inclusion Criteria includes, but is not limited to, the following

1) Histologically confirmed diagnosis of a locally advanced (not amenable to curative therapy such as surgical resection) metastatic: MEL, MCC, TNBC, RCC, CRC, head and neck squamous cell carcinoma (HNSCC), or sarcoma. Further specification per tumor-type cohorts.
2) Life expectancy > 12 weeks as determined by the Investigator.
3) Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1.
4) Measurable disease per RECIST 1.1.
5) Patients enrolled in Cohorts 1-10, Cohort A, Cohort B, and Phase 2 Doublet must be refractory to all therapies known to confer clinical benefit to their disease.
6) Injected lesions (up to two, with preferably one of the lesions injected to be a draining lymph node [if identified as an injectable tumor where an IT injection can be given]) must be between 20 mm and 90 mm in diameter for IT injection; lesions must be accessible for baseline and on-treatment biopsies. Tumors encasing major vascular structures (i.e., carotid artery or tumors close to other vital organs) are not considered appropriate for IT injection. Any liver lesion targeted for injection must not exceed 50 mm at the time of injection.
7) At least one patient in each dose escalation cohort is suggested to have one and preferentially two lesions accessible for repeated biopsy without interventional radiographic (IR) guidance at pre-specified time points.
8) Demonstrated adequate organ function within 14 days of Cycle 1 Day 1 (C1D1) including:
    a. White blood cell (WBC) count ≥ 2000/μL (after at least 7 days without growth factor support or transfusion)
    b. Absolute neutrophil count (ANC) ≥ 1500/μL (after at least 7 days without growth factor support or transfusion)
    c. Platelet count ≥ 100 × 103/μL (no transfusions allowed within 7 days of C1D1 to meet entry criteria)

d. Hemoglobin ≥ 9.0 g/dL (no transfusions allowed within 7 days of C1D1 to meet entry criteria)
e. Serum creatinine ≤ 2 mg/dL (or glomerular filtration rate ≥ 40 mL/min)
f. Aspartate aminotransferase and ALT ≤ 3× upper limit of normal (ULN)
g. Total bilirubin within normal limits unless associated with hepatobiliary metastases or Gilbert's syndrome, in that case total bilirubin ≤ 2× ULN
h. Lipase and amylase ≤ 1.5× ULN. Patients with pancreatic metastases and lipase and/or amylase < 3× ULN may enroll. Patients may not enroll if there are clinical or radiographic signs of pancreatitis

9) On an echocardiogram, documented left ventricular ejection fraction > 45% within 60 days prior to C1D1.
10) Oxygen saturation ≥ 92% on room air.
11) Clinically significant toxic effect(s) of the most recent prior anti-cancer therapy must be Grade 1 or resolved (except alopecia and sensory neuropathy); patients with Grade 2 adrenal insufficiency related to prior anti-cancer therapy (defined as requiring medical intervention, such as concomitant steroids) or Grade 2 hypothyroidism (defined as requiring hormone replacement therapy) may be enrolled provided that clinical symptoms are adequately controlled and the daily dose is 10 mg or less of prednisone or equivalent. If the patient received major surgery or radiation therapy of > 30 Gy, they must have recovered from the toxicity and/or complications from the intervention.
12) Sample of archival tumor tissue or fresh baseline tumor biopsies (fresh baseline biopsy is defined as a biopsy specimen taken within 28 days prior to C1D1) are desirable, except if inaccessible and with Medical Monitor approval. Patients must consent to allow acquisition of existing formalin-fixed paraffin-embedded (FFPE) material, either a block or unstained slides for performance of correlative studies. For archival biopsy tissue it is desirable to have been collected within 6 months of C1D1.
13) Patients with stable brain metastases may be enrolled, provided:
    a. No prior brain metastasis lesion greater than 2 cm. Patients with prior brain metastasis lesions greater than 2 cm that have been removed by surgical

and/or radiotherapy may be enrolled if the lesion has been stable since surgery or radiotherapy.

b. No new or progressing brain metastasis of any size
c. No stereotactic radiation or craniotomy within 4 weeks of C1D1
d. No new central nervous system lesions on repeat radiographic imaging 4 weeks or more from last treatment
e. No treatment with systemic steroids (> 10 mg of prednisone daily or equivalent) within 2 weeks of C1D1
f. No clinically significant symptoms secondary to brain metastases

14) Palliative radiotherapy must have been completed > 14 days before administration of first dose of study drug(s).

Inclusion Criteria for Specific Tumor Types

[0143] Frontline patients will only be enrolled in the cohorts that include nivolumab as a treatment backbone.

[0144] **Melanoma**

- Patients must have histologically confirmed metastatic MEL with measurable, Stage III (lymph node or in transit lesions) or Stage IVA, IVB, or IVC disease per American Joint Committee on Cancer (AJCC) staging system. "Advanced" is defined as either locally advanced unresectable cancer or metastatic disease.
- Patients must consent to BRAF testing or have known BRAF status as per regionally acceptable V600 mutational status testing.
- Uveal MEL is excluded.
- "Isolated limb perfusion" is not considered systemic chemotherapy.

[0145] Phase 1 Cohorts and Phase 2 Doublet

- Patients that have undergone treatment with Cytotoxic T-Lymphocyte Antigen 4 (anti-CTLA-4) antibody must have at least 6 weeks from last dose of CTLA-4 antibody and evidence of tumor progression before they can be enrolled into this study.
- Patients that have undergone treatment with anti-PD-1 or anti-PD-L1 antibody must have at least 4 weeks from last dose of antibody and evidence of tumor progression before enrollment to the study.

- Patients with V600E mutations are eligible if they have progressed on treatment with an approved BRAF inhibitor or MEK inhibitor therapy, were unable to tolerate an approved BRAF inhibitor or MEK inhibitor, or have refused treatment with an approved BRAF inhibitor or MEK inhibitor.

[0146] Phase 2 Triplet Regimen (1L)

- Prior to disease metastasis, patients must not have received adjuvant or neoadjuvant therapy with any I-O regimens.

[0147] **Merkel Cell Carcinoma**

- Patients must have histologically confirmed metastatic MCC and must consent to assess Merkel cell polyomavirus (MCPyV) antibody levels tested by central laboratory at screening or provide historical assessment results if available. "Advanced" is defined as either locally advanced unresectable cancer or metastatic disease.

[0148] Phase 1 Cohorts and Phase 2 Doublet

- Patients must have received at least 1 line of therapy (I-O or chemotherapy) for metastatic MCC and refused any additional anti-cancer treatment.
- Patients that have undergone treatment with anti-CTLA-4 antibody must have at least 6 weeks from last dose of CTLA-4 antibody and evidence of tumor progression before they can be enrolled into this study.
- Patients that have undergone treatment with anti-PD-1 or anti-PD-L1 antibody must have at least 4 weeks from last dose of antibody and evidence of tumor progression before they can be enrolled into this study.

[0149] Phase 2 Triplet Regimen (2L)

- Patients must have received only 1 prior line of therapy with an anti-PD-1 or anti-PD-L1 containing regimen, which must be their most recent anti-cancer treatment.
- Prior to disease metastasis, patients must not have received adjuvant or neoadjuvant therapy with any I-O regimens.

[0150] Triple-Negative Breast Cancer

- Patients must have diagnosis of advanced TNBC. "Advanced" is defined as either locally advanced breast cancer not amenable to curative surgery or radiotherapy or with distant metastases.
- Patients must have metastatic disease and histologically confirmed estrogen-receptor-negative (ER-), progesterone-receptor-negative (PR-) and human epidermal growth factor receptor 2 negative (HER2-) as determined by local pathologist using local institutional guidelines

[0151] Phase 1 Cohorts and Phase 2 Doublet

- Patients must have received 2 and no more than 3 lines of therapy with chemotherapy, neoadjuvant and adjuvant setting excluded

[0152] Phase 2 Triplet Regimen (1-2L)

- Patients may have received or been intolerant to 1 prior line of chemotherapy (neo-adjuvant and adjuvant setting excluded).
- Patients must not have received any prior I-O regimens, including but not limited to checkpoint inhibitors such as anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T cell co -stimulation or checkpoint pathways, indoleamine 2,3 -dioxygenase pathway inhibitors, cancer vaccines, adoptive cell therapies, or other cytokine therapies.
- Prior to disease metastasis, patients must not have received adjuvant or neoadjuvant therapy with any I-O regimens.

[0153] Head and Neck Cancer

- Patients must have histologically or cytologically confirmed recurrent or metastatic HNSCC that could not be treated with curative intent. "Advanced" is defined as either locally advanced HNSCC not amenable to curative surgery or radiotherapy or with distant metastases.
- HNSCC patients requiring treatment on anticoagulation therapy are not eligible.

[0154] Phase 1 Cohorts and Phase 2 Doublet

- Progression to first line platinum-based or any second/third line chemotherapy.

- Patients that have undergone treatment with anti-PD-1or anti-PD-L1 antibody must have at least 4 weeks from last dose of antibody and evidence of tumor progression before they can be enrolled into this study.

[0155] Phase 2 Triplet Regimen (2L)

- Patients must have received at least 1 line of therapy (I-O or chemotherapy) for metastatic HNSCC and refused any additional anti-cancer treatment.
    - Documented PD after platinum-based (either cisplatin or carboplatin) concurrent chemoradiation including induction chemotherapy for curative aim or
    - Documented PD after platinum-based (either cisplatin or carboplatin) chemotherapy for palliative aim or
    - Ineligible to platinum-based (either cisplatin or carboplatin) chemotherapy or chemoradiation due to decline in renal function and patient's intolerance or
    - Prior therapy with an anti PD 1/L1 agent.

[0156] **Renal Cell Carcinoma (RCC)**

- Advanced (not amenable to curative surgery or radiation therapy) or metastatic (AJCC version 8 Stage IV) RCC. "Advanced" is defined as either locally advanced unresectable cancer or metastatic disease.
- Histologically confirmed RCC with a clear-cell component.

[0157] Phase 1 Cohorts and Phase 2 Doublet

- Patients must have received both of the following:
    - Only 1 prior line of therapy with a checkpoint inhibitor (anti-PD-1 or anti-PD-L1).
    - Patients must have received at least 1 prior anti-angiogenic therapy or cytotoxic chemotherapy regimen.

[0158] Phase 2 Triplet Regimen (1L)

- Patients must not have received any anti-angiogenic therapy or any prior I-O regimens, including but not limited to checkpoint inhibitors such as anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T cell co-stimulation or checkpoint

pathways, indoleamine 2,3-dioxygenase pathway inhibitors, cancer vaccines, adoptive cell therapies, or other cytokine therapies.

- Prior to disease metastasis, patients must not have received adjuvant or neoadjuvant therapy with any I-O regimens.

[0159] **Colorectal Cancer**

- Patients must have a diagnosis of advanced CRC. "Advanced" is defined as either locally advanced unresectable cancer or metastatic disease.

[0160] Phase 1 Cohorts and Phase 2 Doublet; microsatellite instability (MSI)-non high

- Patients must have received or were intolerant to 2 prior cancer therapy regimens administered for metastatic disease.

[0161] Phase 2 Triplet Regimen (I-O therapy naïve) (2L); MSI-non high

- Patients must have received or been intolerant to 1 prior cancer therapy regimen administered for metastatic disease.
- Must not have received any prior I-O regimens, including but not limited to checkpoint inhibitors such as anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T cell co-stimulation or checkpoint pathways, indoleamine 2,3-dioxygenase pathway inhibitors, cancer vaccines, adoptive cell therapies, or other cytokine therapies.

[0162] Phase 1 Cohorts and Phase 2 Doublet; MSI-high

- Patients must have either archival or fresh tumor biopsy that demonstrates microsatellite instability high or mismatch repair deficient disease.
- Patients must have received or were intolerant to 2 prior cancer therapy regimens administered for metastatic disease including, checkpoint inhibitors such as anti-PD-1, anti-PD-L1 or anti-CTLA-4 antibody.

[0163] Phase 2 Triplet Regimen Colorectal Carcinoma (2-3L+, I-O therapy naïve); MSI-high

- Patients must have either archival or fresh tumor biopsy that demonstrates microsatellite instability high or mismatch repair deficient disease.
- Must not have received any prior I-O regimens, including, but not limited to, checkpoint inhibitors such as anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or

anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T cell co-stimulation or checkpoint pathways, indoleamine 2,3-dioxygenase pathway inhibitors, cancer vaccines, adoptive cell therapies, or other cytokine therapies.

[0164] **Sarcoma**

- Histologically confirmed metastatic and/or locally advanced osteosarcoma, chondrosarcoma, undifferentiated pleomorphic sarcoma/malignant fibrous histocytoma, dedifferentiated/pleomorphic liposarcoma, angiosarcoma, and leiomyosarcoma. "Advanced" is defined as either locally advanced unresectable cancer or metastatic disease.
- Sarcoma patients who refused standard of care or alternative therapies that would confer clinical benefit are eligible for the study

[0165] Phase 1 Cohorts and Phase 2 Doublet

- Patients must have received or been intolerant to at least 2 and no more than 3 prior cancer therapy regimens administered for metastatic disease.

[0166] Phase 2 Triplet Regimen (I-O therapy naïve) (2L)

- Patients must have received or been intolerant to at least 1 prior cancer therapy regimen administered for metastatic disease.
- Patients must not have received any prior I-O regimens, including but not limited to checkpoint inhibitors such as anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T cell co-stimulation or checkpoint pathways, indoleamine 2,3-dioxygenase pathway inhibitors, cancer vaccines, adoptive cell therapies, or other cytokine therapies.

Exclusion Criteria include, but are not limited to:

- Use of an investigational agent or an investigational device within 21 days before administration of first dose of study drug(s).
- Patients who have an active, known or suspected autoimmune disease. Patients requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease that requires systemic corticosteroids or immunosuppressive agents. (Exceptions include any patient on 10 mg or less of prednisone or equivalent, patients with vitiligo, hypothyroidism stable on hormone

replacement, Type I diabetes, Graves' disease, Hashimoto's disease, alopecia areata, eczema, psoriasis, or with Medical Monitor approval.)

- Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin that has undergone potentially curative therapy, or in situ cervical cancer. An incidental finding of prostate cancer (identified upon resection of the prostate) is acceptable, provided that the following criteria are met: Stage T2N0M0 or lower; Gleason score ≤ 6, and prostate specific antigen (PSA) below lower limit of normal by local laboratory.
- History of organ or tissue transplant that requires use of immune suppressive agents.
- Use of warfarin within 14 days of initiating study drug(s). (Note: Low molecular weight heparin is allowed on the study.)
- Evidence of clinically significant interstitial lung disease or active, noninfectious pneumonitis.
- Prior surgery or radiotherapy within 14 days of initiating study drug(s). Patients must have recovered from all radiation-related toxicities, not required corticosteroids and have not had radiation pneumonitis.
- Patients who have been previously treated with prior IL-2.
- Patients who have been previously treated with a TLR agonist (excluding adjuvant, neo adjuvant and topical agents) and patients who have received experimental cancer vaccines are not eligible.
- Patients who have received systemic IFNα within the previous 6 months prior to enrolling into this study.
- Patients who have had < 28 days since the last chemotherapy, biological therapy, or < 14 days from approved tyrosine kinase inhibitor therapy (sunitinib, sorafenib, vemurafenib, dabrafenib, cobimetinib), < 14 days from last dose of hormonal therapy (for patients with breast cancer) or systemic or inhaled steroid therapy at doses greater than 10 mg of prednisone or equivalent before administration of the first dose of study drug(s).

- Active infection requiring systemic therapy.
- Has known hepatitis B virus infection (e.g., hepatitis B surface antigen [HBsAg] reactive) or HCV infection (e.g., HCV RNA qualitative is detected).
- Has known immunodeficiency or active human immunodeficiency virus (HIV 1/2 antibodies).
- Prolonged QTcF > 450 ms for men and > 470 ms for women at Screening.
- History of unstable or deteriorating cardiac disease within the previous 6 months prior to screening including but not limited to the following:
    - Unstable angina or myocardial infarction.
    - Congestive heart failure (New York Heart Association [NYHA] Class III or IV).
    - Uncontrolled clinically significant arrhythmias.
- Patients with hypertension must be on a stable anti-hypertensive regimen for the 14 days prior to enrolment; screening blood pressure must be < 150 mm Hg for systolic blood pressure and < 90 mm Hg for diastolic blood pressure (using the mean of 3 observations taken during the Screening period).
- Patients with a history of any retinal disorders (e.g., retinal detachment, diabetic retinopathy, retinal hemorrhage, macular degeneration).
- History of pulmonary embolism, deep vein thrombosis, or prior clinically significant venous or non-CVA/transient ischemic attack (TIA) arterial thromboembolic event (e.g., internal jugular vein thrombosis) within 3 months prior to enrollment.
- Patients with a history of a venous or arterial thromboembolic event must be asymptomatic for at least 2 weeks prior to enrollment and must be receiving a stable regimen of therapeutic anticoagulation (preferably low molecular weight heparin [LMWH] or direct oral anticoagulation [DOAC]).
- Unless there is a new medical contraindication observed after C1D1, a patient with a history of venous or arterial thromboembolic event must be maintained on therapeutic anticoagulation throughout the patient's participation in the study (i.e., through the end of-treatment [EOT] visit).

- Need for > 2 antihypertensive medications for management of hypertension (including diuretics). Patients with hypertension must be on a stable antihypertensive regimen (defined as no dose adjustments to antihypertensive medications) for the 14 days prior to C1D1. Note: An antihypertensive medication that contains 2 drugs with antihypertensive effects in one formulation is counted as 2 antihypertensive drugs (e.g., angiotensin-converting-enzyme [ACE] inhibitor plus diuretic; calcium channel blocker plus ACE inhibitor).
- Patients with uncontrolled adrenal insufficiency (Grade ≥ 3 or Grade 2 without adequately controlled clinical symptoms or Grade 2 requiring > 10 mg/day prednisone equivalent).
- Patients with tumors that invade the superior vena cava or other major blood vessels.

TREATMENT PLAN

Overview

[0167] This is a Phase 1/2, open-label, multicenter, dose escalation and dose expansion study of the TLR7/8 agonist 4-arm-PEG20k-CM-Gly-N-R848 in combination with RSLAIL-2 and in combination with RSLAIL-2 plus nivolumab in patients with locally advanced or metastatic solid tumor malignancies of MEL, MCC, TNBC, HNSCC, RCC, CRC, or sarcoma. The study is divided into a Screening period, Treatment period, EOT period, and Long-Term Follow-up period.

[0168] TLR7/8 agonist: The TLR7/8 agonist starting dose is 0.03 mg. Dose modifications of TLR7/8 agonist (either dose escalation or de-escalation to previous dose level or to an interim dose level) may be adjusted based on clinical observations as per the sample dose escalation scheme in Table 1. No intra-patient dose escalation is allowed. TLR7/8 agonist is administered to a maximum of two lesions in three 3-week cycles. Patients may receive additional cycles of TLR7/8 agonist IT injection in cases of SD, PR, or PD with treatment beyond progression or after consultation with the Medical Monitor. During TLR7/8 agonist dose escalation, the second and third doses of TLR7/8 agonist is followed by treatment with RSLAIL-2 administered intravenously 2 days after the TLR7/8 agonist dose (C2D3 and C3D3) for Cohorts 1 and 2. Same-day administration of TLR7/8 agonist and RSLAIL-2 in Cohort 3 is tested at the same dose level as in Cohort

2 starting from Cycle 2, and further escalation of TLR7/8 agonist will occur in Cohorts 4 and above. Cohort A will enroll patients receiving administration of the TLR7/8 agonist (3.84 mg) and RSLAIL-2 (0.006 mg/kg q3w). Cohort B will enroll patients receiving administration of the TLR7/8 agonist (3.84 mg), RSLAIL-2 (0.006 mg/kg q3w), and nivolumab (360 mg q3w).

[0169] TLR7/8 agonist at the RP2D (3.84 mg) co-administered with RSLAIL-2, and co-administered with RSLAIL-2 and nivolumab, respectively for the first 3 cycles. Patients will continue to receive RSLAIL-2 +/- nivolumab in 3-week cycles. The RSLAIL-2 dose of 0.006 mg/kg is supported by the safety observed in the ongoing monotherapy trial with RSLAIL-2. The proposed doses for RSLAIL-2 and nivolumab to be used in triplet combination with TLR7/8 agonist are supported by the ongoing combination study of RSLAIL-2 and nivolumab. A sample proposed dose escalation schema for the Phase 1 part of, is provided in Table 1.

[0170] Results of the assessments must be reviewed and documented before administering the first dose of the next cycle. Every effort should be made to schedule visits within the protocol-specified windows.

Treatment Period

[0171] Patients are treated until disease progression per RECIST 1.1, death, unacceptable toxicity, symptomatic deterioration, achievement of maximal response, the Investigator's decision to discontinue treatment, the patient withdraws consent, loss to follow up, or a decision to terminate the trial.

[0172] TLR7/8 agonist injected and non-injected tumor lesions for biopsy are identified and recorded prior to treatment in each cycle.

[0173] Patients with PD per RECIST 1.1 but with otherwise stable or improved performance and clinical status may continue to be treated in the event of a perceived benefit per Investigator. Patients with a PR or SD will continue to receive treatment until achievement of a confirmed CR, disease progression, or intolerability to therapy or decision of the Investigator in consultation with the Medical Monitor. It is at the discretion of the Investigator to continue treating patients with a confirmed CR.

Administration of Study Drug(s)

[0174] The starting dose of TLR7/8 agonist is 0.03 mg IT injection. TLR7/8 agonist is administered in 3-week cycles. Patients may receive additional cycles of TLR7/8 agonist IT injection after assessment of anti-tumor effect at each designated disease staging in cases of SD, PR, or PD with treatment beyond progression or in consultation with the Medical Monitor. Requirement for IR guidance is assessed at Investigator discretion. Up to two tumor non-target lesions are selected for injection throughout study cycles and are designated as the "injected tumor." The TLR7/8 agonist dose is divided and not exceed the total dose amount assigned for the respective cohort dose level. Subcutaneous tumors are recommended for injection over deep lesions. Tumors encasing major vascular structures (i.e., carotid artery or tumors close to other vital organs) are not considered appropriate for IT injection. The injected lesions at all cycles should measure between 20 mm and 90 mm. Any liver lesion targeted for injection must not exceed 50 mm at the time of injection. The assigned dose of TLR7/8 agonist is administered into the "injected tumor" by qualified medical personnel using an appropriate method for needle localization. Total injected dose should remain constant; in the event a full dose can no longer be practically administered into the "injected tumor," another lesion may be selected. It is recommended that injection of TLR7/8 agonist be rotated between different non target lesions. Please see Pharmacy Manual for details. In consultation with the Medical Monitor, if more than 1 target lesion is available, the target lesions may be injected with TLR7/8 agonist after Cycle 1. TLR7/8 agonist is administered before RSLAIL-2 and before RSLAIL-2 plus nivolumab.

[0175] It is recommended that the tumors be injected with a 28-gauge or smaller needle. TLR7/8 agonist should be thoroughly distributed within the injected tumor while avoiding necrotic areas using a "fanning method" (spread the injection across several angles throughout the lesion to maximize the spread of TLR7/8 agonist in the tumor).

[0176] At the time of the second injection, if the injected lesion becomes non-accessible or nonexistent, a different lesion should be selected for injection and recorded.

[0177] It is recommended that two lesions should be injected at each cycle with TLR7/8 agonist. The total dosing volume remains unchanged (1 mL), when administering TLR7/8 agonist, thereby dividing the dose equally (0.5 mL) per lesion. It is also

recommended, that one of the lesions injected should be a draining lymph node (if identified as an injectable tumor where an IT injection can be given) which contains immune cell rich tissue. This recommendation is based on preliminary data that shows a concentration-dependent effect when delivering greater than or equal to 1 mg TLR7/8 agonist IT.

[0178] Each patient's RSLAIL-2 dose is 0.006 mg/kg. The patient's weight in kilograms is determined before the start of each cycle. RSLAIL-2 is administered after TLR7/8 agonist and before nivolumab. RSLAIL-2 is administered q3w as an IV infusion over 30 ± 5 minutes.

[0179] Nivolumab is administered at a fixed dose of 360 mg q3w as an IV infusion over 30 ± 5 minutes. Nivolumab should be administered within 30 ± 5 minutes after completion of RSLAIL-2 administration.

[0180] Flat Dose Regimens with Nivolumab

[0181] Population PK (PPK) analyses have shown that the PK of nivolumab is linear with proportional exposure over a dose range of 0.1 to 10 mg/kg, and no differences in PK across ethnicities and tumor types were observed. As the PK of nivolumab is linear, the corresponding flat dose for a q3w dosing regimen is nivolumab 360 mg. Using the PPK model developed, the exposures following administration of several dosing regimens of nivolumab administered as a flat dose were simulated, including 360 mg administered q3w. The simulated steady-state average concentrations following administration of nivolumab 360 mg q3w are expected to be similar to those following administration of nivolumab 3 mg/kg every 2 weeks (q2w) to subjects weighing 80 kg, the approximate median weight of subjects used in the PPK analyses. The predicted steady state peak concentrations following nivolumab 360 mg q3w are predicted to be less than those following the administration of nivolumab 10 mg/kg q2w providing sufficient safety margins.

[0182] Based on these supportive data, nivolumab 360 mg q3w alternative schedule is examined in the current study with TLR7/8 agonist and RSLAIL-2.

[0183] Nivolumab Shorter Infusion Duration

[0184] Establishing that nivolumab can be safely administered using a shorter infusion time (30 minutes) is under investigation. Previous clinical studies of nivolumab

monotherapy have used a 60 minute infusion duration, and nivolumab has been safely administered up to 10 mg/kg over long treatment periods. Infusion reactions including high-grade hypersensitivity reactions have been uncommon across the nivolumab clinical program. In study CA209-010 (NCT01354431), a dose association was observed for infusion site reactions and hypersensitivity reactions (1.7% at 0.3 mg/kg, 3.7% at 2 mg/kg, and 18.5% at 10 mg/kg). All of the events were Grade 1-2 and were manageable. An infusion duration of 30 minutes for 3 mg/kg nivolumab (30% of the 10 mg/kg dose) is not expected to present any safety concerns based on the prior experience of 10 mg/kg infused over 60 minutes. The safety of 3 mg/kg nivolumab administered as a 30 minute infusion was assessed in patients (n = 322) with previously treated advanced NSCLC. Overall, there were no clinically meaningful differences in the frequency of hypersensitivity/infusion-related reactions (of any cause or treatment related) between patients infused over 30 minutes versus the frequency reported for 60 minute infusions. Thus, nivolumab is considered safe to infuse over 30 minutes.

Dose Escalation

[0185] A sample dose escalation schema is provided in Table 1.

[0186] TLR7/8 agonist: The TLR7/8 agonist starting dose is 0.03 mg. Dose modifications of TLR7/8 agonist may be adjusted based on clinical observations as per Table 1. The dose may be divided and injected in up to 2 lesions using the fanning method. Safety and tolerability as a single agent are assessed during the first 21 days. The first patient (a sentinel patient) of each escalating TLR7/8 agonist dose cohort and Cohort B is monitored for safety and tolerability on Days 1 through 5 after the first administration of TLR7/8 agonist before additional patients are dosed within the same cohort. Subsequently, two days after the second and third doses of TLR7/8 agonist, patients will receive RSLAIL-2 (0.006 mg/kg) in 3-week cycles for patients enrolled in Cohorts 1 and 2. Following completion of enrollment in Cohort 2, same-day administration of TLR7/8 agonist and RSLAIL-2 is tested at the same dose level in Cohort 3 starting at Cycle 2, with continued escalating TLR7/8 agonist doses in subsequent cohorts. Of note, with the exception of the Cohort 1 dose, dose levels for subsequent cohorts may be adjusted (escalated or de-escalated) based on clinical observations and the decision of declaring the RP2D of TLR7/8 agonist in combination with RSLAIL-2 can

occur at any given dose level based on PK or immunological effects without reaching the MTD. Patients may receive an additional 3 cycles of TLR7/8 agonist IT injection after assessment of anti-tumor effect at each designated disease staging in consultation with the Medical Monitor.

[0187] RSLAIL-2: The dose is a 0.006 mg/kg IV infusion administered over 30 ± 5 minutes q3w following the second and third dose administrations of TLR7/8 agonist.

[0188] Nivolumab: The dose is a 360 mg IV infusion administered over 30 (± 5) minutes q3w. The treatment regimen schema for the combination of nivolumab, RSLAIL-2 and TLR7/8 agonist will start with the first dose of TLR7/8 agonist assuming same-day dose administration of TLR7/8 agonist and RSLAIL-2 is safe (Cohort A). RSLAIL-2 and nivolumab will continue to be administered q3w after the third and/or last TLR7/8 agonist dose.

[0189] Cohorts A and B will enroll patients to receive TLR7/8 agonist at the RP2D (3.84 mg) co administered, respectively, with RSLAIL-2 (0.006 mg/kg) or with RSLAIL-2 (0.006 mg/kg) and nivolumab (360 mg) in 3-week cycles.

[0190] Dose-limiting toxicity evaluation for the combination will occur in the first dosing cycle.

Table 1. SAMPLE Dose Escalation Scheme (3+3 Design)[a]

	TLR7/8 agonist	RSLAIL-2	Nivolumab
Cohort -2	0.008 mg	0.003 mg/kg q3w	-
Cohort -1	0.015 mg	0.006 mg/kg q3w	-
Cohort 1	0.03 mg	0.006 mg/kg q3w	-
Cohort 2[b]	0.06 mg	0.006 mg/kg q3w	-
Cohort 3[b]	0.06 mg	0.006 mg/kg q3w	-
Cohort 4[b]	0.12 mg	0.006 mg/kg q3w	-
Cohort 5[b]	0.24 mg	0.006 mg/kg q3w	-
Cohort 6[b]	0.48 mg	0.006 mg/kg q3w	-
Cohort 7[b]	0.96 mg	0.006 mg/kg q3w	-
Cohort 8[b]	1.92 mg	0.006 mg/kg q3w	-
Cohort 9	3.84 mg	0.006 mg/kg q3w	-
Cohort 10	7.68 mg	0.006 mg/kg q3w	-

	TLR7/8 agonist	RSLAIL-2	Nivolumab
Cohort A[c]	3.84 mg	0.006 mg/kg q3w	-
Cohort B [c]	3.84 mg	0.006 mg/kg q3w	360 mg q3w

a. With the exception of the Cohort 1 dose, dose levels for subsequent cohorts may be adjusted (escalated or de-escalated) based on clinical observations. Dose escalation will not exceed double the prior dose of TLR7/8 agonist. Dose may be rounded off to one decimal point based on study drug reconstitution calculations. Exact dose is confirmed based on review of safety and PK data after a minimum of 3 patients have been enrolled in each cohort, by the Safety Review Committee. Dosing beyond Cohort 5 will further characterize safety and define optimal PK and PD. Cohort -1 and -2 will only open in the event of a dose limiting toxicity (DLT) in Cohort 1 of TLR7/8 agonist. If dose limiting toxicities are observed at the starting dose, TLR7/8 agonist and RSLAIL-2 may be de-escalated as shown in Cohort -1 and Cohort -2. If DLTs are observed in a subsequent Cohort, previous Cohort may be reopened to enrollment or dose de-escalation done in the same cohort to ensure safety, PK/PD data for a minimum of 6 subjects for RP2D decision. DLT defining period is 21 days for TLR7/8 agonist monotherapy and an additional 9 days in combination with RSLAIL-2 (Cohort -2 up to Cohort 2). Cohort -1 provides for de-escalation of TLR7/8 agonist only; Cohort -2 provides for de-escalation of TLR7/8 agonist and RSLAIL-2.

b. Starting at Cycle 2, numbered Cohorts 3 and greater will explore same-day administration of TLR7/8 agonist and RSLAIL-2 at the dose level of Cohort 2. DLT defining period is 21 days for TLR7/8 agonist monotherapy and an additional 7 days in combination with RSLAIL-2 (for same day dosing). If the same-day dosing is not tolerated at the established TLR7/8 agonist dose level, TLR7/8 agonist is dose-reduced in an additional cohort with the same RSLAIL-2 dose level. If toxicity still occurs, RSLAIL-2 will also be dose-reduced.

c. Starting at the RP2D of TLR7/8 agonist (3.84 mg), Cohort B (triplet) will explore same-day administration of TLR7/8 agonist plus RSLAIL-2 with nivolumab starting at Cycle 1. The doublet and triplet regimen for Phase 2 may initiate enrollment before completion of Cohorts A (TLR7/8 agonist plus RSLAIL-2) and B (TLR7/8 agonist plus RSLAIL-2 + nivolumab). The DLT defining period is 7 days during the first cycle for both cohorts.

Duration of Treatment

[0191] Patients are treated until any one of the following:

- Disease progression per RECIST 1.1
- Death
- Unacceptable toxicity
- Symptomatic deterioration
- Achievement of maximal response
- Investigator's decision to discontinue treatment
- Patient decision to discontinue treatment
- Patient withdraws consent
- Female pregnancy
- Lost to follow up
- A decision to terminate the study

Treatment Beyond Progression

[0192] Accumulating evidence indicates that a minority of patients with solid tumors treated with immunotherapy may derive clinical benefit despite initial evidence of PD. Patients are permitted to continue on treatment beyond initial RECIST 1.1-defined PD as long as they meet the following criteria:

- Investigator-assessed clinical benefit and without rapid disease progression.
- Continue to meet all other study protocol eligibility criteria.
- Patient tolerates study drug(s).
- Patient has stable ECOG performance status of 0 or 1.

[0193] Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (e.g., central nervous system metastases).

[0194] The assessment of clinical benefit should take into account whether the patient is clinically deteriorating and unlikely to receive further benefit from continued treatment. All decisions to continue treatment beyond initial progression must be discussed with the Medical Monitor, and an assessment of the risk/benefit of continuing with study drug(s) must be documented in the study records.

[0195] For patients who stay on treatment beyond RECIST 1.1-defined PD, all study procedures should continue to be performed, including radiographic assessment by CT (preferred) or magnetic resonance imaging (MRI) every 9 weeks. Patients are discontinued from treatment upon further evidence of disease progression, defined as an additional 10% or greater increase in the total tumor burden from the time of initial disease progression. The total tumor burden is calculated as the sum of the longest diameters (SLD) of all target tumors and SLD of all new measurable lesions. A new lesion is measurable if the longest diameter is at least 10 mm except for pathological lymph nodes which must have a short axis of at least 15 mm. Any new lesions that are non-measurable at the time of initial appearance and become measurable later are included in the calculation of total tumor burden. The total tumor burden from the time of initial RECIST 1.1-defined progression is used as the reference baseline for comparison with all post-progression assessments.

[0196] Patient remain on treatment phase until discontinuation of all study drugs, unless discussed with study Medical Monitor.

End of Study

[0197] End of study is defined as no more than 3 years after the last patient received his or her first dose of TLR7/8 agonist or a decision to terminate the study, whichever comes first. Survival data may be collected beyond end of study for a maximum of 5 years.

[0198] Tumor assessments for all patients (Phase 1 and Phase 2) is performed at Screening and every 9 weeks (± 7 days) until the patient withdraws consent or starts a new antineoplastic regimen, or till the end of study. Assessments become less frequent during the long-term follow up period. Tumor response is evaluated using RECIST 1.1 as the primary and irRECIST as an exploratory measure.

[0199] Radiographic assessments (chest/abdomen/pelvis, and other known affected anatomical areas) are required for all patients for tumor measurements. Additional scan assessments may be collected based on clinical symptoms, as appropriate.

[0200] Documented tumor measurements are required using CT scans, MRI, physical examination, and/or digital photography, as appropriate. Any imaging used to assess disease at any time point is submitted for an independent radiology review.

[0201] The same method of assessment (CT or MRI and/or digital photography) and the same technique for acquisition of images must be used for all study assessments (contrast must be used unless medically contraindicated). Baseline imaging should be done at the same institution/facility which is used to measure response during the patient's participation in the study. Radiographic assessments and efficacy analyses are conducted by the Investigator site as well as the independent radiology review committee.

Pharmacokinetic Measurements

[0202] Blood samples for PK analyses are collected from all patients. Urine samples are collected from patients enrolled in numbered Cohorts 5 and greater, and for a minimum of 6 patients in Cohort A. Serial PK samples are collected at multiple scheduled sampling times. Concentrations of TLR7/8 agonist, RSLAIL-2, and their metabolites, as well as nivolumab are measured using validated or qualified method(s). Pharmacokinetic

parameters such as maximum concentration (Cmax), time to Cmax (Tmax), area under the curve (AUC), clearance (CL/F), volume of distribution (Vd/F), and half-life (t1/2) are estimated from plasma concentration-time data where possible.

[0203] Blood and urine samples for PK analysis are collected and processed as outlined in the Laboratory Manual that is provided to the sites. All on-treatment time points are intended to align with days on which study drug is administered; if dosing occurs on a different day, the PK sampling should be adjusted accordingly. If it is known that a dose is going to be delayed, then the pre-dose sample should be collected just prior to the delayed dose. However, if a pre-dose sample is collected but the dose is subsequently delayed, an additional pre-dose sample should be collected.

[0204] For all PK blood and urine samples, the date and actual time collected must be recorded. For patients whose only peripheral access is via a venous access device or peripherally inserted central catheter, refer to the Laboratory Manual for the proper technique to ensure undiluted whole blood for PK assessments.

Immunogenicity Measurements

[0205] Immunogenicity samples for RSLAIL-2 are drawn at pre-dose C1D1, pre-dose on Cycle 2 (Cohorts A and B and Phase 2 only), and pre-dose on Day 1 of all odd-numbered cycles thereafter (Cycles 3, 5, 7, etc.), follow-up visits, and EOT.

[0206] Immunogenicity samples for nivolumab are collected as follows for Cohort B: pre-dose C1D1, and pre-dose Cycle 2 Day 1, Cycle 5 Day 1, Cycle 11 Day 1, Cycle 16 Day 1, Cycle 24 Day 1, Cycle 32 Day 1. If dosing occurs on a different day, the immunogenicity sampling should be adjusted accordingly.

Biomarker Measurements (Blood and Tumor Collection)

[0207] Blood and tumor tissue biopsies are collected and processed as outlined in a Laboratory Manual provided to the sites. For biomarker blood samples, at each designated sampling time, collect specified volume of blood to analyze immune cell populations by flow cytometry, cytokines secretion in the plasma, mutations, profile RNA expression, and for PBMC isolation. For tumor biopsies, collect core biopsies or biopsies with a volume ≥ 100 mm3, and divide into 2 portions. One portion is FFPE for immunohistochemistry (IHC) and mutation analysis and one portion is used for RNA isolation and molecular characterization.

Exploratory Biomarker Tests to Characterize CVA Events

[0208] Blood samples for exploratory biomarker analyses are collected before study drug administration on C1D1, C3D1, at an unscheduled visit, and at the time of a new CVA event for patients with a baseline C1D1 sample. Exploratory CVA biomarker samples are obtained only from participants from whom a C1D1 biomarker sample has been collected.

[0209] The exploratory biomarkers may include, but are not limited to, markers of eosinophil activation, vascular damage, and/or coagulation.

Blood-Based Mechanism of Action Analysis

[0210] Blood for MOA analysis are collected as described in the Schedule of Events. The whole blood or PBMCs isolated from whole blood correlative pharmacodynamic samples are analyzed by RNA expression analysis, including assessment of genes indicative of TRL7 and TRL8 engagement (type 1 IFN-dependent genes and production of proinflammatory cytokines downstream of the NFκB pathway). In addition, genomic profiling of cancer- and immune-system related genes may be assessed.

Blood-Based Biomarker Analysis

[0211] Blood for biomarker analysis are collected. The whole blood or PBMCs isolated from whole blood correlative pharmacodynamic samples are analyzed by flow cytometry for changes in markers of immune cell populations, including, but not limited to, functional markers on T and B lymphocytes, dendritic cells, NK cells, and monocyte subsets. In addition, whole blood samples or PBMCs may be used for evaluation of other immune functions (assessed by cytokine secretion, proliferation, etc.).

Tumor Tissue Based Biopsy Analysis

[0212] Fresh tumor biopsies (defined as a biopsy specimen taken within approximately 28 days prior to the first dose of study treatment) are required prior to C1D1. If available, archival tumor sample should be collected. For archival samples, a tumor block, or 20 slides should be submitted (no more than 30 days after first dose). Exceptions may be made in cases where fresh tumor tissue samples are inaccessible. A biopsy specimen from a sample taken since completion of the most recent prior systemic treatment but no more than 6 months prior may be acceptable in lieu of a fresh biopsy during Phase 2. Biopsies should be performed on lesions that have not been exposed to

prior radiation. As per RECIST, biopsies should be obtained from non-target lesions, unless there are no other lesions suitable for biopsy. If a target lesion is used for biopsy, there must be more than 1 target lesion, the lesion must be ≥ 2 cm in the longest diameter, and the lesion used for tumor evaluation cannot be used. Pre and post-treatment tumor tissue biopsies should be taken from the same lesion, if feasible. In addition, biopsies may be taken from a distant, non-injected lesion to serve as a control biopsy. Tumor tissue biopsies are used for RNA expression analysis, including assessment of genes indicative of TRL7 and TRL8 engagement (including NF-kB- and type I interferon dependent genes) and to characterize infiltrating immune cell populations with IHC using a panel of markers (including, but not limited to, CD3, CD4, CD8, CD25, FoxP3, CD56, CD11c, CD14, CD16, HLA-DR, and PD-L1). Biopsy samples may also be used to investigate molecular signatures, and DNA may be extracted from these samples for somatic mutation analysis. Genes to be assayed may include, but are not limited to, those with known driver mutations in solid tumors.

Management Guidelines for Immune-mediated Adverse Events

[0213] Immune-oncology agents are associated with AEs that can differ in severity and duration from AEs caused by other therapeutic classes. Early recognition and management of AEs associated with I-O agents may mitigate severe toxicity. TLR7/8 agonist, RSLAIL-2, and nivolumab, are considered I-O agents in this protocol

Cytokine Release Syndrome/Hypersensitivity Reactions

[0214] The mechanism of action of TLR7/8 agonist involves TLR7/8 activation and stimulation of cytokine release; therefore, CRS, and/or hypersensitivity reactions may occur. These reactions may be allergic or non-allergic in nature and may be associated with the release of cytokines. Mild-to-moderate presentations of CRS may include symptoms such as influenza-like illness, fever, headache, rash and myalgia, and may be treated symptomatically with analgesics, anti-pyretics, and antihistamines as indicated. Severe or life-threatening presentations of CRS, such as hypotension, tachycardia, dyspnea, chest discomfort, wheezing, angioedema, and urticaria should be treated aggressively with supportive and resuscitative measures as indicated, including the use of corticosteroids, IV fluids, and other supportive measures per institutional practice and published guidelines (Lee, 2014).

Permanent Treatment Discontinuation Criteria

[0215] Patients meeting any of the following criteria are required to permanently discontinue all assigned study drug(s). However, with approval, RSLAIL-2 or TLR7/8 agonist treatment may continue if the toxicities listed below are considered related to nivolumab only.

- Progressive disease (see details regarding continuing treatment beyond initial assessment of progression per RECIST 1.1).
- Clinical deterioration, as assessed by the Investigator.
- Grade 3 or 4 pneumonitis
- AST or ALT greater than 5 × ULN or total bilirubin greater than 3 × ULN
- Grade 4 diarrhea or colitis
- Grade 4 hypophysitis
- Myasthenic syndrome/myasthenia gravis, Guillain-Barre, or meningoencephalitis (all grades)
- Grade 3 or 4 ocular inflammatory toxicity
- Grade 3 or 4 CRS (Lee, 2014)
- Grade 4 pancreatitis, or any grade of recurrent pancreatitis
- Grade 3 or 4 infusion-related reactions
- Grade ≥ 3 adrenal insufficiency or Grade ≥ 4 hypophysitis require discontinuation regardless of control with hormone replacement.
- Grade 4 rash
- Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except Grade 3 drug-related thrombocytopenia > 7 days associated with clinically significant bleeding requires discontinuation.
- Any Grade 4 drug-related AE or laboratory abnormality, except for the following events, which do not require discontinuation:
    - Grade 4 neutropenia ≤ 7 days
    - Grade 4 lymphopenia or leukopenia
    - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to

Grade < 4 after consultation with the Medical Monitor (or designee)

- Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset

- Any AE, laboratory abnormality, or intercurrent illness, which, in the judgment of the Investigator, presents a substantial clinical risk to the patient with continued treatment.
- Any new CVA event confirmed by imaging (DWI MRI preferred unless otherwise contraindicated) regardless of neurological symptoms (e.g. cryptogenic CVA).
- For a suspected TIA event without clear alternative etiology, study treatment may be continued only after careful risk-benefit assessment by the Investigator.
- Any dosing delay lasting > 2 weeks after completion of the prior cycle, with the following exceptions:
  - Dosing delays to allow for prolonged steroid tapers to manage drug-related AEs are allowed. Tumor assessments should continue as per protocol even if dosing is delayed.
  - The Investigator and the Medical Monitor may agree to a longer dose delay following a clinically significant AE if the patient is deriving clinical benefit from the regimen as defined by RECIST 1.1 CR, PR, or SD, up to 6 weeks from last dose of any of the three investigational agents.

[0216] A dosing delay lasting more than 2 weeks should be discussed with the Medical Monitor and patient may resume the study if provided approval based on clinical assessment.

[0217] Tumor assessments should continue as per protocol even if dosing is delayed, and patients must otherwise meet the criteria for continued treatment at the time re initiation of study therapy is considered.

Prior and Concomitant Medications

[0218] Prophylaxis for flu-like symptoms with either acetaminophen or ibuprofen is permitted on study per the Investigator's discretion. Prophylaxis for flu-like symptoms can be initiated on either Day 1 or Day 2 of the dosing cycle and may continue through Day 5 or longer as needed.

[0219] All medications (prescription and OTC), vitamin and mineral supplements, and/or herbs taken by the patient from Screening through the EOT visit are documented and recorded, including start and stop date, dose and route of administration, frequency, and indication. Medications taken for a procedure (e.g., biopsy) should also be included.

Thromboembolism Prophylaxis and Treatment

[0220] Patients with a history of a venous or arterial thromboembolic event must be receiving a stable regimen of therapeutic anticoagulation (LMWH or DOAC). Additionally:

[0221] Use of warfarin (Coumadin) is permitted; however, therapeutic dosing should target a specific INR stable for at least 4 weeks prior to enrollment. RSLAIL-2 has the potential to down-regulate metabolizing enzymes for warfarin for approximately 1 week after administration of each dose of RSLAIL-2. Due to the possibility of drug-drug interactions between warfarin and RSLAIL-2, frequent monitoring of INR and ongoing consideration of dose adjustments are warranted throughout the patient's participation in the study.

[0222] Unless there is a new medical contraindication observed after C1D1, a patient with a history of venous or arterial thromboembolic event must be maintained on therapeutic anticoagulation throughout the patient's participation in the study (i.e., through the EOT visit).

Effect of RSLAIL-2 *on Concomitant Medications*

[0223] RSLAIL-2 may have the potential to affect the clearance of co-administered drugs based on its ability to modulate immune function. RSLAIL-2 causes increases in circulating cytokines typical of those associated with an acute inflammatory response to infection or tissue injury. The increases in inflammatory cytokines induced by RSLAIL-2 are generally moderate, persist for approximately one week after RSLAIL-2 administration, and return to baseline levels prior to the next dose. Several of these cytokines (IFN-γ, IL-6, IL 10, etc.) have the potential to decrease the activity of multiple enzymes and drug transporters, and the suppressive effects can be additive (Haas, 2005; Zidek, 2009). Similar to changes that occur during a typical inflammatory response, RSLAIL-2 treatment may lead to downregulation of drug metabolizing enzymes, such as cytochrome P450 (CYP) enzymes, hepatic flavin monooxygenases, UDP-glucuronosyltransferases, sulfotransferases, and glutathione S transferases.

Consequently, treatment with RSLAIL-2 may lead to temporary decrease in clearance of drugs that are substrates of drug metabolizing enzymes or drug transporters. Investigators should carefully monitor for the occurrence of adverse effects in patients receiving drugs with narrow therapeutic indices or drugs that are sensitive substrates of drug metabolizing enzymes or drug transporters and adjust the dose of these drugs if needed.

Interaction of RSLAIL-2 *and Warfarin*

[0224] For patients receiving warfarin, therapeutic dosing should target a specific INR that is stable for at least 4 weeks prior to RSLAIL-2 administration. RSLAIL-2 has the potential to down regulate metabolizing enzymes for warfarin for approximately 1 week after administration of each dose of RSLAIL-2. Due to the possibility of drug-drug interactions between warfarin and RSLAIL-2, frequent monitoring of INR and ongoing consideration of dose adjustments are warranted during RSLAIL-2 administration

Prohibited Medications

[0225] Strong cytochrome P450 3A4 (CYP3A4) inhibitors or inducers: Concomitant administration of strong inhibitors (e.g., ketoconazole) or inducers (e.g., rifampicin, carbamazepine, phenobarbital, phenytoin, St. John's wort) of CYP3A4 should be avoided. If use of one of these medications cannot be avoided, the risks and benefits should be discussed with the Medical Monitor prior to its concomitant administration with TLR7/8 agonist. For a list of these agents, see the Food and Drug Administration (FDA) Drug Development and Drug Interactions Table.

[0226] Any antineoplastic therapy (i.e., chemotherapy, hormonal therapy, immunotherapy, extensive non-palliative radiation therapy, investigational agent, or radiation therapy) is prohibited during the study. Palliative radiation is permitted to ≤ 2 non target lesions at a time, provided it is completed 14 days before dosing of TLR7/8 agonist, RSLAIL-2 and nivolumab.

[0227] Consideration should be given to withholding antihypertensive medications including diuretics, as well as other drugs with hypotensive properties (e.g., alpha blockers for benign prostatic hypertrophy), particularly when therapy involves multiple antihypertensive drugs and classes other than thiazide diuretics. Study subjects who are on medications with antihypertensive effects for the treatment of coronary artery disease

(e.g., beta-blockers, calcium channel blockers, nitrates, etc.) should be able to temporarily discontinue these drugs. If withholding antihypertensive medications, withhold no less than 12 hours and no more than 48 hours prior to each dose of TLR7/8 agonist and RSLAIL-2. Antihypertensive medications may be reinstituted in between doses of TLR7/8 agonist and RSLAIL-2 at any time as clinically indicated (e.g., based on blood pressure monitoring result).

[0228] Investigators should follow their institutional guidelines for discontinuation of anti-coagulant therapy (e.g., aspirin and Plavix) before IT injections. Investigators should understand current best-practice recommendations for pre and post procedure management of antithrombotic therapy, balancing the risk/benefit of modifying antithrombotic therapy in IR patients.

ASSESSMENT OF EFFICACY

[0229] Response and progression are determined using RECIST 1.1 (Eisenhauer, 2009) as the primary and irRECIST (Nishino, 2013; Nishino, 2015) as exploratory measures.

Measurable Disease

[0230] Target tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of: 10 mm by CT scan (CT scan slice thickness no greater than 5 mm); when CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

[0231] Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis is measured and followed.

Non-measurable Disease

[0232] All other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes ≥ 10 to < 15 mm short axis) as well as truly non measurable lesions, are considered non measurable disease. Lesions considered truly non measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion,

lymphangitic involvement of skin or lung, or abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Specifications by Methods of Measurements

[0233] The same method of assessment and the same technique should be used to characterize each lesion at baseline and during follow-up. Imaging-based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

[0234] Clinical Lesions: Clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm diameter as assessed using calipers (e.g., skin nodules). When lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study. For cutaneous lesions that are included in target lesions, and injected non-target lesions, digital photographs should be obtained and utilized for measurement and data reporting.

[0235] CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. If a slice thickness > 5 mm is used for CT scanning, then the minimum longest diameter for a target lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans).

[0236] Tumor Markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the ULN, however, they must normalize for a patient to be considered in CR.

[0237] Cytology, Histology: These techniques can be used to differentiate between PR and CR in rare cases when the nature of a residual lesion is in question. The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or SD in order to differentiate between response (or SD) and PD.

Tumor Response Evaluation

[0238] Assessment of Overall Tumor Burden at Baseline and Measurable Disease

[0239] To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for

subsequent measurements. Measurable disease is defined by the presence of at least one measurable lesion.

[0240] When more than one measurable lesion is present at baseline, all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and are recorded and measured at baseline (this means in instances where patients have only 1 or 2 organ sites involved, a maximum of 2 and 4 lesions, respectively, are recorded). Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

[0241] Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered non target lesions. Nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed.

[0242] A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions are calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters are used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

[0243] All other lesions (or sites of disease) including injected lesions and pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression'. All non-target lesions chosen for injection should be measured and measurement recorded in EDC.

[0244] For assessment of abscopal response, any of the target lesions identified at baseline are measured and followed for abscopal response. This target lesion should not

be a target of injection or biopsy. The abscopal response is defined as a shrinkage of ≥ 20% from baseline in any target non-manipulated metastatic lesion identified at baseline.

[0245] Lymph nodes: Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if CR criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm. For PR, SD, and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

[0246] Target lesions that become 'too small to measure': While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). However, if the lesion is believed to be present and is faintly seen but too small to measure with any accuracy, a default value of 5 mm should be assigned as per RECIST 1.1.

Response Criteria using RECIST 1.1

[0247] Evaluation of Target Lesions

[0248] Table 2 provides the definitions of the criteria used to determine objective tumor response for target lesions.

Table 2. Criteria to Determine Objective Tumor Response for Target Lesions Per RECIST 1.1

Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of the longest diameters (SLD) of target lesions, taking as reference the baseline SLD.
Progressive Disease (PD)	At least a 20% increase in the SLD of target lesions, taking as reference the smallest SLD on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm relative to nadir. (Note: the appearance of one or more new lesions is considered progression.)
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Evaluation of Non-target Lesions

[0249] Table 3 provides the definitions of the criteria used to determine the tumor response for the group of non-target lesions. Measurements for injected non-target lesions are required both at baseline and after each subsequent injection, and at each tumor assessment visit.

Table 3. Criteria to Determine Tumor Response for Non-Target Lesions Per RECIST 1.1

Complete Response (CR)	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).
Non-CR/Non-PD	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
Progressive Disease (PD)	Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).[a]

NOTE: If tumor markers are assessed for a given patient and are initially above the ULN, they must normalize for a patient to be considered in complete CR.

a. In this setting, when a patient has measurable disease, to achieve "unequivocal progression" on the basis of the non-target disease, there must be an overall level of substantial worsening in the non-target disease such that, even in the presence of SD or PR in the target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest increase in size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR will therefore be extremely rare.

Confirmatory Measurement/Duration of Response

[0250] Confirmation of response (either PR or CR) is required. Changes in tumor measurements must be confirmed by repeat assessments that should be performed ≥ 4 weeks after the criteria for response are first met.

[0251] Table 4 provides RECIST 1.1 definitions and time points used to determine BOR when confirmation of CR and PR are required.

Table 4. Best Overall Response When Confirmation of CR and PR Required

Overall Response First Time Point	Overall Response Subsequent Time Point	BEST Overall Response
CR	CR	CR
CR	PR	SD, PD, or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise. PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise. PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise. NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise. PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise. NE
NE	NE	NE

CR = complete response; NE = inevaluable; PD = progressive disease; PR = partial response; SD = stable disease.

a. If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR. (Eisenhauer, 2009).

Response Criteria using irRECIST

[0252] Target lesions are selected based on RECIST 1.1. The irRECIST is adopted to evaluate target tumor response. Table 5 provides the definitions of the criteria used to determine objective tumor response for target lesions.

Table 5. Criteria to Determine Objective Tumor Response for Target Lesions using irRECIST

Immune-related Complete Response (irCR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
Immune-related Partial Response (irPR)	At least a 30% decrease in total tumor burden, taking as reference the baseline sum of the longest diameters (SLD).
Immune-related Progressive Disease (irPD)	At least a 20% increase in the total tumor burden, taking as reference the smallest total tumor burden on study (this includes the baseline total tumor burden if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm relative to nadir. (Note: the appearance of one or more new lesions is not considered progression. The new measurable lesions are included in the calculation of total tumor burden.)
Immune-related Stable Disease (irSD)	Neither sufficient shrinkage to qualify for irPR nor sufficient increase to qualify for irPD, taking as reference the smallest sum diameters while on study.

Evaluation of Non-Target Lesions

[0253] Table 6 provides the definitions of the criteria used to determine the tumor response for the group of non-target lesions. Non target lesions are assessed only qualitatively to determine tumor response for non-target lesions per irRECIST at the time points of radiographic assessments. Measurements for injected non-target lesions are required both at baseline and after each subsequent injections, and at each tumor assessment visit.

Table 6. Criteria to Determine Tumor Response for Non-Target Lesions using irRECIST

Absent	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).
Stable	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
Unequivocal Progression	Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is not considered progression).

NOTE: If tumor markers are assessed for a given patient and are initially above the ULN, they must normalize for a patient to be considered a CR.

Evaluation of New Lesions

[0254] Both new measurable lesions and new non measurable lesions do not define progression (but they preclude immune-related complete response (irCR)). They are incorporated into tumor burden as defined above for baseline disease progression.

Confirmatory Measurement/Duration of Response

[0255] Confirmation of response (irCR and irPR) is required. Changes in tumor measurements must be confirmed by repeat assessments that should be performed ≥ 4 weeks after the criteria for response are first met.

[0256] Table 7 describes irRECIST overall response derivation.

Table 7. Derivation of irRECIST Overall Responses

Measurable Response	Nonmeasurable Response		Overall Response
Target and new, measurable lesions (tumor burden),a %	**Non-Target Lesions**	**New, nonmeasurable lesions**	**Using irRECIST**
↓ 100	Absent	Absent	irCR[b]
↓ 100	Stable	Any	irPR[b]
↓ 100	Unequivocal Progression	Any	irPR[b]
↓ ≥ 30	Absent/Stable	Any	irPR[b]
↓ ≥ 30	Unequivocal progression	Any	irPR[b]
↓ < 30 to < 20 ↑	Absent/Stable	Any	irSD
↓ < 30 to < 20 ↑	Unequivocal progression	Any	irSD
≥ 20 ↑	Any	Any	irPD[b]

a. Decreases assessed relative to baseline, including measurable lesions (> 10 mm).
b. Assuming response (irCR, irPR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart.
Abbreviations: irCR = immune-related complete response; irPD = immune-related progressive disease; irPR = immune-related partial response; irRECIST = immune-related Response Evaluation Criteria in Solid Tumors; irSD = immune-related stable disease.

General Considerations

[0257] In general, continuous data is summarized by descriptive statistics, including number of patients, mean, standard deviation, median, minimum, and maximum. Categorical data is summarized by the number and percentage of patients. Unless

otherwise specified, data collected during the Phase 1 part is summarized by dose cohort, and data collected during the Phase 2 part is summarized by tumor indication.

[0258] All efficacy endpoints, except PFS and OS, are analyzed using the response evaluable population and modified response evaluable population. Certain efficacy endpoints will also be summarized for all treated patients as sensitivity analyses. All safety endpoints are summarized using the safety population.

Determination of Sample Size

[0259] This is a Phase 1/2 dose escalation and dose expansion study. During the Phase 1 part, cohorts of at least 3 patients are enrolled at each dose level unless a DLT occurs and additional patients are added to each dose cohort based on the scheme and rules or Sponsor determination of the need for additional data to further evaluate the benefit/risk profile.

[0260] During the Phase 2 part, patients are enrolled. The sample size for each cohort is strictly based on efficacy, assuming target and historical ORRs in Table 8. The Fleming 2 stage design framework is used to justify the sample size per cohort and determine appropriate stopping rules for lack of efficacy based on a normal approximation to provide a reasonable false-positive rate (FPR ≤ 10%) and false-negative rate (FNR ≤ 10%) for each indication:

Table 8. Sample Sizes and Stopping Rules for Phase 2 Dose Expansion Cohorts

Indication	Objective Response Rate		Sample Size[a]			Futility		Efficacy		Reference for Historical ORR
	Historical	Target	N1	N2	Total	S1	S2	T1	T2	
Melanoma (1L)	40	65	13	15	28	≤ 5	≤ 14	≥ 10	≥ 15	Robert, 2015
Melanoma (R/R) I-O R/R	5	25	13	7	20	≤ 0	≤ 2	≥ 3	≥ 3	Unmet medical need
MCC (2L) only 1 prior I-O allowed	15	35	18	20	38	≤ 3	≤ 8	≥ 8	≥ 9	Unmet medical need
MCC (R/R) I-O R/R	5	25	13	7	20	≤ 0	≤ 2	≥ 3	≥ 3	Unmet medical need

Indication	Objective Response Rate		Sample Size[a]			Futility		Efficacy		Reference for Historical ORR
	Historical	Target	N1	N2	Total	S1	S2	T1	T2	
TNBC (1-2L) I-O naïve	10	35	10	11	21	≤ 0	≤ 4	≥ 4	≥ 5	Schmid, 2017
TNBC (R/R)	5	25	13	7	20	≤ 0	≤ 2	≥ 3	≥ 3	Unmet medical need
HNSCC 2+L Platinum-recurrent HNSCC	10	25	22	18	40	≤ 1	≤ 6	≥ 6	≥ 7	Ferris, 2016
HNSCC (R/R) I-O R/R	30	50	26	13	39	≤ 7	≤ 15	≥ 13	≥ 16	Unmet medical need
RCC (1L)	25	50	11	15	26	≤ 2	≤ 9	≥ 6	≥ 10	Choueiri, 2017; Topalian, 2012
RCC (R/R)	5	25	13	7	20	≤ 0	≤ 2	≥ 3	≥ 3	Unmet medical need
Colorectal Cancer (2L) MSI-non high	5	25	13	7	20	≤ 0	≤ 2	≥ 3	≥ 3	Le, 2015
Colorectal Cancer (R/R) MSI-non high	5	25	13	7	20	≤ 0	≤ 2	≥ 3	≥ 3	Unmet medical need
Colorectal Cancer 2-3L MSI-high I-O naive	30	50	26	13	39	≤ 7	≤ 15	≥ 13	≥ 16	Overman, 2017
Colorectal Cancer (R/R) MSI-high	5	25	13	7	20	≤ 0	≤ 2	≥ 3	≥ 3	Unmet medical need
Sarcoma (2L)	10	35	10	11	21	≤ 0	≤ 4	≥ 4	≥ 5	Tawbi, 2017
Sarcoma (R/R)	5	20	16	16	32	≤ 0	≤ 3	≥ 3	≥ 4	Unmet medical need

Abbreviations: ORR = objective response rate; L = line; I-O = immune oncology therapy; R/R = relapsed refractory; MCC = Merkel cell carcinoma; MSI = microsatellite instability; TNBC = triple negative breast cancer; HNSCC = head and neck squamous cell carcinoma; RCC = Renal cell carcinoma.

a. Total sample size for each expansion cohort is calculated using a normal approximation to provide a reasonable false positive rate (FPR ≤ 10%) and false-negative rate (FNR ≤ 10%).

Expansion Cohorts Safety Monitoring

[0261] Bayesian sequential monitoring is employed to perform ongoing safety monitoring targeting a toxicity rate of no more than 30% (where toxicity follows the same definition as a DLT defined for Phase 1 Escalation) for the first 80 subjects enrolled in the Phase 2 Expansion. Subjects are monitored across indications after a minimum of 6 subjects have been treated at the RP2D for the Phase 2 Expansion. During the Phase 1 portion of the trial, safety and efficacy data are collected in Cohorts A and B. The decision to enroll relapsed refractory patients in the doublet or triplet regimen in the Phase 2 portion of the trial is based on Safety Review Committee data, Investigator assessment in consultation with the Medical Monitor.

Analysis Sets

[0262] Safety Population: All patients who receive at least 1 dose (or partial dose) of study drug are included in the analysis of safety.

[0263] DLT Population: All patients who complete at least the DLT observation period or discontinue from the study treatment due to a DLT are included.

[0264] Pharmacokinetic Population: All patients in the Safety Population who have evaluable analyte concentration-time profiles that allow for the computation of meaningful PK parameter values.

[0265] PK/ECG Population: All patients who have at least 1 baseline adjusted QTcF interval (dQTcF) with time-matched TLR7/8 agonist plasma concentrations available.

[0266] Response Evaluable Population: Patients who have measurable disease (per RECIST 1.1) at baseline and also have at least one post-baseline assessment of tumor response.

[0267] Modified Response Evaluable Population: Patients who have measurable disease (per RECIST 1.1) at baseline and also have at least one post-baseline assessment of tumor response or are withdrawn due to PD/death prior to first response assessment.

Analyses

[0268] Efficacy analyses is performed on data provided by the Investigator sites as well as from an independent radiology review for the following efficacy outcomes:

- Objective response rate (ORR) using RECIST 1.1

- Best overall response (BOR) using RECIST 1.1
- Duration of response (DOR) using RECIST 1.1
- Clinical benefit rate (CBR) using RECIST 1.1
- Time to response (TTR) using RECIST 1.1
- Progression-free survival (PFS) using RECIST 1.1
- Overall survival (OS)
- Abscopal response using RECIST 1.1

[0269] The primary efficacy measurement is ORR per RECIST 1.1 based on data provided by the Investigator's assessment using the response evaluable population and modified response evaluable population. The number and percentage of patients with CR or PR as their BOR is calculated. The ninety-five percent (95%) CI is calculated using the exact binomial method. BOR is summarized similarly using the response evaluable population and modified response evaluable population. In addition, the ORR analysis are performed in all treated patients. All tumor assessments and response data are listed.

[0270] TTR is defined for patients who had confirmed CR or confirmed PR as the time from the date of first dose to the date of first documented CR or PR. TTR is summarized using descriptive statistics.

[0271] DOR is defined for patients who have confirmed CR or confirmed PR as the date from first documented CR or PR to the date of the first objectively documented disease progression per RECIST 1.1 or death due to any cause, whichever is earlier. Patients who do not have disease progression per RECIST 1.1 are censored on the date of last evaluable tumor assessment. Patients who started any subsequent antineoplastic regimen, including tumor-directed radiotherapy and tumor-directed surgery, without a prior reported progression are censored at the last evaluable tumor assessment prior to initiation of the subsequent antineoplastic regimen. The DOR is estimated using the Kaplan-Meier method. The median and its 95% CI, along with the 25% and 75% quartiles are summarized.

[0272] CBR, defined as the number of patients with confirmed CR, confirmed PR, or SD, is summarized similarly to ORR.

[0273] PFS is defined as the time from the date of first dose to the date of the first objectively documented PD per RECIST 1.1 or death, whichever is earlier. Patients who

do not have the date of disease progression per RECIST 1.1 or date of death are censored on the date of the last evaluable tumor assessment. Patients who started a new antineoplastic regimen prior to disease progression per RECIST 1.1 are censored on the date of the last evaluable tumor assessment prior to receiving the new antineoplastic regimen. Patients whose disease progression or death appears after missing two consecutive tumor assessments are censored on the date of the last evaluable tumor assessment. Patients who are lost to follow up are censored on the date of their last evaluable tumor assessment.

[0274] PFS is estimated using the Kaplan-Meier method. The median and its 95% CI, along with the 25% and 75% quartiles are summarized for all treated patients.

[0275] OS is defined as the date of first dose to the date of death. Patients who do not have a date of death are censored on the last date for which the patient was known to be alive. OS is analyzed similarly to PFS.

[0276] Abscopal response is defined as the number of patients with a shrinkage of ≥ 20% from baseline in any target, non-manipulated metastatic lesion identified at baseline. Results are summarized similarly to ORR.

[0277] Response per an independent radiology review committee is analyzed as a sensitivity analysis of the related analysis based on Investigator's assessment. Final statistical considerations and analyses are detailed in the SAP.

[0278] ORR per irRECIST is evaluated as an exploratory endpoint. In addition, characterization of immune system biomarkers in pre-dose blood and tumor biopsies, as well as changes in immune system biomarkers after treatment with TLR7/8 agonist plus RSLAIL-2.

[0279] TLR7/8 agonist plus RSLAIL-2 and nivolumab and their relationship with efficacy are exploratory endpoints. Samples may be used for additional research purposes related to the development of RSLAIL-2.

[0280] Plasma concentrations of TLR7/8 agonist, RSLAIL-2 and their metabolites, and serum concentrations of nivolumab are measured using validated or qualified method(s). Before analysis of samples, assay sensitivity, specificity, linearity, and reproducibility are determined. Pharmacokinetic parameters such as Cmax, Tmax, area AUC, clearance (CL/F), Vd/F, and t1/2 are estimated from concentration-time data where

possible. Pharmacokinetic data from this study may also be pooled with data from other clinical studies for the purpose of PK modeling. Pharmacokinetic parameters are tabulated and summarized with descriptive statistics. Select PK parameter values are correlated with select safety and response measurements for assessment of exposure-response relationships.

Example 2

CD11c Biomarker Measurements and Associations with Patient Tumor Responses

[0281] The goal of this study was to evaluate the relationship between baseline tumor biomarkers and patient responses to treatment with the TLR 7/8 agonist, 4-arm-PEG20k-CM-Gly-N-R848. The analysis included patient cohorts 1-9, A, and B from the dose-escalation and expansion phases of the phase 1/2 clinical trial as described in Example 1. Tumor biopsies collected from patients prior to and 24 hours following administration of 4-arm-PEG20k-CM-Gly-N-R848 were analyzed for expression of selected genes and proteins.

Protein expression

[0282] An immunohistochemistry (IHC) assay was performed to evaluate expression of CD11c on the surface of cells in the tumor biopsy. Multiplex IHC was performed on 4 μm formalin-fixed paraffin-embedded sections of tumor biopsies. Sections were incubated with anti-CD11c (EP1347Y, Abcam, Cambridge, UK) and anti-Ki67 (MIB-1, Dako, Carpinteria, CA). CD11c was visualized with red chromogen (Biocare Medical, Concord, CA), and Ki67 with DAB (Dako). Slides were scanned using an Aperio AT Turbo system (Aperio, Vista, CA). Image analysis was performed with a Halo Multiplex algorithm (Halo v2.3.2089.27 and multiplex IHC v1.2) from Indica (Corrales, NM) with modification. Cells were categorized as CD11c positive ($CD11c^+$) or negative.

Gene expression

[0283] RNA expression analysis was performed to evaluate expression of >800 genes, and the fold change in gene expression was calculated between 24 hours post-treatment and pre-treatment samples. NanoString nCounter gene expression assay was performed on RNA extracted from tumor biopsies using the RNeasy Micro Kit (Qiagen). Samples were hybridized with Human PanCancer Immune Profiling Panel CodeSets, and then scanned using the nCounter Digital Analyzer as per the manufacturer's instructions

(NanoString Technologies, Seattle WA). Gene expression was analyzed using nSolver software (NanoString Technologies), with the expression levels of each gene normalized to those of control genes.

Results

[0284] In patient biopsies taken after administration of ≥0.06 mg 4-arm-PEG20k-CM-Gly-N-R848, several genes previously shown to be induced by TLR7/8 agonism were increased in response to the administration and expressed at higher levels compared to baseline biopsies, consistent with the proposed mechanism of action. Notably, the magnitude of induction of multiple TLR7/8 target genes associated with the density of $CD11c^+$ cells in baseline biopsies, providing a link between the presence of $CD11c^+$ cells in tumors and the ability of the TLR7/8 agonist to drive signaling through TLR7/8 (FIGs. 6A-6C). The correlation analysis included patients in cohorts 6-9, and A/B with available IHC and gene induction data (N=15 melanoma).

[0285] Additionally, analysis of melanoma patient samples with both baseline IHC data and clinical response information showed a trend of higher $CD11c^+$ cell density associated with enriched disease control rate (DCR, defined as stable disease (SD) or partial response (PR)) in melanoma compared to other tumor types (FIGs. 4, 7A), suggesting that melanoma may be more responsive to the treatment. No association between $CD11c^+$ density and tissue histology or dose level was observed (FIG 7B-7C). Because the treatment regimen included RSLAIL-2 in cycles ≥2, clinical response is not entirely attributable to the activity of the TLR7/8 agonist. However, the higher mean $CD11c^+$ density in DCR patients, particularly in the cohort that received the highest TLR7/8 agonist dose (3.84 mg for cohort 9), supports the association between baseline $CD11c^+$ cell density and responsiveness to treatment with the TLR7/8 agonist. These results suggest that a higher $CD11c^+$ density at baseline may be necessary to achieve the full complement of TLR7/8 signaling, and thus maximal TLR7/8 agonist activity, required to achieve the desired modulation of a cytotoxic T cell response in tumors treated with TLR7/8 agonist in combination with RSLAIL-2.

Conclusion

[0286] The above detailed description of embodiments of the technology are not intended to be exhaustive or to limit the technology to the precise forms disclosed above. Although specific embodiments of, and examples for, the technology are described above for illustrative purposes, various equivalent modifications are possible within the scope of the technology as those skilled in the relevant art will recognize. For example, although steps are presented in a given order, alternative embodiments may perform steps in a different order. The various embodiments described herein may also be combined to provide further embodiments.

[0287] From the foregoing, it will be appreciated that specific embodiments of the technology have been described herein for purposes of illustration, but well-known components and functions have not been shown or described in detail to avoid unnecessarily obscuring the description of the embodiments of the technology. Where the context permits, singular or plural terms may also include the plural or singular term, respectively. Further, while advantages associated with some embodiments of the technology have been described in the context of those embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the technology. Accordingly, the disclosure and associated technology can encompass other embodiments not expressly shown or described herein.

[0288] All articles, books, patents, patent publications and other publications referenced herein are incorporated by reference in their entireties. In the event of an inconsistency between the teachings of this specification and the art incorporated by reference, the meaning of the teachings in this specification shall prevail.

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CLAIMS

1. A method of treating a subject having a solid cancer, comprising:

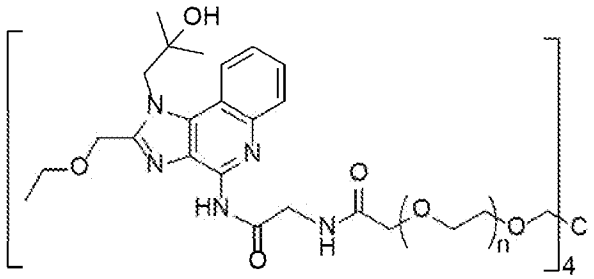
evaluating a baseline cluster of differentiation (CD)11c expression level in a solid tumor sample obtained from the subject; and

administering a therapeutically effective amount of a TLR7/8 agonist to a subject having a CD11c+ baseline expression level of at least about 500 to about 2000 CD11c positive cells/mm^2.

2. The method of claim 1, wherein the subject has a CD11c+ baseline expression level of at least about 500 to about 1000 CD11c positive cells/mm^2.

3. The method of claim 1 or claim 2, wherein the subject has a CD11c+ baseline expression level of about 1000 CD11c positive cells/mm^2.

4. The method of any one of claims 1-3, wherein the TLR7/8 agonist has a structure:



wherein each n is independently an integer from about 40 to about 350.

5. The method of claim 4, wherein each n is independently an integer from about 100 to about 250.

6. The method of claim 4 or 5, wherein the TLR7/8 agonist is administered at a dose of about 30 μg to about 4 mg.

7. The method of claim 4 or 5, wherein the TLR7/8 agonist is administered at a dose of about 0.03 mg to 7.68 mg.

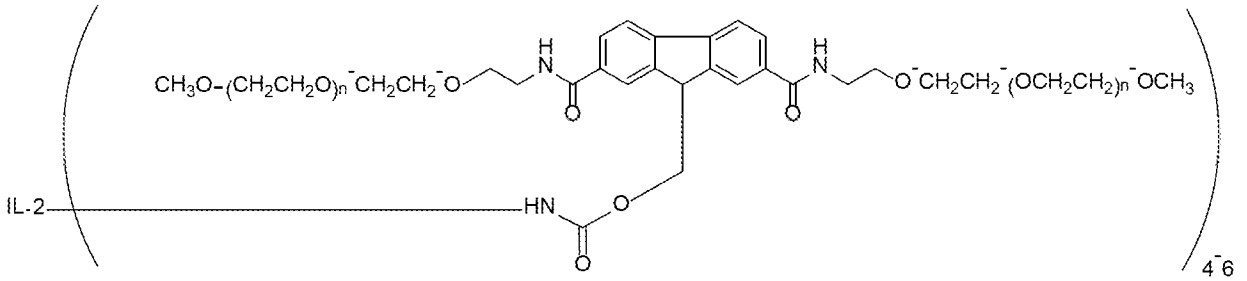
8. The method of claim 4 or 5, wherein the TLR7/8 agonist is administered at a dose of about 3.84 mg.

9. The method of any one of claims 1-8, wherein the TLR7/8 agonist is administered locally.

10. The method of claim 9, wherein the TLR7/8 agonist is administered intratumorally.

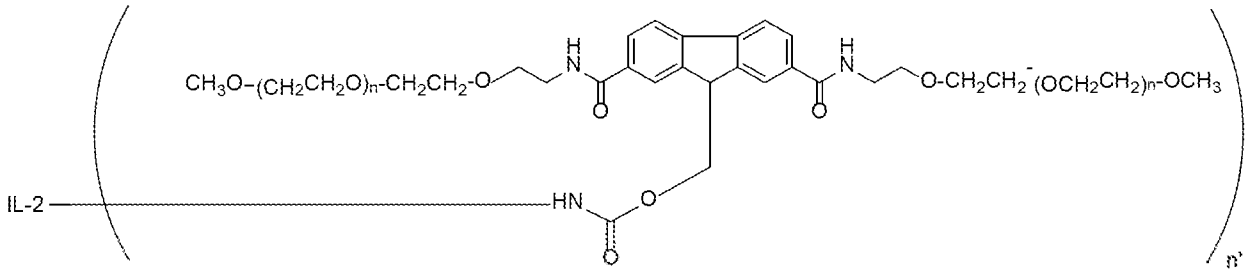
11. The method of any one of claims 1-10, further comprising administering a therapeutically effective amount of a CD-122 preferential agonist to the subject.

12. The method of claim 11, wherein the CD-122 preferential agonist comprises compounds encompassed by the formula:



wherein IL-2 is an interleukin-2, where n is an integer from about 200 to about 300, or is on average about 227, or about 228 pharmaceutically acceptable salts thereof.

13. The method of claim 11 or 12, wherein the CD-122 preferential agonist is comprised in a composition comprising no more than 10% (based on a molar amount) of compounds encompassed by the following formula:



wherein IL-2 is interleukin-2, n' is an integer selected from the group consisting of 1, 2, 3, 7 and >7, or pharmaceutically acceptable salts thereof.

14. The method of any one of claims 11-13, wherein the CD-122 preferential agonist is administered at a dose of about 0.006 mg/kg.

15. The method of any one of claims 11-14, wherein the CD-122 preferential agonist is administered parenterally.

16. The method of any one of claims 11-15, wherein the TLR7/8 agonist and the CD-122 preferential agonist are administered concurrently or sequentially.

17. The method of claim 16, wherein the TLR7/8 agonist and the CD-122

preferential agonist are administered sequentially in any order.

18. The method of any one of claims 11-17, wherein the method comprises a single cycle of administering at least one of the TLR7/8 agonist or the CD-122 preferential agonist.

19. The method of any one of claims 1-18, further comprising administering a therapeutically effective amount of one or more additional therapeutic agents to the subject.

20. The method of claim 19, wherein the one or more additional therapeutic agents comprises an immunotherapy agent.

21. The method of claim 20, wherein the immunotherapy agent is nivolumab.

22. The method of claim 21, wherein the nivolumab is administered at a dose of about 360 mg.

23. The method of any one of claims 1-22, wherein the solid tumor is selected from the group consisting of melanoma, sarcoma, Merkel cell carcinoma, colorectal cancer, head and neck cancer, renal cell carcinoma, and breast cancer.

24. The method of claim 23, wherein the sarcoma is selected from osteosarcoma, chondrosarcoma, undifferentiated pleomorphic sarcoma, fibrous histocytoma, liposarcoma, angiosarcoma and leiomyosarcoma.

25. The method of any one of claims 1-24, wherein the sample is obtained by a biopsy of the solid tumor.

26. The method of any one of claims 1-25, wherein the CD11c expression level is determined by an immunohistochemistry assay.

27. The method of claim 26, wherein the immunohistochemistry assay comprises use of an anti-CD11c antibody.

28. The method of any one of claims 1-27, wherein treating or preventing cancer in a subject comprises promoting an anti-antitumor immune response in the tumor microenvironment in the subject.

29. The method of any one of claims 1-28, wherein treating or preventing cancer in a subject comprises reducing tumor size or tumor cell number in the subject.

30. The method of any one of claims 1-29, wherein said treating comprises reducing tumor burden or loci in the subject.

31. The method of claim 30, wherein the tumor burden is a metastatic tumor burden.

32. The method of any one of claims 1-31, wherein said treating comprises at least one of increasing cancer remission or increasing cancer survival rate in the subject.

33. A method of identifying a subject likely to respond positively to treatment with a TLR7/8 agonist, wherein the subject has one or more solid tumors, optionally followed by treatment, the method comprises:

evaluating a baseline cluster of differentiation (CD)11c expression level in a solid tumor sample obtained from the subject; and

if the subject has a CD11c+ baseline expression of at least about 500 to about 2000 CD11c positive cells/mm^2, administering to the subject a therapeutically effective amount of the TLR7/8 agonist.

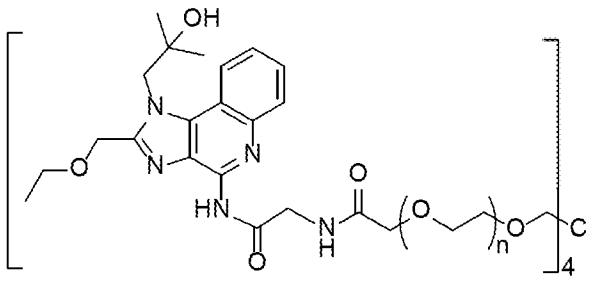
34. The method of claim 33, wherein the subject has a CD11c+ baseline expression level of at least about 500 to about 1000 CD11c positive cells/mm^2.

35. The method of claim 33, wherein the subject has a CD11c+ baseline expression level of at least about 1000 CD11c positive cells/mm^2.

36. The method of any one of claims 33-35, wherein a subject having a CD11c+ baseline expression level of at least about 500 to about 2000 CD11c positive cells/mm^2 is more likely to respond more favorably to treatment with the TLR7/8 agonist than a subject having a CD11c+ baseline expression level of less than about 500 CD11c positive cells/mm^2.

37. The method of claim 36, wherein a more favorable response to treatment is measured by one or more of increased survival rate, decreased tumor size, decreased tumor numbers, and reduced tumor burden or loci.

38. The method of any one of claims 33-37, wherein the TLR7/8 agonist has formula:



wherein each n is independently an integer from about 40 to about 350.

39. The method of claim 38, wherein each n is independently selected from 100 to 250.

40. The method of claim 38 or 39, wherein the TLR7/8 agonist is administered at a dose of about 30 μg to about 4 mg.

41. The method of claim 38 or 39, wherein the TLR7/8 agonist is administered at a dose of about 0.03 mg to 7.68 mg.

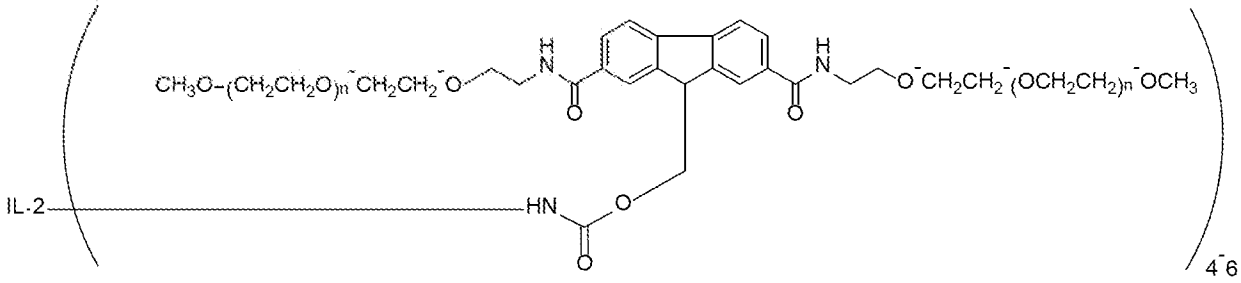
42. The method of claim 38 or 39, wherein the TLR7/8 agonist is administered at a dose of about 3.84 mg.

43. The method of any one of claims 33-42, wherein the TLR7/8 agonist is administered locally.

44. The method of claim 43, wherein the TLR7/8 agonist is administered intratumorally.

45. The method of any one of claims 33-44, further comprising administering a therapeutically effective amount of a CD-122 preferential agonist to the subject.

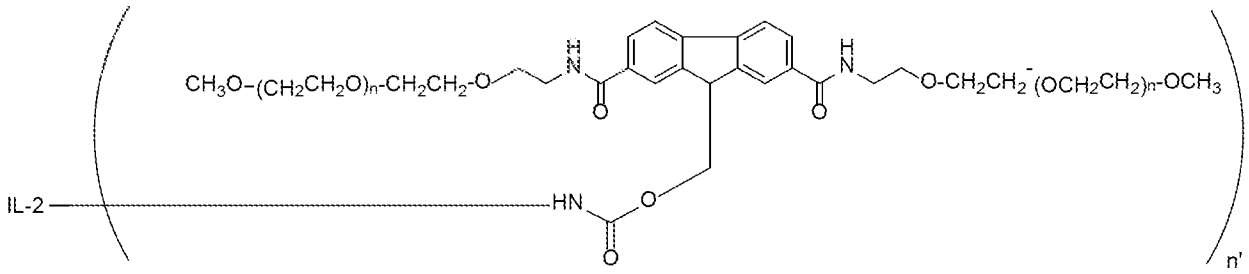
46. The method of claim 45, wherein the CD-122 preferential agonist comprises compounds encompassed by the formula:



wherein IL-2 is an interleukin-2, where n is an integer from about 200 to about 300, or is on average about 227 or about 228, and pharmaceutically acceptable salts thereof.

47. The method of claim 45 or 46, wherein the CD-122 preferential agonist is

comprised in a composition comprising no more than 10% (based on a molar amount) of compounds encompassed by the following formula:



wherein IL-2 is interleukin-2, n' is an integer selected from the group consisting of 1, 2, 3, 7 and >7, or pharmaceutically acceptable salts thereof.

48. The method of any one of claims 45-47, wherein the CD-122 preferential agonist is administered at a dose of about 0.006 mg/kg.

49. The method of any one of claims 45-48, wherein the CD-122 preferential agonist is administered parenterally.

50. The method of any one of claims 45-49, wherein the TLR7/8 agonist and the CD-122 preferential agonist are administered concurrently or sequentially.

51. The method of claim 50, wherein the TLR7/8 agonist and the CD-122 preferential agonist are administered sequentially in any order.

52. The method of any one of claims 45-51, wherein the method comprises a single cycle of administering at least one of the TLR7/8 agonist or the CD-122 preferential agonist.

53. The method of any one of claims 33-52, further comprising administering a therapeutically effective amount of one or more additional therapeutic agents to the subject.

54. The method of claim 53, wherein the one or more additional therapeutic agents comprises an immunotherapy agent.

55. The method of claim 54, wherein the immunotherapy agent is nivolumab.

56. The method of claim 55, wherein the nivolumab is administered at a dose of about 360 mg.

57. The method of any one of claims 33-56, wherein the solid tumor is selected from the group consisting of melanoma, sarcoma, Merkel cell carcinoma, colorectal

cancer, head and neck cancer, renal cell carcinoma, and breast cancer.

58. The method of claim 57, wherein the sarcoma is selected from osteosarcoma, chondrosarcoma, undifferentiated pleomorphic sarcoma, fibrous histocytoma, liposarcoma, angiosarcoma and leiomyosarcoma.

59. The method of any one of claims 33-58, wherein the sample is obtained by a biopsy of the solid tumor.

60. The method of any one of claims 33-59, wherein the CD11c expression level of the sample is determined by an immunohistochemistry assay.

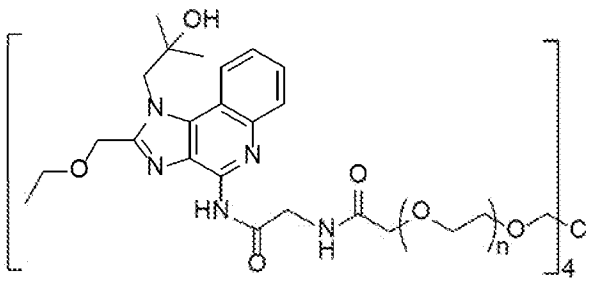
61. The method of claim 60, wherein the immunohistochemistry assay comprises use of an anti-CD11c antibody.

62. An improved method of treating a subject having a solid tumor by administering to the subject a TLR7/8 agonist, the improvement comprising prior to the administering step, evaluating a baseline cluster of differentiation (CD)11c expression level in a solid tumor sample obtained from the subject, wherein said expression level is predictive of a favorable response of the subject to treatment.

63. The method of claim 62, wherein the baseline CD11c expression level is at least about 500 to about 1000 CD11c positive cells/mm^2.

64. The method of claim 62 or 63, wherein the baseline CD11c expression level is at least about 1000 CD11c positive cells/mm^2.

65. The method of any one of claims 62-64, wherein the TLR7/8 agonist has a structure:



wherein each n is independently an integer from about 40 to about 350.

66. The method of claim 65, wherein each n is independently selected from about 100 to about 250.

67. The method of claim 65 or 66, wherein the TLR7/8 agonist is administered

at a dose of about 30 μg to about 4 mg.

68. The method of claim 65 or 66, wherein the TLR7/8 agonist is administered at a dose of about 0.03 mg to 7.68 mg.

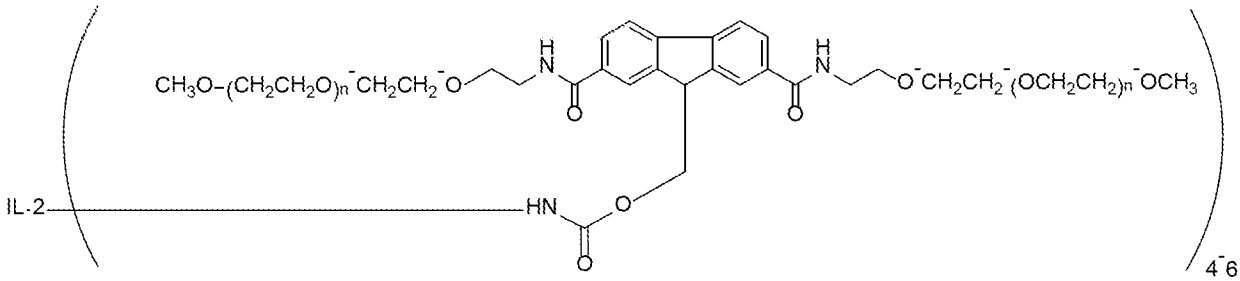
69. The method of claim 65 or 66, wherein the TLR7/8 agonist is administered at a dose of about 3.84 mg.

70. The method of any one of claims 62-69, wherein the TLR7/8 agonist is administered locally.

71. The method of claim 70, wherein the TLR7/8 agonist is administered intratumorally.

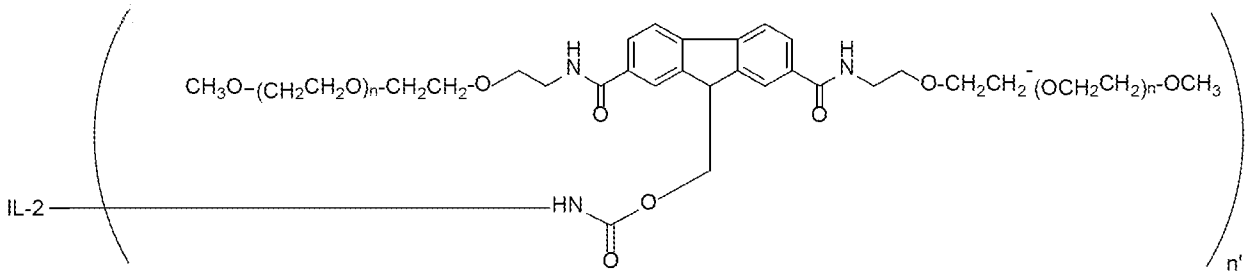
72. The method of any one of claims 65-71, further comprising administering a therapeutically effective amount of a CD-122 preferential agonist to the subject.

73. The method of claim 72, wherein the CD-122 preferential agonist comprises compounds encompassed by the formula:



wherein IL-2 is an interleukin-2, where n is an integer from about 200 to about 300, or is on average about 227, or about 228 or pharmaceutically acceptable salts thereof.

74. The method of claim 72 or 73, wherein the CD-122 preferential agonist is comprised in a composition comprising no more than 10% (based on a molar amount) of compounds encompassed by the following formula:



wherein IL-2 is an interleukin-2, n' is an integer selected from the group consisting of 1, 2, 3, 7 and >7, or pharmaceutically acceptable salts thereof.

75. The method of any one of claims 72-74, wherein the CD-122 preferential agonist is administered at a dose of about 0.006 mg/kg.

76. The method of any one of claims 72-75, wherein the CD-122 preferential agonist is administered parenterally.

77. The method of any one of claims 72-76, wherein the TLR7/8 agonist and the CD-122 preferential agonist are administered concurrently or sequentially.

78. The method of claim 77, wherein the TLR7/8 agonist and the CD-122 preferential agonist are administered sequentially in any order.

79. The method of any one of claims 72-78, wherein the method comprises a single cycle of administering at least one of the TLR7/8 agonist or the CD-122 preferential agonist.

80. The method of any one of claims 62-79, further comprising administering a therapeutically effective amount of one or more additional therapeutic agents to the subject.

81. The method of claim 80, wherein the one or more additional therapeutic agents comprises an immunotherapy agent.

82. The method of claim 81, wherein the immunotherapy agent is nivolumab.

83. The method of claim 82, wherein the nivolumab is administered at a dose of about 360 mg.

84. The method of any one of claims 62-83, wherein the solid tumor is selected from the group consisting of melanoma, sarcoma, Merkel cell carcinoma, colorectal cancer, head and neck cancer, renal cell carcinoma, and breast cancer.

85. The method of claim 84, wherein the sarcoma is selected from osteosarcoma, chondrosarcoma, undifferentiated pleomorphic sarcoma, fibrous histocytoma, liposarcoma, angiosarcoma and leiomyosarcoma.

86. The method of any one of claims 62-85, wherein the sample is obtained by a biopsy of the solid tumor.

87. The method of any one of claims 92-86, wherein the CD11c expression level is determined by an immunohistochemistry assay.

88. The method of claim 87, wherein the immunohistochemistry assay comprises use of an anti-CD11c antibody.

89. A method of treating a subject having a solid cancer, comprising:

administering a TLR7/8 agonist to a subject having a solid tumor that is positive for expression of cluster of differentiation (CD)11c.

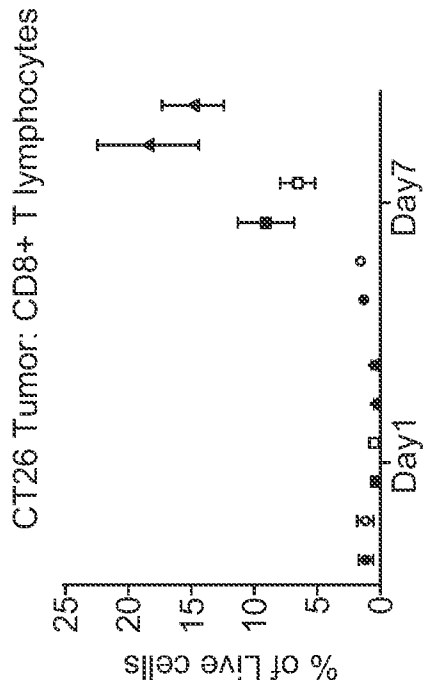


FIG. 1C

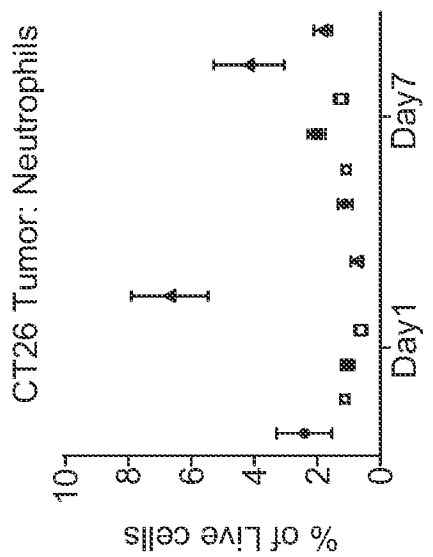


FIG. 1B

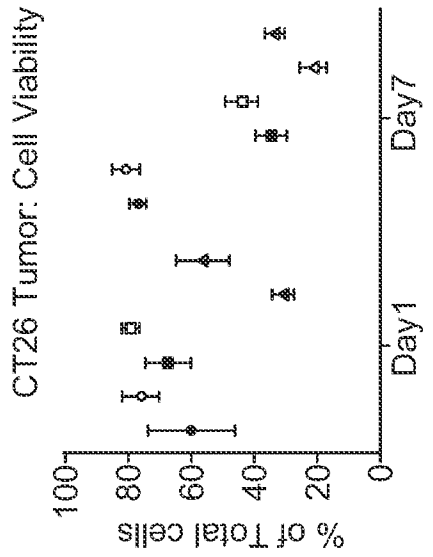


FIG. 1A

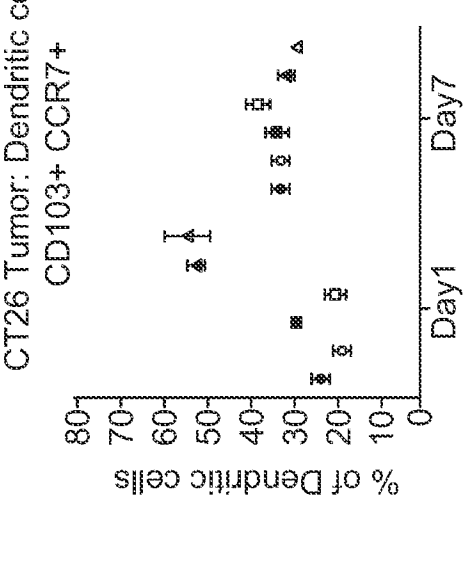


FIG. 1E

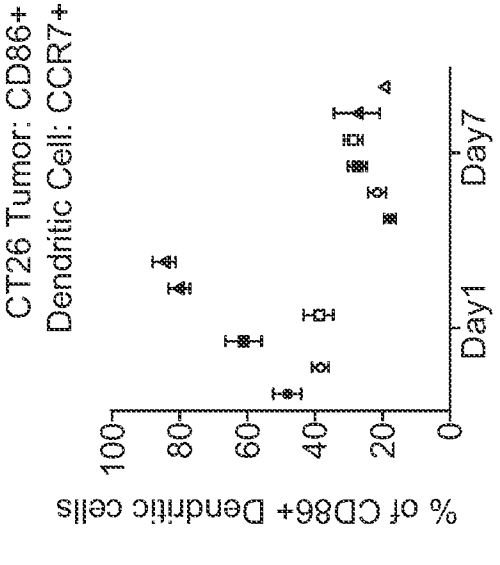


FIG. 1D

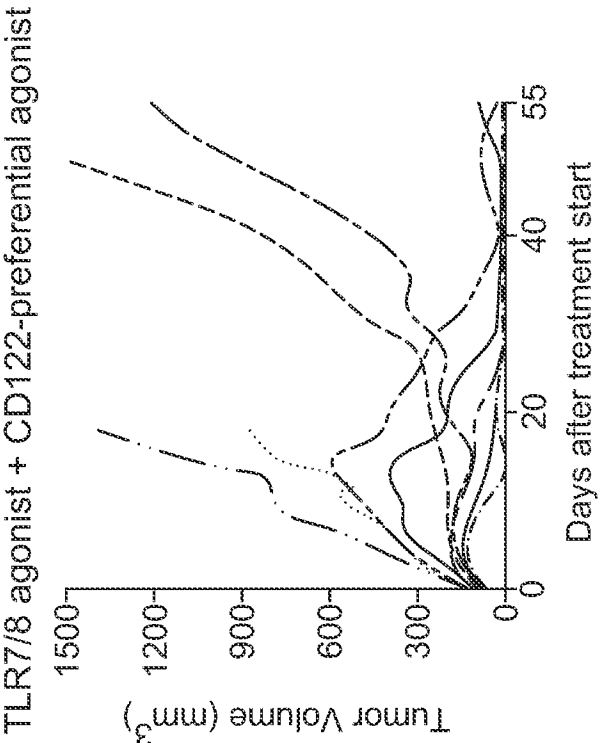


FIG. 2A

FIG. 2B

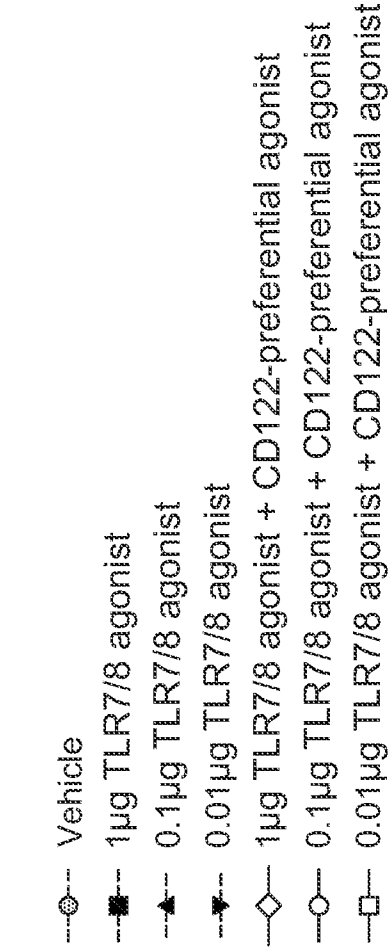


FIG. 2C

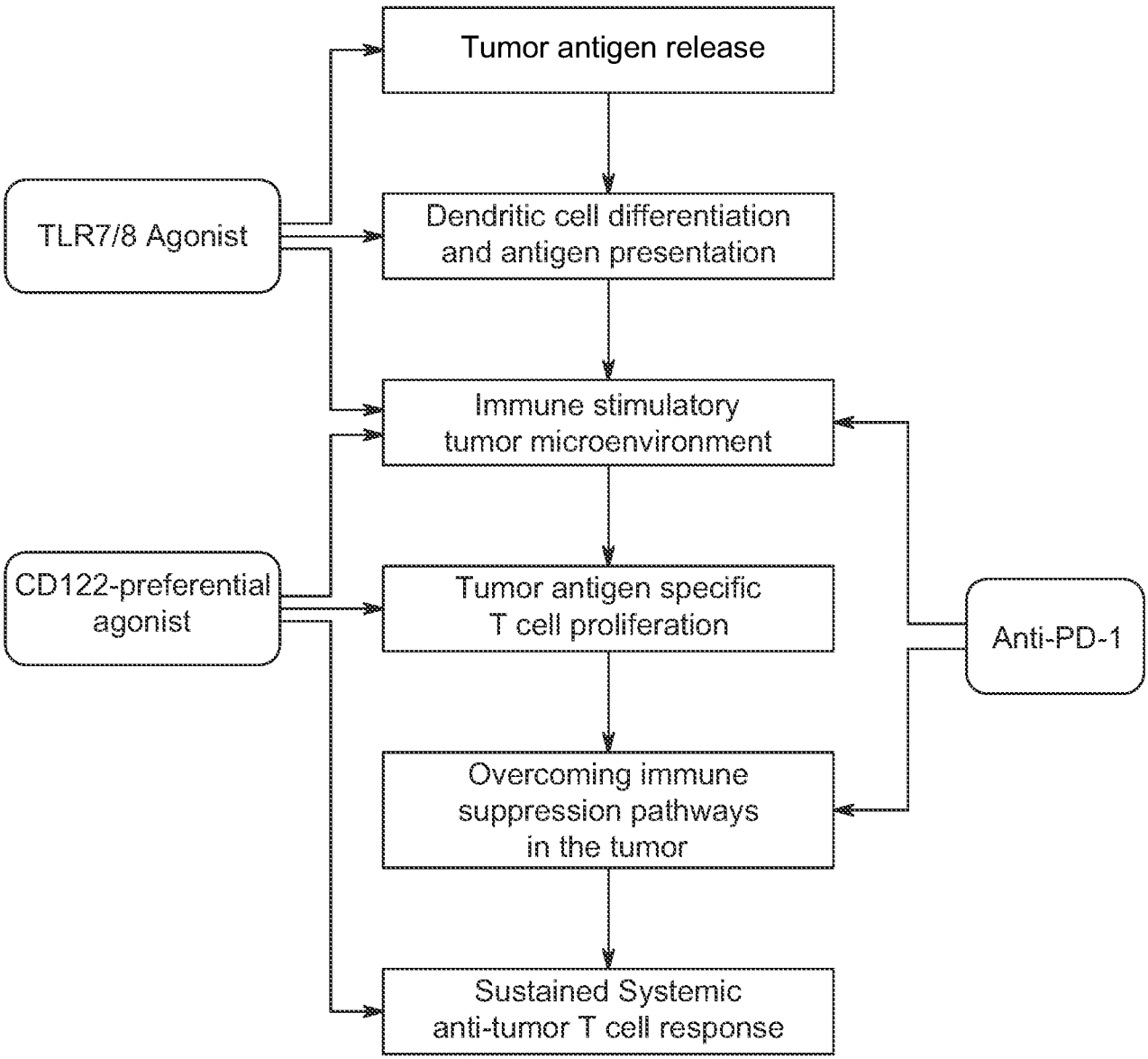

Tumor antigen release
TLR7/8 Agonist
Dendritic cell differentiation and antigen presentation
Immune stimulatory tumor microenvironment
CD122-preferential agonist
Tumor antigen specific T cell proliferation
Anti-PD-1
Overcoming immune suppression pathways in the tumor
Sustained Systemic anti-tumor T cell response

FIG. 3

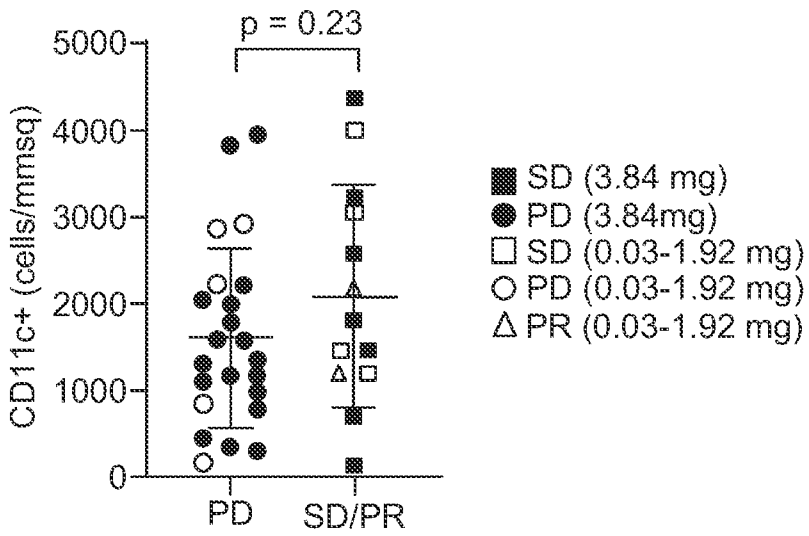

p = 0.23
5000
4000
3000
2000
1000
0
CD11c+ (cells/mmsq)
PD
SD/PR
SD (3.84 mg)
PD (3.84mg)
SD (0.03-1.92 mg)
PD (0.03-1.92 mg)
PR (0.03-1.92 mg)

FIG. 4

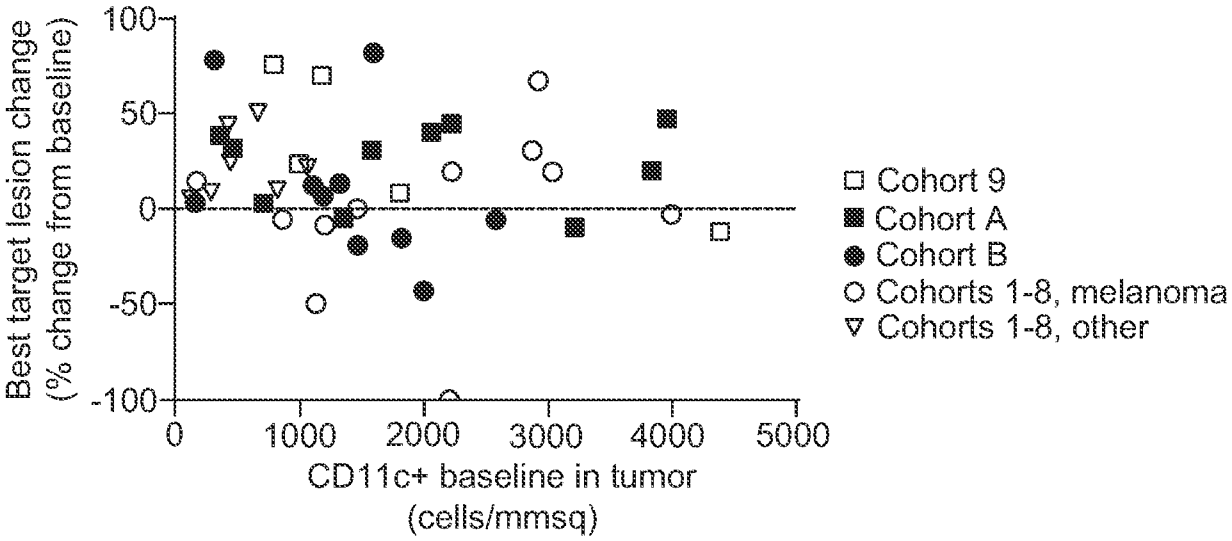

100
50
0
-50
-100
Best target lesion change
(% change from baseline)
0
1000
2000
3000
4000
5000
CD11c+ baseline in tumor
(cells/mmsq)
Cohort 9
Cohort A
Cohort B
Cohorts 1-8, melanoma
Cohorts 1-8, other

FIG. 5

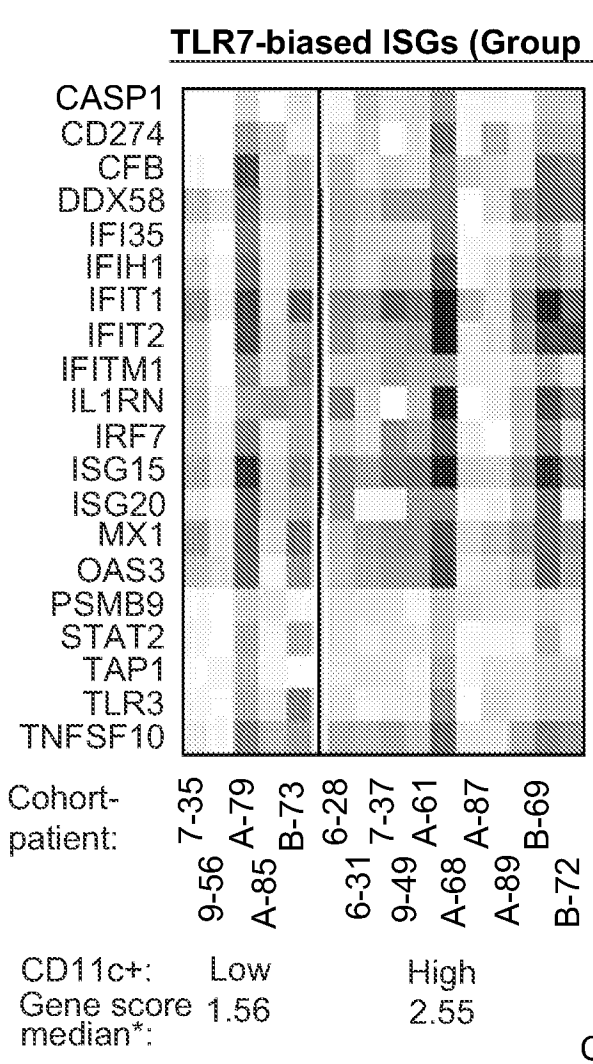


FIG. 6A

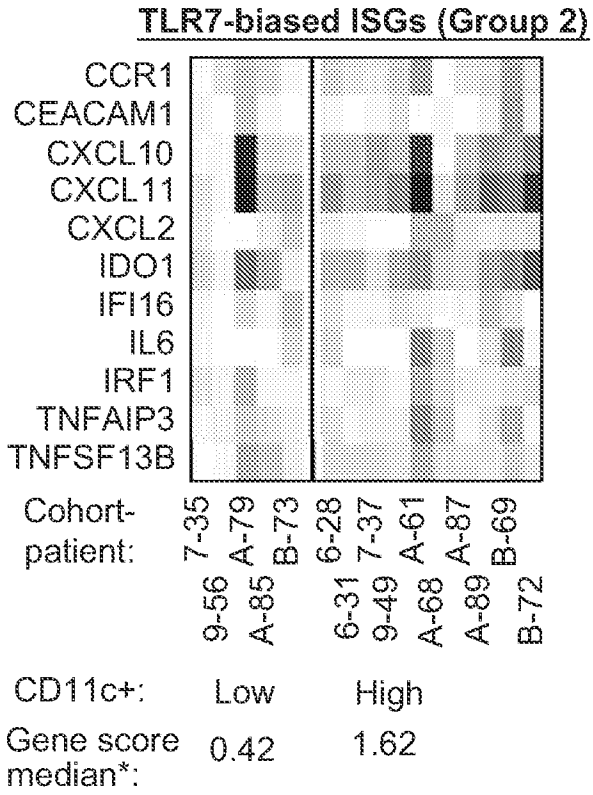


FIG. 6B

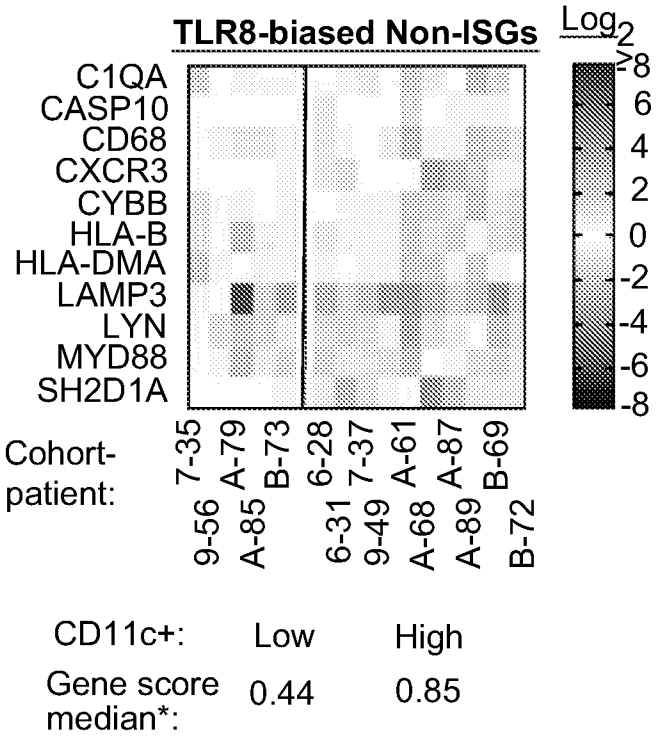


FIG. 6C

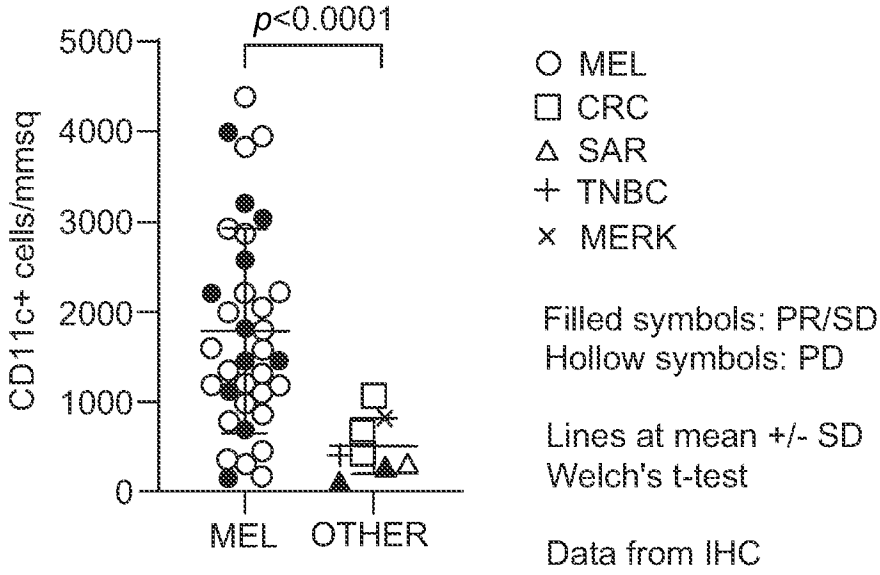

p<0.0001
5000
4000
3000
2000
1000
0
CD11c+ cells/mmsq
MEL
OTHER
MEL
CRC
SAR
TNBC
MERK
Filled symbols: PR/SD
Hollow symbols: PD
Lines at mean +/- SD
Welch's t-test
Data from IHC

FIG. 7A

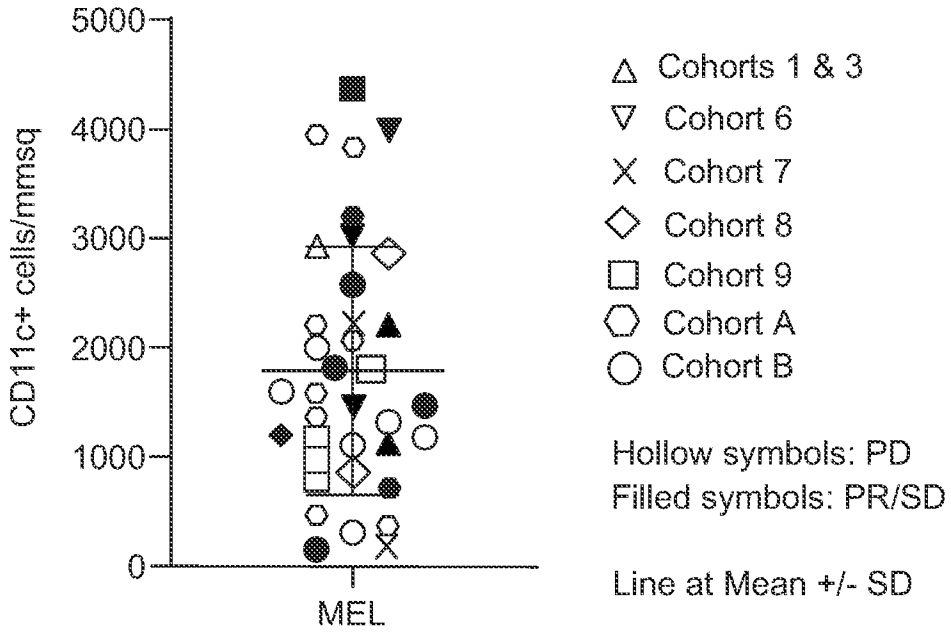

5000
4000
3000
2000
1000
0
CD11c+ cells/mmsq
MEL
Cohorts 1 & 3
Cohort 6
Cohort 7
Cohort 8
Cohort 9
Cohort A
Cohort B
Hollow symbols: PD
Filled symbols: PR/SD
Line at Mean +/- SD

FIG. 7B

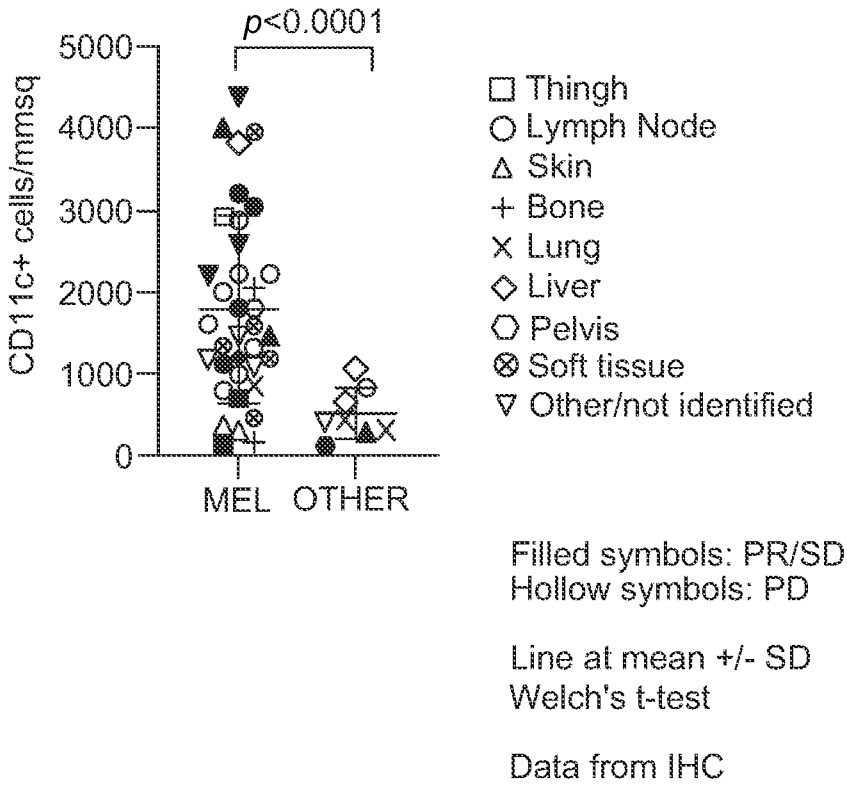

p<0.0001
5000
4000
3000
2000
1000
0
CD11c+ cells/mmsq
MEL
OTHER
Thingh
Lymph Node
Skin
Bone
Lung
Liver
Pelvis
Soft tissue
Other/not identified
Filled symbols: PR/SD
Hollow symbols: PD
Line at mean +/- SD
Welch's t-test
Data from IHC

FIG. 7C

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/058187

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4745 A61K38/20 A61K45/06 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KIM HYUNJOON ET AL: "Polymeric nanoparticles encapsulating novel TLR7/8 agonists as immunostimulatory adjuvants for enhanced cancer immunotherapy", BIOMATERIALS, ELSEVIER, AMSTERDAM, NL, vol. 164, 17 February 2018 (2018-02-17), pages 38-53, XP085357438, ISSN: 0142-9612, DOI: 10.1016/J.BIOMATERIALS.2018.02.034 page 44, column 2 page 48, column 1, last paragraph ----- -/--	1-89

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 2022	11/02/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Büttner, Ulf

1

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/058187

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TAKEDA YOHEI ET AL: "Tumoricidal efficacy coincides with CD11c up-regulation in antigen-specific CD8+ T cells during vaccine immunotherapy", JOURNAL OF EXPERIMENTAL & CLINICAL CANCER RESEARCH, vol. 35, no. 1, 1 December 2016 (2016-12-01), XP055884088, DOI: 10.1186/s13046-016-0416-x Retrieved from the Internet: URL:https://jeccr.biomedcentral.com/track/pdf/10.1186/s13046-016-0416-x.pdf> page 13, column 1 page 14, column 1	1-89

X	ANNAH S ROLIG: "NKTR-214 (CD122-biased agonist) and NKTR-262 (TLR7/8 agonist) combination treatment pairs local innate immune activation with systemic CD8+ T cell expansion to enhance antitumor immunity", PROVIDENCE ST. JOSEPH HEALTH DIGITAL COMMONS, 7 November 2018 (2018-11-07), XP055675097,	89
Y	the whole document	1-88

X	WO 2018/132496 A1 (NEKTAR THERAPEUTICS [US]) 19 July 2018 (2018-07-19) cited in the application	89
Y	claims table 5 page 25; examples 24-30	1-88

X,P	ZEIGLER STAD ET AL: "623 Immuno-STATs: Leveraging protein engineering to expand and track antigen-specific T cells in vivo", JOURNAL FOR IMMUNOTHERAPY OF CANCER, vol. 8, no. Suppl 3, 9 November 2020 (2020-11-09), pages A374-A375, XP055847202, GB ISSN: 2051-1426, DOI: 10.1136/jitc-2020-SITC2020.0622 Retrieved from the Internet: URL:https://jitc.bmj.com/content/jitc/8/Suppl_3/A374.2.full.pdf> claims	1-89

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/058187

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CA 3049254 A1	19-07-2018
		CN 110167598 A	23-08-2019
		EP 3568162 A1	20-11-2019
		JP 2020514300 A	21-05-2020
		KR 20190104377 A	09-09-2019
		US 2021128737 A1	06-05-2021
		WO 2018132496 A1	19-07-2018