METHODS OF TREATING CLEAR CELL RENAL CELL CARCINOMA WITH BISPECIFIC ANTI-PSMA x ANTI-CD28 ANTIBODIES

REFERENCE TO A SEQUENCE LISTING

[0001] This application incorporates by reference a computer readable Sequence Listing in ST.26 XML format, titled 11742WO01_Sequence, created on August 1, 2024 and containing 58,834 bytes.

FIELD OF THE INVENTION

[0002] The present disclosure relates to methods for treating clear cell renal cell carcinoma (ccRCC), including metastatic ccRCC, comprising administering to a subject in need thereof a therapeutically effective amount of a bispecific antibody that specifically binds to prostate-specific membrane antigen (PSMA) and CD28, as well as methods of treating ccRCC via administration of the bispecific antibody in combination with an antibody that specifically binds to programmed death receptor-1 (PD-1).

BACKGROUND

[0003] Clear cell renal cell carcinoma (ccRCC) represents the most common subtype of kidney cancer with a frequency of 75-80% of initial diagnoses (Makhov *et al., Mol Cancer Ther.*, 17(7):1355-1364, 2018). Prostate-specific membrane antigen (PSMA), also known as FOLH1, glutamate carboxypeptidase II (GCPII), N-acetyl-L-aspartyl-L-glutamate peptidase I (NAALADase I), or N-acetyl-aspartylglutamate (NAAG) peptidase, is a homodimeric, enzymatic type II transmembrane protein encoded by the folate hydrolase 1 (FOLH1) gene. PSMA is expressed in all subtypes of renal cell carcinoma, and significantly more so in ccRCC (Baccala *et al., Urology*, 70(2):385-390, 2017). In-house immunohistochemistry(IHC) confirmed that >90% of patients with ccRCC express PSMA, and this expression is generally limited to the tumor neovasculature, though not expressed on normal vasculature (Chang *et al., Cancer Res.*, 59(13):3192-3198, 1999). PSMA PET imaging studies have been performed in patients with metastatic ccRCC and have demonstrated higher sensitivity for detecting metastases than conventional imaging via CT/MRI (94.7% vs 78.9%) (Rowe *et al., Ann Nucl Med.,* 29(10):877-882, 2015), which could have important implications for staging, patient management, and informing treatment decisions.

[0004] CD28 is a type I transmembrane protein, which has a single extracellular Ig-V-like domain assembled as a homodimer and which is expressed on the surface of T cells. CD28 is the receptor for the CD80 (B7.1) and CD86 (B7.2) proteins and is activated by CD80 or CD86 expressed on antigen-presenting cells (APCs). The binding of CD28 to CD80 or CD86 provides co-stimulatory signals important for T cell activation and survival. T cell stimulation through CD28, in addition to the T-cell receptor (TCR), provides a potent signal for the production of various interleukins. CD28

also potentiates cellular signals such as pathways controlled by the NFκB transcription factor after TCR activation. The CD28 co-signal is important for effective T-cell activation such as T cell differentiation, proliferation, cytokine release and cell-death. Anti-CD28 antibodies have been proposed for therapeutic purposes involving the activation of T cells. One particular anti-CD28 antibody, TGN1412 (anti-CD28 superagonist), was used in a clinical trial in 2006, in which six healthy volunteers were dosed intravenously with TGN1412 (anti-CD28 superagonist) at a dose of 0.1 mg/kg. Within two hours, all six patients had significant inflammatory responses (cytokine storm), and all patients were in multi-organ failure within sixteen hours. Subjects were treated with corticosteroids, and cytokine levels returned to normal within 2-3 days (Suntharalingam, *et al.*, Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412, NEJM 355:1018-1028 (2006)).

[0005] Programmed death receptor-1 (PD-1) signaling in the tumor microenvironment plays a key role in allowing tumor cells to escape immune surveillance by the host immune system. Blockade of the PD-1 signaling pathway has demonstrated clinical activity in patients with multiple tumor types, and antibody therapeutics that block PD-1 (*e.g.*, nivolumab and pembrolizumab) have been approved for the treatment of metastatic melanoma and metastatic squamous non-small cell lung cancer. Recent data has demonstrated the clinical activity of PD-1 blockade in patients with aggressive NHL and Hodgkin's lymphoma (Lesokhin, *et al.* 2014, Abstract 291, 56th ASH Annual Meeting and Exposition, San Francisco, Calif.; Ansell *et al.* 2015, *N. Engl. J. Med.* 372(4):311-9).

[0006] Patients with metastatic ccRCC are treated with systemic therapies including a combination of both targeted and immunotherapeutic agents. Both anti-PD-1 monotherapies and combination of immune checkpoint inhibitors (ICIs) with nivolumab and ipilimumab have improved overall survival (OS) (Motzer *et al., New England Journal of Medicine*, 373(19):1803-1813, 2015). Standard of care for first line therapies is stratified by patients' risk group and includes either dual ICIs or a combination of an anti-PD1 therapy and an antiangiogenic agent (Powles *et al., Ann Oncol.*, 32(12):1511-1519, , 2021). There is no standard treatment in the second line setting and this remains an area of high unmet need. Most often, patients are treated with a tyrosine kinase inhibitor in the second line setting tailored upon which prior therapy was received by the patient in the first line setting (Tannir *et al., Oncologist*, 23(5):540-*555, 2018).

SUMMARY

[0007] According to certain embodiments, the present disclosure provides methods for treating, ameliorating at least one symptom or indication, or inhibiting the growth of a PSMA-expressing clear cell renal cell carcinoma in a subject (*e.g.*, metastatic clear cell renal cell carcinoma). The methods according to this aspect of the disclosure comprise administering a therapeutically

effective amount of a bispecific antibody or antigen-binding fragment thereof that specifically binds to prostate specific membrane antigen (PSMA) and CD28 to a subject in need thereof. In some embodiments, the bispecific antibody is administered in combination with an antibody or antigen-binding fragment thereof that specifically binds to programmed death receptor-1 (PD-1).

[0008] In one aspect, the present disclosure provides a method of treating clear cell renal cell carcinoma in a subject in need thereof, comprising administering to the subject a bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds prostate specific membrane antigen (PSMA), and a second antigen-binding domain that specifically binds human CD28.

[0009] In some embodiments, the bispecific antibody is administered to the subject at a dose of at least 30 mg once weekly (QW) for at least three weeks.

[0010] In some embodiments, the clear cell renal cell carcinoma is metastatic clear cell renal cell carcinoma.

[0011] In some embodiments, the subject has received at least one prior therapy for clear cell renal cell carcinoma. In some cases, the subject has received an anti-PD-1 therapy. In some cases, the subject has received an anti-CTLA-4 therapy and/or a tyrosine kinase inhibitor. In some cases, the anti-CTLA-4 therapy is ipilimumab.

[0012] In some embodiments, the bispecific antibody or antigen-binding fragment thereof is administered for at least three weeks, at least four weeks, at least five weeks, or at least six weeks as a monotherapy.

[0013] In some embodiments, the methods discussed above or herein further comprise administering an antibody or antigen-binding fragment thereof that specifically binds programmed death receptor-1 (PD-1), wherein the antibody or antigen-binding fragment is administered at a dose of from 3 mg to 100 mg. In some embodiments, the methods discussed above or herein further comprise administering an antibody or antigen-binding fragment thereof that specifically binds programmed death receptor-1 (PD-1), wherein the antibody or antigen-binding fragment is administered at a dose of from 3 mg to 350 mg. In some embodiments, the methods discussed above or herein further comprise administering an antibody or antigen-binding fragment thereof that specifically binds programmed death receptor-1 (PD-1), wherein the antibody or antigen-binding fragment is administered at a dose of from 3 mg to 1000 mg.

[0014] In some embodiments, the first antigen-binding domain of the bispecific antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 1; and three light chain complementarity determining

regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 9, and the second antigen-binding domain of the bispecific antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 5; and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 9.

[0015] In some cases, the first antigen-binding domain comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO: 2, a HCDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 4. In some cases, the second antigen-binding domain comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO: 6, a HCDR2 comprising the amino acid sequence of SEQ ID NO: 7, and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 8.

[0016] In some cases, the first antigen-binding domain and the second antigen-binding domain comprise a LCDR1 comprising the amino acid sequence of SEQ ID NO: 10, a LCDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 12.

[0017] In some cases, the first antigen-binding domain comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 1, and a LCVR comprising the amino acid sequence of SEQ ID NO: 9. In some cases, the second antigen-binding domain comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 5, and a LCVR comprising the amino acid sequence of SEQ ID NO: 9.

[0018] In some embodiments, the bispecific antibody comprises a human IgG heavy chain constant region. In some cases, the human IgG heavy chain constant region is isotype IgG1. In some cases, the human IgG heavy chain constant region is isotype IgG4.

[0019] In some embodiments, the bispecific antibody comprises a chimeric hinge that reduces Fcγ receptor binding relative to a wild-type hinge of the same isotype.

[0020] In some embodiments, the first heavy chain or the second heavy chain of the bispecific antibody, but not both, comprises a CH3 domain comprising a H435R (EU numbering) modification and a Y436F (EU numbering) modification.

[0021] In some embodiments, the bispecific antibody comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, the bispecific antibody comprises a second heavy chain comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the bispecific antibody comprises a first heavy chain comprising the amino acid

sequence of SEQ ID NO: 13, a second heavy chain comprising the amino acid sequence of SEQ ID NO: 14, and a common light chain comprising the amino acid sequence of SEQ ID NO: 15.

[0022] In some embodiments, the antibody or antigen-binding fragment thereof that binds PD-1 comprises: (a) three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 36; and (b) three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 40.

[0023] In some embodiments, the antibody or antigen-binding fragment thereof that binds PD-1 comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO: 37, a HCDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 39. In some embodiments, the antibody or antigen-binding fragment thereof that binds PD-1 comprises a LCDR1 comprising the amino acid sequence of SEQ ID NO: 41, a LCDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 43.

[0024] In some embodiments, the antibody or antigen-binding fragment thereof that binds PD-1 comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 36, and a LCVR comprising the amino acid sequence of SEQ ID NO: 40.

[0025] In some embodiments, the antibody or antigen-binding fragment thereof that binds PD-1 is an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 44 and a light chain comprising the amino acid sequence of SEQ ID NO: 45.

[0026] In some embodiments, the bispecific antibody or antigen-binding fragment thereof is administered to the subject at a dose of from 30 mg to 900 mg once weekly for at least three weeks. In some embodiments, the bispecific antibody or antigen-binding fragment thereof is administered to the subject at a dose of from 100 mg to 900 mg once weekly for at least three weeks. In some embodiments, the bispecific antibody or antigen-binding fragment thereof is administered to the subject at a dose of from 300 mg to 900 mg once weekly for at least three weeks. In some embodiments, the bispecific antibody or antigen-binding fragment thereof is administered to the subject at a dose of 30 mg, 100 mg, 300 mg, 600 mg or 900 mg once weekly for at least three weeks.

[0027] The present disclosure also encompasses the use of the bispecific antibodies and/or the anti-PD-1 antibodies (and antigen-binding fragment of either) in the manufacture of a medicament for treating a PSMA-expressing cancer as set forth in any of the embodiments of the methods discussed above or herein The present disclosure also encompasses bispecific antibodies and/or

anti-PD-1 antibodies (and antigen-binding fragments of either) for use in any of the embodiments of the methods discussed above or herein. The present disclosure also encompasses pharmaceutical compositions comprising the bispecific antibodies and/or anti-PD-1 antibodies (and antigen-binding fragments of either) for use in any of the embodiments of the methods discussed above or herein.

[0028] In various embodiments, any of the features or components of embodiments discussed above or herein may be combined, and such combinations are encompassed within the scope of the present disclosure. Any specific value discussed above or herein may be combined with another related value discussed above or herein to recite a range with the values representing the upper and lower ends of the range, and such ranges are encompassed within the scope of the present disclosure.

[0029] Other embodiments of the present disclosure will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0030] Figure 1 illustrates an embodiment of a study flow diagram for QW dosing of REGN5678 monotherapy, as discussed in Example 2. Participants with evidence of progressive disease after a minimum of 6 weeks of REGN5678 monotherapy may receive low dose cemiplimab (3 mg IV Q3W). Participants may also switch to REGN5678 Q3W dosing after 12 months on treatment.

DETAILED DESCRIPTION

[0031] It is to be understood that this disclosure is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims. Any embodiments or features of embodiments can be combined with one another, and such combinations are expressly encompassed within the scope of the present disclosure. Any specific value discussed above or herein may be combined with another related value discussed above or herein to recite a range with the values representing the upper and lower ends of the range, and such ranges are encompassed within the scope of the present disclosure.

[0032] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as

used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0033] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. All patents, applications and non-patent publications mentioned in this specification are incorporated herein by reference in their entireties.

Methods for Treating or Inhibiting the Growth of Cancers

[0034] The present disclosure includes methods for treating, ameliorating or reducing the severity of at least one symptom or indication, or inhibiting the growth of a cancer (*e.g.*, metastatic clear cell renal cell carcinoma) in a subject. The methods according to this aspect of the disclosure comprise administering a therapeutically effective amount of a bispecific antibody against PSMA and CD28 to a subject in need thereof. As used herein, the terms "treat", "treating", or the like, mean to alleviate symptoms, eliminate the causation of symptoms either on a temporary or permanent basis, to delay or inhibit tumor growth, to reduce tumor cell load or tumor burden, to promote tumor regression, to cause tumor shrinkage, necrosis and/or disappearance, to prevent tumor recurrence, and/or to increase duration of survival of the subject. In some cases, the methods further comprise administration of a therapeutically effective amount of an antibody or antigen-binding fragment thereof that binds human PD-1.

[0035] As used herein, the expressions “a subject” or "a subject in need thereof" means a human or non-human mammal that exhibits one or more symptoms or indications of cancer, and/or who has been diagnosed with cancer, including clear cell renal cell carcinoma (ccRCC) (*e.g.*, metastatic ccRCC) and who needs treatment for the same. In many embodiments, the term "subject" may be interchangeably used with the term "patient". For example, a human subject may be diagnosed with a primary or a metastatic tumor and/or with one or more symptoms or indications including, but not limited to, enlarged lymph node(s), swollen abdomen, unexplained pain, unexplained weight loss, fever, night sweats, persistent fatigue, loss of appetite, and/or enlargement of spleen. The expression includes subjects with primary or established renal cell carcinoma tumors with a clear cell component. In specific embodiments, the expression includes human subjects that have and need treatment for a PSMA-expressing ccRCC. In other specific embodiments, the expression includes subjects with PSMA+ tumors (*e.g.*, a tumor with PSMA expression as determined by flow cytometry). In certain embodiments, the expression "a subject in need thereof" includes patients with a ccRCC that is resistant to or refractory to or is inadequately controlled by prior therapy (*e.g.*, treatment with a conventional anti-cancer agent, including anti-PD-1 therapy, anti-CTLA-4 therapy, or treatment with a tyrosine kinase inhibitor). For example, the expression includes subjects who

have been treated with chemotherapy, or anti-PD-1 therapy, or anti-PD-1 therapy and anti-CTLA-4 therapy (*e.g.*, ipilimumab), or anti-PD-1 therapy and a tyrosine kinase inhibitor, or anti-PD-1 therapy, anti-CTLA-4 therapy and a tyrosine kinase inhibitor. The expression also includes subjects with a ccRCC tumor for which conventional anti-cancer therapy is inadvisable, for example, due to toxic side effects. For example, the expression includes patients who have received one or more cycles of chemotherapy or other anti-cancer therapy with toxic side effects. In certain embodiments, the expression "a subject in need thereof" includes patients with a ccRCC tumor which has been treated but which has subsequently relapsed or metastasized. For example, patients with a ccRCC tumor that may have received treatment with one or more anti-cancer agents leading to tumor regression; however, subsequently have relapsed with cancer resistant to the one or more anti-cancer agents are treated with the methods of the present disclosure.

[0036] In certain embodiments, the methods of the present disclosure may be used to treat patients that have histologically or cytologically confirmed renal cell carcinoma with a clear cell component. The terms "tumor", "cancer" and "malignancy" are interchangeably used herein. The term "clear cell renal cell carcinoma", as used herein, refers to tumors of the kidney, including metastatic tumors originating in the kidney with a clear cell component.

[0037] According to certain embodiments, the present disclosure includes methods for treating, or delaying or inhibiting the growth of a tumor. In certain embodiments, the present disclosure includes methods to promote tumor regression. In certain embodiments, the present disclosure includes methods to reduce tumor cell load or to reduce tumor burden. In certain embodiments, the present disclosure includes methods to prevent tumor recurrence. The methods, according to this aspect of the disclosure, comprise administering a therapeutically effective amount of a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, optionally in combination with an anti-PD-1 antibody or antigen-binding fragment thereof to a subject in need thereof, wherein each antibody or fragment is administered to the subject in multiple doses, *e.g.*, as part of a specific therapeutic dosing regimen. For example, the therapeutic dosing regimen may comprise administering one or more doses of an anti-PSMA x CD28 antibody or antigen-binding fragment thereof to the subject at a frequency of about once a day, once every two days, once every three days, once every four days, once every five days, once every six days, once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every two months, once every three months, once every four months, or less frequently. In certain embodiments, the anti-PSMA x anti-CD28 antibody or antigen-binding fragment thereof is administered once a week. In certain embodiments, the anti-PSMA x anti-CD28 antibody or antigen-binding fragment thereof is administered once every three weeks. In certain embodiments, the one or more doses of the anti-

PD-1 antibody or antigen-binding fragment thereof are administered to the subject at a frequency of about once a day, once every two days, once every three days, once every four days, once every five days, once every six days, once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every two months, once every three months, once every four months, or less frequently. In certain embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof is administered to the subject once every three weeks.

[0038] In certain embodiments, the present disclosure includes methods to inhibit, retard or stop tumor metastasis or tumor infiltration into peripheral organs. The methods, according to this aspect, comprise administering a therapeutically effective amount of a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, optionally in combination with an anti-PD-1 antibody or antigen-binding fragment thereof to a subject in need thereof.

[0039] In specific embodiments in which a combination therapy is administered, the anti-PSMA/CD28 bispecific antibody or antigen-binding fragment thereof is administered to the subject prior to the anti-PD-1 antibody or antigen-binding fragment thereof. In some cases, the anti-PSMA/CD28 antibody or antigen-binding fragment thereof may be administered about 1 day, more than 1 day, more than 2 days, more than 3 days, more than 4 days, more than 5 days, more than 6 days, more than 7 days, 2 weeks, 3 weeks or more prior to the anti-PD-1 antibody or antigen-binding fragment thereof. In some cases, the anti-PSMA/CD28 antibody or antigen-binding fragment is administered for three weeks, for four weeks, for five weeks, or for six weeks as monotherapy prior to administration of the anti-PD-1 antibody or antigen-binding fragment thereof.

[0040] In certain embodiments, the methods of the present disclosure are used to treat a patient with a MRD-positive disease. Minimum residual disease (MRD) refers to small numbers of cancer cells that remain in the patient during or after treatment, wherein the patient may or may not show symptoms or signs of the disease. Such residual cancer cells, if not eliminated, frequently lead to relapse of the disease. The present disclosure includes methods to inhibit and/or eliminate residual cancer cells in a patient upon MRD testing. MRD may be assayed according to methods known in the art (*e.g.*, MRD flow cytometry). The methods, according to this aspect of the disclosure, comprise administering a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, optionally in combination with an anti-PD-1 antibody or antigen-binding fragment thereof to a subject in need thereof.

[0041] The methods of the present disclosure, according to certain embodiments, comprise administering to a subject a therapeutically effective amount of a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, optionally in combination with an anti-PD-1 antibody or antigen-binding fragment thereof and, optionally, a third therapeutic agent or therapy. The third

therapeutic agent or therapy may be an agent or therapy selected from the group consisting of, *e.g.*, radiation, chemotherapy, surgery, a cancer vaccine, a PD-L1 inhibitor (*e.g.*, an anti-PD-L1 antibody), a LAG3 inhibitor (*e.g.*, an anti-LAG3 antibody), a CTLA-4 inhibitor (*e.g.*, an anti-CTLA-4 antibody), a TIM3 inhibitor, a BTLA inhibitor, a TIGIT inhibitor, a CD47 inhibitor, an indoleamine-2,3-dioxygenase (IDO) inhibitor, a vascular endothelial growth factor (VEGF) antagonist, an Ang2 inhibitor, a transforming growth factor beta (TGF.beta.) inhibitor, an epidermal growth factor receptor (EGFR) inhibitor, an antibody to a tumor-specific antigen, a cytotoxin, a chemotherapeutic agent, anti-androgen therapy, an IL-6R inhibitor, an IL-4R inhibitor, an IL-10 inhibitor, a cytokine such as IL-2, IL-7, IL-21, and IL-15, an anti-inflammatory drug such as corticosteroids, and non-steroidal anti-inflammatory drugs, and a dietary supplement such as anti-oxidants. In certain embodiments, the antibodies may be administered in combination with therapy including a chemotherapeutic agent, radiation and surgery. As used herein, the phrase “in combination with" means that the antibody or antibodies are administered to the subject at the same time as, just before, or just after administration of the third therapeutic agent. In certain embodiments, the antibody or antibodies and the third therapeutic agent are administered in separate formulations.

[0042] In certain embodiments, the methods of the present disclosure comprise administering to a subject in need thereof a therapeutically effective amount of a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, optionally in combination with an anti-PD-1 antibody or antigen-binding fragment thereof. In certain embodiments, administration of the bispecific antibody, or optionally the combination, results in tumor growth inhibition by at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70% or about 80% as compared to an untreated subject. In certain embodiments, the administration of the bispecific antibody , or optionally the combination, leads to increased tumor regression, tumor shrinkage and/or disappearance. In certain embodiments, the administration of the bispecific antibody, or optionally the combination, leads to delay in tumor growth and development, *e.g.*, tumor growth may be delayed by about 3 days, more than 3 days, about 7 days, more than 7 days, more than 15 days, more than 1 month, more than 3 months, more than 6 months, more than 1 year, more than 2 years, or more than 3 years as compared to an untreated subject. In certain embodiments, administration of the bispecific antibody, or optionally the combination, prevents tumor recurrence and/or increases duration of survival of the subject, *e.g.*, increases duration of survival by more than 15 days, more than 1 month, more than 3 months, more than 6 months, more than 12 months, more than 18 months, more than 24 months, more than 36 months, or more than 48 months relative to an untreated subject. In certain embodiments, administration of the bispecific antibody, or optionally the combination, increases progression-free survival or overall survival. In certain

embodiments, administration of the bispecific antibody, or optionally the combination, increases response and duration of response in a subject, *e.g.*, by more than 2%, more than 3%, more than 4%, more than 5%, more than 6%, more than 7%, more than 8%, more than 9%, more than 10%, more than 20%, more than 30%, more than 40% or more than 50% over an untreated subject. In certain embodiments, administration of the bispecific antibody, or optionally the combination, to a subject with ccRCC leads to complete disappearance of all evidence of tumor cells ("complete response"). In certain embodiments, administration of the bispecific antibody, or optionally the combination, to a subject with ccRCC leads to at least 30% or more decrease in tumor cells or tumor size ("partial response"). In certain embodiments, administration of the bispecific antibody, or optionally the combination, to a subject with ccRCC leads to complete or partial disappearance of tumor cells/lesions including new measurable lesions. Tumor reduction can be measured by any of the methods known in the art, *e.g.*, X-rays, positron emission tomography (PET), computed tomography (CT), magnetic resonance imaging (MRI), cytology, histology, or molecular genetic analyses.

[0043] In certain cases, the response of a subject to therapy is categorized as a complete response (CR), a partial response (PR), progressive disease (PD), or as stable disease (SD). A CR is defined as disappearance of all target lesions, and a reduction in short axis of any pathological lymph nodes (whether target or non-target) to <10 mm (<1 cm). A PR is defined as an at least 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters. PD is defined as an at least 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum 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 (0.5 cm). (Note: the appearance of one or more new lesions is also considered a progression). SD is defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Bispecific Anti-PSMA x Anti-CD28 Antibodies

[0044] According to certain exemplary embodiments of the present disclosure, the methods comprise administering a therapeutically effective amount of a bispecific antibody that specifically binds CD28 and PSMA or antigen-binding fragment thereof. Such antibodies and fragments may be referred to herein as, *e.g.*, "anti-PSMA/anti-CD28," or "anti-PSMA x CD28" or "PSMA x CD28" bispecific antibodies or antigen-binding fragments thereof, or other similar terminology.

[0045] As used herein, the expression "bispecific antibody" refers to an immunoglobulin protein comprising at least a first antigen-binding domain and a second antigen-binding domain. In the context of the present disclosure, the first antigen-binding domain specifically binds a first antigen

(*e.g.*, PSMA), and the second antigen-binding domain specifically binds a second, distinct antigen (*e.g.*, CD28). Each antigen-binding domain of a bispecific antibody comprises a heavy chain variable domain (HCVR) and a light chain variable domain (LCVR), each comprising three CDRs. In the context of a bispecific antibody, the CDRs of the first antigen-binding domain may be designated with the prefix "A" and the CDRs of the second antigen-binding domain may be designated with the prefix "B". Thus, the CDRs of the first antigen-binding domain may be referred to herein as A-HCDR1, A-HCDR2, and A-HCDR3; and the CDRs of the second antigen-binding domain may be referred to herein as B-HCDR1, B-HCDR2, and B-HCDR3.

[0046] The first antigen-binding domain and the second antigen-binding domain are each connected to a separate multimerizing domain. As used herein, a "multimerizing domain" is any macromolecule, protein, polypeptide, peptide, or amino acid that has the ability to associate with a second multimerizing domain of the same or similar structure or constitution. In the context of the present disclosure, the multimerizing component is an Fc portion of an immunoglobulin (comprising a C_{H2}-C_{H3} domain), *e.g.*, an Fc domain of an IgG selected from the isotypes IgG1, IgG2, IgG3, and IgG4, as well as any allotype within each isotype group.

[0047] Bispecific antibodies of the present disclosure typically comprise two multimerizing domains, *e.g.*, two Fc domains that are each individually part of a separate antibody heavy chain. The first and second multimerizing domains may be of the same IgG isotype such as, *e.g.*, IgG1/IgG1, IgG2/IgG2, IgG4/IgG4. Alternatively, the first and second multimerizing domains may be of different IgG isotypes such as, *e.g.*, IgG1/IgG2, IgG1/IgG4, IgG2/IgG4, etc.

[0048] Any bispecific antibody format or technology may be used to make the bispecific antibodies of the present disclosure. For example, an antibody or fragment thereof having a first antigen binding specificity can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment having a second antigen-binding specificity to produce a bispecific antibody. Specific exemplary bispecific formats that can be used in the context of the present disclosure include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab_2 bispecific formats (see, *e.g.*, Klein et al. 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats).

[0049] In the context of bispecific antibodies of the present disclosure, Fc domains may comprise one or more amino acid changes (*e.g.*, insertions, deletions or substitutions) as compared to the wild-type, naturally occurring version of the Fc domain. For example, the disclosure includes

bispecific antibodies comprising one or more modifications in the Fc domain that results in a modified Fc domain having a modified binding interaction (*e.g.*, enhanced or diminished) between Fc and FcRn. In one embodiment, the bispecific antibody comprises a modification in a C_{H2} or a C_{H3} region, wherein the modification increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0). Non-limiting examples of such Fc modifications are disclosed in US Patent Publication No. 20150266966, incorporated herein in its entirety.

[0050] The present disclosure also includes bispecific antibodies comprising a first C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). See, for example, US Patent No. 8,586,713. Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies.

[0051] In certain embodiments, the Fc domain may be chimeric, combining Fc sequences derived from more than one immunoglobulin isotype. For example, a chimeric Fc domain can comprise part or all of a C_H2 sequence derived from a human IgG1, human IgG2 or human IgG4 C_H2 region, and part or all of a C_H3 sequence derived from a human IgG1, human IgG2 or human IgG4. A chimeric Fc domain can also contain a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. A particular example of a chimeric Fc domain that can be included in any of the antibodies set forth herein comprises, from N- to C-terminus: [IgG4 C_H1]-[IgG4 upper hinge]-[IgG2 lower hinge]-[IgG4 CH2]-[IgG4 CH3]. Another example of a chimeric Fc domain that can be included in any of the antibodies set forth herein comprises, from N- to C-terminus: [IgG1 C_H1]-[IgG1 upper hinge]-[IgG2 lower hinge]-[IgG4 CH2]-[IgG1 CH3]. These and other examples of chimeric Fc domains or chimeric heavy chain constant regions that can be included in any of the

antibodies of the present disclosure are described in US Patent Publication No. 20140243504, which is herein incorporated in its entirety. Chimeric Fc domains and chimeric heavy chain constant regions having these general structural arrangements, and variants thereof, can have altered Fc receptor binding, which in turn affects Fc effector function.

[0052] According to certain exemplary embodiments of the present disclosure, the bispecific anti-PSMA/anti-CD28 antibody, or antigen-binding fragment thereof comprises heavy chain variable regions (A-HCVR and B-HCVR), light chain variable regions (A-LCVR and B-LCVR), and/or complementarity determining regions (CDRs) comprising any of the amino acid sequences of the bispecific anti-PSMA/anti-CD28 antibodies as set forth in WO 2019/246514. In certain exemplary embodiments, the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof that can be used in the context of the methods of the present disclosure comprises: (a) a first antigen-binding arm that specifically binds PSMA comprising the heavy chain complementarity determining regions (A-HCDR1, A-HCDR2 and A-HCDR3) of a heavy chain variable region (A-HCVR) comprising the amino acid sequence of SEQ ID NO: 1 and the light chain complementarity determining regions (A-LCDR1, A-LCDR2 and A-LCDR3) of a light chain variable region (A-LCVR) comprising the amino acid sequence of SEQ ID NO: 9; and (b) a second antigen-binding arm that specifically binds CD28 comprising the heavy chain CDRs (B-HCDR1, B-HCDR2 and B-HCDR3) of a HCVR (B-HCVR) comprising an amino acid sequence of SEQ ID NO: 5, and the light chain CDRs (B-LCDR1, B-LCDR2 and B-LCDR3) of a LCVR (B-LCVR) comprising the amino acid sequence of SEQ ID NO: 9. According to certain embodiments, the A-HCDR1 comprises the amino acid sequence of SEQ ID NO: 2; the A-HCDR2 comprises the amino acid sequence of SEQ ID NO: 3; the A-HCDR3 comprises the amino acid sequence of SEQ ID NO: 4; the A-LCDR1 comprises the amino acid sequence of SEQ ID NO: 10; the A-LCDR2 comprises the amino acid sequence of SEQ ID NO: 11; the A-LCDR3 comprises the amino acid sequence of SEQ ID NO: 12; the B-HCDR1 comprises the amino acid sequence of SEQ ID NO: 6; the B-HCDR2 comprises the amino acid sequence of SEQ ID NO: 7; and the B-HCDR3 comprises the amino acid sequence of SEQ ID NO: 8; and the B-LCDR1 comprises the amino acid sequence of SEQ ID NO: 10; the B-LCDR2 comprises the amino acid sequence of SEQ ID NO: 11; the B-LCDR3 comprises the amino acid sequence of SEQ ID NO: 12. In yet other embodiments, the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof comprises: (a) a first antigen-binding arm comprising a HCVR (A-HCVR) comprising SEQ ID NO: 1 and a LCVR (A-LCVR) comprising SEQ ID NO: 9; and (b) a second antigen-binding arm comprising a HCVR (B-HCVR) comprising SEQ ID NO: 5, and a LCVR (B-LCVR) comprising SEQ ID NO: 9. In certain exemplary embodiments, the bispecific anti-PSMA x CD28 antibody comprises a PSMA-binding arm comprising a heavy chain comprising the

amino acid sequence of SEQ ID NO: 13 and a light chain comprising the amino acid sequence of SEQ ID NO: 15, and a CD28-binding arm comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 14 and a light chain comprising the amino acid sequence of SEQ ID NO: 15.

[0053] In certain exemplary embodiments, the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof that can be used in the context of the methods of the present disclosure comprises: (a) a first antigen-binding arm that specifically binds PSMA comprising the heavy chain complementarity determining regions (A-HCDR1, A-HCDR2 and A-HCDR3) of a heavy chain variable region (A-HCVR) comprising the amino acid sequence of SEQ ID NO: 16 and the light chain complementarity determining regions (A-LCDR1, A-LCDR2 and A-LCDR3) of a light chain variable region (A-LCVR) comprising the amino acid sequence of SEQ ID NO: 28; and (b) a second antigen-binding arm that specifically binds CD28 comprising the heavy chain CDRs (B-HCDR1, B-HCDR2 and B-HCDR3) of a HCVR (B-HCVR) comprising an amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 24, and the light chain CDRs (B-LCDR1, B-LCDR2 and B-LCDR3) of a LCVR (B-LCVR) comprising the amino acid sequence of SEQ ID NO: 28. According to certain embodiments, the A-HCDR1 comprises the amino acid sequence of SEQ ID NO: 17; the A-HCDR2 comprises the amino acid sequence of SEQ ID NO: 18; the A-HCDR3 comprises the amino acid sequence of SEQ ID NO: 19; the A-LCDR1 comprises the amino acid sequence of SEQ ID NO: 29; the A-LCDR2 comprises the amino acid sequence of SEQ ID NO: 30; the A-LCDR3 comprises the amino acid sequence of SEQ ID NO: 31; the B-HCDR1 comprises the amino acid sequence of SEQ ID NO: 21, or SEQ ID NO: 25; the B-HCDR2 comprises the amino acid sequence of SEQ ID NO: 22, or SEQ ID NO: 26; and the B-HCDR3 comprises the amino acid sequence of SEQ ID NO: 23, or SEQ ID NO: 27; and the B-LCDR1 comprises the amino acid sequence of SEQ ID NO: 29; the B-LCDR2 comprises the amino acid sequence of SEQ ID NO: 30; the B-LCDR3 comprises the amino acid sequence of SEQ ID NO: 31. In yet other embodiments, the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof comprises: (a) a first antigen-binding arm comprising a HCVR (A-HCVR) comprising SEQ ID NO: 16 and a LCVR (A-LCVR) comprising SEQ ID NO: 28; and (b) a second antigen-binding arm comprising a HCVR (B-HCVR) comprising SEQ ID NO: 20 or SEQ ID NO: 24, and a LCVR (B-LCVR) comprising SEQ ID NO: 28. In certain exemplary embodiments, the bispecific anti-PSMA x CD28 antibody comprises a PSMA-binding arm comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 32 and a light chain comprising the amino acid sequence of SEQ ID NO: 35, and a CD28-binding arm comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 33 and a light chain comprising the amino acid sequence of SEQ ID NO: 35. In certain exemplary embodiments, the bispecific ant-

PSMA x CD28 antibody comprises a PSMA-binding arm comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 32 and a light chain comprising the amino acid sequence of SEQ ID NO: 35, and a CD28-binding arm comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 34 and a light chain comprising the amino acid sequence of SEQ ID NO: 35.

[0054] The methods of the present disclosure also encompass use of a bioequivalent to the bispecific antibodies discussed herein. The term "bioequivalent", as used herein, refers to antibodies or fragments thereof that are pharmaceutical equivalents or pharmaceutical alternatives whose rate and/or extent of absorption do not show a significant difference with that of the bispecific antibodies discussed above when administered at the same molar dose under similar experimental conditions, either single dose or multiple dose. In the context of the disclosure, the term refers to antigen-binding proteins that bind to PSMA and CD28, which do not have clinically meaningful differences to the bispecific antibodies discussed herein in their safety, purity and/or potency.

Anti-PD-1 Antibodies and Antigen-Binding Fragments Thereof

[0055] According to certain exemplary embodiments of the present disclosure, the methods comprise administering a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds PD-1. The term "antibody," as used herein, includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). In a typical antibody, each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the disclosure, the FRs of the anti-IL-4R antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0056] The term "antibody," as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically

obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0057] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (e.g. monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0058] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0059] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present disclosure include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the

variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present disclosure may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0060] The antibodies used in the methods of the present disclosure may be human antibodies. The term "human antibody," as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the disclosure may nonetheless include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0061] The antibodies used in the methods of the present disclosure may be recombinant human antibodies. The term "recombinant human antibody," as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire in vivo.

[0062] According to certain embodiments, the antibodies used in the methods of the present

disclosure specifically bind PD-1. The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Methods for determining whether an antibody specifically binds to an antigen are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. For example, an antibody that "specifically binds" PD-1, as used in the context of the present disclosure, includes antibodies that bind PD-1 or portion thereof with a K_D of less than about 500 nM, less than about 300 nM, less than about 200 nM, less than about 100 nM, less than about 90 nM, less than about 80 nM, less than about 70 nM, less than about 60 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 20 nM, less than about 10 nM, less than about 5 nM, less than about 4 nM, less than about 3 nM, less than about 2 nM, less than about 1 nM or less than about 0.5 nM, as measured in a surface plasmon resonance assay. An isolated antibody that specifically binds human PD-1 may, however, have cross-reactivity to other antigens, such as PD-1 molecules from other (non-human) species.

[0063] According to certain exemplary embodiments of the present disclosure, the anti-PD-1 antibody, or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR), light chain variable region (LCVR), and/or complementarity determining regions (CDRs) comprising any of the amino acid sequences of the anti-PD-1 antibodies as set forth in US Patent No. 9,987,500. In certain exemplary embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof that can be used in the context of the methods of the present disclosure comprises the heavy chain complementarity determining regions (HCDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 36 and the light chain complementarity determining regions (LCDRs) of a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 40. According to certain embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof comprises three HCDRs (HCDR1, HCDR2 and HCDR3) and three LCDRs (LCDR1, LCDR2 and LCDR3), wherein the HCDR1 comprises the amino acid sequence of SEQ ID NO: 37; the HCDR2 comprises the amino acid sequence of SEQ ID NO: 38; the HCDR3 comprises the amino acid sequence of SEQ ID NO: 39; the LCDR1 comprises the amino acid sequence of SEQ ID NO: 41; the LCDR2 comprises the amino acid sequence of SEQ ID NO: 42; and the LCDR3 comprises the amino acid sequence of SEQ ID NO: 43. In yet other embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof comprises an HCVR comprising SEQ ID NO: 36 and an LCVR comprising SEQ ID NO: 40. In certain embodiments, the methods of the present disclosure comprise the use of an anti-PD-1 antibody, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 44. In some embodiments, the anti-PD-1 antibody comprises a light chain comprising

the amino acid sequence of SEQ ID NO: 45. An exemplary antibody comprising a HCVR comprising the amino acid sequence of SEQ ID NO: 36 and a LCVR comprising the amino acid sequence of SEQ ID NO: 40 is the fully human anti-PD-1 antibody known as REGN2810 (also known as cemiplimab, LIBTAYO®). According to certain exemplary embodiments, the methods of the present disclosure comprise the use of REGN2810, or a bioequivalent thereof. The term "bioequivalent", as used herein, refers to anti-PD-1 antibodies or PD-1-binding proteins or fragments thereof that are pharmaceutical equivalents or pharmaceutical alternatives whose rate and/or extent of absorption do not show a significant difference with that of REGN2810 when administered at the same molar dose under similar experimental conditions, either single dose or multiple dose. In the context of the disclosure, the term refers to antigen-binding proteins that bind to PD-1 which do not have clinically meaningful differences with REGN2810 in their safety, purity and/or potency.

[0064] Other anti-PD-1 antibodies that can be used in the context of the methods of the present disclosure include, *e.g.*, the antibodies referred to and known in the art as nivolumab (U.S. Pat. No. 8,008,449), pembrolizumab (U.S. Pat. No. 8,354,509), MEDI0608 (U.S. Pat. No. 8,609,089), pidilizumab (U.S. Pat. No. 8,686,119), or any of the anti-PD-1 antibodies as set forth in U.S. Pat. Nos. 6,808,710, 7,488,802, 8,168,757, 8,354,509, 8,779,105, or 8,900,587.

[0065] The anti-PD-1 antibodies used in the context of the methods of the present disclosure may have pH-dependent binding characteristics. For example, an anti-PD-1 antibody for use in the methods of the present disclosure may exhibit reduced binding to PD-1 at acidic pH as compared to neutral pH. Alternatively, an anti-PD-1 antibody of the disclosure may exhibit enhanced binding to its antigen at acidic pH as compared to neutral pH. The expression "acidic pH" includes pH values less than about 6.2, e.g., about 6.0, 5.95, 5.9, 5.85, 5.8, 5.75, 5.7, 5.65, 5.6, 5.55, 5.5, 5.45, 5.4, 5.35, 5.3, 5.25, 5.2, 5.15, 5.1, 5.05, 5.0, or less. As used herein, the expression "neutral pH" means a pH of about 7.0 to about 7.4. The expression "neutral pH" includes pH values of about 7.0, 7.05, 7.1, 7.15, 7.2, 7.25, 7.3, 7.35, and 7.4.

[0066] In certain instances, "reduced binding to PD-1 at acidic pH as compared to neutral pH" is expressed in terms of a ratio of the K_D value of the antibody binding to PD-1 at acidic pH to the K_D value of the antibody binding to PD-1 at neutral pH (or vice versa). For example, an antibody or antigen-binding fragment thereof may be regarded as exhibiting "reduced binding to PD-1 at acidic pH as compared to neutral pH" for purposes of the present disclosure if the antibody or antigen-binding fragment thereof exhibits an acidic/neutral K_D ratio of about 3.0 or greater. In certain exemplary embodiments, the acidic/neutral K_D ratio for an antibody or antigen-binding fragment of the present disclosure can be about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5,

10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0, 60.0, 70.0, 100.0, or greater.

[0067] Antibodies with pH-dependent binding characteristics may be obtained, *e.g.*, by screening a population of antibodies for reduced (or enhanced) binding to a particular antigen at acidic pH as compared to neutral pH. Additionally, modifications of the antigen-binding domain at the amino acid level may yield antibodies with pH-dependent characteristics. For example, by substituting one or more amino acids of an antigen-binding domain (*e.g.*, within a CDR) with a histidine residue, an antibody with reduced antigen-binding at acidic pH relative to neutral pH may be obtained. As used herein, the expression "acidic pH" means a pH of 6.0 or less.

Combination Therapies

[0068] The methods of the present disclosure, according to certain embodiments, comprise administering to the subject an anti-PSMA/anti-CD28 bispecific antibody or antigen-binding fragment thereof in combination with an anti-PD-1 antibody or antigen-binding fragment thereof. In certain embodiments, the methods of the present disclosure comprise administering the antibodies for additive or synergistic activity to treat a PSMA-expressing cancer, preferably ccRCC. In some embodiments, the combination of anti-PSMA x CD28 bispecific antibody (*e.g.*, mAb1) and anti-PD-1 antibody (*e.g.*, cemiplimab) produces a synergistic therapeutic effect in the treatment of metastatic clear cell renal cell carcinoma. As used herein, the expression "in combination with" means that the anti-PSMA/anti-CD28 bispecific antibody or antigen-binding fragment thereof is administered before, after, or concurrent with the anti-PD-1 antibody or antigen-binding fragment thereof. The term "in combination with" also includes sequential or concomitant administration of anti-PD-1 antibody or antigen-binding fragment thereof and a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof. For example, when administered "before" the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, the anti-PD-1 antibody or antigen-binding fragment thereof may be administered more than 72 hours, about 72 hours, about 60 hours, about 48 hours, about 36 hours, about 24 hours, about 12 hours, about 10 hours, about 8 hours, about 6 hours, about 4 hours, about 2 hours, about 1 hour, or about 30 minutes prior to the administration of the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof. When administered "after" the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, the anti-PD-1 antibody or antigen-binding fragment thereof may be administered about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours, about 72 hours, or more than 72 hours after the administration of the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof. Administration "concurrent" with the bispecific anti-PSMA/anti-

CD28 antibody or antigen-binding fragment thereof means that the anti-PD-1 antibody or antigen-binding fragment thereof is administered to the subject in a separate dosage form within less than 30 minutes (before, after, or at the same time) of administration of the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, or administered to the subject as a single combined dosage formulation comprising both the anti-PD-1 antibody or antigen-binding fragment thereof and the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof.

[0069] In certain embodiments, the methods of the present disclosure comprise administration of a second (*i.e.*, when the bispecific antibody is administered without the anti-PD-1 antibody) or third therapeutic agent (*i.e.*, when a combination of the bispecific antibody and the anti-PD-1 antibody are administered) wherein the second or third therapeutic agent is an anti-cancer drug or anti-cancer therapy. In certain embodiments, the methods of the disclosure comprise administering an anti-PSMA/anti-CD28 bispecific antibody or antigen-binding fragment thereof, and optionally an anti-PD-1 antibody or antigen-binding fragment thereof, in combination with radiation therapy, surgery or other anti-cancer therapy to generate long-term durable anti-tumor responses and/or enhance survival of patients with a PSMA-expressing cancer.

[0070] In some embodiments, the methods of the disclosure comprise administering radiation therapy prior to, concomitantly or after administering a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, and optionally an anti-PD-1 antibody or antigen-binding fragment thereof, to a cancer patient. For example, radiation therapy may be administered in one or more doses to tumor lesions after administration of one or more doses of the antibodies. In some embodiments, radiation therapy may be administered locally to a tumor lesion to enhance the local immunogenicity of a patient's tumor (adjuvinating radiation) and/or to kill tumor cells (ablative radiation) after systemic administration of a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, and optionally an anti-PD-1 antibody or antigen-binding fragment thereof.

Efficacy and Monitoring

[0071] The methods discussed in the present disclosure may further comprise tumor biopsies, imaging, and cytokine release syndrome (CRS) monitoring and management to evaluate efficacy and safety within individual subjects or populations of subjects.

Tumor Biopsy

[0072] Patients with soft tissue disease may undergo a core or excisional biopsy from a soft tissue lesion if clinically accessible at screening and/or during treatment as discussed herein. For patients without soft tissue disease that is clinically accessible, a bone biopsy may be performed if feasible. Any available tissue from samples (*e.g.*, formalin-fixed paraffin-embedded, or preserved in

block for molecular extraction) collected at various time points, as well as archival specimens from previous treatment, in addition to clinical diagnostic uses, may be utilized for biomarker assays. Specifically, these samples may be assessed using *in situ* imaging with probes for gene targets relevant to REGN5678 (PSMA, CD28) and optionally cemiplimab (PD-L1), as well as markers of immune activation, suppression, and function, and tumor cell phenotype.

[0073] Tumor tissue biopsies, if available, may also be subjected to gene expression profiling (using RNA sequencing or other methods) as a measure of composite tumor microenvironmental phenotype, whole exome sequencing or other mutational profiling, and targeted study of gene variants (tumor mutations) such as those affecting DNA repair pathways. They may also be profiled using next-generation sequencing for the T cell receptor repertoire, as a measure of tumor-associated T cell clonal proliferation.

Imaging

[0074] Prostate-specific membrane antigen (PSMA) PET/CT has been shown to provide a sensitive measure of both PSMA expression and tumor burden in PSMA-expressing cancer patients. It allows detection of tumor lesions and improves the effectiveness of tumor response assessment and treatment strategy decision-making.

CRS Monitoring and Management

[0075] Cytokine release has been observed with superagonist anti-CD28 bivalent antibodies, bispecific antibodies, and similar molecules. Cytokine release syndrome (CRS) has often resulted in clinical symptoms during infusion or within hours to days of infusion. In a clinical study of 6 patients treated with a bivalent anti-CD28 superagonist antibody (TGN1412), life-threatening CRS occurred acutely, and patients became critically ill within 12 to 16 hours. Prior experience with bispecific antibodies targeting a tumor antigen and CD3 has shown that when CRS occurred, the events were most prominent following the first 1 or 2 weekly doses of study treatment and were typically transient, even when higher doses were administered in subsequent weeks. This has also been observed in combination with cemiplimab. CRS typically occurs more frequently with the first 2 weekly doses for any given patient and decreases in frequency upon subsequent exposure. Based on these findings, the risk of an initial episode of CRS occurring after third dose or later is considered to be low.

[0076] Subcutaneous administration of bispecific antibodies has recently been evaluated in preclinical and early-phase clinical studies. Subcutaneous administration of a bispecific antibody targeting a tumor antigen and CD3 was tolerated without severe CRS events (no Grade ≥3 CRS) in a B-cell tumor. In cynomolgus monkeys, SC administration of the same bispecific antibody resulted

in lower C_{max}, delayed T_{max}, and lower plasma cytokine levels compared to IV administration. The methods discussed herein may include measures to address potential safety issues resulting from cytokine release, including:

(1) Cytokine monitoring;

(2) Use of anti-IL-6 pathway therapies (*e.g.*, sarilumab or tocilizumab) and corticosteroids for management of CRS; and

(3) Provisions for premedication and use of lower dose at initial dosing visits before stepping up to the full dose at the dose level (DL) in the event of observed CRS.

[0077] Patients who develop symptoms consistent with severe CRS, including but not limited to, persistent fevers, neurologic disorders (including mental status changes, obtundation, and seizures), clinical signs of toxicity (hypotension requiring at least 1 IV vasoactive pressor or hypoxia [PO_2 <90%]) may be considered for pharmacologic intervention with anti-IL-6 pathway therapies (*e.g.*, sarilumab or tocilizumab) and/or high dose steroids. Such additions to the methods discussed herein are contemplated by this disclosure.

[0078] Corticosteroids may also be utilized in the management of CRS, particularly in cases with neurologic symptoms. In general, corticosteroids should be used when: 1) IRR/CRS does not respond adequately to anti-IL-6 pathway therapies (*e.g.*, sarilumab or tocilizumab), or 2) anti-IL-6 pathway therapies are not in the best interest of the patient.

Pharmaceutical Compositions and Administration

[0079] The present disclosure includes methods which comprise administering a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, optionally in combination with an anti-PD-1 antibody or antigen-binding fragment thereof, to a subject wherein the antibody or antibodies (or fragments) are contained within separate or a combined (single) pharmaceutical composition. The pharmaceutical compositions of the disclosure may be formulated with suitable carriers, excipients, and other agents that provide suitable transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) *J Pharm Sci Technol* 52:238-311.

[0080] Various delivery systems are known and can be used to administer the pharmaceutical

composition of the disclosure, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu *et al.*, 1987, *J. Biol. Chem.* 262: 4429-4432). Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, or by injection, and may be administered together with other biologically active agents.

[0081] A pharmaceutical composition of the present disclosure can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present disclosure. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0082] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present disclosure. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present disclosure include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0083] In certain situations, the pharmaceutical composition can be delivered in a controlled

release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used; see, Medical Applications of Controlled Release, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Fla. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249:1527-1533.

[0084] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by known methods. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent. The injection thus prepared is preferably filled in an appropriate ampoule.

[0085] Advantageously, the pharmaceutical compositions for use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, a vial or a prefilled syringe.

Administration Regimens

[0086] The present disclosure includes methods comprising administering to a subject a bispecific anti-PSMA x CD28 antibody or antigen-binding fragment thereof, optionally in combination with an anti-PD-1 antibody or antigen-binding fragment thereof at a dosing frequency of about four times a week, twice a week, once a week, once every two weeks, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every eight weeks, once every twelve weeks, or less frequently so long as a therapeutic response is achieved.

[0087] According to certain embodiments of the present disclosure, multiple doses of a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, optionally in combination with an anti-PD-1 antibody or antigen-binding fragment thereof, may be administered to a subject over a defined time course. The methods according to this aspect of the disclosure comprise administering to a subject one or more doses of a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, optionally in sequential administration with one or more doses of an anti-PD-1 antibody or antigen-binding fragment thereof. As used herein, "sequential administering" means that each dose of the antibody is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present

disclosure includes methods which comprise sequential administration to the patient a single initial dose of an antibody (or fragment), followed by one or more secondary doses of the antibody (or fragment), and optionally followed by one or more tertiary doses of the antibody (or fragment).

[0088] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of the antibody or antigen-binding fragment thereof (*e.g.*, the bispecific antibody). In certain embodiments, however, the amount contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, one or more (e.g., 1, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[0089] In one exemplary embodiment of the present disclosure, each secondary and/or tertiary dose is administered 1/2 to 14 or more (*e.g.*, 1/2, 1, 11/2, 2, 21/2, 3, 31/2, 4, 41/2, 5, 51/2, 6, 61/2, 7, 71/2, 8, 81/2, 9, 91/2, 10, 101/2, 11, 111/2, 12, 121/2, 13, 131/2, 14, 141/2, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of a bispecific anti-PSMA/anti-CD28 or antigen-binding fragment thereof (and optionally an anti-PD-1 antibody or antigen-binding fragment thereof), which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0090] The methods according to this aspect of the disclosure may comprise administering to a patient any number of secondary and/or tertiary doses of bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof and optionally an anti-PD-1 antibody or antigen-binding fragment thereof. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0091] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1, 2 or 3 weeks (*e.g.*, 1 week or 3 weeks) after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each

tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 1 to 4 weeks (*e.g.*, 1 week or 3 weeks) after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

[0092] In certain embodiments, one or more doses of a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, and optionally an anti-PD-1 antibody or antigen-binding fragment thereof, are administered at the beginning of a treatment regimen as "induction doses" on a more frequent basis (twice a week, once a week, once in 2 weeks, once in 3 weeks, once in four weeks, once in five weeks, or once in six weeks) followed by subsequent doses ("consolidation doses" or "maintenance doses") that are administered on the same or a less frequent basis (*e.g.*, once in 4-12 weeks).

[0093] The present disclosure includes methods comprising administering to a subject in need thereof a bispecific antibody or antigen-binding fragment thereof at a dose of at least 30 mg once weekly (QW) for at least three weeks in a monotherapy regimen. In various embodiments, the monotherapy regimen may last from three weeks to 36 weeks or longer. In some cases, the monotherapy regimen is at least four weeks, at least five weeks, or at least six weeks in duration. In some cases, the monotherapy regimen is, or is at least, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 weeks. In some cases, the methods further comprise administration of an antibody or antigen-binding fragment thereof that specifically binds programmed death receptor-1 (PD-1), wherein the antibody or antigen-binding fragment is administered at a dose of from 3 mg to 100 mg once every three weeks (Q3W). In some cases, the methods further comprise administration of an antibody or antigen-binding fragment thereof that specifically binds programmed death receptor-1 (PD-1), wherein the antibody or antigen-binding fragment is administered at a dose of from 3 mg to 350 mg once every three weeks (Q3W). In some cases, the methods further comprise administration of an antibody or antigen-binding fragment thereof that specifically binds programmed death receptor-1 (PD-1), wherein the antibody or antigen-binding fragment is administered at a dose of from 3 mg to 1000 mg (*e.g.*, Q3W, Q4W, Q5W, or Q6W). In some cases, the administration of the anti-PD-1 antibody or antigen-binding fragment thereof follows the monotherapy regimen.

[0094] The present disclosure also includes methods comprising administering to the subject a combination of a bispecific antibody or antigen-binding fragment thereof, and an antibody or antigen-binding fragment thereof that specifically binds programmed death receptor-1 (PD-1),

wherein the bispecific antibody is administered to the subject at a dose of at least 30 mg once weekly (QW) for at least three weeks. In some cases, the bispecific antibody is administered as monotherapy during a lead-in regimen that is of a duration of at least three weeks, at least four weeks, at least five weeks, or at least six weeks. In some cases, the lead-in regimen is, or is at least, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 weeks.

Dosage

[0095] The amount of bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, and optionally the anti-PD-1 antibody or antigen-binding fragment thereof, administered to a subject according to the methods of the present disclosure is, generally, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means an amount of antibody (a bispecific anti-PSMA/anti-CD28 antibody and optionally an anti-PD-1 antibody) or antigen-binding fragment thereof that results in one or more of: (a) a reduction in the severity or duration of a symptom of a cancer (*e.g.*, ccRCC); (b) inhibition of tumor growth, or an increase in tumor necrosis, tumor shrinkage and/or tumor disappearance; (c) delay in tumor growth and development; (d) inhibit or retard or stop tumor metastasis; (e) prevention of recurrence of tumor growth; (f) increase in survival of a subject with cancer (*e.g.*, ccRCC); and/or (g) a reduction in the use or need for conventional anti-cancer therapy (*e.g.*, reduced or eliminated use of chemotherapeutic or cytotoxic agents) as compared to an untreated subject.

[0096] In the case of a bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof, a therapeutically effective amount can be from about 0.3 milligrams (mg) to about 2000 mg, *e.g.*, about 0.3 mg, about 0.5 mg, about 1 mg, about 3 mg, about 5 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 75 mg, about 100 mg, about 125 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, or about 1000 mg of the bispecific anti-PSMA/anti-CD28 antibody or antigen-binding fragment thereof. In certain embodiments, 30 mg, 100 mg, 300 mg, 600 mg, or 900 mg of the bispecific anti-PSMA x anti-CD28 antibody or antigen-binding fragment thereof is administered (*e.g.*, once weekly or once every three weeks) to the subject to treat a PSMA-expressing cancer or a PSMA-expressing clear cell renal cell carcinoma (*e.g.*, metastatic ccRCC).

[0097] In the case of an anti-PD-1 antibody or antigen-binding fragment thereof, a therapeutically effective amount can be from about 3 mg to about 100 mg (when following the monotherapy regimen of the bispecific antibody). Thus, in certain embodiments, the effective amount of the anti-

PD-1 antibody (or fragment) may be about 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 mg. In certain embodiments, 3 mg to 99 mg of the anti-PD-1 antibody or antigen-binding fragment thereof is administered (*e.g.*, once every three weeks) to the subject in combination with the bispecific antibody or antigen-binding fragment thereof to treat a PSMA-expressing cancer or a PSMA-expressing clear cell renal cell carcinoma cancer (*e.g.*, metastatic ccRCC). In certain embodiments, 300 mg to 400 mg, or 650 mg to 750 mg of the anti-PD-1 antibody or antigen-binding fragment thereof is administered (*e.g.*, once every three weeks or once every six weeks) to the subject to treat a PSMA-expressing cancer or a PSMA-expressing clear cell renal cell carcinoma cancer (*e.g.*, metastatic ccRCC). In some embodiments, the anti-PD-1 antibody or antigen-binding fragment thereof can be administered at a dose of about 3 mg to about 1000 mg. Thus, in certain embodiments, the effective amount of the anti-PD-1 antibody (or fragment) may be about 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 mg (*e.g.*, administered once every three weeks or once every six weeks).

[0098] A summary of the sequences and the corresponding SEQ ID NOs referenced herein is shown in Table 1, below.

Table 1: Summary of Sequences

SEQ ID NO:	Description
1	Anti-PSMA Heavy Chain Variable Region (-001)
2	Anti-PSMA HCDR1 (-001)
3	Anti-PSMA HCDR2 (-001)
4	Anti-PSMA HCDR3 (-001)
5	Anti-CD28 Heavy Chain Variable Region (-001)
6	Anti-CD28 HCDR1 (-001)
7	Anti-CD28 HCDR2 (-001)
8	Anti-CD28 HCDR3 (-001)
9	Anti-PSMA and Anti-CD28 Light Chain Variable Region (-001)
10	Anti-PSMA and Anti-CD28 LCDR1 (-001)
11	Anti-PSMA and Anti-CD28 LCDR2 (-001)

12	Anti-PSMA and Anti-CD28 LCDR3 (-001)
13	Anti-PSMA Heavy Chain (-001)
14	Anti-CD28 Heavy Chain (-001)
15	Anti-PSMA and Anti-CD28 Light Chain (-001)
16	Anti-PSMA Heavy Chain Variable Region (-002 and -003)
17	Anti-PSMA HCDR1 (-002 and -003)
18	Anti-PSMA HCDR2 (-002 and -003)
19	Anti-PSMA HCDR3 (-002 and -003)
20	Anti-CD28 Heavy Chain Variable Region (-002)
21	Anti-CD28 HCDR1 (-002)
22	Anti-CD28 HCDR2 (-002)
23	Anti-CD28 HCDR3 (-002)
24	Anti-CD28 Heavy Chain Variable Region (-003)
25	Anti-CD28 HCDR1 (-003)
26	Anti-CD28 HCDR2 (-003)
27	Anti-CD28 HCDR3 (-003)
28	Anti-PSMA and Anti-CD28 Light Chain Variable Region (-002 and -003)
29	Anti-PSMA and Anti-CD28 LCDR1 (-002 and -003)
30	Anti-PSMA and Anti-CD28 LCDR2 (-002 and -003)
31	Anti-PSMA and Anti-CD28 LCDR3 (-002 and -003)
32	Anti-PSMA Heavy Chain (-002 and -003)
33	Anti-CD28 Heavy Chain (-002)
34	Anti-CD28 Heavy Chain (-003)
35	Anti-MUC16 and Anti-CD28 Light Chain (-002 and -003)
36	Anti-PD-1 Heavy Chain Variable Region
37	Anti-PD-1 HCDR1
38	Anti-PD-1 HCDR2
39	Anti-PD-1 HCDR3
40	Anti-PD-1 Light Chain Variable Region
41	Anti-PD-1 LCDR1
42	Anti-PD-1 LCDR2
43	Anti-PD-1 LCDR3
44	Anti-PD-1 Heavy Chain

45	Anti-PD-1 Light Chain

EXAMPLES

[0099] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the present disclosure, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Generation of Bispecific Antibodies that Bind Prostate-Specific Membrane Antigen (PSMA) and CD28

[0100] Bispecific antibodies comprising an anti-PSMA-specific binding domain and an anti-CD28-specific binding domain were constructed using standard methodologies, wherein the anti-PSMA antigen binding domain and the anti-CD28 antigen binding domain each comprise different, distinct HCVRs paired with a common LCVR. In some instances the bispecific antibodies were constructed utilizing a heavy chain from an anti-CD28 antibody, a heavy chain from an anti-PSMA antibody and a common light chain (See Table 2).

[0101] The bispecific antibodies created in accordance with the present Example comprise two separate antigen-binding domains (*i.e.,* binding arms). The first antigen-binding domain comprises a heavy chain variable region derived from an anti-CD28 antibody ("CD28-VH"), and the second antigen-binding domain comprises a heavy chain variable region derived from an anti-PSMA antibody ("PSMA-VH"). Both the anti-PSMA and the anti-CD28 share a common light chain. The CD28-VH/PSMA-VH pairing creates antigen-binding domains that specifically recognize CD28 on T cells and PSMA on tumor cells.

[0102] A summary of the component parts of the antigen-binding domains of the various anti-PSMAxCD28 bispecific antibodies constructed is set forth in Table 2. The corresponding CDR sequences and full-length heavy and light chain sequences are identified in Table 1 (with reference to the “-001,” “-002,” and “-003” bispecific antibodies of Table 2).

Table 2: Summary of Component Parts of Anti-PSMAxCD28 Bispecific Antibodies

Bispecific Antibody Identifier	Anti-PSMA	Anti-CD28	

	Antigen-Binding Domain	Antigen-Binding Domain	Common Light Chain Variable Region
	Heavy Chain Variable Region	***Heavy Chain Variable Region***	
BSPSMA/CD28-001 (also referred to as mAb1 or REGN5678)	(SEQ ID NO: 1)	(SEQ ID NO: 5)	(SEQ ID NO: 9)
BSPSMA/CD28-002	(SEQ ID NO: 16)	(SEQ ID NO: 20)	(SEQ ID NO: 28)
BSPSMA/CD28-003	(SEQ ID NO: 16)	(SEQ ID NO: 24)	(SEQ ID NO: 28)

Example 2: A Phase 1/2 Study of a Bispecific Anti-PSMA x Anti-CD28 Antibody in Patients with Clear Cell Renal Cell Carcinoma

[0103] This is an open-label, phase 1/2, first-in-human study evaluating safety, tolerability, pharmacokinetics (PK), and anti-tumor activity of mAb1 (REGN5678) in treatment-experienced clear cell renal cell carcinoma (ccRCC).

[0104] Study Objectives

[0105] The primary objectives of the study are:

- To evaluate safety, tolerability and pharmacokinetics (PK) of mAb1 alone and in combination with cemiplimab (dose escalation)
- To assess efficacy, as measured by objective response rate (ORR) per response evaluation criteria in solid tumors (RESIST) 1.1 criteria, of mAb1 alone and in combination with cemiplimab (dose expansion)

[0106] The secondary objectives of the study are:

- To assess efficacy, as measured by ORR per RESIST 1.1 criteria, of mAb1 alone and in combination with cemiplimab (dose escalation and dose expansion)
- To characterize the safety profile in each expansion cohort (dose expansion)
- To characterize the PK of mAb1 alone and in combination with cemiplimab (dose expansion)
- To assess immunogenicity of mAb1 (dose escalation and dose expansion)

[0107] Study Design

[0108] This is an open-label, phase 1/2, first-in-human study evaluating safety, tolerability, PK, and anti-tumor activity of mAb1 (anti-PSMAxCD28) in treatment-experienced clear cell renal cell carcinoma (ccRCC). Upon disease progression, low dose cemiplimab (3 mg IV Q3W) may be

added after a minimum 6 weeks of mAb1 monotherapy in both dose escalation and dose expansion cohorts. There are 2 parts of the study: Dose escalation; and Dose expansion.

[0109] During dose escalation, patients will receive monotherapy mAb1 up to a dose of 900 mg intravenously (IV) once weekly (QW).

[0110] During dose expansion, participants will receive mAb1 at the MTD/ presumptive RP2D.

[0111] Study Duration

[0112] The total duration of study participation for each patient will vary based on the occurrence of 1 or more of the following: disease progression, intolerable adverse events (AEs), withdrawal of consent, or study withdrawal criterion is met. For participants in dose escalation and expansion cohorts, the study consists of 4 periods: a screening period of up to 28 days; a treatment period with mAb1 monotherapy consisting of a series of 6-week (42 day) cycles of treatment; for participants with progressive disease after a minimum of 6 weeks of mAb1 monotherapy, a combination treatment period with low dose cemiplimab (3 mg IV Q3W) consisting of a series of 6-week (42 day) cycles of treatment; and a follow-up period (90 days) for participants who discontinue therapy, following which participants will be followed for survival.

[0113] Dose escalation of mAb1 will proceed QW from 30 mg up to 900 mg (at doses of 30 mg, 100 mg, 300 mg, 600 mg, and 900 mg).

[0114] Study Population

[0115] The study population includes men and women with metastatic ccRCC who have progressed on or after ≥1 line prior systemic therapy approved in the metastatic setting. Prior treatment must include an anti-PD-1/PD-L1 therapy and either ipilimumab and/or a tyrosine kinase inhibitor.

[0116] Inclusion Criteria: A patient must meet the following criteria to be eligible for inclusion in the study:

1. Men and women ≥18 years of age.
2. Histologically or cytologically confirmed renal cell carcinoma with a clear-cell component.
3. Diagnosis of metastatic clear cell renal cell carcinoma (ccRCC) with at least one measurable lesion via response evaluation criteria in solid tumors (RESIST) 1.1 criteria.
4. Has progressed on or after ≥1 line prior systemic therapy approved in the metastatic setting. Prior treatment must include an anti-PD-1/PD-L1 therapy and either ipilimumab and/or a tyrosine kinase inhibitor.
5. Able and willing to provide tumor tissue, either archival or newly obtained. If archival or fresh tissue is not available, a pathology report that confirms diagnosis may be submitted.
6. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1

7. Adequate organ and bone marrow function documented by:
    a. Hemoglobin ≥8.5 g/dL
    b. Absolute neutrophil count ≥1.5 x 10^9/L)
    c. Platelet count ≥100 x 10^9/L
8. Serum creatinine ≤1.5 x ULN or estimated glomerular filtration rate >50 mL/min/1.73 m^2. A 24-hour urine creatinine collection may substitute for the calculated creatinine clearance to meet eligibility criteria
9. Adequate hepatic function:
    a. Total bilirubin ≤1.5 x ULN (≤3 x ULN if tumor liver involvement)
    b. AST ≤2.5 x ULN (≤5 x ULN if tumor liver involvement)
    c. ALT ≤2.5 x ULN (≤5 x ULN if tumor liver involvement)
    d. Alkaline Phosphatase (ALP) ≤2.5 x ULN (≤5 x ULN if tumor liver or bone involvement)
    NOTES:
    - In patients with tumor liver involvement if levels of AST ≥3 x ULN or ALT ≥3 x ULN, and bilirubin levels ≥2 x ULN will be excluded regardless of the above criteria
    - Patients with Gilbert's syndrome do not need to meet total bilirubin requirements provided their total bilirubin is not greater than their historical level. Gilbert's syndrome must be documented appropriately as past medical history
10. Willing and able to comply with clinic visits and study-related procedures
11. Provide informed consent signed by study patient
12. Able to understand and complete study-related questionnaires

[0117] Exclusion Criteria: A patient who meets any of the following criteria will be excluded from the study:

1. Currently receiving treatment in another study
2. Has participated in a study of an investigational agent or an investigational device within 4 weeks of first dose of study therapy
3. Has received treatment with an approved systemic therapy (including sipuleucel-T) within 3 weeks of dosing or has not yet recovered (*i.e.*, grade ≤1 or baseline) from any acute toxicities except for laboratory changes as described in inclusion criteria and as below:
    a. Patients with grade 2 ≤ neuropathy
    b. alopecia
4. Has received radiation therapy or major surgery within 14 days of first administration of study drug or has not recovered (*i.e.*, grade ≤1 or baseline) from AEs, except for laboratory changes as described in inclusion criteria and as below:

a. Patients with grade ≤2 neuropathy

5. Has received any previous systemic biologic therapy within 5 half-lives of first dose of study therapy, except that anti-PD-1 therapy, which requires washout of 3 half-lives
   Exception: Patients previously treated with bevacizumab, or other non-immunomodulatory antibodies with half-lives longer than 7 days are permitted after a discussion with the sponsor if at least 30 days have elapsed since last treatment.
6. Has received prior PSMA-targeting therapy
7. **Dose Escalation:** Has had prior anti-cancer immunotherapy (other than sipuleucel-T) within 5 half-lives prior to study therapy. Examples of immune modulating agents include blockers of CTLA-4, 4-1BB (CD137), or OX-40, therapeutic vaccines, anti-PD-1/PD-L1, phosphoinositide 3-kinase (PI3K) delta inhibitors, or cytokine anticancer treatments. NOTE: Patients who have received prior investigational cell-based therapies (*e.g.*, CAR-T cells) are excluded. Exception is approved anti-PD-1 therapy following washout of 3 half-lives
8. **Dose Expansion:** Has had prior anti-cancer immunotherapy. Examples of immune modulating agents include blockers of CTLA-4, 4-1BB (CD137), or OX-40, therapeutic vaccines, anti-PD-1/PD-L1, PI3Kdelta inhibitors, CAR-T cells, or cytokine anticancer treatments. NOTE: Prior treatment with sipuleucel-T is permitted
9. Participants who have not recovered (*i.e.*, grade ≤1 or baseline) from immune-mediated AEs 3 months prior to initiation of study drug therapy except for endocrinopathies adequately managed with hormone replacement
10. Participants who have permanently discontinued anti-cancer immune modulating therapies due to immune-related AEs
11. Another malignancy that is progressing or requires active treatment, except:
    a. Non-melanoma skin cancer that has undergone potentially curative therapy
    b. Any tumor that has been deemed to be effectively treated with definitive local control (with or without continued adjuvant hormonal therapy)
12. Any condition that requires ongoing/continuous corticosteroid therapy (>10 mg prednisone/day or anti-inflammatory equivalent) within 1 week prior to the first dose of study therapy. Patients who require a brief course of steroids (up to 2 days in the week before enrollment) or physiologic replacement are not excluded
13. Ongoing or recent (within 5 years) evidence of significant autoimmune disease that required treatment with systemic immunosuppressive treatments. The following are not exclusionary: vitiligo, childhood asthma that has resolved, endocrinopathies (such as hypothyroidism or

type 1 diabetes) that require only hormone replacement, or psoriasis that does not require systemic treatment

14. History of CNS metastases, including previously treated metastases
15. Encephalitis, meningitis, neurodegenerative disease (with the exception of mild dementia that does not interfere with activities of daily living [ADLs]) or uncontrolled seizures in the year prior to first dose of study therapy
16. Known history of, or any evidence of interstitial lung disease, or active, non-infectious pneumonitis (past 5 years). A history of radiation pneumonitis in the radiation field is permitted
17. Uncontrolled infection with human immunodeficiency virus (HIV), hepatitis B or hepatitis C infection; or diagnosis of immunodeficiency

    NOTES:

    - Patients will be tested for hepatitis C virus (HCV) and hepatitis B virus (HBV) at screening.
    - Patients with HIV who have controlled infection (undetectable viral load (HIV RNA polymerase chain reaction [PCR]) and CD4 count above 350 either spontaneously or on a stable antiviral regimen) are permitted.
    - Patients with hepatitis B surface antigen positive (HepBsAg+) who have controlled infection (serum hepatitis B virus DNA PCR that is below the limit of detection AND receiving antiviral therapy for hepatitis B) are permitted.
    - Participants with HBsAg negative but total HBV core antibody positive (HBcAb+) are permitted with the following requirements: If serum HBV DNA PCR is above the limit of detection at screening, antiviral therapy for HBV must be initiated prior to study entry. If serum HBV DNA PCR is below the limit of detection, periodic monitoring of HBsAg must be performed.
    - Patients who are hepatitis C antibody positive (HCV Ab+) who have controlled infection (undetectable HCV RNA by PCR either spontaneously or in response to a successful prior course of anti-HCV therapy) are permitted.
18. Any infection requiring hospitalization or treatment with IV anti-infectives within 2 weeks of first dose of study therapy
19. Receipt of a live vaccine within 4 weeks of planned start of study medication.
20. Prior allogeneic stem cell transplantation or recipients of organ transplants at any time, or autologous stem cell transplantation within 12 weeks of the start of study treatment
21. Has known allergy or hypersensitivity to cemiplimab or components of study drugs.

22. Known psychiatric or substance abuse disorders that would interfere with participation with the requirements of the study
23. Any medical condition, co-morbidity, physical examination finding, metabolic dysfunction, or clinical laboratory abnormality that, in the opinion of the investigator, renders the patient unsuitable for participation in a clinical trial due to high safety risks and/or potential to affect interpretation of results of the study including, but not limited to, significant cardiovascular disease (*e.g.*, New York Heart Association Class III or IV cardiac disease, myocardial infarction within the previous 6 months, unstable arrhythmias or unstable angina) and/or significant pulmonary disease (*e.g.*, obstructive pulmonary disease and history of symptomatic bronchospasm).
24. Active infection including
    a. Infection requiring hospitalization or treatment with IV anti-infectives within 2 weeks of start of study therapy
    b. Known active tuberculosis or history of incompletely treated active or latent tuberculosis. Acceptable treatments for latent tuberculosis would be 9 months of isoniazid 300 mg by mouth daily or equivalent proven regimen)
25. History of invasive opportunistic infections, including but not limited to histoplasmosis, coccidioidomycosis, Pneumocystic jirovecii, or aspergillosis, or John Cunningham virus (progressive multifocal leukoencephalopathy).

[0118] Study Treatments

[0119] mAb1 at the assigned dose level will be administered QW or Q3W either by IV infusion over 30 minutes to 2 hours or by SC injection. Cemiplimab (for designated cohorts) will be administered by IV infusion over 30 minutes Q3W. Low dose cemiplimab (3 mg) will be administered by either IV infusion or IV injection as a bolus of drug Q3W to eligible participants. When both mAb1 and cemiplimab are administered on the same day, mAb1 will be administered first.

[0120] Study Endpoints

[0121] The study's primary endpoints are assessed for both mAb1 alone and in combination with cemiplimab:

Dose Escalation:

- Safety, as measured by the incidence and severity of treatment-emergent adverse events (TEAEs)/adverse events of special interest (AESIs)/serious adverse events (SAEs) and grade ≥3 laboratory abnormalities during the treatment period and up to 90 days after the

last dose of study treatment or until the start of new therapy for treatment of the patient's tumor, whichever occurs first.

- Tolerability, as measured by the incidence of dose-limiting toxicities (DLTs) from the first dose through the end of the DLT observation period for mAb1 alone and in combination with cemiplimab (if applicable)
- mAb1 concentrations in serum over time

Dose Expansion:

- ORR per RESIST 1.1 criteria based on confirmed radiologic complete response (CR) or partial response (PR).

[0122] **Results** – the administration of ccRCC patients with mAb1 or a combination of mAb1 and cemiplimab will be well tolerated and treat ccRCC.

[0123] The present disclosure is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the disclosure in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Sequences

SEQ ID NO: 1
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFMSYDGSNKFYSDSVKGRFTISRD
NSRKMLFLQMNNLRAEDTAVYYCARDQYYDFLTDHGVFDYWGQGTLVTVSS

SEQ ID NO: 2
GFTFSSYG

SEQ ID NO: 3
MSYDGSNK

SEQ ID NO: 4
ARDQYYDFLTDHGVFDY

SEQ ID NO: 5
QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGITHYNPSLKSRVTISVDT
SKIQFSLKLSSVTAADTAVYYCARWGVRRDYYYYGMDVWGQGTTVTVSS

SEQ ID NO: 6
GGSISSYY

SEQ ID NO: 7
IYYSGIT

SEQ ID NO: 8
ARWGVRRDYYYYGMDV

SEQ ID NO: 9
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFT
LTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK

SEQ ID NO: 10
RASQSVSSSYLA

SEQ ID NO: 11
GASSRAT

SEQ ID NO: 12
QQYGSSPWT

SEQ ID NO: 13
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFMSYDGSNKFYSDSVKGRFTISRD
NSRKMLFLQMNNLRAEDTAVYYCARDQYYDFLTDHGVFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSEST
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ
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YTQKSLSLSLGK

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SKIQFSLKLSSVTAADTAVYYCARWGVRRDYYYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKR
VESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNRFT
QKSLSLSPGK

SEQ ID NO: 15
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFT
LTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 16
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYAGNNKYYADSVKGRFTVSRD
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SEQ ID NO: 17
GFTFSSYG

SEQ ID NO: 18
ISYAGNNK

SEQ ID NO: 19
AKDSYYDFLTDPDVLDI

SEQ ID NO: 20
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GFTFSTYA

SEQ ID NO: 22
ISGSGDNT

SEQ ID NO: 23
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SEQ ID NO: 24
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SEQ ID NO: 25
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SEQ ID NO: 26
ISSNGGRT

SEQ ID NO: 27
TRDDELLSFDY

SEQ ID NO: 28
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TISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIK

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QSISSY

SEQ ID NO: 30
AAS

SEQ ID NO: 31
QQSYSTPPIT

SEQ ID NO: 32
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NSKKTLYLQMNSLRSEDTAVYYCAKDSYYDFLTDPDVLDIWGQGTMVTVSSASTKGPSVFPLAPCSRSTSEST
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNH
YTQKSLSLSLGK

SEQ ID NO: 33
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NSKNTLYLQMNSLRADDSAIYHCAKDSGYCSSGGCSLYFYYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPS
NTKVDKRVESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE

MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE
ALHNRFTQKSLSLSPGK

SEQ ID NO: 34
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VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESK
YGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNRFTQKSL
SLSPGK

SEQ ID NO: 35
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTL
TISSLQPEDFATYYCQQSYSTPPITFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 36
EVQLLESGGVLVQPGGSLRLSCAASGFTFSNFGMTWVRQAPGKGLEWVSGISGGGRDTYFADSVKGRFTISRD
NSKNTLYLQMNSLKGEDTAVYYCVKWGNIYFDYWGQGTLVTVSS

SEQ ID NO: 37
GFTFSNFG

SEQ ID NO: 38
ISGGGRDT

SEQ ID NO: 39
VKWGNIYFDY

SEQ ID NO: 40
DIQMTQSPSSLSASVGDSITITCRASLSINTFLNWYQQKPGKAPNLLIYAASSLHGGVPSRFSGSGSGTDFTL
TIRTLQPEDFATYYCQQSSNTPFTFGPGTVVDFR

SEQ ID NO: 41
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SEQ ID NO: 42
AAS

SEQ ID NO: 43
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SEQ ID NO: 44
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KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKY
GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLGK

SEQ ID NO: 45

DIQMTQSPSSLSASVGDSITITCRASLSINTFLNWYQQKPGKAPNLLIYAASSLHGGVPSRFSGSGSGTDFTL
TIRTLQPEDFATYYCQQSSNTPFTFGPGTVVDFRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

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What is claimed is:

1. A method of treating clear cell renal cell carcinoma in a subject in need thereof, comprising administering to the subject a bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds prostate specific membrane antigen (PSMA), and a second antigen-binding domain that specifically binds human CD28.

2. The method of claim 1, wherein the bispecific antibody is administered to the subject at a dose of at least 30 mg once weekly (QW) for at least three weeks.

3. The method of claim 1 or 2, wherein the clear cell renal cell carcinoma is metastatic clear cell renal cell carcinoma.

4. The method of any one of claims 1-3, wherein the subject has received at least one prior therapy for clear cell renal cell carcinoma.

5. The method of claim 4, wherein the subject has received an anti-PD-1 therapy.

6. The method of claim 4 or 5, wherein the subject has received an anti-CTLA-4 therapy and/or a tyrosine kinase inhibitor.

7. The method of claim 6, wherein the anti-CTLA-4 therapy is ipilimumab.

8. The method of any one of claims 2-7, wherein the bispecific antibody or antigen-binding fragment thereof is administered for at least three weeks, at least four weeks, at least five weeks, or at least six weeks as a monotherapy.

9. The method of any one of claims 1-8, further comprising administering an antibody or antigen-binding fragment thereof that specifically binds programmed death receptor-1 (PD-1), wherein the antibody or antigen-binding fragment is administered at a dose of from 3 mg to 100 mg.

10. The method of any one of claims 1-8, further comprising administering an antibody or antigen-binding fragment thereof that specifically binds programmed death receptor-1 (PD-1), wherein the antibody or antigen-binding fragment is administered at a dose of from 3 mg to 350 mg.

11. The method of any one of claims 1-10:

(a) wherein the first antigen-binding domain comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 1; and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 9, and

(b) wherein the second antigen-binding domain comprises three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 5; and three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 9.

12. The method of claim 11, wherein the first antigen-binding domain comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO: 2, a HCDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 4.

13. The method of claim 11 or 12, wherein the second antigen-binding domain comprises a HCDR1 comprising the amino acid sequence of SEQ ID NO: 6, a HCDR2 comprising the amino acid sequence of SEQ ID NO: 7, and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 8.

14. The method of any one of claims 11-13, wherein the first antigen-binding domain and the second antigen-binding domain comprise a LCDR1 comprising the amino acid sequence of SEQ ID NO: 10, a LCDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 12.

15. The method of any one of claims 11-14, wherein the first antigen-binding domain comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 1, and a LCVR comprising the amino acid sequence of SEQ ID NO: 9.

16. The method of any one of claims 11-15, wherein the second antigen-binding domain comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 5, and a LCVR comprising the amino acid sequence of SEQ ID NO: 9.

17. The method of any one of claims 1-16, wherein the bispecific antibody comprises a human IgG heavy chain constant region.

18. The method of claim 17, wherein the human IgG heavy chain constant region is isotype IgG1.

19. The method of claim 17, wherein the human IgG heavy chain constant region is isotype IgG4.

20. The method of claim 18 or 19, wherein the bispecific antibody comprises a chimeric hinge that reduces Fcγ receptor binding relative to a wild-type hinge of the same isotype.

21. The method of any one of claims 17-20, wherein the first heavy chain or the second heavy chain, but not both, comprises a CH3 domain comprising a H435R (EU numbering) modification and a Y436F (EU numbering) modification.

22. The method of any one of claims 1-16, wherein the bispecific antibody comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO: 13.

23. The method of any one of claims 1-16, wherein the bispecific antibody comprises a second heavy chain comprising the amino acid sequence of SEQ ID NO: 14.

24. The method of any one of claims 1-16, wherein the bispecific antibody comprises a first heavy chain comprising the amino acid sequence of SEQ ID NO: 13, a second heavy chain comprising the amino acid sequence of SEQ ID NO: 14, and a common light chain comprising the amino acid sequence of SEQ ID NO: 15.

25. The method of any one of claims 9-24, wherein the antibody or antigen-binding fragment thereof that binds PD-1 comprises:

(a) three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 36; and

(b) three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 40.

26. The method of claim 25, wherein the antibody or antigen-binding fragment thereof that binds PD-1 comprises a HCDR1 comprising the amino acid sequence of SEQ ID

NO: 37, a HCDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a HCDR3 comprising the amino acid sequence of SEQ ID NO: 39.

27. The method of claim 25 or 26, wherein the antibody or antigen-binding fragment thereof that binds PD-1 comprises a LCDR1 comprising the amino acid sequence of SEQ ID NO: 41, a LCDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a LCDR3 comprising the amino acid sequence of SEQ ID NO: 43.

28. The method of any one of claims 25-27, wherein the antibody or antigen-binding fragment thereof that binds PD-1 comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 36, and a LCVR comprising the amino acid sequence of SEQ ID NO: 40.

29. The method of claim 28, wherein the antibody or antigen-binding fragment thereof that binds PD-1 is an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 44 and a light chain comprising the amino acid sequence of SEQ ID NO: 45.

30. The method of any one of claims 1-29, wherein the bispecific antibody or antigen-binding fragment thereof is administered to the subject at a dose of from 30 mg to 900 mg once weekly for at least three weeks.

31. The method of claim 30, wherein the bispecific antibody or antigen-binding fragment thereof is administered to the subject at a dose of from 100 mg to 900 mg once weekly for at least three weeks.

32. The method of claim 30, wherein the bispecific antibody or antigen-binding fragment thereof is administered to the subject at a dose of from 300 mg to 900 mg once weekly for at least three weeks.

33. The method of claim 30, wherein the bispecific antibody or antigen-binding fragment thereof is administered to the subject at a dose of 30 mg, 100 mg, 300 mg, 600 mg or 900 mg once weekly for at least three weeks.