(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 April 2023 (06.04.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/056461 A1

(51) International Patent Classification:
*A61K 39/395* (2006.01) *A61P 35/00* (2006.01)
*C07K 14/42* (2006.01) *A61P 35/04* (2006.01)

(21) International Application Number:
PCT/US2022/077408

(22) International Filing Date:
30 September 2022 (30.09.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/251,227 01 October 2021 (01.10.2021) US
63/277,384 09 November 2021 (09.11.2021) US

(71) Applicant: **PURETECH LYT, INC.** [US/US]; 6 Tide Street, Suite 400, Boston, Massachusetts 02210 (US).

(72) Inventors: **FILIPOVIC, Aleksandra**; 6 Tide Street, Suite 400, Boston, Massachusetts 02210 (US). **ELENKO, Eric**; 6 Tide Street, Suite 400, Boston, Massachusetts 02210 (US). **BOLEN, Joseph**; 6 Tide Street, Suite 400, Boston, Massachusetts 02210 (US). **PADEN, Heather**; 6 Tide Street, Suite 400, Boston, Massachusetts 02210 (US). **KORTH, Christopher**; 6 Tide Street, Suite 400, Boston, Massachusetts 02210 (US).

(74) Agent: **CHEN, Yahua**; McDermott Will & Emery LLP, 500 North Capitol Street, NW, Washington, District of Columbia 20001-1531 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: ANTI-GALECTIN-9 ANTIBODIES AND THERAPEUTIC USES THEREOF

(57) Abstract: Methods for treating solid tumors (*e.g.*, head and neck cancer, urothelial carcinoma, *etc.*) using an anti-Galectin-9 antibody (e.g., G9.2-17(IgG4)) in combination with tislelizumab.

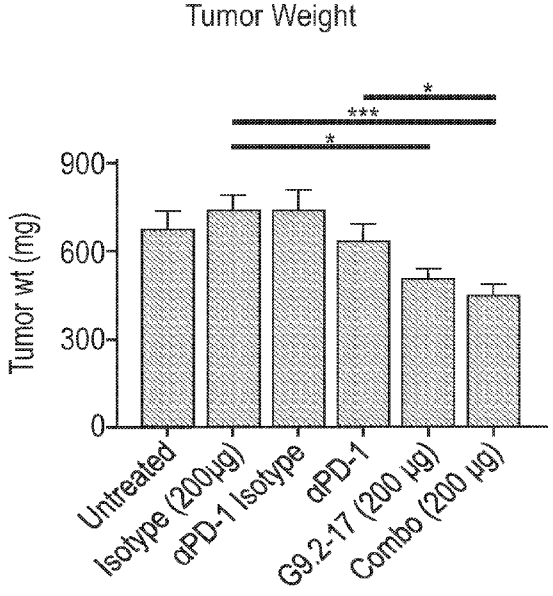



Figure 1

WO 2023/056461 A1

[Continued on next page]

**Published:**

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

# ANTI-GALECTIN-9 ANTIBODIES AND THERAPEUTIC USES THEREOF

## CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 63/251,227, filed October 1, 2021, and U.S. Provisional Application No. 63/277,384, filed November 9, 2021, the contents of each of which are incorporated by reference herein in their entirety.

## SEQUENCE LISTING

The instant application contains a Sequence Listing which has been filed electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on September 30, 2022, is named 112174-0234-NP003WO01_SEQ.xml and is 25,891 bytes in size.

## BACKGROUND OF INVENTION

The immune system holds remarkable potential to recognize and destroy cancer cells, but the complex network governing tumor immune escape is an obstacle to broadly effective immune modulation (Martinez-Bosch N, et al., Immune Evasion in Pancreatic Cancer: From Mechanisms to Therapy. Cancers (Basel). 2018;10 (1)). Approved immuno-oncology (IO) agents deliver incremental survival improvements to many tumor types (*e.g.*, melanoma, lung, renal, bladder cancer, some colon cancers etc.), and are being rapidly integrated as standard of care in addition to and in conjunction with surgery, chemotherapy, and radiotherapy. However, there is still a major gap in the treatment and survivorship of multiple other aggressive malignancies. For example, metastatic pancreatic ductal adenocarcinoma (PDAC or PDA)), cholangiocarcinoma (CCA) and colorectal cancer (CRC) still have 5-year survival rates of < 9%, < 5 % and < 15%, respectively. These gastrointestinal tumors are very aggressive, many patients have advanced-stage disease at presentation, and the effectiveness of approved immunotherapies is suboptimal (Rizvi, et al., Cholangiocarcinoma - evolving concepts and therapeutic strategies; Nat Rev Clin Oncol. 2018;15(2):95-111; Kalyan, et al., Updates on immunotherapy for colorectal cancer; J Gastrointest Oncol. 2018;9(1):160-169).

The success of first-generation checkpoint inhibitors (anti-PD-1, anti-PD-L1, and anti-CTLA4) has led to an explosion of new IO clinical trial efficacy and differentiation (Holl et al., Examining Peripheral and Tumor Cellular Immunome in Patients with Cancer; Front Immunol.

2019; 10:1767). However, among successes, there have also been many unfortunate development failures, consequently, there is still a need for more novel and efficacious treatments.

Galectin-9 is a tandem-repeat lectin consisting of two carbohydrate recognition domains (CRDs) and was discovered and described for the first time in 1997 in patients suffering from Hodgkin's lymphoma (HL) (Tureci et al., J. Biol. Chem. 1997, 272, 6416–6422). Three isoforms exist and can be located within the cell or extracellularly. Elevated Galectin-9 levels have been in observed a wide range of cancers, including melanoma, Hodgkin's lymphoma, hepatocellular, pancreatic, gastric, colon and clear cell renal cell cancers (Wdowiak et al. Int. J. Mol. Sci. 2018, 19, 210). In renal cancer, patients with high Galectin-9 expression showed more advanced progression of the disease with larger tumor size (Kawashima et al.; BJU Int. 2014;113:320–332). In melanoma, Galectin-9 was expressed in 57% of tumors and was significantly increased in the plasma of patients with advanced melanoma compared to healthy controls (Enninga et al., Melanoma Res. 2016 Oct; 26(5): 429–441). A number of studies have shown utility for Galectin-9 as a prognostic marker, and more recently as a potential new drug target (Enninga et al., 2016; Kawashima et al. BJU Int 2014; 113: 320–332; Kageshita et al., Int J Cancer. 2002 Jun 20;99(6):809-16, and references therein).

Galectin-9 has been described to play an important role in in a number of cellular processes such as adhesion, cancer cell aggregation, apoptosis, and chemotaxis. Recent studies have shown a role for Galectin-9 in immune modulation in support of the tumor, e.g., through negative regulation of Th1 type responses, Th2 polarization and polarization of macrophages to the M2 phenotype. This work also includes studies that have shown that Galectin-9 participates in direct inactivation of T cells through interactions with the T-cell immunoglobulin and mucin protein 3 (TIM-3) receptor (Dardalhon et al., *J Immunol.*, 2010, 185, 1383-1392; Sanchez-Fueyo et al., *Nat Immunol.*, 2003, 4, 1093-1101).

Galectin-9 has also been found to play a role in polarizing T cell differentiation into tumor suppressive phenotypes), as well as promoting tolerogenic macrophage programming and adaptive immune suppression (Daley et al., *Nat Med.*, 2017, 23, 556-567). In mouse models of pancreatic ductal adenocarcinoma (PDAC), blockade of the checkpoint interaction between Galectin-9 and the receptor Dectin-1 found on innate immune cells in the tumor microenvironment (TME) has been shown to increase anti-tumor immune responses in the TME and to slow tumor progression (Daley et al., *Nat Med., 2017, 23, 556-567*). Galectin-9

also has been found to bind to CD206, a surface marker of M2 type macrophages, resulting in a reduced secretion of CVL22 (MDC), a macrophage derived chemokine which has been associated with longer survival and lower recurrence risk in lung cancer (Enninga et al, J Pathol. 2018 Aug;245(4):468-477).

## SUMMARY OF INVENTION

The present disclosure is based, at least in part, on the development of treatment regimen for solid tumors (*e.g.*, metastatic solid tumors) such as head and neck cancer or urothelial carcinomar, either alone or in combination with a checkpoint inhibitor such as an anti-PD-1 antibody (e.g., tislelizumab).

Accordingly, the present disclosure provides, in some aspects, a method for treating a solid tumor, the method comprising administering to a subject in need thereof (a) an effective amount of an antibody that binds human Galectin-9 (anti-Galectin-9 antibody) and (b) an effective amount of an anti-PD-1 antibody such as tislelizumab. In some embodiments, the anti-Galectin-9 antibody may comprise: (i) a light chain variable region ($V_L$) comprising a light chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 1, a light chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 2, and a light chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 3, and (ii) a heavy chain variable region comprising a heavy chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 4, a heavy chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 5, and a heavy chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 6. In some instances, the anti-Galectin-9 antibody (*e.g.*, G9.2-17(IgG4) as disclosed herein) may be administered to the subject at a dose of about 0.2 mg/kg to about 18 mg/kg. In some examples, the anti-Galectin-9 antibody may be administered to the subject once a week.

In some embodiments, the solid tumor is head and neck cancer, urothelial cancer, gastric esophageal cancer, or non-small cell lung cancer. In some embodiments, the solid tumor is a metastatic tumor (*e.g.*, locally advanced or metastatic solid tumor). In some embodiments, the solid tumor is refractory and/or relapsed. In some embodiments, the subject to be treated by any of the methods disclosed herein is a human patient having the solid tumor.

In some embodiments, the anti-Galectin-9 antibody (*e.g.*, G9.2-17(IgG4) as disclosed herein) may be administered to the subject at a dose of about 4 mg/kg to about 18 mg/kg, for

example, the anti-Galectin-9 antibody is administered to the subject at a dose of about 4 mg/kg, about 6.3 mg/kg, about 10 mg/kg, about 12 mg/kg, about 14 mg/kg, about 16 mg/kg, or about 18 mg/kg. In one example, the dose of the anti-Galecti-9 antibody is about 6.3 mg/kg. In another example, the dose of the anti-Galectin-9 antibody is about 10 mg/kg. In yet another example, the dose of the anti-Galectin-9 antibody is about 16 mg/kg.

In some specific examples, the anti-Galectin-9 antibody (*e.g.*, G9.2-17(IgG4) as disclosed herein) may be administered to the subject at a dose of about 6.3 mg/kg once a week. In some specific examples, the anti-Galectin-9 antibody (*e.g.*, G9.2-17(IgG4) as disclosed herein) may be administered to the subject at a dose of about 10 mg/kg once a week. In other specific examples, the anti-Galectin-9 antibody (*e.g.*, G9.2-17(IgG4) as disclosed herein) may be administered to the subject at a dose of about 16 mg/kg once a week. Alternatively or in addition, the anti-Galectin-9 antibody may be administered to the subject by intravenous infusion.

In some embodiments, tislelizumab is administered to the subject at a dose of about 200 mg once every 3 weeks, at a dose of about 300 mg every 4 weeks, or at a dose of about 400 mg every six weeks. In one example, tislelizumab is administered to the subject at a dose of about 300 mg every 4 weeks. Alternatively or in addition, the tislelizumab is administered to the subject by intravenous infusion.

In one example, the method disclosed herein comprise administration of the the anti-Galectin-9 antibody (*e.g.*, G9.2-17(IgG4)) at a dose of about 6.3 mg/kg once a week and administration of tislelizumab at a dose of about 300 mg every 4 weeks. Both antibodies may be administered via intravenous infusion.

In one example, the method disclosed herein comprise administration of the the anti-Galectin-9 antibody (*e.g.*, G9.2-17(IgG4)) at a dose of about 10 mg/kg once a week and administration of tislelizumab at a dose of about 300 mg every 4 weeks. Both antibodies may be administered via intravenous infusion.

In one example, the method disclosed herein comprise administration of the the anti-Galectin-9 antibody (*e.g.*, G9.2-17(IgG4)) at a dose of about 16 mg/kg once a week and administration of tislelizumab at a dose of about 300 mg every 4 weeks. Both antibodies may be administered via intravenous infusion.

In some examples, tislelizumab is administered to the subject on a day when the subject receives the anti-Galectin 9 antibody. Alternatively, the administration of tislelizumab and the administration of the anti-Galectin 9 antibody are on two consecutive

days. In some examples, the administration of tislelizumab is performed prior to the administration of the anti-Galectin 9 antibody.

In any of the methods disclosed herein, the anti-Galectin-9 antibody may comprise a $V_L$ chain comprising the amino acid sequence of SEQ ID NO: 8, and a $V_H$ chain comprising the amino acid sequence of SEQ ID NO: 7. In some instances, the anti-Galectin-9 antibody is an IgG1 or IgG4 molecule. For example, the anti-Galectin-9 antibody is an IgG4 molecule having a modified Fc region of human IgG4. In some examples, the modified Fc region of human IgG4 comprises the amino acid sequence of SEQ ID NO: 14. In one example, the anti-Galectin-9 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 19 and a light chain comprising the amino acid sequence of SEQ ID NO: 15.

In any of the methods disclosed herein, the subject has undergone one or more prior anti-cancer therapies. In some examples, the one or more prior anti-cancer therapies comprise chemotherapy, immunotherapy, radiation therapy, a therapy involving a biologic agent, or a combination thereof. In some instances, the subject has progressed disease through the one or more prior anti-cancer therapies or is resistant to the one or more prior therapies.

In some embodiments, the subject is a human patient having an elevated level of Galectin-9 relative to a control value. For example, the human patient has an elevated serum or plasma level of Galectin-9 relative to the control value. In some instances, the human patient has cancer cells expressing Galectin-9, immune cells expressing Galectin-9, or both.

Any of the methods disclosed herein may further comprise monitoring occurrence of adverse effects in the subject. Alternatively or in addition, the method may further comprise reducing the dose of the anti-Galectin-9 antibody, the dose of tislelizumab, or both, when an adverse effect occurs.

Also within the scope of the present disclosure are pharmaceutical compositions for use in treating a solid tumor (*e.g.*, those described herein and including metastatic solid tumors), and uses of any of the anti-Galectin-9 antibodies and the anti-PD-1 antibody such as tislelizumab for manufacturing a medicament for treating the solid tumor, wherein the uses disclosed herein, in some embodiments, involve one or more of the treatment conditions (*e.g.*, dose, dosing regimen, administration route, etc.) as also disclosed herein.

The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention are apparent from the following drawing and detailed description of several embodiments, and also from the appended claims.

## BRIEF DESCRIPTION OF DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to the drawing in combination with the detailed description of specific embodiments presented herein.

***FIGURE 1*** depict graphs showing results of a study in which mice treated with G9.2-17 mIgG2a alone or in combination with αPD-1 mAb. Mice (n=10/group) with orthotopically implanted KPC tumors were treated with commercial αPD-1 (200μg) mAb or G9.2-17 mIg2a (200μg), or a combination of G9.2-17 and αPD-1, or matched isotype once weekly for three weeks. Tumors were removed and weighed. Each point represents one mouse; $*p<0.05$; $**p<0.01$; $***p<0.001$; $****p<0.0001$; by unpaired Student's *t*-test.

***FIGURES 2A*** and ***2B*** depict graphs showing the effect of G9.2-17 in a B16F10 subcutaneous syngeneic model. Tumors were engrafted subcutaneously and treated with G9.2-17 IgG1 mouse mAb, anti-PD-1 antibody or a combination of G9.2-17 IgG1 mouse mAb and anti-PD-1 antibody. *Figure 9A* depicts a graph showing the effect on tumor volume. *Figure 9B* depicts a graph showing intratumoral CD8 T cell infiltration. Results show that intra-tumoral presence effector T cells were enhanced in the combination arm.

***FIGURES 3A and 3B*** include charts showing cholangiocarcinoma patient-derived tumor cultures *ex vivo* (organoids) treated with G9.2-17. Patient derived tumor cultures ex vivo (organoids) were treated with G9.2-17 or isotype control for three days. Expression of CD44 (*Figure 3A*), and TNFα (*Figure 3B*) in CD3+ T cells from PDOTS was assessed.

## DETAILED DESCRIPTION OF INVENTION

Provided herein are methods of using anti-Galectin-9 antibodies, *e.g.*, G9.2-17, for treating solid tumors, for example, head and neck cancer, urothelial carcinoma, and other solid tumors as disclosed herein, in combination with a checkpoint inhibitor such as an anti-PD-1 antibody (*e.g.*, tisleilizumab). In some embodiments, the cancers are metastatic. In some embodiments, the methods disclosed herein provide specific doses and/or dosing schedules. In some instances, the methods disclosed herein target specific patient populations, for example, patients who have undergone prior treatment and show disease progression through the prior treatment, or patients who are resistant (de novo or acquired) to the prior treatment.

Galectin-9, a tandem-repeat lectin, is a beta-galactoside-binding protein, which has been shown to have a role in modulating cell-cell and cell-matrix interactions. It is found to be

strongly overexpressed in Hodgkin's disease tissue and in other pathologic states. It has in some instances also been found circulating in the tumor microenvironment (TME).

Galectin-9 is found to interact with Dectin-1, an innate immune receptor which is highly expressed on macrophages in PDAC, as well as on cancer cells (Daley, et al. *Nat Med.* 2017;23(5):556-6). Regardless of the source of Galectin-9, disruption of its interaction with Dectin-1 has been shown to lead to the reprogramming of $CD4^+$ and $CD8^+$ cells into indispensable mediators of anti-tumor immunity. Thus, Galectin-9 serves as a valuable therapeutic target for blocking the signaling mediated by Dectin-1. Accordingly, in some embodiments, the anti-Galectin-9 antibodies describe herein disrupt the interaction between Galectin-9 and Dectin-1.

Galectin-9 is also found to interact with TIM-3, a type I cell surface glycoprotein expressed on the surface of leukemic stem cells in all varieties of acute myeloid leukemia (except for M3 (acute promyelocytic leukemia)), but not expressed in normal human hematopoietic stem cells (HSCs). TIM-3 signaling resulting from Galectin-9 ligation has been found to have a pleiotropic effect on immune cells, inducing apoptosis in Th1 cells (Zhu et al., *Nat Immunol.*, 2005, 6:1245-1252) and stimulating the secretion of tumor necrosis factor-α (TNF-α), leading to the maturation of monocytes into dendritic cells, resulting in inflammation by innate immunity (Kuchroo et al., *Nat Rev Immunol.*, 2008, 8:577-580). Further Galectin-9/TIM-3 signaling has been found to co-activate NF-κB and β-catenin signaling, two pathways that promote LSC self-renewal (Kikushige et al., *Cell Stem Cell,* 2015, 17(3):341-352). An anti-Galectin-9 antibody that interferes with Galectin-9/TIM-3 binding could have a therapeutic effect, especially with respect to leukemia and other hematological malignancies. Accordingly, in some embodiments, the anti-Galectin-9 antibodies described herein disrupt the interaction between Galectin-9 and TIM-3.

Further, Galectin-9 is found to interact with CD206, a mannose receptor highly expressed on M2 polarized macrophages, thereby promoting tumor survival (Enninga et al., *J Pathol.* 2018 Aug;245(4):468-477). Tumor-associated macrophages expressing CD206 are mediators of tumor immunosuppression, angiogenesis, metastasis, and relapse (see, e.g., Scodeller et al., *Sci Rep.* 2017 Nov 7;7(1):14655, and references therein). Specifically, M1 (also termed classically activated macrophages) are trigged by Th1-related cytokines and bacterial products, express high levels of IL-12, and are tumoricidal. By contrast, M2 (so-called alternatively activated macrophages) are activated by Th2-related factors, express high level of anti-inflammatory cytokines, such as IL-10, and facilitate tumor progression (Biswas

and Mantovani; *Nat Immunol.* 2010 Oct; 11(10):889-96). The pro-tumoral effects of M2 include the promotion of angiogenesis, advancement of invasion and metastasis, and the protection of the tumor cells from chemotherapy-induced apoptosis (Hu et al., *Tumour Biol.* 2015 Dec; 36(12): 9119–9126, and references therein). Tumor-associated macrophages are thought be of M2-like phenotype and have a protumor role. Galectin-9 has been shown to mediate myeloid cell differentiation toward an M2 phenotype (Enninga et al., *Melanoma Res.* 2016 Oct; 26(5):429-41). It is possible that Galectin-9 binding CD206 may result in reprogramming TAMs towards the M2 phenotype, similar to what has been previously shown for Dectin-1. Without wishing to be bound by theory, blocking the interaction of Galectin-9 with CD206 may provide one mechanism by which an anti-Galectin-9 antibody, *e.g.*, a G9.2-17 antibody, can be therapeutically beneficial. Accordingly, in some embodiments, the anti-Galectin-9 antibodies described herein disrupt the interaction between Galectin-9 and CD206.

Galectin-9 has also been shown to interact with protein disulfide isomerase (PDI) and 4-1BB (Bi S, et al. *Proc Natl Acad Sci U S A.* 2011; 108(26):10650-5; Madireddi et al. *J Exp Med.* 2014;211(7):1433-48).

Anti-Galectin-9 antibodies can serve as therapeutic agents for treating diseases associated with Galectin-9 (*e.g.*, those in which a Galectin-9 signaling plays a role). Without being bound by theory, an anti-Galectin-9 antibody may block a signaling pathway mediated by Galectin-9. For example, the antibody may interfere with the interaction between Galectin-9 and its binding partner (*e.g.*, Dectin-1, TIM-3 or CD206), thereby blocking the signaling triggered by the Galectin-9/Ligand interaction. Alternatively, or in addition, an anti-Galectin-9 antibody may also exert its therapeutic effect by inducing blockade and/or cytotoxicity, for example, ADCC, CDC, or ADCP against pathologic cells that express Galectin-9. A pathologic cell refers to a cell that contributes to the initiation and/or development of a disease, either directly or indirectly.

The anti-Galectin-9 antibodies disclosed herein are capable of suppressing the signaling mediated by Galectin-9 (*e.g.*, the signaling pathway mediated by Galectin-9/Dectin-1 or Galectin-9/Tim-3) or eliminating pathologic cells expressing Galectin-9 via, *e.g.*, ADCC. Accordingly, the anti-Galectin-9 antibodies described herein can be used for inhibiting any of the Galectin-9 signaling and/or eliminating Galectin-9 positive pathologic cells, thereby benefiting treatment of diseases associated with Galectin-9.

Anti-Galectin-9 antibodies such as G9.2-17 were found to be effective in inducing apoptosis against cells expressing Galectin-9. Further, the anti-tumor effects of anti-Galectin-9

antibodies such as G9.2-17 were demonstrated in a mouse model, either by itself, or in combination with a checkpoint inhibitor (*e.g.*, an anti-PD-1 antibody). As reported herein, the efficacy of G9.2-17 was tested in mouse models of PDAC and melanoma as well as in patient derived organoid tumor models (PDOTs). The orthotopic PDAC KPC mouse model (LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx-1-Cre) that was used recapitulates many features of human disease, including unresponsiveness to approved checkpoint inhibitors (Bisht and Feldmann G; Animal models for modeling pancreatic cancer and novel drug discovery; Expert Opin Drug Discov. 2019;14(2):127-142; Weidenhofer et al., Animal models of pancreatic cancer and their application in clinical research; Gastrointestinal Cancer: Targets and Therapy 2016;6). The B16F10 melanoma mouse model has been a long-standing standard to test immunotherapies (Curran et al., PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors; Proc Natl Acad Sci U S A. 2010; 107(9):4275-4280).

PDOTs isolated from fresh human tumor samples retain autologous lymphoid and myeloid cell populations, including antigen-experienced tumor infiltrating CD4 and CD8 T lymphocytes, and respond to immune therapies in short-term ex vivo culture (Jenkins et al. Ex Vivo Profiling of PD-1 Blockade Using Organotypic Tumor Spheroids. Cancer Discov. 2018;8(2):196-215; Aref et al., 3D microfluidic ex vivo culture of organotypic tumor spheroids to model immune checkpoint blockade; Lab Chip. 2018;18(20):3129-3143). As reported herein, expression of Galectin-9 on cancer cells was observed in patient-derived organoid assays.

*In vivo* studies were performed with G9.2-17 mouse IgG1 (G9.2-17 mIgG1 contains the exact same binding epitope as G9.2-17 human IgG4 and has the same effector function), which achieves significant reduction of tumor growth already as a single agent in the orthotopic KPC model, where approved checkpoint inhibitors do not work. In the B16F10 model G9.2-17 significantly exceeds the efficacy of anti-PD-1. In both models, modulation of the intra-tumoral immune microenvironment using G9.2-17 mIgG1 through the upregulation of effector T cell activity and inhibition of immunosuppressive signals, as well as the augmentation of intra-tumoral CD8 T cell infiltration was demonstrated.

These results demonstrate that the anti-tumor methods disclosed herein, involving an anti-Galectin-9 antibody, optionally in combination the checkpoint inhibitor, would achieve superior therapeutic efficacy against the target solid tumors.

Accordingly, described herein are therapeutic uses of anti-Galectin-9 antibodies for

treating certain cancers as disclosed herein.

**Antibodies Binding to Galectin-9**

The present disclosure provides anti-Galectin-9 antibody G9.2-17 and functional variants thereof for use in the treatment methods disclosed herein.

An antibody (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term "antibody", *e.g.*, anti-Galectin-9 antibody, encompasses not only intact (*e.g.*, full-length) polyclonal or monoclonal antibodies, but also antigen-binding fragments thereof (such as Fab, Fab', F(ab')2, Fv), single chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, chimeric antibodies, diabodies, nanobodies, linear antibodies, single chain antibodies, multispecific antibodies (*e.g.*, bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. An antibody, e.g., anti-Galectin-9 antibody, includes an antibody of any class, such as IgD, IgE, IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

A typical antibody molecule comprises a heavy chain variable region ($V_H$) and a light chain variable region ($V_L$), which are usually involved in antigen binding. The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, also known as "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, which are known as "framework regions" ("FR"). Each $V_H$ and $V_L$ is typically 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. The extent of the framework

region and CDRs can be precisely identified using methodology known in the art, for example, by the Kabat definition, the Chothia definition, the AbM definition, the EU definition, the "Contact" numbering scheme, the IMGT" numbering scheme, the "AHo" numbering scheme, and/or the contact definition, all of which are well known in the art. See, *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia et al., (1989) *Nature* 342:877; Chothia, C. et al. (1987) *J. Mol. Biol.* 196:901-917, Al-lazikani et al (1997) *J. Molec. Biol.* 273:927-948; Edelman et al., *Proc Natl Acad Sci U S A.* 1969 May;63(1):78-85; and Almagro, *J. Mol. Recognit.* 17:132-143 (2004); MacCallum et al., *J. Mol. Biol.* 262:732-745 (1996), Lefranc M P et al., *Dev Comp Immunol*, 2003 January; 27(1):55-77; and Honegger A and Pluckthun A, J Mol Biol, 2001 Jun. 8; 309(3):657-70. See also hgmp.mrc.ac.uk and bioinf.org.uk/abs).

In some embodiments, the anti-Galectin-9 antibody described herein is a full-length antibody, which contains two heavy chains and two light chains, each including a variable domain and a constant domain. Alternatively, the anti-Galectin-9 antibody can be an antigen-binding fragment of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding fragment" of a full length antibody include (i) a Fab fragment, a monovalent fragment consisting of the $V_L$, $V_H$, $C_L$ and $C_H1$ domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the $V_H$ and $C_H1$ domains; (iv) a Fv fragment consisting of the $V_L$ and $V_H$ domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a $V_H$ domain; and (vi) an isolated complementarity determining region (CDR) that retains functionality. Furthermore, although the two domains of the Fv fragment, $V_L$ and $V_H$, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the $V_L$ and $V_H$ regions pair to form monovalent molecules known as single chain Fv (scFv). See *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883.

Any of the antibodies described herein, *e.g.*, anti-Galectin-9 antibody, can be either monoclonal or polyclonal. A "monoclonal antibody" refers to a homogenous antibody population and a "polyclonal antibody" refers to a heterogeneous antibody population. These two terms do not limit the source of an antibody or the manner in which it is made.

Reference antibody G9.2-17 refers to an antibody capable of binding to human Galectin-9 and comprises a heavy chain variable region of SEQ ID NO: 7 and a light chain variable domain of SEQ ID NO: 8, both of which are provided below. In some embodiments, the anti-Galectin-9 antibody for use in the methods disclosed herein is the G9.2-17 antibody. In some embodiments, the anti-Galectin-9 antibody for use in the methods disclosed herein is an antibody having the same heavy chain complementarity determining regions (CDRs) as reference antibody G9.2-17 and/or the same light chain complementarity determining regions as reference antibody G9.2-17. Two antibodies having the same $V_H$ and/or $V_L$ CDRs means that their CDRs are identical when determined by the same approach (*e.g.*, the Kabat approach, the Chothia approach, the AbM approach, the Contact approach, or the IMGT approach as known in the art. See, *e.g.*, bioinf.org.uk/abs/).

The heavy and light chain CDRs of reference antibody G9.2-17 is provided in **Table 1** below (determined using the Kabat methodology):

**Table 1. Heavy and Light Chain CDRs of G9.2-17**

| | | | |
|---|---|---|---|
| G9.2-17 | $V_L$ CDR1 | RASQSVSSAVA | SEQ ID NO: 1 |
| | $V_L$ CDR2 | SASSLYS | SEQ ID NO: 2 |
| | $V_L$ CDR3 | QQSSTDPIT | SEQ ID NO: 3 |
| | $V_H$ CDR1 | FTVSSSSIH | SEQ ID NO: 4 |
| | $V_H$ CDR2 | YISSSSGYTYYADSVKG | SEQ ID NO: 5 |
| | $V_H$ CDR3 | YWSYPSWWPYRGMDY | SEQ ID NO: 6 |

In some examples, the anti-Galectin-9 antibody for use in the methods disclosed herein may comprise (following the Kabat scheme) a heavy chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 4, a heavy chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 5, and a heavy chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 6 and/or may comprise a light chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 1, a light chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 2, and a light chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 3. The anti- Galectin-9 antibody, including the reference antibody G9.2-17, can be in any format as disclosed herein, for example, a full-length antibody or a Fab. The term "G9.2-17(IgG4)" used herein refers to a G9.2-17 antibody which is an IgG4 molecule (*e.g.*, having a heavy chain comprising SEQ ID NO. 19 and a light chain comprising SEQ ID NO: 15). Likewise, the term "G9.2-17 (Fab)" refers to a G9.2-17 antibody, which is a Fab molecule.

In some embodiments, the anti-Galectin-9 antibody or binding portion thereof comprises heavy and light chain variable regions, wherein the light chain variable region CDR1, CDR2, and CDR3 amino acid sequences have at least 80% (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to the light chain variable region CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NOs: 1, 2, and 3, respectively. In some embodiments, the anti-Galectin-9 antibody or binding portion thereof comprises heavy and light chain variable regions, wherein the heavy chain variable region CDR1, CDR2, and CDR3 amino acid sequences have at least 80% (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to the heavy chain variable region CDR1, CDR2, and CDR3 amino acid sequences set forth in SEQ ID NO: 4, 5, and 6, respectively.

Additional Galectin-9 antibodies, e.g., which bind to the CRD1 and/or CRD2 region of Galectin-9 are described in co-owned, co-pending US Patent Application 16/173,970 and in co-owned, co-pending International Patent Applications PCT/US18/58028 and PCT/US2020/024767, the contents of each of which are herein incorporated by reference in their entireties.

In some embodiments, the anti-Galectin-9 antibody disclosed herein comprises light chain CDRs that have at least 80% (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity, individually or collectively, as compared with the corresponding $V_L$ CDRs of reference antibody G9.2-17. Alternatively or in addition, in some embodiments, the anti-Galectin-9 antibody comprises heavy chain CDRs that have at least 80% (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity, individually or collectively, as compared with the corresponding $V_H$ CDRs of reference antibody G9.2-17.

The "percent identity" of two amino acid sequences is determined using the algorithm of Karlin and Altschul Proc. Natl. Acad. Sci. USA 87:2264-68, 1990, modified as in Karlin and Altschul Proc. Natl. Acad. Sci. USA 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. J. Mol. Biol. 215:403-10, 1990. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of the invention. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul et al., Nucleic Acids Res. 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g.,

XBLAST and NBLAST) can be used.

In other embodiments, the anti-Galectin-9 antibody described herein comprises a $V_H$ that comprises the HC CDR1, HC CDR2, and HC CDR3, which collectively contain up to 8 amino acid residue variations (8, 7, 6, 5, 4, 3, 2, or 1 variation(s), including additions, deletions, and/or substitutions) relative to the HC CDR1, HC CDR2, and HC CDR3 of reference antibody G9.2-17. Alternatively or in addition, in some embodiments, the anti-Galectin-9 antibody described herein comprises a $V_H$ that comprises the LC CDR1, LC CDR2, and LC CDR3, which collectively contain up to 8 amino acid residue variations (8, 7, 6, 5, 4, 3, 2, or 1 variation(s) including additions, deletions, and/or substitutions) relative to the LC CDR1, LC CDR2, and LC CDR3 of reference antibody G9.2-17.

In one example, the amino acid residue variations are conservative amino acid residue substitutions. As used herein, a "conservative amino acid substitution" refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, *e.g.*, Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

In some embodiments, the anti-Galectin-9 antibodies disclosed herein, having the heavy chain CDRs disclosed herein, contains framework regions derived from a subclass of germline $V_H$ fragment. Such germline $V_H$ regions are well known in the art. See, *e.g.*, the IMGT database (www.imgt.org) or at www.vbase2.org/vbstat.php. Examples include the IGHV1 subfamily (*e.g.*, IGHV1-2, IGHV1-3, IGHV1-8, IGHV1-18, IGHV1-24, IGHV1-45, IGHV1-46, IGHV1-58, and IGHV1-69), the IGHV2 subfamily (*e.g.*, IGHV2-5, IGHV2-26, and IGHV2-70), the IGHV3 subfamily (*e.g.*, IGHV3-7, IGHV3-9, IGHV3-11, IGHV3-13, IGHV3-15, IGHV3-20, IGHV3-21, IGHV3-23, IGHV3-30, IGHV3-33, IGHV3-43, IGHV3-48, IGHV3-49, IGHV3-53, IGHV3-64, IGHV3-66, IGHV3-72, and IGHV3-73, IGHV3-74), the IGHV4 subfamily (*e.g.*, IGHV4-4, IGHV4-28, IGHV4-31, IGHV4-34, IGHV4-39, IGHV4-59, IGHV4-61, and IGHV4-B), the IGHV subfamily (*e.g.*, IGHV5-51, or IGHV6-1), and the IGHV7 subfamily (*e.g.*, IGHV7-4-1).

Alternatively or in addition, in some embodiments, the anti-Galectin-9 antibody, having the light chain CDRs disclosed herein, contains framework regions derived from a germline Vκ fragment. Examples include an IGKV1 framework (*e.g.*, IGKV1-05, IGKV1-12, IGKV1-27, IGKV1-33, or IGKV1-39), an IGKV2 framework (*e.g.*, IGKV2-28), an IGKV3 framework (*e.g.*, IGKV3-11, IGKV3-15, or IGKV3-20), and an IGKV4 framework (*e.g.*, IGKV4-1). In other instances, the anti-Galectin-9 antibody comprises a light chain variable region that contains a framework derived from a germline Vλ fragment. Examples include an IGλ1 framework (*e.g.*, IGλV1-36, IGλV1-40, IGλV1-44, IGλV1-47, IGλV1-51), an IGλ2 framework (e.g., IGλV2-8, IGλV2-11, IGλV2-14, IGλV2-18, IGλV2-23,), an IGλ3 framework (e.g., IGλV3-1, IGλV3-9, IGλV3-10, IGλV3-12, IGλV3-16, IGλV3-19, IGλV3-21, IGλV3-25, IGλV3-27,), an IGλ4 framework (e.g., IGλV4-3, IGλV4-60, IGλV4-69,), an IGλ5 framework (e.g., IGλV5-39, IGλV5-45,), an IGλ6 framework (e.g., IGλV6-57,), an IGλ7 framework (e.g., IGλV7-43, IGλV7-46, ), an IGλ8 framework (*e.g.*, IGλV8-61), an IGλ9 framework (*e.g.*, IGλV9-49), or an IGλ10 framework (*e.g.*, IGλV10-54).

In some embodiments, the anti-Galectin-9 antibody for use in the method disclosed herein can be an antibody having the same heavy chain variable region ($V_H$) and/or the same light chain variable region ($V_L$) as reference antibody G9.2-17, the $V_H$ and $V_L$ region amino acid sequences are provided below:

$V_H$:

EVQLVESGGGLVQPGGSLRLSCAASG**FTVSSSSIH**WVRQAPGKGLEWVA**YISSSSGYTYYADSVKG**RFT ISADTSKNTAYLQMNSLRAEDTAVYYCAR**YWSYPSWWPYRGMDY**WGQGTLVTVSS (SEQ ID NO: 7)

$V_L$:

DIQMTQSPSSLSASVGDRVTITC**RASQSVSSAVA**WYQQKPGKAPKLLIY**SASSLYS**GVPSRFSGSRSGT DFTLTISSLQPEDFATYYC**QQSSTDPIT**FGQGTKVEIKR (SEQ ID NO: 8)

In some embodiments, the anti-Galectin-9 antibody has at least 80% sequence identity (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%identity) to the heavy chain variable region of SEQ ID NO: 7. Alternatively or in addition, the anti-Galectin-9 antibody has at least 80% sequence identity (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%identity) to the light chain variable region of SEQ ID NO: 8.

In some instances, the anti-Galectin-9 antibody disclosed herein is a functional variant of reference antibody G9.2-17. A functional variant can be structurally similar as the reference antibody (e.g., comprising the limited number of amino acid residue variations in one or more

of the heavy chain and/or light chain CDRs as G9.2-17 as disclosed herein, or the sequence identity relative to the heavy chain and/or light chain CDRs of G9.2-17, or the VH and/or VL of G9.2-17 as disclosed herein) with substantially similar binding affinity (e.g., having a $K_D$ value in the same order) to human Galectin-9.

In some embodiments, the anti-Galectin-9 antibody as described herein can bind and inhibit the activity of Galectin-9 by at least 20% (*e.g.*, 31%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). The apparent inhibition constant ($Ki^{app}$ or $K_{i,app}$), which provides a measure of inhibitor potency, is related to the concentration of inhibitor required to reduce enzyme activity and is not dependent on enzyme concentrations. The inhibitory activity of an anti-Galectin-9 antibody described herein can be determined by routine methods known in the art.

The $K_i^{app}$ value of an antibody may be determined by measuring the inhibitory effect of different concentrations of the antibody on the extent of the reaction (*e.g.*, enzyme activity); fitting the change in pseudo-first order rate constant ($v$) as a function of inhibitor concentration to the modified Morrison equation (Equation 1) yields an estimate of the apparent Ki value. For a competitive inhibitor, the $Ki^{app}$ can be obtained from the y-intercept extracted from a linear regression analysis of a plot of $K_i^{app}$ versus substrate concentration.

$$v = A \cdot \frac{([E]-[I]-K_i^{app}) + \sqrt{([E]-[I]-K_i^{app})^2 + 4[E]\cdot K_i^{app}}}{2} \qquad \text{(Equation 1)}$$

Where $A$ is equivalent to $v_o/E$, the initial velocity ($v_o$) of the enzymatic reaction in the absence of inhibitor ($I$) divided by the total enzyme concentration ($E$). In some embodiments, the anti-Galectin-9 antibody described herein has a $Ki^{app}$ value of 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 pM or less for the target antigen or antigen epitope. In some embodiments, the anti-Galectin-9 antibody has a lower $Ki^{app}$ for a first target (*e.g.*, the CRD2 of Galectin-9) relative to a second target (*e.g.*, CRD1 of the Galectin-9). Differences in $Ki^{app}$ (*e.g.*, for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 91, 100, 500, 1000, 10,000 or $10^5$ fold. In some examples, the anti-Galectin-9 antibody inhibits a first antigen (*e.g.*, a first protein in a first conformation or mimic thereof) greater relative to a second antigen (*e.g.*, the same first protein in a second conformation or mimic thereof; or a second protein). In some embodiments, any of the anti-Galectin-9 antibodies is further affinity matured to reduce the $Ki^{app}$ of the antibody to the target antigen or antigenic epitope thereof.

In some embodiments, the anti-Galectin-9 antibody suppresses Dectin-1 signaling, *e.g.*, in tumor infiltrating immune cells, such as macrophages. In some embodiments, the anti-Galectin-9 antibody suppresses Dectin-1 signaling triggered by Galectin-9 by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). Such inhibitory activity can be determined by conventional methods, such as routine assays. Alternatively or in addition, the anti-Galectin-9 antibody suppresses the T cell immunoglobulin mucin-3 (TIM-3) signaling initiated by Galectin-9. In some embodiments, the anti-Galectin-9 antibody suppresses the T cell immunoglobulin mucin-3 (TIM-3) signaling, e.g., in tumor infiltrating immune cells, e.g., in some embodiments, by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). Such inhibitory activity can be determined by conventional methods, such as routine assays.

In some embodiments, the anti-Galectin-9 antibody suppresses the CD206 signaling, *e.g.*, in tumor infiltrating immune cells. In some embodiments, the anti-Galectin-9 antibody suppresses the CD206 signaling triggered by Galectin-9 by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). Such inhibitory activity can be determined by conventional methods, such as routine assays. In some embodiments, the anti-Galectin-9 antibody blocks or prevents binding of Galectin-9 to CD206 by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). Such inhibitory activity can be determined by conventional methods, such as routine assays.

In some embodiments, the anti-Galectin-9 antibody induces cell cytotoxicity, such as ADCC, in target cells expressing Galectin-9, e.g., wherein the target cells are cancer cells or immune suppressive immune cells. In some embodiments, the anti-Galectin-9 antibody induces apoptosis in immune cells, such as T cells, or cancer cells by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). Such inhibitory activity can be determined by conventional methods, such as routine assays. In some embodiments, any of the anti-Galectin-9 antibodies described herein induce cell cytotoxicity such as complement-dependent cytotoxicity (CDC) against target cells expressing Galectin-9.

Antibody-dependent cell-mediated phagocytosis (ADCP) is an important mechanism of action for antibodies that mediate part or all of their action though phagocytosis. In that case, antibodies mediate uptake of specific antigens by antigen presenting cells. ADCP can be mediated by monocytes, macrophages, neutrophils, and dendritic cells, through FcγRIIa, FcγRI, and FcγRIIIa, of which FcγRIIa (CD32a) on macrophages represent the predominant

pathway.

In some embodiments, the anti-Galectin-9 antibody induces cell phagocytosis of target cells, *e.g.*, cancer cells or immune suppressive immune cells expressing Galectin-9 (ADCP). In some embodiments, the anti-Galectin-9 antibody increases phagocytosis of target cells, e.g., cancer cells or immune suppressive immune cells, by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein).

In some embodiments, the anti-Galectin-9 antibody described herein induces cell cytotoxicity such as complement-dependent cytotoxicity (CDC) against target cells, e.g., cancer cells or immune suppressive immune cells. In some embodiments, the anti-Galectin-9 antibody increases CDC against target cells by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein).

In some embodiments, the anti-Galectin-9 antibody induces T cell activation, e.g., in tumor infiltrating T cells, i.e., suppress Galectin-9 mediated inhibition of T cell activation, either directly or indirectly. In some embodiments, the anti-Galectin-9 antibody promotes T cell activation by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). T cell activation can be determined by conventional methods, such as using well-known assays for measuring cytokines and checkpoint inhibitors (*e.g.*, measurement of CD44, TNF alpha, IFNgamma, and/or PD-1). In some embodiments, the anti-Galectin-9 antibody promotes CD4+ cell activation by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). In a non-limiting example, the anti-Galectin antibody induces CD44 expression in CD4+ cells. In some embodiments, the anti-Galectin-9 antibody increases CD44 expression in CD4+ cells by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). In a non-limiting example, the anti-Galectin antibody induces IFNgamma expression in CD4+ cells. In some embodiments, the anti-Galectin-9 antibody increases IFNgamma expression in CD4+ cells by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). In a non-limiting example, the anti-Galectin antibody induces TNFalpha expression in CD4+ cells. In some embodiments, the anti-Galectin-9 antibody increases TNFalpha expression in CD4+ cells by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein).

In some embodiments, the anti-Galectin-9 antibody promotes CD8+ cell activation by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater), including any

increment therein). In a non-limiting example, the anti-Galectin antibody induces CD44 expression in CD8+ cells. In some embodiments, the anti-Galectin-9 antibody increases CD44 expression in CD8+ cells by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). In a non-limiting example, the anti-Galectin antibody induces IFNgamma expression in CD8+ cells. In some embodiments, the anti-Galectin-9 antibody increases IFNgamma expression in CD8+ cells by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein). In a non-limiting example, the anti-Galectin antibody induces TNFalpha expression in CD8+ cells. In some embodiments, the anti-Galectin-9 antibody increases TNFalpha expression in CD8+ cells by at least 30% (*e.g.*, 31%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or greater, including any increment therein).

In some embodiments, an anti-Galectin-9 antibody as described herein has a suitable binding affinity for the target antigen (*e.g.*, Galectin-9) or antigenic epitopes thereof. As used herein, "binding affinity" refers to the apparent association constant or $K_A$. The $K_A$ is the reciprocal of the dissociation constant ($K_D$). The anti-Galectin-9 antibody described herein may have a binding affinity ($K_D$) of at least $10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$, $10^{-10}$ M, or lower for the target antigen or antigenic epitope. An increased binding affinity corresponds to a decreased $K_D$. Binding affinity (or binding specificity) can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (*e.g.*, using a fluorescence assay). Exemplary conditions for evaluating binding affinity are in HBS-P buffer (10 mM HEPES pH7.4, 150 mM NaCl, 0.005% (v/v) Surfactant P20).

These techniques can be used to measure the concentration of bound binding protein as a function of target protein concentration. Under certain conditions, the fractional concentration of bound binding protein ([Bound]/[Total]) is generally related to the concentration of total target protein ([Target]) by the following equation:

$$[Bound]/[Total] = [Target]/(Kd+[Target])$$

It is not always necessary to make an exact determination of $K_A$, though, since sometimes it is sufficient to obtain a quantitative measurement of affinity, *e.g.*, determined using a method such as ELISA or FACS analysis, is proportional to $K_A$, and thus can be used for comparisons, such as determining whether a higher affinity is, *e.g.*, 2-fold higher, to obtain a qualitative measurement of affinity, or to obtain an inference of affinity, *e.g.*, by activity in a

functional assay, *e.g.*, an *in vitro* or *in vivo* assay. In some cases, the *in vitro* binding assay is indicative of *in vivo* activity. In other cases, the *in vitro* binding assay is not necessarily indicative of *in vivo* activity. In some cases, tight binding is beneficial, but in other cases tight binding is not as desirable *in vivo*, and an antibody with lower binding affinity is more desirable.

In some embodiments, the heavy chain of any of any of the anti-Galectin-9 antibodies as described herein further comprise a heavy chain constant region (CH) or a portion thereof (*e.g.*, CH1, CH2, CH3, or a combination thereof). The heavy chain constant region can be of any suitable origin, *e.g.*, human, mouse, rat, or rabbit. In one specific example, the heavy chain constant region is from a human IgG (a gamma heavy chain) of any IgG subfamily as described herein.

In some embodiments, the heavy chain constant region of the antibodies described herein comprise a single domain (*e.g.*, CH1, CH2, or CH3) or a combination of any of the single domains, of a constant region (*e.g.*, SEQ ID NO: 4, 5, 6). In some embodiments, the light chain constant region of the antibodies described herein comprise a single domain (*e.g.*, CL), of a constant region. Exemplary light and heavy chain sequences are listed below. Exemplary light and heavy chain sequences are listed below. The hIgG1 LALA sequence includes two mutations, L234A and L235A (EU numbering), which suppress FcgR binding as well as a P329G mutation (EU numbering) to abolish complement C1q binding, thus abolishing all immune effector functions. The hIgG4 Fab Arm Exchange Mutant sequence includes a mutation to suppress Fab Arm Exchange (S228P; EU numbering). An IL2 signal sequence (MYRMQLLSCIALSLALVTNS; SEQ ID NO: 9) can be located N-terminally of the variable region. It is used in expression vectors, which is cleaved during secretion and thus not in the mature antibody molecule. The mature protein (after secretion) starts with "EVQ" for the heavy chain and "DIM" for the light chain. Amino acid sequences of exemplary heavy chain constant regions are provided below:

**hIgG1 Heavy Chain Constant Region (SEQ ID NO: 10)**

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**hIgG1 LALA Heavy Chain Constant Region (SEQ ID NO: 12)**

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKAL**G**APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**hIgG4 Heavy Chain Constant Region (SEQ ID NO: 13)**

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**hIgG4 Heavy Chain Constant Region (SEQ ID NO: 20)**

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK*

**hIgG4 mut Heavy Chain Constant Region (SEQ ID NO: 14)**

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCP**P**CPAPEFLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**hIgG4 mut Heavy Chain Constant Region (SEQ ID NO: 21)**

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCP**P**CPAPEFLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK*

In some embodiments, anti-Galectin-9 antibodies having any of the above heavy chain constant regions are paired with a light chain having the following light chain constant region:

**Light Chain Constant Region (SEQ ID NO: 11)**

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS
STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Exemplary full length anti-Galectin-9 antibodies are provided below:

**G9.2-17 hIgG1 Heavy Chain (SEQ ID NO: 16)**

**EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF**
**TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSS**ASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**G9.2-17 hIgG1 LALA Heavy Chain (SEQ ID NO: 17)**

**EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF**
**TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSS**ASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL**G**APIE
KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**G9.2-17 hIgG4 Heavy Chain (SEQ ID NO: 18)**

**EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF**
**TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSS**ASTKGPSVFPLA
PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT
YTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI
SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**G9.2-17 hIgG4 Heavy Chain (SEQ ID NO: 22)**

**EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF**
**TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSS**ASTKGPSVFPLA
PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT
YTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI
SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK*

**G9.2-17 hIgG4 Fab Arm Exchange mut Heavy Chain (SEQ ID NO: 19)**

**EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF**
**TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSS**ASTKGPSVFPLA
PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT
YTCNVDHKPSNTKVDKRVESKYGPPCP**P**CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI
SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK*

**G9.2-17 hIgG4 Fab Arm Exchange mut Heavy Chain (SEQ ID NO: 23)**

**EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF**
**TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSS**ASTKGPSVFPLA
PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKT
YTCNVDHKPSNTKVDKRVESKYGPPCP**S**CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQF**N**STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI
SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK*

Any of the above heavy chain can be paired with a Light Chain of (SEQ ID NO: 15) shown below:

**DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSG**
**TDFTLTISSLQPEDFATYYCQQSSTDPITFGQGTKVEIKR**TVAAPSVFIFPPSDEQLKSGTASVVCLL
NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC*

In some embodiments, the anti-Galectin-9 antibody comprises a heavy chain IgG1 constant region that has at least 80% (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 10. In one embodiment, the constant region of the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region comprising SEQ ID NO: 10. In one embodiment, the constant region of the anti-Galectin-9 antibody comprises a heavy chain IgG1 constant region consisting of SEQ ID NO: 10.

In some embodiments, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region that has at least 80% (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 20. In one embodiment, the constant region of the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region comprising SEQ ID NO: 20. In one embodiment, the constant region of the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region consisting of SEQ ID NO: 20.

In some embodiments, the constant region is from human IgG4. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region that has at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 13. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region comprising SEQ ID NO: 13. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region

consisting of SEQ ID NO: 13.

In some embodiments, the constant region is from human IgG4. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region that has at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 20. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region comprising SEQ ID NO: 20. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region consisting of SEQ ID NO: 20.

In any of these embodiments, the anti-Galectin-9 antibody comprises a light chain constant region that has at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 11. In some embodiments, the anti-Galectin-9 antibody comprises a light chain constant region comprising SEQ ID NO: 11. In some embodiments, the anti-Galectin-9 antibody comprises a light chain constant region consisting of SEQ ID NO: 11.

In some embodiments, the IgG is a mutant with minimal Fc receptor engagement. In one example, the constant region is from a human IgG1 LALA. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG1 constant region that has at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 12. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG1 constant region comprising SEQ ID NO: 12. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG1 constant region consisting of SEQ ID NO: 12.

In some embodiments, the anti-Galectin-9 antibody comprises a modified constant region. In some embodiments, the anti-Galectin-9 antibody comprise a modified constant region that is immunologically inert, *e.g.*, does not trigger complement mediated lysis, or does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC). ADCC activity can be assessed using methods disclosed in U.S. Pat. No. 5,500,362. In other embodiments, the constant region is modified as described in *Eur. J. Immunol.* (1999) 29:2613-2624; PCT Application No. PCT/GB99/01441; and/or UK Patent Application No. 9809951.8. In some embodiments, the IgG4 constant region is a mutant with reduced heavy chain exchange. In some embodiments, the constant region is from a human IgG4 Fab Arm Exchange mutant S228P.

In one embodiment, the constant region of the anti-Galectin-9 antibody comprises a

heavy chain IgG4 constant region that has at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 14. In one embodiment, the constant region of the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region comprising SEQ ID NO: 14. In one embodiment, the constant region of the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region consisting of SEQ ID NO: 14.

In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region that has at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 21. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region comprising SEQ ID NO: 21. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain IgG4 constant region consisting of SEQ ID NO: 21.

In some embodiments, the anti-Galectin -9 antibody has chains corresponding to SEQ ID NO: 15 for the light chains; and the amino acid sequences of exemplary heavy chains correspond to SEQ ID NO: 10 (hIgG1); 12 (hIgG1 LALA); 13 (hIgG4); 20 (hIgG4); 14 (hIgG4 mut); and 21 (hIgG4 mut).

In some embodiments, the anti-Galectin-9 antibody has a light chain comprising, consisting essentially of, or consisting of SEQ ID NO: 15. In some embodiments, the anti-Galectin-9 antibody has a heavy chain comprising, consisting essentially of, or consisting of any one of the sequences selected from the group consisting of SEQ ID NO: 16-19, 22 and 23. In some embodiments, the anti-Galectin-9 antibody has a light chain comprising, consisting essentially of, or consisting of SEQ ID NO: 15 and a heavy chain comprising, consisting essentially of, or consisting of any one of the sequences selected from the group consisting of SEQ ID NO: 16-19. In some embodiments, the anti-Galectin-9 antibody has a light chain comprising SEQ ID NO: 15 and a heavy chain comprising any one of the sequences selected from the group consisting of SEQ ID NO: 16-19, 22 and 23. In some embodiments, the anti-Galectin-9 antibody has a light chain consisting essentially of SEQ ID NO: 15 and a heavy chain consisting essentially of any one of the sequences selected from the group consisting of SEQ ID NO: 16-19, 22 and 23. In some embodiments, the anti-Galectin-9 antibody has a light chain consisting of SEQ ID NO: 15 and a heavy chain consisting of any one of the sequences selected from the group consisting of SEQ ID NO: 16-19, 22 and 23. In one specific embodiment, the anti-Galectin-9 antibody has a light chain consisting essentially of SEQ ID NO: 15 and a heavy chain consisting essentially of SEQ ID NO: 19. In another specific

embodiment, the anti-Galectin-9 antibody has a light chain consisting essentially of SEQ ID NO: 15 and a heavy chain consisting essentially of SEQ ID NO: 20.

In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence having at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 16. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence comprising SEQ ID NO: 16. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence consisting of SEQ ID NO: 16.

In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence having at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 17. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence comprising SEQ ID NO: 17. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence consisting of SEQ ID NO: 17.

In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence having at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 18. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence comprising SEQ ID NO: 18. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence consisting of SEQ ID NO: 18.

In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence having at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 22. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence comprising SEQ ID NO: 22. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence consisting of SEQ ID NO: 22.

In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence having at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 19. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence comprising SEQ ID NO: 19. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence consisting of SEQ ID NO: 19.

In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence

having at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 23. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence comprising SEQ ID NO: 23. In one embodiment, the anti-Galectin-9 antibody comprises a heavy chain sequence consisting of SEQ ID NO: 23.

In any of these embodiments, the anti-Galectin-9 antibody comprises a light chain sequence having at least 80% (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% and any increment therein) sequence identity to SEQ ID NO: 15. In some embodiments, the anti-Galectin-9 antibody comprises a light chain sequence comprising SEQ ID NO: 15. In some embodiments, the anti-Galectin-9 antibody comprises a light chain sequence consisting of SEQ ID NO: 15.

In specific examples, the anti-Galectin-9 antibody used in the treatment methods disclosed herein has a heavy chain of SEQ ID NO:19 and a light chain of SEQ ID NO:15. In some embodiments, the the anti-Galectin-9 antibody used in the treatment methods disclosed herein is G9.2-17 IgG4.

In some embodiments, any of the anti-Galectin-9 antibody disclosed herein (*e.g.*, G9.2-17(IgG4)) may have the C-terminus lysine residue of the heavy chain deleted.

**Preparation of Anti-Galectin-9 Antibodies**

Antibodies capable of binding Galectin-9 as described herein can be made by any method known in the art, including but not limited to, recombinant technology. One example is provided below.

Nucleic acids encoding the heavy and light chain of an anti-Galectin-9 antibody as described herein can be cloned into one expression vector, each nucleotide sequence being in operable linkage to a suitable promoter. In one example, each of the nucleotide sequences encoding the heavy chain and light chain is in operable linkage to a distinct promoter. Alternatively, the nucleotide sequences encoding the heavy chain and the light chain can be in operable linkage with a single promoter, such that both heavy and light chains are expressed from the same promoter. When necessary, an internal ribosomal entry site (IRES) can be inserted between the heavy chain and light chain encoding sequences.

In some examples, the nucleotide sequences encoding the two chains of the antibody are cloned into two vectors, which can be introduced into the same or different cells. When the two chains are expressed in different cells, each of them can be isolated from the host cells

expressing such and the isolated heavy chains and light chains can be mixed and incubated under suitable conditions allowing for the formation of the antibody.

Generally, a nucleic acid sequence encoding one or all chains of an antibody can be cloned into a suitable expression vector in operable linkage with a suitable promoter using methods known in the art. For example, the nucleotide sequence and vector can be contacted, under suitable conditions, with a restriction enzyme to create complementarity ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of a gene. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector. The selection of expression vectors/promoter would depend on the type of host cells for use in producing the antibodies.

A variety of promoters can be used for expression of the antibodies described herein, including, but not limited to, cytomegalovirus (CMV) intermediate early promoter, a viral LTR such as the *Rous sarcoma* virus LTR, HIV-LTR, HTLV-1 LTR, the simian virus 40 (SV40) early promoter, *E. coli* lac UV5 promoter, and the herpes simplex tk virus promoter.

Regulatable promoters can also be used. Such regulatable promoters include those using the lac repressor from *E. coli* as a transcription modulator to regulate transcription from lac operator-bearing mammalian cell promoters [Brown, M. et al., *Cell*, 49:603-612 (1987)], those using the tetracycline repressor (tetR) [Gossen, M., and Bujard, H., *Proc. Natl. Acad. Sci. USA* 89:5547-5551 (1992); Yao, F. et al., *Human Gene Therapy*, 9:1939-1950 (1998); Shockelt, P., et al., *Proc. Natl. Acad. Sci. USA*, 92:6522-6526 (1995)]. Other systems include FK506 dimer, VP16 or p65 using astradiol, RU486, diphenol murislerone, or rapamycin. Inducible systems are available from Invitrogen, Clontech and Ariad.

Regulatable promoters that include a repressor with the operon can be used. In one embodiment, the lac repressor from *E. coli* can function as a transcriptional modulator to regulate transcription from lac operator-bearing mammalian cell promoters (M. Brown et al., *Cell*, 49:603-612 (1987); Gossen and Bujard (1992); M. Gossen et al., *Natl. Acad. Sci. USA*, 89:5547-5551 (1992)) combined the tetracycline repressor (tetR) with the transcription activator (VP 16) to create a tetR-mammalian cell transcription activator fusion protein, tTa (tetR-VP 16), with the tetO-bearing minimal promoter derived from the human cytomegalovirus (hCMV) major immediate-early promoter to create a tetR-tet operator system to control gene expression in mammalian cells. In one embodiment, a tetracycline inducible switch is used. The tetracycline repressor (tetR) alone, rather than the tetR-mammalian cell

transcription factor fusion derivatives can function as potent trans-modulator to regulate gene expression in mammalian cells when the tetracycline operator is properly positioned downstream for the TATA element of the CMVIE promoter (Yao et al., *Human Gene Therapy*, 10(16):1392-1399 (2003)). One particular advantage of this tetracycline inducible switch is that it does not require the use of a tetracycline repressor-mammalian cells transactivator or repressor fusion protein, which in some instances can be toxic to cells (Gossen et al., *Natl. Acad. Sci. USA*, 89:5547-5551 (1992); Shockett et al., *Proc. Natl. Acad. Sci. USA*, 92:6522-6526 (1995)), to achieve its regulatable effects.

Additionally, the vector can contain, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; internal ribosome binding sites (IRESes), versatile multiple cloning sites; and T7 and SP6 RNA promoters for *in vitro* transcription of sense and antisense RNA. Suitable vectors and methods for producing vectors containing transgenes are well known and available in the art.

Examples of polyadenylation signals useful to practice the methods described herein include, but are not limited to, human collagen I polyadenylation signal, human collagen II polyadenylation signal, and SV40 polyadenylation signal.

One or more vectors (*e.g.*, expression vectors) comprising nucleic acids encoding any of the antibodies may be introduced into suitable host cells for producing the antibodies. The host cells can be cultured under suitable conditions for expression of the antibody or any polypeptide chain thereof. Such antibodies or polypeptide chains thereof can be recovered by the cultured cells (*e.g.*, from the cells or the culture supernatant) via a conventional method, *e.g.*, affinity purification. If necessary, polypeptide chains of the antibody can be incubated under suitable conditions for a suitable period of time allowing for production of the antibody.

In some embodiments, methods for preparing an antibody described herein involve a recombinant expression vector that encodes both the heavy chain and the light chain of an anti-Galectin-9 antibody, as also described herein. The recombinant expression vector can be introduced into a suitable host cell (*e.g.*, a dhfr- CHO cell) by a conventional method, *e.g.*, calcium phosphate-mediated transfection. Positive transformant host cells can be selected and cultured under suitable conditions allowing for the expression of the two polypeptide chains

that form the antibody, which can be recovered from the cells or from the culture medium. When necessary, the two chains recovered from the host cells can be incubated under suitable conditions allowing for the formation of the antibody.

In one example, two recombinant expression vectors are provided, one encoding the heavy chain of the anti-Galectin-9 antibody and the other encoding the light chain of the anti-Galectin-9 antibody. Both of the two recombinant expression vectors can be introduced into a suitable host cell (*e.g.*, dhfr- CHO cell) by a conventional method, *e.g.*, calcium phosphate-mediated transfection. Alternatively, each of the expression vectors can be introduced into a suitable host cell. Positive transformants can be selected and cultured under suitable conditions allowing for the expression of the polypeptide chains of the antibody. When the two expression vectors are introduced into the same host cells, the antibody produced therein can be recovered from the host cells or from the culture medium. If necessary, the polypeptide chains can be recovered from the host cells or from the culture medium and then incubated under suitable conditions allowing for formation of the antibody. When the two expression vectors are introduced into different host cells, each of them can be recovered from the corresponding host cells or from the corresponding culture media. The two polypeptide chains can then be incubated under suitable conditions for formation of the antibody.

Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recovery of the antibodies from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G coupled matrix.

Any of the nucleic acids encoding the heavy chain, the light chain, or both of an anti-Galectin-9 antibody as described herein, vectors (*e.g.*, expression vectors) containing such; and host cells comprising the vectors are within the scope of the present disclosure.

Anti-Galectin-9 antibodies thus prepared can be characterized using methods known in the art, whereby reduction, amelioration, or neutralization of Galectin-9 biological activity is detected and/or measured. For example, in some embodiments, an ELISA-type assay is suitable for qualitative or quantitative measurement of Galectin-9 inhibition of Dectin-1 or TIM-3 signaling.

The bioactivity of an anti-Galectin-9 antibody can verified by incubating a candidate antibody with Dectin-1 and Galectin-9, and monitoring any one or more of the following characteristics: (a) binding between Dectin-1 and Galectin-9 and inhibition of the signaling transduction mediated by the binding; (b) preventing, ameliorating, or treating any aspect of a

solid tumor; (c) blocking or decreasing Dectin-1 activation; (d) inhibiting (reducing) synthesis, production or release of Galectin-9. Alternatively, TIM-3 can be used to verify the bioactivity of an anti-Galectin-9 antibody using the protocol described above. Alternatively, CD206 can be used to verify the bioactivity of an anti-Galectin-9 antibody using the protocol described above.

In some embodiments, bioactivity or efficacy is assessed in a subject, *e.g.*, by measuring peripheral and intra-tumoral T cell ratios, T cell activation, or by macrophage phenotyping.

Additional assays to determine bioactivity of an anti-Galectin-9 antibody include measurement of CD8+ and CD4+ (conventional) T-cell activation (in an in vitro or in vivo assay, e.g., by measuring inflammatory cytokine levels, e.g., IFNgamma, TNFalpha, CD44, ICOS granzymeB, Perforin, IL2 (upregulation); CD26L and IL-10 (downregulation)); measurement of reprogramming of macrophages (in vitro or in vivo), e.g., from the M2 to the M1 phenotype (e.g., increased MHCII, reduced CD206, increased TNF-alpha and iNOS), Alternatively, levels of ADCC can be assessed, e.g., in an in vitro assay, as described herein.

**Methods of Treatment**

The present disclosure provides methods for treating solid tumors including, but not limited to, head and neck cancer, urothelial carcinoma, gastric esophageal cancer, or non-small cell lung cancer, using any of the anti-Galectin antibodies, for example G9.2-17, e.g., G9.2-17 IgG4, either alone or in combination with a checkpoint inhibitor such as an anti-PD-1 antibody, for example, tislelizumab. Additional target solid tumors for treatment by the method disclosed herein may include pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), hepatocellular carcinoma (HCC), cholangiocarcinoma, renal cell carcinoma, and breast cancer.

Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the pharmaceutical composition to the subject, depending upon the type of disease to be treated or the site of the disease. In some embodiments, the anti-Galectin-9 antibody and/or the anti-PD-1 antibody can be administered to a subject by intravenous infusion.

Injectable compositions may contain various carriers such as vegetable oils, dimethylactamide, dimethylformamide, ethyl lactate, ethyl carbonate, isopropyl myristate, ethanol, and polyols (glycerol, propylene glycol, liquid polyethylene glycol, and the like). For intravenous infusion, water soluble antibodies can be administered by the drip method,

whereby a pharmaceutical formulation containing the antibody and a physiologically acceptable excipient is infused. Physiologically acceptable excipients may include, for example, 5% dextrose, 0.9% saline, Ringer's solution or other suitable excipients. Intramuscular preparations, *e.g.*, a sterile formulation of a suitable soluble salt form of the antibody, can be dissolved and administered in a pharmaceutical excipient such as Water-for-Injection, 0.9% saline, or 5% glucose solution.

As used herein, the term "treating" refers to the application or administration of a composition including one or more active agents to a subject, who has a target disease or disorder, a symptom of the disease/disorder, or a predisposition toward the disease/disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disorder, a symptom of the disease or disorder, or the predisposition toward the disease or disorder.

Alleviating a target disease/disorder includes delaying the development or progression of the disease or reducing disease severity or prolonging survival. Alleviating the disease or prolonging survival does not necessarily require curative results. As used therein, "delaying" the development of a target disease or disorder means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

"Development" or "progression" of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of a target disease or disorder includes initial onset and/or recurrence.

*(i) Treatment with Anti-Galectin 9 Antibodies*

Any of the anti-Galectin-9 antibodies described herein can be used in any of the

methods described herein. In some embodiments, the anti-Galectin-9 antibody is G9.2-17, *e.g.*, G9.2-17(IgG4). Such antibodies can be used for treating diseases associated with Galectin-9. In some aspects, the invention provides methods of treating cancer. In some embodiments, the present disclosure methods for reducing, ameliorating, or eliminating one or more symptom(s) associated with cancer.

In some embodiments, the anti-Galectin-9 antibody is an antibody having the same heavy chain CDR sequences and/or the same light chain CDR sequences as reference antibody G9.2-17. In some embodiments, the anti-Galectin-9 antibody is an antibody having the same VH and VL sequences as reference antibody G9.2-17. In some embodiments, such an antibody is an IgG1 molecule (*e.g.*, having a wild-type IgG1 constant region or a mutant thereof as those disclosed herein). Alternatively, the antibody is an IgG4 molecule (e.g., having a wild-type IgG4 constant region or a mutant thereof as those described herein). In some embodiments, the antibody comprises a light chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 1, a light chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 2, and a light chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 3 and/or comprises a heavy chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 4, a heavy chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 5, and a heavy chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 6. In some embodiments, the antibody comprises a heavy chain variable region comprising SEQ ID NO: 7. In some embodiments, the antibody comprises a light chain variable region comprising SEQ ID NO: 8. In some embodiments, the antibody comprises a heavy chain variable region comprising SEQ ID NO: 7 and a light chain variable region comprising SEQ ID NO: 8. In some embodiments, the antibody comprises a heavy chain comprising SEQ ID NO: 19. In some embodiments, the antibody comprises a light chain comprising SEQ ID NO: 15. In specific examples, the anti-Galectin-9 antibody used herein (G9.2-17(IgG4)) has a heavy chain of SEQ ID NO:19 and a light chain of SEQ ID NO:15.

An effective amount of the anti-Galectin-9 antibody described herein (e.g., G9.2-17(IgG4)) can be administered to a subject (*e.g.*, a human) in need of the treatment via a suitable route, systemically or locally. In some embodiments, the anti-Galectin-9 antibodies are administered by intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-arterial, intra-articular, intrasynovial, intrathecal, intratumoral, sub-urothelial, oral, inhalation or topical routes. In one embodiment, the anti-Galectin-9 antibody is administered to the

subject by intravenous infusion. In one embodiment, the anti-galectin-9 antibody is administered to the subject intraperitoneally.

As used herein, "an effective amount" refers to the amount of each active agent required to confer therapeutic effect on the subject, either alone or in combination with one or more other active agents. In some embodiments, the therapeutic effect is reduced Galectin-9 activity and/or amount/expression, reduced Dectin-1 signaling, reduced TIM-3 signaling, reduced CD206 signaling, or increased anti-tumor immune responses in the tumor microenvironment. Non-limiting examples of increased anti-tumor responses include increased activation levels of effector T cells or switching of the TAMs from the M2 to the M1 phenotype. In some cases, the anti-tumor response includes increased ADCC responses. Determination of whether an amount of the antibody achieved the therapeutic effect would be evident to one of skill in the art. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment.

Empirical considerations, such as the half-life, generally contribute to the determination of the dosage. For example, antibodies that are compatible with the human immune system, such as humanized antibodies or fully human antibodies, are in some instances used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a target disease/disorder. Alternatively, sustained continuous release formulations of an antibody may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

In one example, dosages for an antibody as described herein are determined empirically in individuals who have been given one or more administration(s) of the antibody. Individuals are given incremental dosages of the antagonist. To assess efficacy of the antagonist, an indicator of the disease/disorder can be followed.

In some embodiments, the antibodies described herein, *e.g.*, G9.2-17 such as G9.2-17(IgG4), are administered to a subject in need of the treatment at an amount sufficient to inhibit the activity of Galectin-9 (and/or Dectin-1 or TIM-3 or CD206) in immune suppressive immune cells in a tumor by at least 20% (*e.g.*, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater) *in vivo*. In other embodiments, the antibodies described herein, e.g, G9.2-17, are administered in an amount effective in reducing the activity level of Galectin-9 (and/or Dectin-1 or TIM-3 or CD206) in immune suppressive immune cells in a tumor by at least 20% (*e.g.*, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater) (as compared to levels prior to treatment or in a control subject). In some embodiments, the antibodies described herein, e.g., G9.2-17, are administered to a subject in need of the treatment at an amount sufficient to promote M1-like programming in TAMs by at least 20% (*e.g.*, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater) *in vivo* (as compared to levels prior to treatment or in a control subject).

The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which are dependent in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within an acceptable standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to ± 20 %, preferably up to ± 10 %, more preferably up to ± 5 %, and more preferably still up to ± 1 % of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated, the term "about" is implicit and in this context means within an acceptable error range for the particular value.

In some embodiments, the antibody is G9.2-17 IgG4. In some embodiments, the anti-Galectin-9 antibody is administered to the subject at a dose of about 0.2 mg/kg to about 32 mg/kg, e.g., 0.2 mg/kg, 0.63 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 6.3 mg/kg, 8 mg/kg, 10 mg/kg, 12 mg/kg, and 16 mg/kg or a higher dose level. In some embodiments, the anti-Galectin-9 antibody is administered to the subject at a dose of about 1 mg/kg to about 32 mg/kg, e.g., the dose may be selected from 2 mg/kg, 4 mg/kg, 8 mg/kg, 12 mg/kg, and 16 mg/kg or a higher dose level. In some embodiments, the anti-Galectin-9 antibody is administered to the subject at a dose of about 0.2 mg/kg to about 32 mg/kg, e.g., the dose may be selected from 0.2 mg/kg, 0.63 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 6.3 mg/kg, 10 mg/kg, and 16 mg/kg or a higher dose level. In some embodiments, the antibody is administered once

every two weeks, e.g., via intravenous infusion.

In some embodiments, the anti-Galectin 9 antibody disclosed herein (*e.g.*, G9.2-17 IgG4) is administered *via* a 30 minute to 6-hour infusion intravenously. In some examples the intravenous infusion of the anti-Galectin 9 antibody may be performed for 30 minutes to 2 hours. In other examples, the the anti-Galectin 9 antibody may be administered *via* a long infusion period, for example, about 2-6 hours, *e.g.*, about 2-4 hours or about 4-6 hours. In specific examples, examples anti-Galectin 9 antibody may be infused intravenous in a period of about 3 hours, about 4 hours, about 5 hours, or about 6 hours.

In some embodiments, the anti-Galectin-9 antibody for use in any of the methods disclosed herein (e.g., G9.2-17(IgG4) as disclosed herein) may be administered to the subject at a dose of about 0.2 mg/kg to about 32 mg/kg, e.g., the dose may be selected from 0.2 mg/kg, 0.63 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 6.3 mg/kg, 8 mg/kg, 10 mg/kg, 12 mg/kg, and 16 mg/kg or a higher dose level. In some embodiments, the anti-Galectin-9 antibody is administered to the subject at a dose of about 1 mg/kg to about 32 mg/kg, e.g., the dose may be selected from 2 mg/kg, 4 mg/kg, 8 mg/kg, 12 mg/kg, and 16 mg/kg or a higher dose level. In some embodiments, the anti-Galectin-9 antibody is administered to the subject at a dose of about 0.2 mg/kg to about 32 mg/kg, e.g., the dose may be selected from 0.2 mg/kg, 0.63 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, 6.3 mg/kg, 10 mg/kg, or 16 mg/kg or a higher dose level.

In some embodiments, the anti-Galectin-9 antibody (e.g., G9.2-17(IgG4) as disclosed herein) for use in any of the methods disclosed herein may be administered to a patient in need of the treatment once every week, *e.g.*, via intravenous infusion. Alternatively, the anti-Galectin-9 antibody may be administered to the patient once every two weeks, e.g., via intravenous infusion. In some embodiments, the anti-Galectin-9 antibody is administered once every week for one cycle, once every week for two cycles, once every week for 3 cycles, once every week for 4 cycles, or once every week for more than 4 cycles. In other embodiments, the anti-Galectin-9 antibody is administered once every 2 weeks for one cycle, once every 2 weeks for two cycles, once every 2 weeks for 3 cycles, once every 2 weeks for 4 cycles, or once every 2 weeks for more than 4 cycles.

In some embodiments, the duration of treatment is 12-24 months or longer. In some embodiments, the cycles extend for a duration of 3 months to 6 months, or 6 months to 12 months or 12 months to 24 months or longer. In some embodiments, the cycle length is modified, e.g., temporarily or permanently to a longer duration, e.g., 3 weeks or 4 weeks.

Given that pro-tumor action of Galectin-9 is mediated through interaction with immune

cells (*e.g.*, interactions with lymphoid cells via TIM-3, CD44, and 41BB, and with macrophages via dectin-1 and CD206) and given that Galectin-9 is expressed in a large number of tumors, targeting Galectin-9, *e.g.*, using a Galectin-9 binding antibody to inhibit interaction with its receptors provides a therapeutic approach that can be applied across a variety of different tumor types.

*(ii) Combined Treatment with Anti-PD-1 Antibodies*

Any of the methods disclosed herein may further comprise administering to the patient an effective amount of an anti-PD-1 antibody, for example, tislelizumab. Examples of PD-1 inhibitors include anti-PD-1 antibodies, such as pembrolizumab, nivolumab, tislelizumab, dostarlimab, and cemiplimab. Such checkpoint inhibitors can be administered simultaneously or sequentially (in any order) with the anti-Galectin-9 antibody according to the present disclosure. In some embodiments, the checkpoint molecule is PD-L1. Examples of PD-L1 inhibitors include anti-PD-L1 antibodies, such as durvalumab, avelumab, and atezolizumab.

In some embodiments, the antibody that binds PD-1 is tislelizumab. In some embodiments, the methods described herein comprise administration of tislelizumab to the subject at a dose of about 200 mg intravenously once every 3 weeks. In some embodiments, the methods described herein comprise administration of tislelizumab to the subject at a dose of about 400 mg intravenously once every 6 weeks. In some embodiments, the methods described herein comprise administration of tislelizumab to the subject at a dose of about 300 mg every 4 weeks. In some embodiments, tislelizumab is administered at about 300 mg intravenously every 4 weeks, in a 28-day cycle. Alternatively or in addition, tislelizumab is administered as an intravenous infusion, e.g., over approximately 30 minutes. In some embodiments, the antibody that binds PD-1 is dostarlimab. In some embodiments, the methods described herein comprise administration of dostarlimab to the subject at a dose of about 500 mg intravenously every three weeks or about 1000 mg intravenously every six weeks.

In some instances, the checkpoint inhibitor such as any of the anti-PD-1 antibodies (e.g., tislelizumab) disclosed herein and any of the anti-Galectin 9 antibodies disclosed herein such as G9.2-17(IgG4) may have same day administration. In some examples, the checkpoint inhibitor can be administered to a subject prior to administration of the anti-Galectin 9 antibody. In other instances, the administration of the checkpoint inhibitor, e.g., anti-PD-1 antibody, and the administration of the anti-Galectin 9 antibody are performed on two consecutive days. The checkpoint inhibitor, e.g., anti-PD-1 antibody, may be administered to

the subject on the first day of dosing and the anti-Galectin-9 antibody can be administered to the subject on the subsequent day.

In other instances, the checkpoint inhibitor such as any of the anti-PD-1 antibodies disclosed herein may be administered about 1-7 days (*e.g.*, 1 day, 2 day, 3 day, 4 day, 5 day, 6 day, or 7 day) prior to administration of the anti-Galectin 9 antibodies disclosed herein such as G9.2-17.

In some examples, the anti-Galectin 9 antibody can be administered to a subject prior to administration of the checkpoint inhibitor, *e.g.*, an anti-PD-1 antibody. In other instances, the administration of the anti-Galectin 9 antibody and the administration of the checkpoint inhibitor, e.g., anti-PD-1 antibody, are performed on two consecutive days. The anti-Galectin-9 antibody may be administered to the subject on the first day of dosing and checkpoint inhibitor, e.g., anti-PD-1 antibody, can be administered to the subject on the subsequent day.

In other instances, the anti-Galectin-9 antibodies disclosed herein, such as G9.2-17, may be administered about 1-7 days (*e.g.*, 1 day, 2 day, 3 day, 4 day, 5 day, 6 day, or 7 day) prior to administration of the checkpoint inhibitor, such as any of the anti-PD-1 antibodies disclosed herein.

In any of the method embodiments described herein, the anti-galectin-9 antibody can be administered (alone or in combination with an anti-PD-1 antibody such as tislelizumab) once every 2 weeks for one cycle, once every 2 weeks for two cycles, once every 2 weeks for three cycles, once every 2 weeks for four cycles, or once every 2 weeks for more than four cycles. In some embodiments, the treatment is 1 to 3 months, 3 to 6 months, 6 to 12 months, 12 to 24 months, or longer. In some embodiments, the treatment is once every 2 weeks for 1 to 3 months, once every 2 weeks for 3 to 6 months, once every 2 weeks for 6 to 12 months, or once every 2 weeks for 12 to 24 months, or longer.

In some examples, the method provided herein comprises administering to a subject in need of the treatment (e.g., a human patient having head and neck cancer, urothelial carcinoma, or other solid tumurs as disclosed herein) an anti-Galectin-9 antibody such as G9.2-17(IgG4) at a dose of 2 mg/kg to 20 mg/kg once every week and an anti-PD-1 antibody such as tislelizumab at a dose of, e.g., 300 mg once every 4 weeks. In one example, the patient is given G9.2-17(IgG4) at a dose of 4 mg/kg once every week and tislelizumab at a dose of 300 mg once every 4 weeks. In another example, the patient is given G9.2-17(IgG4) at a dose of 6.3 mg/kg once every week and tislelizumab at a dose of 300 mg once every 4 weeks. In yet another example, the patient is given G9.2-17(IgG4) at a dose of 10 mg/kg once every week and

tislelizumab at a dose of 300 mg once every 4 weeks. Alternatively, the patient is given G9.2-17(IgG4) at a dose of 16 mg/kg once every week and tislelizumab at a dose of 300 mg once every 4 weeks.

*(iii) Patients for Treatment*

A subject having any of the above noted cancers can be identified by routine medical examination, *e.g.*, laboratory tests, organ functional tests, genetic tests, interventional procedure (biopsy, surgery) any and all relevant imaging modalities. In some embodiments, the subject to be treated by the method described herein is a human cancer patient who has undergone or is subjected to an anti-cancer therapy regimen delivered systemically and/or locally, for example, chemotherapy, radiotherapy, tumor-treating fields (TTFields), immunotherapy, biological therapy, small molecule inhibitors, anti-hormonal therapy, cell-based therapy, and/or surgery, in any combination or sequence of the outlined therapeutic modalities. In some embodiments, subjects have received prior immune-modulatory or any other anti-tumor agents or treatment modalities listed above. Non-limiting examples of such immune-modulatory agents include, but are not limited to as anti-PD-1, anti-PD-L1, anti-CTLA-4, anti-TIGIT, anti-PVRIG, anti-LAG-3, anti-CD47, anti-CD40, anti-CSFR1, anti-CD73, anti-SIRP, anti-A2AR, anti-OX40, anti-CD137, etc. In some embodiments, the subject shows disease progression through the treatment. In other embodiments, the subject is resistant to the treatment (either de novo or acquired). In some embodiments, such a subject is demonstrated as having advanced malignancies (*e.g.*, inoperable or metastatic). Alternatively or in addition, in some embodiments, the subject has no standard therapeutic options available or ineligible for standard treatment options, which refer to therapies commonly used in clinical settings for treating the corresponding solid tumor.

Tumor-treating fields (TTFields) are a cancer treatment modality that uses alternating electric fields of intermediate frequency (~100-500 kHz) and low intensity (1-3 V/cm) to disrupt cell division. In any of the embodiments described herein, the anti-Galectin-9 antibody, alone or in combination with a checkpoint inhibitor, such as an anti-PD-1 antibody, may be administered prior to, concurrent with, or after a tumor-treating fields (TTFields) regimen.

In some instances, the subject may be a human patient having a refractory disease, for example, a refractory head and neck cancer, or a refractory urothelial carcinoma. As used herein, "refractory" refers to the tumor that does not respond to or becomes resistant to a treatment. In some instances, the subject may be a human patient having a relapsed disease, for

example, a relapsed head and neck cancer, or a relapsed urothelial carcinoma. As used herein, "relapsed" or "relapses" refers to the tumor that returns or progresses following a period of improvement (e.g., a partial or complete response) with treatment.

In some embodiments, the human patient to be treated by the methods disclosed herein meets one or more of the inclusion and exclusion criteria disclosed in **Example 3** below. For example, the human patient may be 18 or older; having histologically confirmed unresectable metastatic or inoperable cancer (*e.g.*, without standard therapeutic options), having a life expectancy > 3 months, having recent archival tumor sample available for biomarker analysis (e.g., an archival species for Galectin-9 tumor tissue expression levels assessed by IHC); having a measurable disease, according to RECIST v1.1, having Eastern Cooperative Oncology Group (ECOG) performance status 0-1 or Karnofsky score >70; having no available standard of care options, having MSI-H (Microsatellite instability high and MSS ( Microsatellite Stable); received at least one line of systemic therapy in the advanced/metastatic setting; having adequate hematologic and end organ function (defined in Example 1 below; e.g., *e.g.*, neutrophil count $\geq 1 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L, for HCC in Part 1 $\geq 50 \times 10^9$/L; hemoglobin $\geq 9.0$ g/dL without transfusion in the previous week, Creatinine $\leq 1.5 \times$ ULN, AST (SGOT) $\leq 3 \times$ ULN ($\leq 5 \times$ ULN when HCC or hepatic metastases are present), ALT (SGPT) $\leq 3 \times$ ULN ($\leq 5 \times$ ULN when HCC or hepatic metastases present), Bilirubin $\leq 1.5 \times$ ULN (patients with known Gilbert's disease may have a bilirubin $\leq 3.0 \times$ ULN), Albumin $\geq 3.0$ g/dL, INR and PTT $\leq 1.5 \times$ ULN; and/or amylase and lipase $\leq 1.5 \times$ ULN)); having completed treatment for brain metastases if any (see Example 1 below); having no evidence of active infection and no serious infection within the past month; having at least four (4) weeks s or 5 half lives (whichever is shorter) since the last dose of anti-cancer therapy before the first anti-Gal-9 antibody administration.

Alternatively or in addition, the subject suitable for the treatment disclosed herein may not have one or more of the following: diagnosed with metastatic cancer of an unknown primary; any active uncontrolled bleeding, and any patients with a bleeding diathesis (e.g., active peptic ulcer disease); receiving any other investigational agents within 4 weeks or 5 half-lives of anti-galectin-9 antibody administration; receiving radiation therapy within 4 weeks of the first dose of the anti-Galectin-9 antibody, except for palliative radiotherapy to a limited field, such as for the treatment of bone pain or a focally painful tumor mass; having fungating tumor masses; having active clinically serious infection > grade 2 NCI-CTCAE version 5.0; having symptomatic or active brain metastases; having $\geq$ CTCAE grade 3 toxicity

(see details and exceptions in Example 1); having history of second malignancy (see exceptions in Example 1); having evidence of severe or uncontrolled systemic diseases, congestive cardiac failure; having serious non-healing wound, active ulcer or untreated bone fracture; having uncontrolled pleural effusion, pericardial effusion, or ascites requiring recurrent drainage procedures; having spinal cord compression not definitively treated with surgery and/or radiation. Leptomeningeal disease, active or previously treated; having significant vascular disease; having active auto-immune disorder (see exceptions in Example 1); require systemic immunosuppressive treatment; having tumor-related pain (> grade 3) unresponsive to broad analgesic interventions (oral and/or patches); having uncontrolled hypercalcemia, despite use of bisphosphonates; having any history of an immune-related Grade 4 adverse event attributed to prior checkpoint inhibitor therapy (CIT); received an organ transplant(s); and/or on undergoing dialysis; and/or having Child-Pugh score ≥7. In some instances, the human patient may not have metastatic hepatocellular carcinoma that progressed while receiving at least one previous line of systemic therapy; have refuse or not tolerated sorafenib; or have had standard therapy considered ineffective, intolerable, or inappropriate or for which no effective standard therapy is available.

Alternatively or in addition, the human patient subject to any treatment disclosed herein may be free of: (i) metastatic cancer of an unknown primary, (ii) clinically significant, active uncontrolled bleeding, any bleeding diathesis (e.g., active peptic ulcer disease); (iii) radiation therapy within 4 weeks of the first dose of the treatment, (iv) with fungating tumor masses; (v) ≥ CTCAE grade 3 toxicity (except alopecia and vitiligo) due to prior cancer therapy; (v) history of second malignancy, (vi) evidence of severe or uncontrolled systemic diseases, congestive cardiac failure > New York Heart Association (NYHA) class 2, or myocardial infarction (MI) within 6 months, (vii) serious non-healing wound, active ulcer, or untreated bone fracture; (viii) uncontrolled pleural effusion, pericardial effusion, or ascites requiring recurrent drainage procedures; (ix) history of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins; (x) significant vascular disease (e.g., aortic aneurysm requiring surgical repair or recent arterial thrombosis) within 6 months of the treatment, history of pulmonary embolism, stroke or transient ischemic attack within 3 months prior to the treatment, and/or history of abdominal fistula or gastrointestinal perforation within 6 months prior to the treatment; (xi) active auto-immune disorder (except type I diabetes, hypothyroidism requiring only hormone replacement, vitiligo, psoriasis, or alopecia); (xii) requires systemic immunosuppressive treatment; (xii)

tumor-related pain (> grade 3) unresponsive to broad analgesic interventions (oral and/or patches); (xiii) uncontrolled hypercalcemia, despite use of bisphosphonates; (xiv) received organ transplant(s).

In some instances, the subject is a human patient having an elevated level of Galectin-9 as relative to a control level. The level of Galectin-9 can be a plasma or serum level of Galectin-9 in the human patient. In other examples, the level of Galectin-9 is the level of Galectin-9 of cancer cells within the tumor. In other examples, the level of Galectin-9 is the level of Galectin-9 of immune cells within the tumor. In other examples, the level of Galectin-9 can be the level of cell-surface Galectin-9, for example the level of Galectin-9 on cancer cells. In one example, the level of Galectin-9 can be the level of Galectin-9 expressed cancer cells, e.g., on the surface of cancer cells, or Galectin-9 expressed in immune cells, measured in patient-derived organotypic tumor spheroids (PDOT), which can be prepared by, *e.g.*, the method disclosed in Examples below. A control level may refer to the level of Galectin-9 in a matched sample of a subject of the same species (e.g., human) who is free of the solid tumor. In some examples, the control level represents the level of Galectin-9 in healthy subjects. In some embodiments, the control level may be a baseline level prior to treatment.

To identify such a subject, a suitable biological sample can be obtained from a subject who is suspected of having the solid tumor and the biological sample can be analyzed to determine the level of Galectin-9 contained therein (*e.g.*, free, cell-surface expressed, or total) using conventional methods, *e.g.*, ELISA or FACS. In some embodiments, organoid cultures are prepared, e.g., as described herein, and used to assess Galectin-9 levels in a subject. Single cells derived from certain fractions obtained as part of the organoid preparation process are also suitable for assessment of Galectin-9 levels in a subject. In some instances, an assay for measuring the level of Galectin-9, either in free form or expressed on cell surface, involves the use of an antibody that specifically binds the Galectin-9 (*e.g.*, specifically binds human Galectin-9). Any of the anti-Galectin-9 antibodies known in the art can be tested for suitability in any of the assays described above and then used in such assays in a routine manner. In some embodiments, an antibody described herein (*e.g.*, a G9.2-17 antibody) can be used in such as assay. In some embodiments, an antibody described in US Patent No. 10,344,091 and WO2019/084553, the relevant disclosures of each of which are incorporated by reference for the purpose and subject matter referenced herein. In some examples, the anti-Galectin-9 antibody is a Fab molecule. Assay methods for determining Galectin-9 levels as disclosed herein are also within the scope of the present disclosure.

*(iv) Responses to Treatment*

Efficacy of the treatment as disclosed herein can be assessed via routine practice. In some embodiments, any of the methods disclosed herein can increase anti-tumor activity (e.g., reduce cell proliferation, tumor growth, tumor volume, and/or tumor burden or load or reduce the number of metastatic lesions over time) by at least about 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or more as compared to levels prior to treatment or in a control subject. In some embodiments, reduction is measured by comparing cell proliferation, tumor growth, and/or tumor volume in a subject before and after administration of the pharmaceutical composition. In some embodiments, the method disclosed herein may improve one or more symptoms of the cancer by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more. In some embodiments, before, during, and after the administration of the pharmaceutical composition, cancerous cells and/or biomarkers in a subject are measured in a biological sample, such as blood, serum, plasma, urine, peritoneal fluid, and/or a biopsy from a tissue or organ. In some embodiments, the methods include administration of the compositions of the invention to reduce tumor volume, size, load or burden in a subject to an undetectable size, or to less than about 1%, 2%, 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, or 90% of the subject's tumor volume, size, load or burden prior to treatment. In other embodiments, methods are provided for reducing the cell proliferation rate or tumor growth rate in a subject to an undetectable rate, or to less than about 1%, 2%, 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, or 90% of the rate prior to treatment. In other embodiments, methods include administration of the compositions of the invention to reduce the development of or the number or size of metastatic lesions in a subject to an undetectable rate, or to less than about 1%, 2%, 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, or 90% of the rate prior to treatment.

A response to treatment, e.g., a treatment of a solid tumor as described herein, can be assessed according to RECIST or the RECIST 1.1 criteria and /or irRC, irRECIST, iRECIST, imRECISTPDAC, as described in Example 1 below and Eisenhower et al., New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1); European Journal Of Cancer 45 (2009) 228 – 247; or Borcoman et al., Annals of Oncology 30: 385–396, 2019;Nishino et al., Clin Cancer Res 2013; 19(14): 3936–3943, the contents of each of which is herein incorporated by reference in its entirety.

In some embodiments, methods are provided for improving and or controlling the

overall response/tumor burden/tumor size (e.g., at approximately 2, 3, 6 or 12 months, or a later time) comprising administering an anti-Galectin-9 antibody described herein, e.g., as compared to a baseline level obtained prior to initiation of G9.2-17 IgG4 treatment regimen. In some embodiments, the methods are for improving and or controlling the overall response/tumor burden/tumor size at approximately 2 months. In some embodiments, where the anti-Galectin-9 antibody (e.g., G9.2-17(IgG4)) is administered in a combination regimen with a checkpoint inhibitor, e.g., an anti-PD-1 antibody such as tislelizumab, can improve or control the overall response /tumor burden/tumor size (*e.g.*, at approximately 2, 3, 6 or 12 months, or a later time), e.g., as compared to a baseline level obtained prior to initiation of treatment. In some embodiments, methods are provided, which result in a complete response, a partial response or stable disease (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), comprising administering an anti-Galectin-9 antibody described herein. Such a response can be temporary over a certain time period or permanent.

In some embodiments, a method as disclosed herein may improve the likelihood of a complete response, a partial response or stable disease (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), e.g., as compared to a baseline level obtained prior to initiation of G9.2-17 IgG4 treatment regimen. Such a response can be temporary over a certain time period or permanent. In some embodiments, treating can result in reduced or attenuated progressive disease (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), e.g., as compared to a baseline level obtained prior to initiation of G9.2-17 IgG4 treatment regimen. Such an attenuation may be temporary or permanent. In any of these embodiments, anti-Galectin-9 antibody may be administered in combination with a checkpoint inhibitor, *e.g.*, an anti-PD-1 antibody.

In some embodiments, a method as disclosed herein may attenuate disease progression or reducing progressive disease (e.g., as measured at approximately 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point). The method comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. In any of these embodiments, the anti-Galectin-9 antibody may be administered in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody.

In any of the methods described herein, partial response, stable disease, complete response, a partial response, stable disease, progressive disease, disease progressing (e.g., as

measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), can be assessed according to irC criteria, RECIST criteria, RECIST1.1., irRECIST or iRECIST, or imRECIST criteria, or other criteria known in the art (see, e.g., Borcoman et al., Annals of Oncology 30: 385–396, 2019' iRC: Hoos et al., J. Immunother. 30 (1): 1–15).

A partial response is a decrease in the size of a tumor, or in the extent of cancer in the body, *i.e.*, the tumor burden, in response to treatment as compared to a baseline level before the initiation of the treatment. For example, according to the RECIST response criteria, a partial response is defined as at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters. Progressive disease is a disease that is growing, spreading, or getting worse. For example, according to the RECIST response criteria, progressive disease includes disease in which at least a 20% increase in the sum of diameters of target lesions is observed, and the sum must also demonstrate an absolute increase of at least 5 mm. Additionally, the appearance of one or more new lesions is also considered progression. A tumor that is neither decreasing nor increasing in extent or severity as compared to a baseline level before initiation of the treatment is considered stable disease. For example, according to the RECIST response criteria, stable disease occurs when there is neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum diameters while on study.

In some embodiments, the disclosure provides methods for reducing or maintaining tumor size in a subject, including a human subject, (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point) either permanently or over a minimum time period, relative to a baseline tumor size prior to initiation of the treatment in the subject, the method comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody alone or in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody.. Tumor size, e.g., the diameters of tumors, can be measured according to methods known in the art, which include measurements from CT and MRI images in combination with various software tools, according to specific measurement protocols, e.g., as described in Eisenhower et al., referenced above. Accordingly, in some embodiments, tumor size is measured in regularly scheduled restaging scans (e.g., CT with/without contrast, MRI with/without contrast, PET-CT (diagnostic CT) and/or X-ray, ultrasound and /or other relevant imaging modality). In some embodiments, tumor size reduction, maintenance of tumor size refers to the size of target lesions. In some

embodiments, tumor size reduction, maintenance of tumor size refers to the size of non-target lesions. According to RECIST 1.1, when more than one measurable lesion is present at baseline, all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions. All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions.

In some embodiments, the disclosure provides methods for increasing the likelihood of reducing or maintaining a tumor burden (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), the methods comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein, alone or in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody such as tislelizumab. In some embodiments, treating can result in in a greater likelihood of a reduction of tumor burden, or maintenance of tumor burden, (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point). As used herein, tumor burden refers to amount of cancer, the size or the volume of the tumor in the body of a subject, accounting for all sites of disease. Tumor burden can be measured using methods known in the art, including but not limited to, FDG positron emission tomography (FDG-PET), magnetic resonance imaging (MRI), and optical imaging, comprising bioluminescence imaging (BLI) and fluorescence imaging (FLI).

In some embodiments, the methods described herein increase in the time to disease progression or in progression free survival (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point post initiation of treatment). Progression free survival can be either permanent or progression free survival over a certain amount of time. In some embodiments, the methods provide a greater likelihood of progression free survival (either permanent progression free survival or progression free survival over a certain amount of time, *e.g.*, 3, 6 or 12 months or e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point post initiation of treatment). Progression-free survival (PFS) is defined as the time from random assignment in a clinical trial, *e.g.*, from initiation of a treatment to disease progression or death from any cause. In some embodiments, the methods achieve longer survival or greater likelihood of survival, *e.g.*, at a certain time, *e.g.*, at 6 or 12 months.

A response to treatment, e.g., a treatment of a solid tumor as described herein, can be assessed according to iRECIST criteria, as described in Seymour et al, iRECIST: guidelines for response criteria for use in trials; The Lancet, Vol18, March 2017, the contents of which is herein incorporated by reference in its entirety. iRECIST was developed for the use of modified RECIST1.1 criteria specifically in cancer immunotherapy trials, to ensure consistent design and data collection and can be used as guidelines to a standard approach to solid tumor measurements and definitions for objective change in tumor size for use in trials in which an immunotherapy is used. iRECIST is based on RECIST 1.1. Responses assigned using iRECIST have a prefix of "i" (ie, immune)—*e.g.*, "immune" complete response (iCR) or partial response (iPR), and unconfirmed progressive disease (iUPD) or confirmed progressive disease (iCPD) or stable disease (iSD) to differentiate them from responses assigned using RECIST 1.1, and all of which are defined in Seymour et al. RECIST 1.1. In some embodiments criteria can be compared to baseline levels prior to initiation of treatment. In any of these embodiments, the anti-Galectin-9 antibody may be administered alone or in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody such as those disclosed herein.

Accordingly, in some embodiments, the disclosure provides methods for improving overall response (iOR) or achieving "immune" complete response (iCR), a partial response (iPR) or stable disease (iSD) (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), as compared to the baseline level of disease prior to initiation of the treatment. The reduction in the "immune" response, e.g., iCR, iPR, or iSD can be temporary over a certain time period or permanent. In some embodiments, treating can improve the likelihood of a complete response (iCR), a partial response (iPR) or stable disease (iSD) (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), e.g., In some embodiments, the disclosure provides methods for attenuating disease progression or reducing progressive disease, e.g., reducing unconfirmed progressive disease (iUPD) or reducing confirmed progressive disease (iCPD)) (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), the method comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. Any of these above mentioned iRECIST criteria can be compared to baseline levels prior to initiation of treatment. In any of these methods the anti-Galectin-9 antibody may be administered alone or in combination with a

checkpoint inhibitor, *e.g.*, an anti-PD-1 antibody.

The reduction in iUPD or iCPD can be temporary over a certain time period or permanent. In some embodiments, treating can result in greater likelihood of overall reduction in unconfirmed progressive disease (iUPD) or confirmed progressive disease (iCPD) (e.g., as measured at approximately 2 months , 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point In some embodiments, the disclosure provides methods for reducing the number of new lesions in a subject, including a human subject, according to iRECIST criteria (e.g., as measured at approximately 2 months , 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), the methods comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. Reduced number of lesions can be relative to baseline levels prior to initiation of treatment, and the reduction can be temporary over a certain time period or permanent. In any of these embodiments, the anti-Galectin-9 antibody may be administered in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody.

Additional criteria can be used to measure a treatment response. For example, tumor burden can be measured according to the irRC criteria (Hoos et al., 2007). In the irRC, tumor burden is measured by combining 'index' lesions with new lesions, i.e., new lesions are considered a change in tumor burden. In the irRC, an immune-related Complete Response (irCR) is the disappearance of all lesions, measured or unmeasured, and no new lesions; an immune-related Partial Response (irPR) is a 50% drop in tumor burden from baseline as defined by the irRC; and immune-related Progressive Disease (irPD) is a 25% increase in tumor burden from the lowest level recorded. Everything else is considered immune-related Stable Disease (irSD).

Immune-related RECIST (irRECIST) is based on unidimensional measurements of RECIST, and Specific immune-related criteria were further redefined in the irRECIST. Recently, new criteria were evaluated based on atezolizumab data in NSCLC, the immune-modified RECIST (imRECIST), requiring a confirmation of disease progression at least 4 weeks after initial assessment (Hodi et al, JCO 2018; 36(9): 850–858). For a comparison of RECIST 1.1., irRC, irRECIST, iRECIST and imRECIST, see, *e.g.*, Figure 4 in Borcoman et al., Annals of Oncology 30: 385–396, 2019; Nishino et al., Clin Cancer Res 2013; 19(14): 3936–3943, the contents of which is herein incorporated by reference in its entirety. Any of these criteria are suitable in determining response rate in any of the methods described herein.

*(v) Monitoring Adverse Events and Modifying Treatment Conditions*

In addition, a subject being treated by any of the anti-galectin-9 antibodies disclosed herein (e.g., G9.2-17), either alone or in combination with a checkpoint inhibitor (e.g., an anti-PD-1 such as tislelizumab) as disclosed herein may be monitored for occurrence of adverse effects (for example, severe adverse effects). Exemplary adverse effects to monitor are provided in **Example 3** below. If occurrence of adverse effects is observed, treatment conditions may be changed for that subject. For example, the dose of the anti-galectin-9 antibody may be reduced and/or the dosing interval may be extended. Suitability and extent of reduction may be assessed by a qualified clinician. In one embodiment, a reduction level of 30 or 50% of the previous dose level is implemented. In one specific example, a reduction level as per clinician's assessment or at least by 30% is implemented (to dose level 1, the level at first dose reduction). If required, one more dose reduction by 30% of dose level -1 is implemented (dose level -2, the level at second dose reduction). In another example, one more dose reduction by 50% of dose level -1 is implemented (dose level -2). In some embodiments, one or more dose reductions by about 10% to about 80% of a previous dose level are implemented. In some embodiments, one or more dose reductions by about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, or about 70% to about 80% of a previous dose level are implemented. In some embodiments, one or more dose reductions by 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, or 70% to 80% of a previous dose level are implemented. In some embodiments, one or more dose reductions by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, or by about 80% of a previous dose level are implemented. In some embodiments, one or more dose reductions by 10%, by 20%, by 30%, by 40%, by 50%, by 60%, by 70%, or by 80% of a previous dose level are implemented. Alternatively or in addition, the dose of the checkpoint inhibitor can be reduced and/or the dosing interval of the checkpoint inhibitor may be extended. In some instances (e.g., occurring of life-threatening adverse effects), the treatment may be terminated.

In some instances, the dose of the anti-Galectin-9 antibody such as G9.2-17(IgG4) and/or the dose of the anti-PD-1 antibody such as tislelizumab may be reduced if an adverse effect is observed in a patient. In some instances, the dose may be reduced by 50%. When needed, the dose may further reduce by 50%. See, *e.g.*, **Example 3** below.

*(vi) Biomarkers for Assessing Response to Treatment*

Response to treatment can also be characterized by one or more of immunophenotype in blood and tumors, cytokine profile (serum), soluble galectin-9 levels in blood (serum or plasma), galectin-9 tumor tissue expression levels and pattern of expression by immunohistochemistry (tumor, stroma, immune cells), tumor mutational burden (TMB), PD-L1 expression ( e.g., by immunohistochemistry), mismatch repair status, or tumor markers relevant for the disease (e.g., as measured at approximately 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point). Examples of such tumor markers include, but are not limited to, CA15-3, CA-125, CEA, CA19-9, alpha fetoprotein. These parameters can be compared to baseline levels prior to initiation of treatment. In any of these embodiments, the anti-Galectin-9 antibody may be administered alone or in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody.

In any of the methods disclosed herein, the subject may examined for one or more of the following features before, during, and/or after the treatment: (a) one or more tumor markers in blood samples from the subject, optionally wherein the one or more tumor markers comprise CA15-3, CA-125, CEA, CA19-9, and/or alpha fetoprotein, and any other tumor -type specific tumor markers; (b) cytokine profile; and (c)galectin 9 serum/plasma levels, d) peripheral blood mononuclear cell immunophenotyping, e) tumor tissue biopsy/excisional specimen multiplex immunophenotyping, f) tumor tissue biopsy/excisional specimen galectin-9 expression levels and pattern, g) any other immune score test such as: PD-L1 immunohistochemistry, tumor mutational burden (TMB), tumor microsatellite instability status, as well as panels such as: Immunoscore®- HalioDx, ImmunoSeq- Adaptive Biotechnologies, TIS, developed on the NanoString nCounter® gene expression system, 18-gene signature, PanCancer IO 360™ assay (NanoString Technologies) etc. Other suitable biomarkers specific to the target tumor such as PDAC may also be used. In one non-limiting example, PD-L1 (SP263) (Roche, Ventana) can be used for detection of PD-L1 in cancer tissues using immunohistochemistry.

In some embodiments, the methods are described herein for changing levels of immune cells and immune cell markers in the blood or in tumors, e.g., immune activation, comprising an anti-Gal-9 antibody is administered alone or in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody. Such changes can be measured in patient blood and tissue samples using methods known in the art, such as multiplex flow cytometry and multiplex immunohistochemistry. For example, a panel of phenotypic and functional PBMC immune

markers can be assessed at baseline prior to commencement of the treatment and at various time point during treatment. **Table 2** lists non-limiting examples of markers useful for these assessment methods. Flow cytometry (FC) is a fast and highly informative method of choice technology to analyze cellular phenotype and function and has gained prominence in immune phenotype monitoring. It allows for the characterization of many subsets of cells, including rare subsets, in a complex mixture such as blood, and represents a rapid method to obtain large amounts of data. Advantages of FC are high speed, sensitivity, and specificity. Standardized antibody panels and procedures can be used to analyze and classify immune cell subtypes. Multiplex IHC is a powerful investigative tool, which provides objective quantitative data describing the tumor immune context in both immune subset number and location and allows for multiple markers to be assessed on a single tissue section. Computer algorithms can be used to quantify IHC-based biomarker content from whole slide images of patient biopsies, combining chromogenic IHC methods and stains with digital pathology approaches.

Accordingly, in some embodiments, methods are described herein, for modulating an immune response, e.g., modulation of immune activation markers such as those in **Table 2** comprising administering an anti-gal9 antibody alone or in combination with a checkpoint inhibitor therapy. In some embodiments, modulation comprises in one or more of (1) an increase in more CD8 cells in plasma or tumor tissue, (2) a reduction in T regulatory cells (Tregs) in plasma or tumor tissue, (3) an increase in M1 macrophages in plasma or tumor tissue and (4) a decrease in MDSCs in plasma or tumor tissue, and (5) a decrease in M2 macrophages in plasma or tumor tissue (e.g., as measured at approximately 2 months , 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point). In some embodiments, the markers that are assessed using the techniques described above or known in the art are selected from CD4, CD8 CD14, CD11b/c, and CD25. These parameters can be compared to baseline levels prior to initiation of treatment.

**Table 2. PBMC phenotyping markers**

| PBMC phenotyping markers | | PBMC phenotyping markers | |
|---|---|---|---|
| CD3 | Total T cells | CD16 | NK cells |
| CD4 | CD4+ T cells | CD11b | Monocytes/macrophages |
| CD8 | CD8+ T cells | CD11c | Monocytes/macrophages, DCs |
| CD25 | Treg activation | CD14 | Monocyte subsets, macrophages |
| CD27 | T cell maturation; B cell naive/memory | CD33 | Total myeloid cells |
| CD38 | T cell maturation; B cell naive/memory | FceR1 a | Antigen presenting DC cells |
| CD45RA | Naive/memory cells | CD19 | Total B cells |
| CD45RO | Naive/memory cells | T-bet | T cells subsets |
| CD56 | NKT/NK cells (T cell subset) | gdTCR | Gamma delta T cells |
| CD127 | T cell subsets | CD274 (PDL-1) | Checkpoint |
| CD152 (CLTA-4) | Checkpoint | Tim-3 | Checkpoint |
| CD279 (PD-1) | Checkpoint | TCRVa24-Ja18 | iNKT cells |
| FoxP3 | Treg cells | Live/dead | General |
| HLA-DR | Activation/Antigen presentation | CD45 | General |

*(vii) Modulating Immune Responses*

In some embodiments, methods are described herein, comprising administering an anti-gal9 alone or in combination with a checkpoint inhibitor therapy, for modulating proinflammatory and anti-inflammatory cytokines. In some embodiments, methods are provided for one or more of (1) increasing levels of IFNgamma in plasma or tumor tissue; (2) increasing levels of TNFalpha in plasma or tumor tissue; (3) decreasing levels of IL-10 in plasma or tumor tissue (e.g., as measured at approximately 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point). These parameters can be compared to baseline levels prior to initiation of treatment.

In some embodiments, cytokine levels or immune cell levels may be assessed between a pre dose 1 tumor biopsy and repeat biopsy conducted at a feasible time. In some embodiments, cytokine levels or immune cell levels may be assessed between 2 repeat biopsies. In some embodiments, methods are provided for modulating one or more of soluble galectin-9 levels in blood (serum or plasma), or galectin-9 tumor tissue expression levels and pattern of expression by immunohistochemistry (tumor, stroma, immune cells), (e.g., as measured at approximately 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point). In some embodiments, the methods decrease soluble galectin-9 levels in blood (serum or plasma), or galectin-9 tumor tissue expression levels or pattern of expression by immunohistochemistry (tumor, stroma, immune cells) (*e.g.*, as measured at

approximately 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point). Galectin-9 levels can be compared to baseline levels prior to initiation of treatment. In some embodiments, Galectin-9 levels may be compared to a control group not receiving the treatment or healthy subjects. In any of these embodiments, the anti-Galectin-9 antibody may be administered alone or in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody. In some embodiments, methods for modulating PD-L1 expression are provided, e.g., as assessed by immunohistochemistry, comprising administering an anti-Galectin-9 antibody, alone or in combination with a checkpoint inhibitor, e.g., an ant-Galectin-9 antibody. In some embodiments, the methods modulate in one or more tumor markers (increase or decrease) relevant for the disease (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point). Examples of such tumor markers include, but are not limited to, CA15-3, CA-125, CEA, CA19-9, alpha fetoprotein. These parameters can be compared to baseline levels prior to initiation of treatment. In any of these embodiments, the anti-Galectin-9 antibody may be administered alone or in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody.

In some embodiments, the disclosure provides methods of modulating an immune response in a subject. As used herein, the term "immune response" includes T cell-mediated and/or B cell-mediated immune responses that are influenced by modulation of immune cell activity, for example, T cell activation. In one embodiment of the disclosure, an immune response is T cell mediated. As used herein, the term "modulating" means changing or altering, and embraces both upmodulating and downmodulating. For example, "modulating an immune response" means changing or altering the status of one or more immune response parameter(s). Exemplary parameters of a T cell mediated immune response include levels of T cells (e.g., an increase or decrease in effector T cells) and levels of T cell activation (e.g., an increase or decrease in the production of certain cytokines). Exemplary parameters of a B cell mediated immune response include an increase in levels of B cells, B cell activation and B cell mediated antibody production.

When an immune response is modulated, some immune response parameters may decrease and others may increase. For example, in some instances, modulating the immune response causes an increase (or upregulation) in one or more immune response parameters and a decrease (or downregulation) in one or more other immune response parameters, and the result is an overall increase in the immune response, e.g., an overall increase in an inflammatory immune response. In another example, modulating the immune response causes

an increase (or upregulation) in one or more immune response parameters and a decrease (or downregulation) in one or more other immune response parameters, and the result is an overall decrease in the immune response, e.g., an overall decrease in an inflammatory response. In some embodiments an increase in an overall immune response, i.e., an increase in an overall inflammatory immune response, is determined by a reduction in tumor weight, tumor size or tumor burden or any RECIST or iRECIST criteria described herein. In some embodiments an increase in an overall immune response is determined by increased level(s) of one or more proinflammatory cytokine(s), e.g., including two or more, three or more, etc. or a majority of proinflammatory cytokines (one or more, two or more, etc. or a majority of anti-inflammatory and/or immune suppressive cytokines and/or one or more of the most potent anti-inflammatory or immune suppressive cytokines either decrease or remain constant). In some embodiments an increase in an overall immune response is determined by increased levels of one or more of the most potent proinflammatory cytokines (one or more anti-inflammatory and/or immune suppressive cytokines including one or more of the most potent cytokines either decrease or remain constant). In some embodiments an increase in an overall immune response is determined by decreased levels of one or more, including a majority of, immune suppressive and/or anti-inflammatory cytokines (the levels of one or more, or a majority of, proinflammatory cytokines, including e.g., the most potent proinflammatory cytokines, either increase or remain constant). In some embodiments, an increase in an overall immune response is determined by increased levels of one or more of the most potent anti-inflammatory and/or immune suppressive cytokines (one or more, or a majority of, proinflammatory cytokines, including, e.g., the most potent proinflammatory cytokines either increase or remain constant). In some embodiments an increase in an overall immune response is determined by a combination of any of the above. Also, an increase (or upregulation) of one type of immune response parameter can lead to a corresponding decrease (or downregulation) in another type of immune response parameter. For example, an increase in the production of certain proinflammatory cytokines can lead to the downregulation of certain anti-inflammatory and/or immune suppressive cytokines and vice versa.

In some embodiments, the disclosure provides methods for modulating an immune response (e.g., as measured at approximately 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point) in a subject, including a human subject, comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. In some embodiments, the disclosure provides

methods for modulating levels of immune cells and immune cell markers, including but not limited to those described herein in **Table 2**, *e.g.*, as compared to baseline levels prior to initiation of treatment, *e.g.*, as compared to a baseline level obtained prior to initiation of the anti-Gal9 antibody treatment regimen, , in the blood or in tumors of a subject, including a human subject, comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. In some embodiments, the overall result of modulation is upregulation of proinflammatory immune cells and/or down regulation of immune-suppressive immune cells. In some embodiments, the disclosure provides methods for modulating levels of immune cells, wherein the modulating encompasses one or more of (1) increasing CD8 cells in plasma or tumor tissue, (2) reducing Tregs in plasma or tumor tissue, (3) increasing M1 macrophages in plasma or tumor tissue and (4) decreasing MDSC in plasma or tumor tissue, and (5) decreasing in M2 macrophages in plasma or tumor tissue, and wherein the methods comprise administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. In some embodiments, the markers to assess levels of such immune cells include but are not limited to CD4, CD8 CD14, CD11b/c, and CD25. In some embodiments, the disclosure provides methods for modulating levels of proinflammatory and immune suppressive cytokines (e.g., as measured at approximately 2 months , 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), e.g., as compared to baseline levels prior to initiation of treatment, in the blood or in tumors of a subject, including a human subject, comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. In some embodiments, the overall result of modulation is upregulation of proinflammatory cytokines and/or down regulation of immune-suppressive cytokines. In some embodiments, the disclosure provides methods for modulating levels of cytokines cells, wherein the modulating encompasses one or more of (1) increasing levels of IFNgamma in plasma or tumor tissue; (2) increasing levels of TNFalpha in plasma or tumor tissue; (3) decreasing levels of IL-10 in plasma or tumor tissue.

In some embodiments, the disclosure provides methods for changing one or more of soluble galectin-9 levels in blood (serum or plasma), or in galectin-9 tumor tissue expression levels and pattern of expression by immunohistochemistry (tumor, stroma, immune cells) (e.g., as measured at 2 weeks, 4 weeks, 1 month, 2 month , 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. In some embodiments of the methods, one or more of soluble galectin-9 levels in blood (serum or

plasma), or in galectin-9 tumor tissue expression levels and pattern of expression by immunohistochemistry (tumor, stroma, immune cells) remain unchanged. In some embodiments, the methods provided herein decrease one or more of soluble galectin-9 levels in blood (serum or plasma), or in galectin-9 tumor tissue expression levels and pattern of expression by immunohistochemistry (tumor, stroma, immune cells) (e.g., e.g., as measured at 2 weeks, 4 weeks, 1 month, 2 month, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point). Galectin-9 levels can be compared to baseline levels prior to initiation oftreatment. In some embodiments, the Galectin-9 levels may be compared to healthy subjects. In some embodiments, treating results in a change in PD-L1 expression, e.g., by immunohistochemistry. 16 mg/kg or higher dose level16 mg/kg or higher dose level16 mg/kg or a higher dose level.

In some embodiments, the disclosure provides methods for changing PD-L1 expression, e.g., as assessed by immunohistochemistry (e.g., as measured at 2 weeks, 4 weeks, 1 month, 2 month, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. In some embodiments of the methods, PD-L1 expression, e.g., as assessed by immunohistochemistry, remains unchanged. PD-L1 levels can be compared to baseline levels prior to initiation of treatment. In some embodiments, the methods provided herein decrease PD-L1 expression, *e.g.*, as assessed by immunohistochemistry. PD-L1 levels may be measured using routine methods known in the art. In one non-limiting example, PD-L1 (SP263) (Roche, Ventana) can be used for detection of PD-L1 in cancer tissues using immunohistochemistry. 16 mg/kg or higher dose level16 mg/kg or higher dose level 16 mg/kg or a higher dose level.

In some embodiments, the disclosure provides methods for changing one or more tumor markers (increasing or decreasing) relevant for the disease (e.g., as measured at 2 weeks, 4 weeks, 1 month, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. In some embodiments of the methods, one or more tumor markers (increasing or decreasing) relevant for the disease, remain unchanged. Examples of such tumor markers include, but not limited to CA15-3, CA-125, CEA, CA19-9, alpha fetoprotein. Levels of tumor markers can be compared to baseline levels prior to initiation of treatment. In some embodiments, the methods provided herein decrease

the occurrence of one or more tumor markers relevant for the disease.

In some embodiments, the disclosure provides methods for changing one or more biomarkers (increasing or decreasing) relevant for the disease (*e.g.*, as measured at 2 weeks, 4 weeks, 1 month, 2 months, 3 months, 6 months or 12 months, or at a later time or at any other clinically indicated time point), comprising administering to the subject a therapeutically effective amount of an anti-Galectin-9 antibody as disclosed herein. Levels of biomarkers in clinical tissues from patients can be measured using routine methods, such as multiplex Immunofluorescence (mIF) technology, as described herein in the examples. An exemplary panel of biomarkers may include CD3, CD4, CD8, CD45RO, FoxP3, CD11b, CD14, CD15, CD16, CD33, CD68, CD163, HLA-DR, Arginase1, Granzyme B, Ki67, PD-1, PD-L1, and PanCK.

**Kits for Use in Treatment of Solid Tumor**

The present disclosure also provides kits for use in treating or alleviating a solid tumor such as those disclosed herein (e.g., head and neck cancer or urothelial carcinoma). Such kits can include one or more containers comprising an anti-Galectin-9 antibody, *e.g.*, any of those described herein (e.g., G9.2-17(IgG4)), and a checkpoint inhibitor such as an anti-PD-1 antibody as disclosed herein, e.g., tislelizumab) to be co-used with the anti-Galectin-9 antibody, which is also described herein.

In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of administration of the anti-Galectin-9 antibody, and the anti-PD-1 antibody, to treat, delay the onset, or alleviate a target disease as those described herein. In some embodiments, the kit further comprises a description of selecting an individual suitable for treatment based on identifying whether that individual has the target disease, *e.g.*, applying the diagnostic method as described herein. In still other embodiments, the instructions comprise a description of administering an antibody to an individual at risk of the target disease.

The instructions relating to the use of an anti-Galectin-9 antibody and an anti-PD-1 antibody as disclosed herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic

or optical storage disk) are also acceptable.

The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (*e.g.*, an atomizer) or an infusion device such as a minipump. In some embodiments, a kit has a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some embodiments, the container also has a sterile access port (for example the container is an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-Galectin-9 antibody as those described herein.

Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiments, the invention provides articles of manufacture comprising contents of the kits described above.

**General Techniques**

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M. J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds., 1993-8) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); Current Protocols in Molecular Biology (F. M. Ausubel, et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis, et al., eds., 1994); Current Protocols in Immunology (J. E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical

approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995).

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

## EXAMPLES

### Example 1: Evaluation of Anti-Gal-9 Antibodies alone or in combination with Checkpoint Inhibition in a Mouse Model of Pancreatic Cancer and Tumor Mass and Immune Profile of Mice Treated with G9.2-17 mIgG1

The effect of G9.2-17 mIgG1 on tumor weight and on immune profile was assessed in a mouse model of pancreatic cancer. 8-week old C57BL/6 male (Jackson Laboratory, Bar Harbor, ME) mice were administered intra-pancreatic injections of FC1242 PDAC cells derived from Pdx1Cre; KrasG12D; Trp53R172H (KPC) mice (Zambirinis CP, et al., TLR9 ligation in pancreatic stellate cells promotes tumorigenesis. J Exp Med. 2015;212:2077-94). Tumor cells were suspended in PBS with 50% Matrigel (BD Biosciences, Franklin Lakes, NJ) and $1x10^5$ tumor cells were injected into the body of the pancreas via laparotomy. Mice (n=10/group) received one pre-treatment dose i.p. followed by 3 doses (q.w.) of commercial αGalectin 9 mAb (RG9-1, 200ug, BioXcell, Lebanon, NH) or G9.2-17 mIgG1 (200µg), or paired isotype, either G9.2-Iso or rat IgG2a (LTF-2, BioXcell, Lebanon, NH) (200µg) (one dose per week for three weeks). Mice were sacrificed 3 weeks later and tumors were harvested for analyses by flow cytometry. Tissue was processed and prepared and flow cytometric analysis was performed following routine practice. See, e.g., U.S. Patent No. 10,450,374.

*Tumor Mass and Immune Profile of Mice Treated with G9.2-17 mIgG2a alone or in combination with αPD-1 mAb*

The effect of G9.2-17 mIgG2a on tumor weight and on immune profile was assessed in a mouse model of pancreatic cancer, alone or in combination with immunotherapy. 8-week old C57BL/6 male mice (Jackson Laboratory, Bar Harbor, ME) were administered intra-

pancreatic injections of FC1242 PDAC cells derived from Pdx1Cre; KrasG12D; Trp53R172H (KPC) mice. Tumor cells were suspended in PBS with 50% Matrigel (BD Biosciences, Franklin Lakes, NJ) and 1x105 tumor cells were injected into the body of the pancreas via laparotomy. Mice received one pre-treatment dose i.p. followed by 3 doses (q.w.) of G9.2-17 mIgG2a (200μg) or a neutralizing αPD-1 mAb (29F.1A12, 200 μg, BioXcell, Lebanon, NH), separately or in combination, or paired isotype (LTF-2 and C1.18.4, BioXcell, Lebanon, NH) as indicated. Mice were sacrificed on day 26 and tumors were harvested for analyses. Tissue was processed and prepared and flow cytometric analysis was performed following routine practice. See, e.g., US 10,450,374. Each point represents one mouse; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; by unpaired Student's t-test. These results show single-agent treatment with G9.2-17 mIgG2a reduces tumor growth at both of the dose levels, whereas anti-PD-1 alone had no effect on tumor size. **Figure 1.**

Significant increases in the level of cytotoxic T cells (CD8) are observed in treatments with anti-galectin-9 mIgG1 200μg + anti-PD-1 ($p < 0.001$) compared to that of anti-galectin-9 mIgG1 200 μg, and between anti-galectin-9 IgG1 200 μg + anti-PD-1 compared to anti-PD-1 alone ($p < 0.01$). Such results suggest that anti-Gal9 antibody and anti-PD-1 antibody in combination would be expected to achieve superior therapeutic effects.

Further, tumor immune responses to treatment with G9.2-17 IgG1 mouse mAb (*a.k.a.* G9.2-17 mIgG), anti-PD-1 antibody, or a combination of the G9.2-17 IgG1 mouse mAb and anti-PD-1 antibody were investigated in the B16F10 subcutaneous syngeneic model described herein. As shown in **Figure 2A** and **Figure 2B**, the G9.2-17 and anti-PD-1 combination showed synergistic effects in reducing tumor volume and in increasing CD8+ cells in the mouse model. **Figures 3A and 3B** show that the G9.2-17 antibody increased CD44 and TNFa expression in intratumoral T cells.

**Example 2: Pharmacokinetics of G9.2-17(IgG4) in Human Studies**

A Phase 1/2, open-label, multicenter study of safety, pharmacokinetics, and anti-tumor activity of G9.2-17(IgG4) alone or in combination with chemotherapy or immune checkpoint inhibitors (e.g., PD1 antagonists) was performed on patients with metastatic solid tumors. This study is evaluating G9.2-17(IgG4) at the dose levels of 0.2, 0.63, 2.0, 6.3, 10, or 16 mg/kg administered every 2 weeks (Q2W), and 10 and 16 mg/kg every week (QW). See WO2020/223702, WO2022/109302, International Patent Application No. PCT/US2022/027127, and International Patent Application No. PCT/US2022/027142, the

relevant disclosures of each of which are incorporated by reference for the subject matter and purpose refereced herein.

Preliminary PK data for 13 subjects from this study were available for analysis. Based on the preliminary data, the beta half-life (*i.e.*, elimination half-life for a 2-compartmental model) of G9.2-17(IgG4) was found to be longer than the non-compartmental analysis (NCA)-derived half-life. These results suggest that the elimination half-life for G9.2-17(IgG4) ranges between 102 and 224 hours (*i.e.*, 4.3 to 9.3 days) over the 0.2 mg/kg to 16 mg/kg dose range, suggesting that weekly dosing of G9.2-17(IgG4) may be an appropriate dosing schedule for this antibody in human treatment.

**Example 3: A Phase 1/2 Open-label, Multi-center Study of the Safety, Pharmacokinetics, and Anti-tumor Activity of G9.2-17(IgG4) as a Single Agent and in Combination with Tislelizumab in Patients with Locally Advanced or Metastatic Solid Tumors**

This is an open-label, non-randomized, multi-center, Phase 1/2 study with a dose escalation phase (Part 1) and a cohort expansion phase (Part 2) in patients with relapsed and/or refractory, unresectable locally advanced or metastatic solid tumors. This study will be conducted at up to 20 sites in the United States. The study duration is estimated to be 12-24 months. Follow-up for survival will continue for up to 2 years.

*Treatment Duration*

Study drug administration is planned to continue until progression of disease, unacceptable toxicity, or withdrawal from the study. Patients who discontinue the study drug prior to disease progression and are not being treated with other anticancer therapy(ies), will be followed on the study until the time of disease progression.

*Treatment Periods*

The study consists of the following periods in both Part 1 and Part 2 (see disclosures below):

Screening period: up to 4 weeks prior to the first dose (Day -28 to Day -1)

Treatment period: 28-day treatment cycles

Post-treatment period: 30 days after the last treatment (End of Treatment Visit/Early Termination Visit)

Immune-Mediated Adverse Reactions (IMAR) follow-up period: All patients treated with G9.2-17 IgG4 + tislelizumab must return 90-days +/- 7 days after the last dose of study drug for an assessment of potential IMARs.

Follow-up period: Long-term follow-up for up to 2 years (follow-up every 3 months).

**Study Design**

***Part 1: Single Agent: Dose Escalation Phase***

A dose-finding study will be conducted using a continuous reassessment method (CRM) to establish dose-limiting toxicities (DLTs) and to help evaluate the potential recommended Phase 2 dose (RP2D). Two to 6 patients per treatment Cohorts 1-6 will be assigned to receive sequentially higher intravenous (IV) infusions of G9.2-17 IgG4 every 2 weeks (Q2W) on Day 1 and Day 15 of each 28-day cycle, starting at a dose of 0.2 mg/kg. Patients assigned to a specific dose escalation cohort will receive the corresponding study dose for that cohort. They receive study drug until progression of disease, unacceptable toxicity, or withdrawal from the study for other reasons.

Part 1, Cohorts 1-8 enroll a maximum of approximately 44 patients. A total of 6 dosage levels is to be evaluated:

- Dose Escalation Cohort 1 = 0.2 mg/kg Q2W
- Dose Escalation Cohort 2 = 0.63 mg/kg Q2W
- Dose Escalation Cohort 3 = 2 mg/kg Q2W
- Dose Escalation Cohort 4 = 6.3 mg/kg Q2W
- Dose Escalation Cohort 5 = 10 mg/kg Q2W
- Dose Escalation Cohort 6 = 16 mg/kg Q2W

If the RP2D is not achieved during Cohorts 1-6, an additional 2 dosage levels may be included for the consideration of RP2D:

- Dose Escalation Cohort 7 = 10 mg/kg QW
- Dose Escalation Cohort 8 = 16 mg/kg QW

In Part 1, sequentially increasing doses of G9.2-17(IgG4) are administered by IV infusion either Q2W (Cohorts 1-6) or QW (Cohorts 7-8).

For Cohorts 1-6, 2 patients at a time are to be dosed. Dose escalations may be initiated based on analysis of patient safety data focusing on occurrences of DLTs at previous dose

levels and other relevant safety and dosing data from previous cohorts. Dose escalations may occur after a minimum of 28 days (1 cycle).

Following the completion of Cohort 6, a once weekly (QW) G9.2-17 IgG4 dosing schema is to be evaluated, provided the RP2D has not been reached. Patients are to be allowed to enter Cohort 7 when safety data from Cohort 6 is analyzed and no DLT has been identified.

Four patients per dose level in Cohorts 7 and 8 are assigned to receive IV infusions of G9.2-17 IgG4 QW on Days 1, 8, 15, and 22 of each 28-day cycle. Starting with the first 4 patients in Cohort 7, dose escalations to the next cohort occurs if no DLTs are identified.

Those patients treated in early cohorts prior to identification of the RP2D are allowed to dose escalate to the RP2D. They can continue be on the RP2D until they are discontinued from treatment for toxicity, disease progression, or other reasons.

Dose escalations are based on the development of DLTs in patients treated at previous dose levels. For each dose cohort, prior DLT probabilities are to be specified from GLP-compliant toxicity studies as well as from preclinical models. For the specified target DLT rate and total number of dose levels, the skeleton for a power model d^exp(a) will be generated according to the approach of Lee and Cheung, using a prior MTD or OBD adjusted by PK/PD data, located at the median dose level and a spacing measure of delta = 0.05 (Lee and Cheung, 2011). The prior distribution on the parameter "a" has a mean zero normal distribution with the least informative prior variance. The RP2D is the OBD/MTD dose derived from Part 1.

For patients who experience toxicities (including IMARs) outside of the DLT window, dose reduction is allowed only clinical benefit is being derived and may continue to be derived with lower doses of G9.2-17 IgG4. The dose of G9.2-17 IgG4 will initially be reduced by 50%, and potentially by a further reduction of 50%, as defined by the dose modification guidance provided in the protocol. No further dose reductions will be allowed, See Appendix 8 for detailed instructions on dose modifications.

***Part 1 Combination Treatment: Dose Escalation***

For the Part 1 Combination Treatment (Cohorts 9-14 listed below), patients are dosed using a 4 + 2 design algorithm. Each disease indication (Cohort 9 and 10- Pancreatic ductal adenocarcinoma [PDAC]; Cohorts 11 and 12- Head and Neck [H/N]; Cohorts 13 and 14- Urothelial carcinoma), is conducted in independent cohorts, as follows:

- Combination Cohort 9 = G9.2-17 IgG4 6.3 mg/kg QW + gemcitabine/nab-paclitaxel (PDAC) (up to n=10)
- Combination Cohort 10 = G9.2-17 IgG4 16.0 mg/kg QW + gemcitabine/nab-paclitaxel (PDAC) (up to n=10)
- Combination Cohort 11 = G9.2-17 IgG4 6.3 mg/kg QW + tislelizumab 300 mg every 4 weeks (Q4W) (H/N) (up to n=6)
- Combination Cohort 12 = G9.2-17 IgG4 16.0 mg/kg QW + tislelizumab 300 mg Q4W (H/N) (up to n=6)
- Combination Cohort 13 = G9.2-17 IgG4 6.3 mg/kg QW + tislelizumab 300 mg Q4W (Urothelial) (up to n=6)
- Combination Cohort 14 = G9.2-17 IgG4 16.0 mg/kg QW + tislelizumab 300 mg Q4W (Urothelial) (up to n=6)

Cohorts 9, 11 and 13 (the 6.3 mg/kg lower dose for PDAC, H/N and urothelial, respectively), can run-in parallel. The higher doses of each of these cohorts (16.0 mg/kg) are open to enrollment DLT occurrences and other safety parameters are evaluated.

Dose De-escalation

In the event of DLTs (s) during Cycle 1, the following occurs. If, during Cycle 1 of Cohort 9, 1 of 4 patients reaches DLT, then 2 additional patients are to be added to the same Cohort. If 2 or more patients reach DLT out of 4-6 patients in Cohort 9, then Cohort 10 may be opened at a lower dose of G9.2-17 IgG4. The same procedure is to be used for Cohort 11-12 and 13-14, respectively.

For patients who experience toxicities (including IMARs) outside of the DLT window, dose reduction is allowed if clinical benefit is being derived and may continue to be derived with lower doses of G9.2-17 IgG4. The dose of G9.2-17 IgG4 may initially be reduced by 50%, and potentially by a further reduction of 50%, as defined by the dose modification guidance provided in the protocol. No further dose reductions is allowed.

*Part 2: Expansion*

Once safety is established in any of the Part 1 cohorts, and a preliminary efficacy and or PD signal is identified, 1 or more expansion cohorts may be launched to further evaluate the safety and efficacy in that particular tumor type. The sample size for each of the expansion arms will be determined based on the point estimates (1) available with standard of

care [null hypothesis] versus (2) anticipated with the proposed combination therapy [alternative hypothesis] for each tumor type investigated in Part 1. A protocol amendment may be submitted with details around the expansion population, treatment regimen, and statistical methods prior to initiating Part 2.

In Part 2, patients receive the RP2D of G9.2-17 IgG4 (as determined in Part 1) as a single agent, or the RP2D-1 in combination with PD-1 tislelizumab in patients having head and neck cancer, urothelial cancer, or other solid tumors.

G9.2-17 IgG4. If for any reason same-day administration cannot be accomplished, tislelizumab may be administered on the first day, and G9.2-17 IgG4 on the subsequent day.

**Study Objectives and Endpoints**

*Part 1 - Dose escalation*

| OBJECTIVES | ENDPOINTS |
|---|---|
| **Primary** | |
| To establish the safety and tolerability of G9.2-17 IgG4 as a single agent treatment and in combination with gemcitabine/nab-paclitaxel or tislelizumab<br><br>To determine the recommended Phase 2 dose (RP2D) for G9.2-17 IgG4 | • Evaluation of safety parameters including adverse events, vital sign measurements, clinical safety laboratory tests, 12-lead electrocardiograms (ECG), echocardiography/cardiac ultrasound (ECHO), physical examinations<br>• Evaluation of DLTs<br>• Determination of RP2D |
| **Secondary** | |
| To characterize the pharmacokinetic (PK) profile of G9.2-17 IgG4 | Evaluation of PK parameters of G9.2-17 IgG4 (including but not limited to area under the curve from time zero until 336 h [$AUC_{0-336h}$], maximum observed serum concentration [$C_{max}$], time to reach $C_{max}$ [$T_{max}$], estimated half-life [$t_{1/2}$]) |
| To assess the pharmacodynamics (PD) of G9.2-17 IgG4 | • Peripheral blood mononucleocyte (PBMC) immunophenotyping by flow cytometry<br>• Pre-specified cytokine profile (serum) by enzyme-linked immunosorbent assay (ELISA)<br>• Soluble gal-9 levels in blood (serum) by ELISA<br>• Gal-9 tumor tissue expression levels, and pattern of expression by immunohistochemistry (IHC) (expression |

| | |
|---|---|
| | on tumor cells and immune cells as well as a combined score)<br>• Tumor tissue multiplex immunophenotyping<br>• Programmed death ligand-1 (PD-L1) expression by IHC<br>• Mismatch repair (MMR) status by IHC |
| To assess the immunogenicity of G9.2-17 IgG4 | Evaluation of anti-drug antibodies (ADA) |
| **Exploratory** | |
| To evaluate the preliminary efficacy of G9.2-17 IgG4 as a single agent treatment and in combination with gemcitabine/nab-paclitaxel or tislelizumab | • Evaluation of confirmed objective response rate (ORR)<br>• Evaluation of disease control rate (DCR)<br>• Evaluation of duration of response (DoR)<br>• Evaluation of progression-free survival (PFS)<br>• Evaluation of overall survival (OS) |

*PART 2 – Cohort Expansion*

| **Objectives** | **Endpoints** |
|---|---|
| **Primary** | |
| To establish the safety and tolerability of G9.2-17 IgG4 as single treatment and in combination with tislelizumab. | • Evaluation of safety parameters including adverse events, vital sign measurements, clinical safety laboratory tests, 12-lead ECG, ECHO, physical examinations<br>• Evaluation of DLTs |
| To assess the ORR in patients with CRC or CCA treated with G9.2-17 IgG4 alone, or in combination with tislelizumab | Evaluation of the confirmed ORR-3 in patients with CRC or CCA |
| To assess PFS in patients with PDAC treated with G9.2-17 IgG4 in combination with | Evaluation of PFS-6 in patients with PDAC |
| **Secondary** | |
| To characterize the pharmacokinetic (PK) profile of G9.2-17 IgG4 | Evaluation of PK parameters of G9.2-17 IgG4 |
| To assess the pharmacodynamics (PD) of G9.2-17 IgG4 | • PBMC immunophenotyping by flow cytometry<br>• Cytokine profile (serum) by ELISA<br>• Soluble gal-9 levels in blood (serum) by ELISA<br>• Gal-9 tumor tissue expression levels, and pattern of expression by IHC (expression |

| | |
|---|---|
| | on tumor cells and immune cells as well as a combined score)<br>• Tumor tissue multiplex immunophenotyping<br>• PD-L1 expression in tissue by IHC<br>• MMR status in tissue by IHC<br>• TMB in tissue |
| To assess the immunogenicity of G9.2-17 IgG4 | Evaluation of ADA |
| To evaluate efficacy of G9.2-17 IgG4 | Confirmed ORR<br>PFS<br>DCR<br>DoR<br>OS |

**Single Agent and Combinted Treatment**

Treatment of single agent cohorts or combination agent cohorts for solid tumor patients (*e.g.*, head and neck cancer or urothelial carcinoma) may be executed in parallel.

*G9.2-17 IgG4 single treatment*

The starting dose of G9.2-17 IgG4 in the single treatment may be the RP2D identified in Part 1. After testing the investigational drug on 23 patients in Stage I, this trial arm may be terminated if ≤ 1 patient responds. If the trial goes on to the Stage II of Simon's optimal design, approximately 33 patients are to be treated additionally in each of the single-agent arms. If the total number of responding patients is ≤ 5, the investigational drug within that arm will be rejected. If ≥ 6 patients have a confirmed ORR-3, the Part 3 expansion cohort for that arm will be activated and described in an amendment to the protocol.

Dose reduction may be allowed if clinical benefit is being derived and may continue to be derived with lower doses of G9.2-17 IgG4. The dose of G9.2-17 IgG4 may initially be reduced by 50%, and potentially by a further reduction of 50%, as defined by the dose modification guidance provided herein.

*G9.2-17 IgG4 + tislelizumab combination treatment*

The dose of G9.2-17 IgG4 in the combination treatment with tislelizumab may be the RP2D-1, which is the dose immediately preceding the RP2D dose identified in Part 1. The optimal two-stage design may also be used to test the null hypothesis that the ORR-3 is ≤ 10% versus the alternative hypothesis that the ORR-3 is ≥ 25%.

To ensure patient safety, a safety run-in will be performed in which the first 8 patients will be dosed. This arm may continue to enroll only if ≤ 2 patients develop a DLT, which will be below the target toxicity level (TTL) of 25%. If 3 or more patients develop a DLT this combination arm may be terminated for the cancer type being treated. If a DLT occurs, in any of the 8 safety run in patients, during the first 28 days of treatment, that patient may be permanently discontinued from study drug administration.

For patients who experience toxicities outside of the DLT window, dose reduction may be allowed when clinical benefit is being derived and may continue to be derived with lower doses of G9.2-17 IgG4. The dose of G9.2-17 IgG4 may initially be reduced by 50%, and potentially by a further reduction of 50%, as defined by the dose modification guidance provided in the protocol. No further dose reductions may be allowed. Dose modifications for tislelizumab may also be allowed as defined by the guidance herein and **Table 7** below.

If an IMAR occurs/recurs that is not managed by dose reduction of either agent, both study medications may be discontinued.

**Dose-limiting Toxicity (DLT) Criteria**

Dose-limiting toxicities assessed in this trial are defined as a clinically significant hematologic and/or non-hematologic AE or abnormal laboratory value assessed as unrelated to metastatic tumor disease progression, intercurrent illness, or concomitant medications and is possibly related or related to the study drug and occurring during the first cycle (28 days) on study. Any patient that experiences a DLT in Part 1 or Part 2 during the first 28 days of treatment will be permanently discontinued from study drug administration.

A DLT is a toxicity that meets any of the following criteria:

- Any death not clearly due to the underlying disease or extraneous causes
- Indications of potential drug induced liver injury (Hy's Law cases) as follows:
    - ALT or AST >3 x the upper limit of normal (ULN) with confirmation by repeat testing 24 hours later, AND
    - Serum total bilirubin (TBL) > 2 x ULN with confirmation by repeat testing 24 hours later
    - No other explanation can be found for the elevated TBL and/or ATs, such as viral hepatitis (A, B or C), alcoholic or autoimmune hepatitis, pre-existing or acute liver disease, gall bladder obstruction or bile duct disease, Gilbert

syndrome, disease progression, or another medication capable of causing the observed effect.

- All Grade 4 non-hematologic and hematological toxicities of any duration
- All Grade 3 non-hematologic and hematological toxicities. Exceptions are as follow:
  - Grade 3 nausea, vomiting and diarrhea that does not require hospitalization or total parenteral nutrition support and can be managed with supportive care to ≤ Grade 2 within 48 h.
  - Grade 3 electrolyte abnormalities that are corrected to ≤ Grade 2 within 24 h.
  - Grade 3 electrolyte abnormality that lasts <24-72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical interventions.
  - ≥ Grade 3 amylase or lipase that is not associated with symptoms or clinical manifestations of pancreatitis.

**Statistical Methods:**

*Sample Size*

Dose escalations will be based on the presence or absence of DLTs in patients treated at previous dose levels. For each dose cohort, prior DLT probabilities are to be specified from Good Laboratory Practice (GLP)-compliant toxicity studies as well as from preclinical models. For the specified target DLT rate and total number of dose levels, the skeleton for a power model d^exp(a) will be generated according to the approach of Lee and Cheung (2011), using a prior MTD adjusted by pharmacokinetic (PK)/pharmacodynamic (PD) data, located at the median dose level and a spacing measure of delta = 0.05. The prior distribution on the parameter "a" has a mean zero normal distribution with the least informative prior variance. The trial is stopped for safety if the lower limit of an Agresti and Coull binomial CI for the lowest study dose level exceeds the target DLT rate.

CRM trial simulation analyses with 1000 iterations suggested an average of approximately 20 patients will be needed to inform the selection of RP2D, which is the largest dose that has an estimated probability of a DLT less than or equal to a TTL of 25%. The CRM used is based upon the first 6 cohorts, but that itself will not necessarily determine the RP2D, as data from Cohorts 7 to 14 are also be used to determine the RP2D.

A total approximate sample size of 80 patients is anticipated for Part 1 of the study. Backfill will provide for the enrollment of additional patients if deemed necessary.

Part 2 of this study (cohort expansion phase) may adopt a Simon's two-stage optimal design to establish safety and efficacy for LYT-200 in patients with tumor types that demonstrated safety and preliminary efficacy in Part 1. In Part 2, the total sample size may depend on the number of expansion cohorts selected as a result of the safety and efficacy findings in the Part 1 single agent and combination cohorts.

*Randomization Stratification:*

This is an open-label study. In Part 1, patients are to be assigned to treatment according to the CRM design of the study. In Part 2, patients are to be assigned to treatment arm, *e.g.*, in line with the inclusion and exclusion criteria.

*Analysis Populations*

The intent-to-treat (ITT) population may be defined as those patients who received at least one dose of the study drug, unless otherwise specified. The primary efficacy analyses may be performed for the ITT. Patient disposition may be performed for the ITT.

The Efficacy Population may be defined as all patients in the ITT and having at least one measurable ORR 3 or PFS 6 assessment. This population may be used for a sensitivity analysis.

The per-protocol (PP) Population may be defined as any patient who received at least one full cycle of G9.2-17(IgG4) and without major protocol deviations.

The safety population (SAF) may be defined as all patients who receive at least one dose of the study drug. The safety analyses may be performed for the SAF.

The PK/PD population may be defined as those patients who have received at least one full cycle of G9.2-17(IgG4).

*General Statistical Plan*

A database lock and primary analysis may be performed after the last patient has had their primary endpoint event. A final study analysis may be performed after study completion. All analyses may be descriptive.

*Safety Analysis*

All safety analyses may be made on the SAF unless otherwise specified and may be analyzed using descriptive statistics

*Efficacy Analysis*

Disease response assessed according to RECIST v1.1 may be summarized descriptively for the ITT and PP. A sensitivity analysis may be performed for the Efficacy Populations.

*Pharmacokinetics, Pharmacodynamics, and Immunogenicity*

PK, PD, and immunogenicity can be summarized descriptively for the PK/PD population.

**Schedule of Assessments**

The Schedule of Assessment in provided in **Table 3**, except that Study drug administration is as follow: G9.2-17 IgG4 treatment may be administered, on C1D1 and C1D15 on every cycle. In Part 2, tislelizumab may be administered on Day 1 of every cycle on the G9.2-17 IgG4 combination regimen. Study drug may be administered on Days 1, 8 and 15 +/- 3 days from C2 onwards. All patients treated with G9.2-17 IgG4 + tislelizumab must return 90-days +/- 7 days after last dose of study drug for an assessment of potential immune-mediated adverse reactions (IMARs).

**Study Population**

Patients are eligible for the study after meeting the following inclusion criteria and not meeting any of the exclusion criteria.

***Inclusion Criteria***

**Part 1**

1. Written Informed Consent (mentally competent patient, able to understand and willing to sign the informed consent form)
2. Age ≥ 18 years, male or non-pregnant female
3. Able to comply with the study protocol, as per Investigator's judgment
4. Histologically confirmed, unresectable locally advanced or metastatic cancer. There are no limits to prior lines of therapies received for the treatment of the cancer condition for which the patient is being enrolled into this study.
    a. For Part 1 combination urothelial carcinoma cohort: histologically or cytologically confirmed diagnosis of unresectable, locally advancer or

metastatic urothelial carcinoma of the renal pelvis, ureter, bladder, or urethra (i.e. transitional cell carcinoma).

b. For Part 1 combination head and neck cancer cohort: histologically confirmed, locally advanced or metastatic SCCHN (oral cavity, oropharynx, hypopharynx, or larynx).

**Table 3. Schedule of Assessments**

| | Pre-Dose | Treatment Phase | | | | | | | | | | | | | | | | Post Treatment | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Screening | Cycle 1 (28 days) | | | | Cycle 2 (28 days) | | | | Cycle 3 (28 days) | | | | | | Cycle 4 (28 days) and beyond | | End of Treatment or Early Termination | IMAR Follo w-UpT | Long Term Follow -Up U Every 3 mons |
| Study Day | -28 to -1 | 1 | 2 | 8 | 15 | 1 | 8 | 15 | 1 | 2 | 8 | 15 | 1 | 2 | 8 | 15 | 1 | 8 | 15 | 30 days after last dose | 90 days after last dose | |
| Cycle Day | | C1 D1 | C1 D2 | C1 D8 | C1 D15 | C2 D1 | C2 D8 | C2 D15 | C3 D1 | C3 D2 | C3 D8 | C3 D15 | CX D1 | CX D8 | CX D15 | | | | | -- | -- | -- |
| +/- days allowable | | | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | | | | | 3 | 7 | n/a |
| Study drug administration A | | X | | X | X | X | X | X | X | | X | X | X | X | X | | | | | | | |
| **Study Procedures & Examinations** | | | | | | | | | | | | | | | | | | | | | | |
| Eligibility assessment and written informed consent | X | | | | | | | | | | | | | | | | | | | | | |
| Demographics B | X | | | | | | | | | | | | | | | | | | | | | |
| Medical history C | X | | | | | | | | | | | | | | | | | | | | | |
| Previous and concomitant medications D | | X | X | X | X | X | | X | X | X | X | X | X | X | X | | | | X | X | | |
| Adverse events E | | X | X | X | X | X | | X | X | X | X | X | X | | | | | | X | X | X | |
| ECHO/MUGA F | X | | | | | | | | | | | | X | | | | | | | | | |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12-lead ECG (QTcF) | X | X | | | X | | X | | X | | | | | | |
| Physical exam G | X | X | | | X | | X | | X | | X | X | X | | |
| ECOG | X | X | | | X | | X | | X | | X | X | | | |
| Vital Signs H | X | X | | | X | | X | | X | | X | X | | | |
| Tumor imaging assessment (CT with contrast preferred) N | X | | | | | | X | | | | | X repeat if end of study is > 8 weeks after last scan | | | X U |
| **Clinical Labs** | | | | | | | | | | | | | | | |
| Pregnancy test I | X | | | | X | | X | | | X | | X | | | |
| Hematology J | X | X | | | X | | X | | | X | X | X | X | | |
| Serum Chemistry K | X | X | | | X | | X | | | X | X | X | X | | |
| TSH, Free T4, serum lipase, amylase, PTH, FSH, LH, free cortisol | X | X | | | X | | X | | | X | | X | X | | |
| Blood Coagulation L | X | X | | | X | | X | | | X | X | X | X | | |
| Urinalysis M | X | X | | | X | | X | | | X | X | X | X | | |
| **Pharmacodynamics and Pharmacokinetics** | | | | | | | | | | | | | | | |
| Tumor biopsy O | X | | | | | | | | | | X ± 7 d | | | | |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tumor type-relevant biomarkers P | X | X | | | | X | X | | X | X | | | X | X | | X | X | | |
| Blood sampling for biomarker PD analysis Q | | X | X | X | X | X | X | | | X | X | X | X | X | | | X | | |
| PK blood sampling R | | X | X | X | X | X | | | | X | X | X | X | X | | | | | |
| Immunogenicity (ADA) S | | X | | | | | X | | | X | | | | X | | | X | | |
| Survival Follow-up | | | | | | | | | | | | | | | | | | | X U |

ADA: anti-drug antibodies; AE: adverse event; ALT: alanine aminotransferase; APTT: activated partial thromboplastin time; AST: aspartate aminotransferase; C: cycle; CPK: creatine phosphokinase; COVID19: Coronavirus SARS-CoV-2; CRP: C-reactive protein; CT: computed tomography; D or d: day(s); ECG: electrocardiogram; ECOG: Eastern Cooperative Oncology Group; ECHO: echocardiography/cardiac ultrasound; FSH: follicle-stimulating hormone; IMAR: immune-mediated adverse reaction; INR: international normalized ratio; LDH: lactate dehydrogenase; LH: lutenizing hormone; min: minute(s); MUGA: multigated acquisition scan; PD: pharmacodynamics; PK: pharmacokinetics; PT: prothrombin time; PTH: parathyroid hormone; PTT: partial thromboplastin time; QTcF: QT interval, Fridericia's Correction Formula; RBC: red blood cell count; SGOT: serum glutamic-oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; TSH: thyroid stimulating hormone; WBC: white blood cell count.

A) Study drug administration: G9.2-17 IgG4 treatment will be administered, on C1D1 and C1D15 on every cycle.

B) Demographics: Data include age, gender, race, and ethnicity.

C) Medical history: In addition to general medical history, data collection also includes oncology history, surgical/transplant and radiation therapy history and COVID-19 history and testing.

D) Previous and concomitant medications (including vaccines and complementary treatments/supplements): Data to include name, indication, dose, route, start and end dates for each. Allergies and intolerances, dose modifications while on study, schedule of dosing changes and reasons for them should also be obtained.

E) Adverse events: Any AEs starting or worsening after study drug administration will be recorded. AEs should be followed until resolved to one of the following: baseline, stabilized, or deemed irreversible. All SAEs are to be collected until 30 days after last dose of study medication. All study-procedure-related SAEs must be collected from the date of patient's written consent.

F) ECHO/MUGA: This assessment of heart function is conducted at Screening and repeated on Day 1 of Cycle 4; the assessment window is +/- 5 days. It should be conducted more frequently when clinically indicated and once every 3 months.

G) Physical exam: Include height at screening for determination of body surface area. Include weight at all scheduled exam times. A Neurological exam will be conducted only on patients who have stable and/or pre-treated brain metastases.

H) Vital Signs: temperature, heart rate, blood pressure, respiratory rate.

I) Pregnancy test (blood or urine): Only for women of childbearing potential with uterus in situ. Test results must be available before scheduled dosing.

J) Hematology: Analysis includes complete blood count, differential, platelets, hemoglobin. Collect blood samples pre-dose.

K) Serum chemistry: Analysis includes albumin, alkaline phosphatase, bilirubin (total, direct), blood urea nitrogen, calcium, CPK, creatinine, electrolytes (sodium, potassium, chloride, magnesium, phosphorus), gamma glutamyl transferase (gamma GT), glucose, hemoglobin A1c (HgbA1c) (only if history of Type 1 or Type 2 diabetes mellitus), LDH, SGPT (ALT) or SGOT (AST), total protein. Fasting glucose to be assessed only if clinically indicated. Collect blood samples pre-dose.

L) Blood Coagulation: Collect blood samples pre-dose. Analysis includes APTT, PT, PTT, and INR (if on allowable anti-coagulants), CRP, and troponin.

M) Urinalysis: Analysis includes color, appearance, dipstick for specific gravity, protein, white blood cell-esterase, glucose, ketones, urobilinogen, nitrite, WBC, RBC, pH. (Urine culture and sensitivity to be run only if patient is clinically symptomatic.)

N) Tumor imaging assessment: For screening, the assessment must be performed within the 28-day screening period. On study, assessments are done every 8 weeks ± 7 days (ie, C3D1, C5D1, C7D1, C9D1, etc.) and at the End of Treatment if not assessed within the previous 4-6 weeks. Assessments may be performed more frequently if clinically indicated. If an objective response is seen on a scan, a confirmation scan will be done 4 weeks (+7 d) later. After this confirmatory scan, the scheduled scans are to be resumed at a frequency of every 8 weeks ± 7 days from the date of the confirmatory scan.

O) Tumor biopsies: If patient MMR/MSI status is unknown at screening, the test should be run at the local laboratory. In Part 2, TMB tissue analysis will be performed. The on-study biopsy is scheduled for C3D15 ± 7 days and should occur only after the tumor imaging scan in Cycle 3. It is recognized that a variety of clinical factors may make it difficult to obtain adequate specimens. Decisions not to perform biopsy on-treatment should be discussed with the Medical Monitor.

P) Tumor type-relevant biomarkers: Blood samples are to be collected at screening and every cycle pre-dose administration as appropriate for the tumor type. Blood sampling may be decreased to every 3rd cycle after 6 months of treatment.

Q) PD blood sampling: Blood samples will be collected pre-dose administration on dosing days. May be decreased to every 3rd cycle after 6 months of treatment.

R) PK blood sampling: Cycle 1 and Cycle 3 Day 1: blood samples will be collected pre-dose and at end of study drug infusion (EOI), 2 and 4 h (± 30 min) post-study drug administration. Cycle 1 and Cycle 3 Day 15, blood samples will be collected pre-dose and at EOI only. Cycle 1 and Cycle 3 Day 2 and 8 (non-dosing days), PK blood samples will be collected at only one time point. Cycle 2 and Cycle 4: blood samples will be collected Day 1 only and should occur pre-dose and at EOI. Blood samples for PK will be collected every 2 cycles thereafter (ie, C6D1, C8D1, etc.) pre-dose and at EOI.

S) ADA blood sampling: Blood samples will be collected Day 1 of Cycles 1-4, pre-dose. Thereafter, it will be collected every 2 cycles, Day 1, pre-dose (ie, C6D1, C8D1, etc.).

T) All patients treated with G9.2-17 IgG4 + tislelizumab must return 90-days +/- 7 days after last dose of study drug for an assessment of potential immune-mediated adverse reactions (IMARs).

U) Long-Term Follow-up: Tumor imaging should continue, where possible, for patients discontinuing treatment due to reasons other than progression of disease and not receiving additional systemic anticancer treatments. Survival data will be collected at a minimum every 3 months. It can be collected more frequently to support data cleaning or regulatory submission efforts. Follow-up can be conducted by telephone, electronic messaging or chart review and will continue for up to 2 years after the patient has the End of Treatment/Early Termination visit.

5. For urothelial and head and neck combination cancer cohorts, prior exposure to immunotherapy is allowed, with standard of care treatment options and /or within a clinical trial context. If the patient received an anti-PD-1 and/or an anti-PD-L1 containing regimen at any point, they must have demonstrated at least stable disease, as per RECIST 1.1. or iRECIST criteria to one of these treatment regimens, if these measurements are available. If RECIST or iRECIST measurements are not available, then clinical PFS of at least 4 months is required to have been achieved on any of the prior anti-PD-1 and/or anti-PD-L1 containing regimens.

6. There is no PD-L1 expression requirement for the Part 1 combination urothelial and head and neck cohorts, however fresh biopsy or archival tissue is required for assessment of PD-L1 by IHC, or a historical PD-L1 expression by IHC must be available. If PD-L1 expression data is already available, this does not override the protocol preference for obtaining a fresh biopsy whenever feasible.

7. For Part 1 combination cohort head and neck cancer patients of oropharynx origin: human papilloma virus (HPV) status needs to be established in the screening period or at any point while patient is on study drug, unless it is historically known. p16+ as a surrogate for HPV+, HPV RNA ISH or DNA PCR are all acceptable. The study accepts both HPV+ and HPV- patients.

8. Life expectancy > 3 months according to Investigator's judgment.

9. ECOG performance status 0-1.

10. Patient able and willing to undergo pre- and on/post treatment biopsies. According to the Investigator's judgment, the planned biopsies should not expose the patient to substantially increased risk of complications. Every effort will be made that the same lesion is biopsied on repeat biopsies. If the patient is eligible according to all other criteria but declines to consent to a biopsy or there are other medical reasons precluding biopsy, this will be discussed with the Sponsor.

11. Measurable disease, according to RECIST v1.1. Note that lesions intended to be biopsied should not be target lesions.

12. Adequate hematologic and end organ function, defined by the following laboratory results obtained prior to first dose of study drug treatment, provided no anticancer treatment was administered within the last 7 days:

a. neutrophil count ≥ 1 x $10^9$/L
b. platelet count ≥ 100 x $10^9$/L; for hepatocellular carcinoma (HCC) in Part 1 ≥ 50 x $10^9$/L
c. hemoglobin ≥ 9.0 g/dL without transfusion in the previous week
d. creatinine ≤ 1.5 x ULN; or eGFR > 50 mg/mmol
e. aspartate aminotransferase AST (SGOT) ≤ 3 x ULN (≤ 5 x ULN when HCC or hepatic metastases are present)
f. alanine aminotransferase (ALT [SGPT]) ≤ 3 x ULN (≤ 5xULN when HCC or hepatic metastases present)
g. bilirubin ≤ 1.5xULN (patients with known Gilbert's disease may have a bilirubin ≤ 3.0xULN)
h. albumin ≥ 3.0 g/dL
i. international normalized ratio (INR) and partial thromboplastin time (PTT) ≤ 1.5xULN, unless patient receiving anticoagulant therapy.

13. No evidence of active serious infection or infections requiring parenteral antibiotics.

14. Women of childbearing potential must have a negative pregnancy test within 72 h prior to start of treatment. For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or to use contraceptive methods that result in a failure rate of < 1% per year during the treatment period and for at least 180 days after the last study treatment.

A woman is of childbearing potential if she is post-menarche, has not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).

Examples of contraceptive methods with a failure rate of < 1% per year include bilateral tubal ligation, male sterilization, hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices and copper intrauterine devices. The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g.,

calendar, ovulation, symptom-thermal, or post ovulation methods) and withdrawal are not acceptable methods of contraception. Fertile men must practice effective contraceptive methods during the study, unless documentation of infertility exists.

15. Four (4) weeks or 5 half-lives (whichever is shorter) since the last dose of anticancer therapy before the first G9.2-17(IgG4) administration.

16. Bisphosphonate treatment (e.g., zoledronic acid) or denosumab are allowed if previously used prior to commencement of clinical trial.

17. Patients:

a. who have already received at least one prior line of systemic therapy for metastatic or locally advanced disease, and/or

b. who have a tumor type for which there are no available standard of care options.

18. Patients who have not previously received a gemcitabine-containing regimen.

*Exclusion Criteria*

1. Patient unwilling or unable to follow protocol requirements

2. Patient diagnosed with metastatic cancer of an unknown primary

3. Current illicit drug addiction (medical and recreational marijuana/cannabidiol [CBD]/ tetrahydrocannabinol [THC] would not be considered "illicit")

4. Clinically significant, active uncontrolled bleeding, and any patients with a bleeding diathesis (e.g., active peptic ulcer disease). Prophylactic or therapeutic use of anticoagulants is allowed.

5. Pregnant and/or lactating females

6. Receiving any other investigational agents or participating in any other clinical trial involving another investigational agent for treatment of solid tumors within 3 weeks or 5 half-lives of the administered drug (whichever is shorter) prior to the first dose of study drug, or major surgery or planned surgery within 4 weeks of the first dose of study drug (this includes dental surgery).

7. Radiation therapy within 4 weeks of the first dose of study drug, except for palliative radiotherapy to a limited field, such as for the treatment of bone pain or a

focally painful tumor mass, and which does not jeopardize required measurable lesions for response assessment (RECIST v1.1).

8. Patients with fungating tumor masses

9. History or current evidence of any condition, therapy, any active infections, or laboratory abnormality that might confound the results of the trial, interfere with the patient's
participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating Investigator

10. Grade 4 immune-mediated toxicities with a prior checkpoint inhibitor. Grade 2 or Grade 3 pneumonitis or any other Grade 3 checkpoint inhibitor-related toxicity that led to immunotherapy treatment discontinuation. Low-grade (< Grade 3) toxicities, such as neuropathy from prior treatments, manageable electrolyte abnormalities and lymphopenia, alopecia and vitiligo are allowed.

11. history of other prior or other concomitant malignancy that requires other active treatment.

12. Active brain, patients with carcinomatous meningitis or leptomeningeal metastases. Patients with brain metastases are eligible provided they have shown clinically and radiographically stable disease for at least 4 weeks after definitive therapy and have not used steroids (> 10 mg/day of prednisone or equivalent) for at least 4 weeks prior to the first dose of study drug

13. Evidence of severe or uncontrolled systemic diseases, congestive heart failure > New York Heart Association (NYHA) class 2, myocardial infarction (MI) within 6 months, or laboratory finding that in the view of the Investigator makes it undesirable for the patient to participate in the trial

14. Any medical condition that the Investigator considers significant to compromise the safety of the patient or that impairs the interpretation of LYT-200 toxicity assessment

15. Serious non-healing wound, active ulcer, or untreated bone fracture unless for e.g., a rib fracture for (which does not elicit treatment)

16. Uncontrolled pleural effusion, pericardial effusion, or ascites requiring recurrent drainage procedures. For the purposes of this study, "recurrent" is defined as ³ 3 drains in the last 30 days.

17. History of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins

18. Significant vascular disease (e.g., aortic aneurysm requiring surgical repair or recent arterial thrombosis) within 6 months of Cycle 1, Day 1

19. History of pulmonary embolism, stroke or transient ischemic attack within 3 months prior to Cycle 1, Day 1

20. Active autoimmune disorder (except type I/II diabetes, hypothyroidism requiring only hormone replacement, vitiligo, psoriasis, or alopecia areata).

21. Requires systemic immunosuppressive treatment, including, but not limited to cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-tumor necrosis factor (anti-TNF) agents. Patients who have received or are receiving acute, low dose systemic immunosuppressant medications (e.g., ≤ 10 mg/day of prednisone or equivalent) may be enrolled. Replacement therapy (e.g., thyroxine, insulin, physiologic corticosteroid replacement therapy [e.g., ≤ 10 mg/day of prednisone equivalent] for adrenal or pituitary insufficiency) is not considered a form of systemic treatment. The use of inhaled corticosteroids and mineralocorticoids (e.g., fludrocortisone), topical steroids, intranasal steroids, intra-articular, and ophthalmic steroids is allowed

22. Severe tumor-related pain (Grade 3, CTCAE] v.5.0 unresponsive to broad analgesic interventions (oral and/or patches)

23. Hypercalcemia (defined as ³ Grade 3, per CTCAE v 5.0) despite use of bisphosphonates

24. Any other diseases, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that may affect the interpretation of the results or render the patient at high risk of treatment complications

25. Received organ transplant(s)

26. Patients undergoing dialysis

27. For Part 1, hormonal androgen deprivation therapy is allowed to continue for patients with metastatic castration-resistant prostate cancer

28. Any ablative therapy (Radio Frequency Ablation or Percutaneous Ethanol Injection) for HCC < 6 weeks prior trial entry

29. Hepatic encephalopathy or severe liver adenoma

30. Child-Pugh score ≥ 7

**Study Drug and Other Interventions**

Study intervention(s) is/are defined as any investigational agent(s), marketed product(s), placebo, or medical device(s) intended to be administered/used to/in a study participant according to the study protocol.

***Agents administered in combination with G9.2-17 IgG4***

*Tislelizumab*

Tislelizumab is a PD-1 inhibiting mAb drug being developed for the treatment of cancer. Tislelizumab is formulated for IV injection in a single-use glass vial (20R glass, USP type I) with a rubber stopper containing a total of 100 mg of tislelizumab mAb in 10 mL of buffered isotonic solution. Tislelizumab is administered as an intravenous infusion over approximately 30 minutes (unless guided otherwise) at 300 mg every 4 weeks, in a 28-day cycle.

The active ingredient of tislelizumab is a humanized IgG4 variant mAb against PD-1, binding to the ECD of human PD- 1 with high specificity and affinity (KD = 0.15 nM). The excipients of tislelizumab include: sodium citrate dihydrate, citric acid monohydrate, L-histidine hydrochloride monohydrate, L-histidine, trehalose dihydrate, polysorbate-20, and WFI. Tislelizumab competitively blocks the binding of both PD-L1 and PD-L2, inhibiting PD-1-mediated negative signaling and enhancing the functional activity in T cells in in vitro cell-based assays. In addition, tislelizumab demonstrated antitumor activity in several human cancer allogeneic xenograft models and a human PD-1 transgenic mouse model.

The IgG4 variant antibody has very low binding affinity to FcγRIIIA and C1q by in vitro assays, suggesting a low or no ADCC and CDC effect in humans. Unlike natural IgG4 antibody, tislelizumab has no observable Fab-arm exchange activity by the in vitro assay, predicting the antibody would be stable in vivo, unlikely forming bispecific antibodies.

Exposure-response (E-R) relationships between tislelizumab exposure and efficacy across a variety of advanced solid tumors support the 300 mg Q4W regimens. 300 mg Q4W regimen is not expected to be clinically different from the 200 mg Q3W in terms of safety or efficacy outcomes.

The safety profile of tislelizumab is consistent with the therapeutic class of the drug with a relatively low rate of treatment-related Grade 3 or above toxicity.

Tislelizumab AEs are presented below in **Table 4** according to their frequency of occurrence. Reported AEs that may be IMAR-related are summarized in **Table 5**.

**Table 4. Adverse Events (non-IMAR-Related) Reported for Tislelizumab According to Frequency**

| ORGAN SITE | ADVERSE EVENT |
|---|---|
| blood and lymphatic system/ febrile | anemia (8-21%, severe 1-8%) |
| | leukopenia (5-12%, severe 2%) |
| neutropenia | lymphopenia (<1%) |
| | neutropenia (3-9%, severe 1-3%) |
| | thrombocytopenia (1-9%, severe 1%) |
| gastrointestinal | *emetogenic potential:* low[3] |
| | constipation (13%, severe <1%) |
| | diarrhea (5-13%, severe 1%) |
| | nausea (4-13%, severe <1%) |
| | stomatitis, aphthous ulcer (3%, severe <1%) |
| | vomiting (10%, severe 1%) |
| general disorders andadministration site conditions | *extravasation hazard:* none[4] |
| | chills (1%, severe 0%) |
| | fatigue, asthenia (1-23%, severe 2%) |
| | pyrexia (5-33%, severe 1%) |
| infections and infestations | pneumonia (3%) |
| injury, poisoning, and procedural complications | ***infusion-related reactions*** (2-29%, severe <2%) |
| investigations | alkaline phosphatase increase (6%, severe 1%) |
| | ALT increase (1-16%, severe 2%) |
| | AST increase (1-17%, severe 3%) |
| | bilirubin increase (5-12%, severe 2%) |

| | |
|---|---|
| | creatine phosphokinase increase (1-6%, severe 1%) |
| | hyperglycemia (2-5%, severe 1%) |
| | hyponatremia (1%, severe 1%) |
| | lipase increase (1%, severe 1%) |
| Clinically important side effects are in ***bold, italics*** | |
| | proteinuria (1-6%) |
| | thyroid hormone (free triiodothyronine and thyroxine) decrease (1%) |
| | thyroid stimulating hormone (TSH) increase (1-7%) |
| metabolism and nutrition | appetite decrease (1-15%, severe <1%) |
| | diabetes mellitus, type 1 (1%, severe 1%) |
| | diabetic ketoacidosis (<1%) |
| musculoskeletal andconnective tissue | arthralgia (2-6%) |
| respiratory, thoracic andmediastinal | cough (13%) |
| | dyspnea (6%, severe 1%) |
| | pruritus (8-14%, severe <1%) |
| | rash (6-18%, severe 1%) |
| | skin reaction, undefined (1%, severe <1%) |

By disrupting PD-1-mediated signaling, tislelizumab acts to restore antitumor immunity and halt progression of tumor growth. This restoration of immune system activity may result in immune related adverse reactions involving 1 or more body systems, which can be life threatening or fatal in rare cases. While these events usually become manifest during treatment with tislelizumab, they can also occur after discontinuation of tislelizumab therapy.

**Table 5. Adverse Events Reported for Tislelizumab to be Treated as IMARs***

| ORGAN SITE | ADVERSE REACTION |
|---|---|
| cardiac | immune-mediated myocarditis (1%, severe <1%) |
| endocrine | immune-mediated adrenal insufficiency (<1%) |
| | immune-mediated hyperthyroidism (3-4%, severe <1%) |
| | immune-mediated hypothyroidism (6-21%, severe 1%) |
| | immune-mediated thyroiditis (1%) |

| | |
|---|---|
| eye | immune-mediated uveitis (<1%) |
| gastrointestinal | immune-mediated colitis (1%, severe 1%) |
| | immune-mediated pancreatitis (1%, severe 1%) |
| | immune-mediated hepatitis (1-3%, severe 1-2%) |
| musculoskeletal and connective tissue | arthralgia (2-6%) |
| | immune-mediated myositis/rhabdomyolysis (1%, severe 1%) |
| | immune-mediated nephritis, renal dysfunction (1%, severe 1%) |
| immune system | immune-mediated adverse events (21-22%, severe 5-11%) |
| respiratory, thoracic and mediastinal | cough (13%) |
| | dyspnea (6%, severe 1%) |
| | immune-mediated pneumonitis, interstitial lung disease (2-7%, severe 1-6%) |
| skin and subcutaneous tissue | immune-mediated dermatitis (1%, severe <1%) |
| | immune-mediated pruritus (2-4%) |
| | immune-mediated rash (3%, severe <1%) |
| | immune-mediated vitiligo (1%) |

See also **Table 6** below for management of IMARs caused by the combination of G9.2-17(IgG4) and tislelizumab.

**Table 6: Management of Immune-Mediated Adverse Reactions (IMARs) Caused by G9.2-17 IgG4 + Tislelizumab Combination Treatment**

| IMAR | Guidance for Management |
|---|---|
| Immune-mediated pneumonitis | • Monitor patients for signs with radiographic imaging and for symptoms of pneumonitis.<br>• Grade 1, consider holding tislelizumab until there is significant improvement in patient's signs and symptoms<br>• Withhold tislelizumab and G9.2-17 IgG4 for moderate (Grade 2) and permanently discontinue for severe (Grade 3) or life-threatening (Grade 4) pneumonitis. Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents for moderate (Grade 2). For more severe (Grade 3-4) pneumonitis, permanently discontinue G9.2-17 IgG4 and |

| IMAR | Guidance for Management |
|---|---|
| | tislelizumab and treatment in a hospital setting with IV methylprednisolone 2 to 4 mg/kg/day is recommended, followed by corticosteroid taper.<br>• Withhold tislelizumab and G9.2-17 IgG4 until resolution to < Grade 2 for moderate (Grade 2) pneumonitis. Resume G9.2-17 IgG4 at a 50% dose reduction.<br>• Tislelizumab may be restarted only if symptoms resolve completely or are controlled on prednisolone ≤ 10 mg/day. Withhold tislelizumab for Grade 2 pneumonitis, and only restart if symptoms resolve completely or are controlled on prednisolone ≤ 10 mg/day. Permanently discontinue treatment with tislelizumab if symptoms persist with the use of corticosteroids.<br>• Upon recurrence of ≥ Grade 2 pneumonitis, permanently discontinue treatment with both the study medication and tislelizumab |
| Immune-mediated colitis and diarrhea | • Monitor patients for signs and symptoms of colitis.<br>• Withhold treatment with tislelizumab and G9.2-17 IgG4 for Grade 2 diarrhea or colitis and start oral prednisolone 0.5 mg/kg/day (nonenteric coated). If worsening or no improvement occurs despite initiation of corticosteroids, increase dose to 1 to 2 mg/kg/day prednisone equivalents.<br>• Taper steroids over 2 to 4 weeks with resolving signs and symptoms. Resume treatment with tislelizumab when signs and symptoms improve or resolve to baseline. Administer prednisolone 1 to 2 mg/kg/day for colitis Grade 3 or Grade 4, converting to oral prednisolone and tapering this dose over at least 4 weeks when improvement is evident. Permanently discontinue tislelizumab and G9.2-17 IgG4 for recurrent severe (Grade 3) or life-threatening Grade 4 diarrhea or colitis.<br>• Resume G9.2-17 IgG4 at a 50% dose reduction when signs and symptoms improve or resolve to baseline. Any dose modifications for tislelizumab are at the Investigator's discretion. |

| IMAR | Guidance for Management |
|---|---|
| | • If ≥ Grade 3 recurs, then permanently discontinue treatment with both the study medication and tislelizumab.<br>• If ≥ Grade 2 colitis recurs, withhold G9.2-17 IgG4 and tislelizumab until colitis resolves to baseline. Apply treatment regimen outlined above. Resume G9.2-17 IgG4 at an additional 50% dose reduction. No further dose reductions for G9.2-17 IgG4 are allowed.<br>• If ≥ Grade 2 colitis recurs, permanently discontinue treatment with both study medications.<br>• Permanently discontinue G9.2-17 IgG4 and tislelizumab for life-threatening (Grade 4). |
| Immune-mediated hepatitis | • Monitor for changes in liver function.<br>• Administer corticosteroids at a dose of 0.5 to 1 mg/kg/day prednisone equivalents for moderate (Grade 2) transaminase elevations.<br>• Withhold tislelizumab and G9.2-17 IgG4 for moderate (Grade 2) immune-mediated hepatitis. When resolved to <Grade 2, and prednisolone tapered to ≤ 10 mg, resume tislelizumab and G9.2-17 IgG4. G9.2-17 IgG4 is to be resumed at a 50 % dose reduction.<br>• If ≥ Grade 2 hepatitis recurs, permanently discontinue treatment with both G9.2-17 IgG4 and tislelizumab.<br>• Administer corticosteroids at a dose of 0.5 to 2 mg/kg/day prednisone equivalents followed by corticosteroid taper for moderate (grade 2), severe (Grade 3) or life-threatening (Grade 4) transaminase elevations, with or without concomitant elevation in total bilirubin.<br>• Permanently discontinue treatment with both G9.2-17 IgG4 and tislelizumab for severe (Grade 3) or life-threatening (grade 4) immune-mediated hepatitis. |
| Immune-mediated endocrinopathies:<br>Hypophysitis | • Administer hormone replacement as clinically indicated and corticosteroids. Withhold G9.2-17 IgG4 and tislelizumab for moderate (Grade 2) hypophysitis. First administer oral prednisolone 0.5 to 1 mg/kg/day and add hormone replacement as clinically indicated until resolved/improved to ≤ Grade 2. Taper corticosteroids over at least 1 month. Resume G9.2-17 IgG4 at a 50% dose reduction when resuming. Discontinuation is usually not necessary for |

| IMAR | Guidance for Management |
|---|---|
| | asymptomatic and manageable patients who are treated by an endocrinologist.<br>• If ≥ Grade 2 toxicity recurs, permanently discontinue treatment with both G9.2-17 IgG4 and tislelizumab.<br>• Permanently discontinue treatment G9.2-17 IgG4 and tislelizumab for severe (grade 3) and life-threatening (Grade 4) hypophysitis. |
| Adrenal insufficiency | • Monitor patients for signs and symptoms of adrenal insufficiency.<br>• Withhold G9.2-17 IgG4 and tislelizumab for moderate (Grade 2) adrenal insufficiency. First administer oral prednisolone 0.5 to 1 mg/kg/day as clinically indicated until resolved/improved to ≤ Grade 2. Taper corticosteroids over at least 1 month. Resume G9.2-17 IgG4 at a 50% dose reduction when resuming. Discontinuation is usually not necessary for asymptomatic and manageable patients who are treated by an endocrinologist.<br>• If ≥ Grade 2 toxicity recurs, permanently discontinue treatment with both G9.2-17 IgG4 and tislelizumab.<br>• Permanently discontinue treatment G9.2-17 IgG4 and tislelizumab for severe (grade 3) and life-threatening (Grade 4) adrenal insufficiency. |
| Hypothyroidism and Hyperthyroidism | • Monitor thyroid function prior to and periodically during tislelizumab treatment.<br>• Administer hormone-replacement therapy for hypothyroidism.<br>• Initiate medical management for control of hyperthyroidism. Administer oral prednisolone 0.5 mg/kg/day in patients with thyroid pain.<br>• Thyrotoxic patients should be treated with a beta blocker and may require additional treatment with a carbimazole.<br>• Hold tislelizumab for thyroiditis Grade 3 or 4 until this condition resolves to a Grade 0-1, then consider resuming treatment.<br>• Discontinue G9.2-17 IgG4 and tislelizumab for ≥Grade 3 and 4 hypothyroidism or hyperthyroidism |
| Type 1 Diabetes Mellitus | • Monitor patients for hyperglycemia or other signs and symptoms of diabetes. |

| IMAR | Guidance for Management |
|---|---|
| | • Withhold G9.2-17 IgG4 and tislelizumab in cases of severe (Grade 3) hyperglycemia until metabolic control is achieved, until blood glucose has been stabilized to baseline or Grade 0 to 1 and the patient is hyperglycemia symptom-free. Administer insulin as clinically indicated for type 1 diabetes.<br>• Follow G9.2-17 IgG4 IMAR guidance for Type 1 Diabetes Mellitus when resolved to ≤ Grade 2. Permanently discontinue treatment with both study medications for life-threatening (Grade 4) hyperglycemia. |
| Immune-mediated nephritis and renal dysfunction<br>(Defined as renal dysfunction or ≥ Grade 2 increased creatinine, requirement for corticosteroids, and no clear alternate etiology) | • Monitor patients for renal function prior to and periodically during treatment. In the event of immune-mediated nephritis, the frequency of renal function tests should be increased.<br>• Withhold G9.2-17 IgG4 and tislelizumab for moderate (Grade 2) or severe (Grade 3) increased nephritis. When resolved to < Grade 2, resume G9.2-17 IgG4 at a 50% dose reduction. If ≥ Grade 2 toxicity recurs, permanently discontinue treatment with both G9.2-17 IgG4 and tislelizumab.<br>• Ensure hydration and administer corticosteroids at a dose of 0.5 to 1 mg/kg/day prednisone equivalents for moderate (Grade 2) or 1 to 2 mg/kg/day prednisone equivalents for severe (Grade 3) nephritis.<br>• Permanently discontinue treatment with both G9.2-17 IgG4 and tislelizumab for life-threatening (Grade 4) nephritis and recurrent Grade 3 nephritis as well. |
| Immune-mediated skin adverse reactions<br>(Tislelizumab can cause immune-mediated rash, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), some cases with fatal outcome.) | • For ≤ Grade 2 rashes, treatment that can be controlled or resolved with moderate strength topical or oral steroids while continuing tislelizumab and G9.2-17 IgG4.<br>• Monitor patients for suspected severe skin reactions and exclude other causes.<br>• Withhold both medications for severe (Grade 3) until signs and symptoms become mild, and permanently discontinued for moderate (Grade 2) to severe (Grade 3) rashes that do not improve or worsen while treated with oral or IV prednisolone, and permanently discontinue for life-threatening (Grade 4) rash.<br>• For symptoms or signs of SJS or TEN, permanently discontinue tislelizumab and G9.2-17 IgG4 and refer |

| IMAR | Guidance for Management |
|---|---|
| | the patient for specialized care for assessment and treatment.<br>• The recommended dose of oral prednisolone is 0.5 to 1 mg/kg/day for 3 days with tapering over 2 to 4 weeks (moderate rashes) and using methyl prednisolone 0.5 to 1 mg/kg/day with converting to oral prednisolone and tapering over at least 4 weeks (severe rashes).<br>• When resolved to < Grade 2, resume G9.2-17 IgG4 at a 50% dose reduction. |
| Immune-mediated encephalitis | • Monitor for changes in neurologic function.<br>• Evaluation of patients with neurologic symptoms may include, but not be limited to, consultation with a neurologist, brain MRI, and lumbar puncture.<br>• Withhold G9.2-17 IgG4 and tislelizumab in patients with new-onset moderate to severe neurologic signs or symptoms and evaluate to rule out infectious or other causes of moderate to severe neurologic deterioration.<br>• If other etiologies are ruled out, administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents for patients with immune-mediated encephalitis, followed by corticosteroid taper.<br>• Permanently discontinue treatment with G9.2-17 IgG4 and tislelizumab for immune-mediated encephalitis. |
| Other Immune-Mediated Adverse Reactions<br>The following immune-mediated adverse reactions occurred in less than 1% of patients treated with tislelizumab: arthritis, encephalitis, rhabdomyolysis, myositis, myocarditis, pancreatitis, and uveitis. | • For any suspected immune-mediated adverse reactions, exclude other causes.<br>• Based on the severity of the adverse reaction, permanently discontinue or withhold G9.2-17 IgG4 and tislelizumab, administer high-dose corticosteroids, and if appropriate, initiate hormone-replacement therapy.<br>• Upon improvement to Grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month.<br>• Consider restarting G9.2-17 IgG4 and tislelizumab after completion of corticosteroid taper based on the severity of the event. Resume G9.2-17 IgG4 at a 50% dose reduction. Immune-mediated adverse reactions may occur after discontinuation of tislelizumab therapy. |
| Infusion reactions<br>(Reported in less than 1.0% of patients in clinical trials.) | • The symptoms of infusion-related reactions that may be observed with tislelizumab include fever, chills/rigor, nausea, pruritus, angioedema, hypotension, headache, bronchospasm, urticaria, rash, vomiting, myalgia, dizziness, or hypertension. |

| IMAR | Guidance for Management |
|---|---|
| | • Patients should be closely monitored for these signs and symptoms during the infusion.<br>• For Grade 1 reactions, decrease infusion rate by 50%; for Grade 2 reactions, stop infusion and only resume with caution at 50% of previous rate if infusion-related reaction has resolved or decreased to Grade 1 in severity. For Grade 3 or Grade 4 infusion-related reactions, stop infusion and permanently discontinue tislelizumab. Subsequent infusions should be given after premedication and at the reduced infusion rate. |
| Complications of Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)<br>(Fatal and other serious complications can occur in patients who receive allogeneic HSCT before or after being treated with a PD-1 receptor blocking antibody.) | • Transplant-related complications include hyperacute graft-versus-host-disease (GVHD), acute GVHD, chronic GVHD, hepatic veno-occlusive disease (VOD) after reduced intensity conditioning, and steroid-requiring febrile syndrome (without an identified infectious cause).<br>• These complications may occur despite intervening therapy between PD-1 blockade and allogeneic HSCT.<br>• Follow patients closely for evidence of transplant-related complications and intervene promptly.<br>• Consider the benefit versus risks of treatment with a PD-1 receptor blocking antibody prior to or after an allogeneic HSCT. |
| Embryo-Fetal Toxicity<br>(Based on its mechanism of action and data from animal studies, tislelizumab can cause fetal harm when administered to a pregnant woman.) | • Advise pregnant women of the potential risk to a fetus.<br>• Advise females of reproductive potential to use effective contraception during treatment with a tislelizumab-containing regimen and for at least 5 months after the last dose of tislelizumab. |

For non-IMAR, hematological and non-hematological AEs that occur in combination arms, upon assessment of causality:

- If G9.2-17 IgG4 related, follow AE management instructions for G9.2-17 IgG4
- If combination agent related (tislelizumab), follow management instructions in **Table 7.** for tislelizumab.

*Dose Modification*

The decision to proceed to the next dose level of G9.2-17 IgG4 in Part 1 may be made based on safety, tolerability, and preliminary PK data obtained in at least 2 patients at the prior dose level.

The dosing schedule may also be adjusted based on PK data obtained. Detailed dose modification instructions are available as described in **Tables 7-9**:

**Table 7: Recommended Dose Modifications for Tislelizumab for AEs (other than IMARs)**

| Adverse Reaction | Severity* | Dose Modification |
|---|---|---|
| AEs | Probably related Grade 3 adverse events | Permanently discontinue |
| | Life-threatening or Grade 4 adverse reaction | |

* Toxicity was graded per NCI CTCAE V5.

**Table 8. Management of Immune-Mediated Adverse Reactions (IMARs) Caused by G9.2-17 IgG4**

| IMAR | Guidance for Management |
|---|---|
| Immune-mediated pneumonitis | • Monitor patients for signs with radiographic imaging and for clinical symptoms of pneumonitis.<br>• Withhold G9.2-17 IGG4 for moderate and permanently discontinue for severe or life-threatening pneumonitis.<br>• Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents for moderate (Grade 2) or more severe (Grade 3-4) pneumonitis, followed by corticosteroid taper.<br>• Withhold G9.2-17 IGG4 until resolution to < Grade 2. For moderate (Grade 2) pneumonitis. Resume treatment at a 50% dose reduction.<br>• Upon recurrence of Grade 2 pneumonitis at the reduced dose of G9.2-17 IGG4, permanently discontinue G9.2-17 IGG4. |
| Immune-mediated colitis | • Monitor patients for signs and symptoms of colitis.<br>• Administer corticosteroids at a dose of 0.5 to 1 mg/kg/day prednisone equivalents followed by corticosteroid taper for moderate (Grade 2) colitis of more than 5 days duration.<br>• If worsening or no improvement occurs despite initiation of corticosteroids, increase dose to 1 to 2 mg/kg/day prednisone equivalents.<br>• When initial Grade 2 colitis resolves to < Grade 2, resume G9.2-17 IGG4 at a 50% dose reduction. |

| IMAR | Guidance for Management |
|---|---|
| | • If Grade ≥2 colitis recurs, withhold G9.2-17 IGG4 until colitis resolves to < Grade 2. Resume G9.2-17 IGG4 at an additional 50% dose reduction. No further dose reductions for G9.2-17 IGG4 are allowed.<br>• Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents followed by corticosteroid taper for severe (Grade 3) or life threatening (Grade 4) colitis.<br>• When Grade 3 colitis resolves to ≤ Grade 2, resume G9.2-17 IGG4 at a 50% dose reduction.<br>• Permanently discontinue G9.2-17 IGG4 for life-threatening (Grade 4) or for recurrent colitis of ≥ Grade 2 upon re-initiation of G9.2-17 IGG4. |
| Immune-mediated hepatitis | • Monitor for changes in liver function.<br>• Administer corticosteroids at a dose of 0.5 to 1 mg/kg/day prednisone equivalents for moderate (Grade 2) transaminase elevations.<br>• Withhold G9.2-17 IGG4 for moderate (Grade 2) immune-mediated hepatitis. When resolved to <Grade 2 resume G9.2-17 IGG4 at a 50% dose reduction.<br>• If ≥ Grade 2 hepatitis recurs, permanently discontinue G9.2-17 IGG4.<br>• Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents followed by corticosteroid taper for severe (Grade 3) or life-threatening (Grade 4) transaminase elevations, with or without concomitant elevation in total bilirubin.<br>• Permanently discontinue G9.2-17 IGG4 for severe (Grade 3) or life-threatening (Grade 4) immune-mediated hepatitis. |
| Immune-mediated endocrinopathies:<br>Hypophysitis | • Administer hormone replacement as clinically indicated and corticosteroids at a dose of 1 mg/kg/day prednisone equivalents followed by corticosteroid taper for moderate (Grade 2) or greater hypophysitis.<br>• Withhold G9.2-17 IG4 for moderate (Grade 2) or severe (Grade 3) and when resolved to <Grade 2, resume G9.2-17 IGG4 at a 50% dose reduction.<br>• If Grade ≥2 toxicity recurs, permanently discontinue G9.2-17 IGG4.<br>• Permanently discontinue G9.2-17 IGG4 for life-threatening (Grade 4) hypophysitis. |
| Adrenal insufficiency | • Monitor patients for signs and symptoms of adrenal insufficiency.<br>  ◦ Withhold G9.2-17 IGG4 for moderate (Grade 2) and once resolved to < Grade 2, resume G9.2-17 IGG4 at a 50% dose reduction.<br>  ◦ If Grade ≥2 toxicity recurs, permanently discontinue G9.2-17 IGG4. |

| IMAR | Guidance for Management |
|---|---|
| | • Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents followed by a corticosteroid taper for severe (Grade 3) or life-threatening (Grade 4) adrenal insufficiency.<br>• Permanently discontinue G9.2-17 IGG4 for severe (Grade 3) or life-threatening (Grade 4) adrenal insufficiency, or for recurrent colitis of ≥ Grade 2 upon re-initiation of G9.2-17 IGG4. |
| Hypothyroidism and Hyperthyroidism | • Monitor thyroid function prior to and periodically during nivolumab treatment.<br>• Administer hormone-replacement therapy for hypothyroidism.<br>• Initiate medical management for control of hyperthyroidism.<br>• There are no recommended dose adjustments of G9.2-17 IGG4 for < Grade 3 hypothyroidism or hyperthyroidism<br>• Discontinue G9.2-17 IGG4 for ≥Grade 3 hypothyroidism or hyperthyroidism |
| Type 1 Diabetes Mellitus | • Withhold G9.2-17 IGG4 in cases of severe (Grade 3) hyperglycemia until metabolic control is achieved.<br>• Upon resolution to ≤Grade 2, resume G9.2-17 IGG4 at a 50% dose reduction.<br>• If Grade ≥2 toxicity recurs, permanently discontinue G9.2-17 IGG4.<br>• Permanently discontinue G9.2-17 IGG4 for life-threatening (Grade 4) hyperglycemia. |
| Immune-mediated nephritis and renal dysfunction<br>(Defined as renal dysfunction or ≥Grade 2 increased creatinine, requirement for corticosteroids, and no clear alternate etiology) | • Monitor patients for elevated serum creatinine prior to and periodically during treatment.<br>• Administer corticosteroids at a dose of 0.5 to 1 mg/kg/day prednisone equivalents for moderate (Grade 2) or severe (Grade 3) increased serum creatinine, if worsening or no improvement occurs, increase dose of corticosteroids to 1 to 2 mg/kg/day prednisone equivalents.<br>• Withhold G9.2-17 IGG4 for moderate (Grade 2) or severe (Grade 3) increased serum creatinine. When resolved to < Grade 2, resume G9.2-17 IGG4 at a 50% dose reduction.<br>• If ≥ Grade 2 toxicity recurs, permanently discontinue G9.2-17 IGG4.<br>• Administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents followed by corticosteroid taper for life-threatening (Grade 4) increased serum creatinine.<br>• Permanently discontinue G9.2-17 IGG4 for life-threatening (Grade 4) increased serum creatinine. |
| Immune-mediated skin adverse reactions<br>(Immune-mediated rash, including Stevens-Johnson syndrome (SJS) | • Withhold for severe and permanently discontinue for life-threatening rash.<br>• For symptoms or signs of SJS or TEN, withhold G9.2-17 IGG4 and refer the patient for specialized care for assessment and treatment. |

| IMAR | Guidance for Management |
|---|---|
| and toxic epidermal necrolysis (TEN)) | • If SJS or TEN is confirmed, permanently discontinue G9.2-17 IGG4.<br>• For immune-mediated rash ≥Grade 2, administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents followed by a corticosteroid taper for severe (Grade 3) or life-threatening (Grade 4) rash.<br>• Withhold G9.2-17 IGG4 for moderate (Grade 2) and severe (Grade 3) rash and when resolved to <Grade 2, resume G9.2-17 IGG4 at a 50% dose reduction.<br>• If ≥Grade 2 rash recurs, withhold G9.2-17 IGG4 until resolved to <Grade 2 and resume G9.2-17 IGG4 at an additional 50% dose reduction. No further dose reductions are allowed.<br>• Permanently discontinue G9.2-17 IGG4 for life threatening (Grade 4) rash. |
| Immune-mediated encephalitis | • Monitor for changes in neurologic function.<br>• Evaluation of patients with neurologic symptoms may include, but not be limited to, consultation with a neurologist, brain MRI, and lumbar puncture.<br>• Withhold G9.2-17 IGG4 in patients with new-onset moderate to severe neurologic signs or symptoms and evaluate to rule out infectious or other causes of moderate to severe neurologic deterioration.<br>• If other etiologies are ruled out, administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents for patients with immune-mediated encephalitis, followed by corticosteroid taper.<br>• Permanently discontinue G9.2-17 IGG4 for immune-mediated encephalitis. |
| Other Immune-Mediated Adverse Reactions<br>(May include: myocarditis, rhabdomyolysis, myositis, uveitis, iritis, pancreatitis, facial and abducens nerve paresis, demyelination, polymyalgia rheumatica, autoimmune neuropathy, Guillain-Barré syndrome, hypopituitarism, systemic inflammatory response syndrome, gastritis, duodenitis, sarcoidosis, histiocytic necrotizing lymphadenitis (Kikuchi lymphadenitis), motor dysfunction, vasculitis, aplastic anemia, pericarditis, and myasthenic syndrome.) | • If uveitis occurs in combination with other immune-mediated adverse reactions, consider a Vogt-Koyanagi-Harada-like syndrome, and may require treatment with systemic steroids to reduce the risk of permanent vision loss.<br>• For any suspected immune-mediated adverse reactions, exclude other causes.<br>• Based on the severity (Grade) of the adverse reaction, permanently discontinue or withhold G9.2-17 IGG4, administer high-dose corticosteroids, and if appropriate, initiate hormone-replacement therapy.<br>• Upon improvement to Grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month.<br>• Consider restarting G9.2-17 IGG4 after completion of corticosteroid taper based on the severity of the event, resuming at a 50% dose reduction. |

**Table 9: Recommended Dose Modifications for G9.2-17 IgG4 (AEs outside the DLT window and other than IMARs)**

| Adverse Reaction | Severity | G9.2-17 IgG4 Dose Modification |
|---|---|---|
| Non-hematologic and hematologic toxicities | ≥ Grade 4 | • Permanently discontinue treatment with G9.2-17 IgG4. |
| Hematologic toxicities | Grade 3 | • Withhold treatment until toxicity resolves to ≤ Grade 2.<br>• Resume G9.2-17 IgG4 at 50% reduction from previous dose.<br>• If recurs again at ≥ Grade 3 severity, hold until resolved to ≤ Grade 2 and implement a further 50% dose reduction and medical monitor.<br>• No further dose reductions are allowed. |
| Non-hematologic toxicities (except those listed in **Table 8**, Management of Immune-Mediated Adverse Reactions (IMARs) Caused by G9.2-17 IgG4) | Grade 3 | • Withhold treatment until toxicity resolves to ≤ Grade 2.<br>• Resume G9.2-17 IgG4 at 50% reduction from previous dose.<br>• If recurs again at ≥ Grade 3 severity, hold until resolved to ≤ Grade 2 and implement a further 50% dose reduction and medical monitor.<br>• No further dose reductions are allowed. |
| Gastrointestinal (Nausea, Vomiting) | Grade 3<br>Not requiring hospitalization or parenteral nutrition support; managed by supportive care | • Withhold G9.2-17 IgG4 until toxicity reverses to ≤ Grade 2<br>• Resume G9.2-17 IgG4 with no dose reduction<br>• If recurrent, withhold G9.2-17 IgG4 until toxicity reverses to ≤ Grade 2<br>• Then resume G9.2-17 IgG4 at a 50% reduction from the previous dose |
| Electrolyte Abnormalities | Grade 3 corrected to ≤ Grade 2 within 24 hours | |
| Electrolyte Abnormalities | ≥ Grade 3 lasting <24-72 hours<br>Not clinically complicated; resolves spontaneously or | |

| Adverse Reaction | Severity | G9.2-17 IgG4 Dose Modification |
|---|---|---|
| | responds to conventional medical interventions | • If recurrent, withhold G9.2-17 IgG4 until toxicity reverses to ≤ Grade 2<br>• Resume G9.2-17 IgG4 at an additional 50% dose reduction<br>• No further dose reductions are allowed |
| Amylase or Lipase Elevation | ≥ Grade 3<br>Not associated with symptoms or clinical manifestations of pancreatitis | |

***Dose Administration and Dose Delay***

If an infusion-related reaction is encountered, interrupt the infusion and if clinically indicated, administer relevant medication(s) (eg, anti-histamine, anti-emetic, steroids, anti-pyretics, beta-blocker(s) etc.). If it is deemed appropriate to resume the infusion, resume at a slower infusion rate.

For subsequent cycles for the same patient, apply the appropriate pre-medications (anti-histamine, anti-emetic, steroids, anti-pyretics, beta-blocker(s) etc., as clinically indicated needed) and consider utilizing a slower infusion rate.

If any clinically meaningful AE ≥ Grade 3 possibly related or related to one or more study drugs occurs, it will be discussed with the Medical Monitor before continuing with dosing. A a dose delay may be necessary for ≥ Grade 3 AE.

***Dose Reduction***

No dose reductions may be allowed for any patient that is being evaluated for DLTs (within the 28 day DLT window). In case a dose reduction is necessary, the study intervention will be administered as follows:

For Parts 1 and 2 G9.2-17 IgG4 alone patients: Dose reduction may be allowed when it is assessed that clinical benefit is being derived and may continue to be derived under dose reduced conditions, see **Table 8** (for IMARs) or **Table 9** (for other AEs).

For Part 2 combination treatment cohorts of G9.2-17 IgG4 or G9.2-17 IgG4 tislelizumab, experience derived from clinical trials of the approved drug, as summarized in the approved

product labeling, will inform the adverse event management including guidance on delaying, dose-reducing and/or withholding entirely tislelizumab, see **Table 6-7** and **9**.

***Dose Modifications for Specific AEs Related to Administration of Tislelizumab***

Recommendations for tislelizumab modifications based on specific AEs are provided in **Table** 6 (for IMARs) **and Table 7** (for other AEs).

***Dose Modification for IMARs***

If an IMAR occurs, refer to **Table 8** and **Table 6** for guidance on dose management of G9.2-17 IgG4 and/or tislelizumab. All relevant medical exam(s)/test(s) in order to confirm that the adverse event is an IMAR.

***Discontinuation of Study Intervention***

In rare instances, it may be necessary for a patient to permanently discontinue study intervention. If study intervention is permanently discontinued due to reasons other than disease progression, and the patient is not being treated with other anti-cancer therapy(ies), the patient will continue to be evaluated for disease progression for up to 2 years. See the Schedule of Assessment table for data to be collected at the time of discontinuation of study intervention and follow-up and for any further evaluations that need to be completed.

Every effort must be made by study personnel to keep patients on study treatment until one of the reasons for study treatment termination are met (disease progression, toxicity related to the study drug, withdrawal of consent). If the patient has radiographic progression but no unequivocal clinical progression and alternate treatment is not initiated, the patient may continue on study treatment. However, if patients have unequivocal clinical progression without radiographic progression, study treatment should be stopped and patients advised regarding available treatment options.

A patient may be discontinued prior to disease progression for any of the following reasons:

- A DLT per definition provided herein
- An AE occurs/recurs outside of the DLT window that requires discontinuation of study treatment(s)

- An IMAR occurs/recurs that requires discontinuation of study treatment(s)
- Intercurrent illness or medical condition that prevents further administration of treatment or may jeopardize the patient's safety if they continue on study treatment
- Pregnancy
- Use of a non-protocol anti-cancer therapy

Patients may also be discontinued prior to disease progression for any of the following reasons:

- Significant deviation from protocol on the part of the patient (includes lack of compliance)

*Concomitant Therapy*

Any medication or vaccine (including over-the-counter or prescription medicines, recreational drugs, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

*Permitted Medications*

The following concomitant medications are allowed:

- Any standard of care pre-medication for patients on a combination treatment regimen.
- Continuation of bisphosphonate treatment (eg, zoledronic acid) or denosumab for bone metastases, which have been stable for at least 6 months before treatment (C1D1),
- The use of inhaled corticosteroids and mineralocorticoids (eg, fludrocortisone), topical steroids, intranasal steroids, intra-articular, and ophthalmic steroids
- Prophylactic or therapeutic use of anticoagulants \

- Vaccination for COVID-19, common flu and/or other common clinically required indications (eg tetanus, pneumococcus, HBV, etc.) is allowed before or during the study period. The timing and type of vaccine must be recorded.

*Prohibited Medications*

Following medications are not allowed while on this study:

- Concomitant administration of other investigational agents, other than G9.2-17 IGG4, for any indication
- Systemic immunosuppressive treatment, including, but not limited to cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-TNF agents. However, patients are allowed to take acute, low dose systemic immunosuppressant medications (eg, ≤ 10 mg/day of prednisone or equivalent).
- Replacement therapy (eg, thyroxine, insulin, physiologic corticosteroid replacement therapy [eg, ≤ 10 mg/day of prednisone equivalent] for adrenal or pituitary insufficiency) is not considered a form of systemic treatment.

**Efficacy Assessments**

Planned time points for all efficacy assessments are provided in the table of Schedule of Assessment.

*RECIST v1.1 Criteria for Tumor Assessment*

At the screening tumor assessment, tumor lesions/lymph nodes will be categorized as measurable or non-measurable with measurable tumor lesions recorded according to the longest diameter in the plane of measurement (except for pathological lymph nodes, which are measured in the shortest axis). When more than one measurable lesion is present at screening all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions. Target lesions should be selected on the basis of their size (lesions with the longest diameter). A sum of the diameters for all target lesions may be calculated and reported as the baseline sum diameters.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at screening. Measurements are not

required, and these lesions should be followed as 'present', 'absent', or 'unequivocal progression'. Tumor target lesions will be assessed according to the RECIST v1.1 Guidelines (Eisenhauer et al., 2009) using the following disease response measures.

- Complete response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
- Partial response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- Stable disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
- Progressive disease: At least a 20% increase in the sum of 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. (Note: the appearance of one or more new lesions is also considered progression).

The following guidelines can be used for evaluating non-target lesions. See also **Table 10** below.

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

The disease response measures at different timepoints will allow for the calculation of the following:

- Disease control rate (DCR), defined as percentage of patients who have achieved CR, PR and SD.
- Objective response rate (ORR), defined as the proportion of patients with tumor size reduction of a predefined amount (tumor shrinkage of ≥ 30%).

- Progression-free survival (PFS), defined as the time from study drug treatment initiation to disease progression (tumor growth by ≥ 30%).
- Duration of response (DoR), defined as the length of time that a tumor continues to respond to treatment without the cancer growing or spreading.
- Overall survival (OS) defined as defined as the time from study drug treatment initiation to death from any cause.

**Table 10. Evaluation of Overall Timepoint Response for Patients with Measurable Disease at Baseline**

| Target Lesions | Non-target Lesions | New Lesions | Overall Response |
|---|---|---|---|
| CR | CR | No | CR |
| CR | Non-CR/Non-PD | No | PR |
| CR | NE | No | PR |
| PR | Non-PD or NE | No | PR |
| SD | Non-PD or NE | No | SD |
| Not all evaluated | Non-PD | No | NE |
| Progressive disease | Any | Yes or No | Progressive disease |
| Any | Progressive disease | Yes or No | Progressive disease |
| Any | Any | Yes | Progressive disease |

CR: Complete Response, Non-PD: Non-progressive Disease, PR: Partial Response, SD: Stable Disease, NE: Non-evaluable

*When target lesions show SD/PR and some subset of non-target lesions is non-evaluable, a careful decision must be made whether to call the overall response at this timepoint SD/PR or NE. This is based on whether the non- evaluable lesions, if they showed growth, could cause an overall response of progressive disease in the context of the other lesion responses seen. If the non-evaluable non-target lesions comprise a significant proportion of the overall disease burden, the appropriate timepoint response is NE.

**Adverse Event Management**

AEs may not be recorded prior to the administration of the first dose of study medication. AEs that start, or symptoms related to medical history that worsen after study drug administration will be recorded. AEs should be followed until they are either resolved, have returned to baseline, or are determined to be a stable or chronic condition. All SAEs are to be collected until 30 days after the last dose of study medication

*Immune-Mediated Adverse Reactions*

Immune-mediated adverse reactions (IMARs) are identified for tislelizumab.

The specific IMARs noted are:

- Immune-Mediated Hepatitis
- Immune-Mediated Nephritis
- Immune-Mediated Pneumonitis
- Immune-Mediated Pneumonitis
- Immune-Mediated Colitis and Diarrhea Immune-Mediated Endocrinopathies
- Immune-Mediated Skin Reactions
- Other Immune-Mediated Adverse Reactions: arthritis, encephalitis, rhabdomyolysis, myositis, myocarditis, pancreatitis, and uveitis.

The monitoring plan is intended to limit the severity and duration of IMARs that occur during combination drug development, and encompass: scheduled visits for a physical exam, vital signs, safety laboratory assessments including blood hematology, biochemistry, assessing endocrine functions each Day 1 of a new dosing cycle (pre-dose), assessing coagulation status and urine analyses. The Schedule of Assessments (see Example 1) also encompasses assessing the ejection fraction once every three months and conducting regular ECGs.

All relevant medical exam(s)/test(s) will be carried out in order to confirm that the adverse event is an IMAR.

Instructions for the management of these IMARs are included in **Table 8** for G9.2-17 IgG4 alone, and **Table 6** for the G9.2-17 IgG4 + tislelizumab combination treatment.

## EQUIVALENTS

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art are readily envision a variety of other means and/or structures for

performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art are readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations are dependent upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art are recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, *i.e.*, elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, *i.e.*, "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus,

as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, *i.e.*, the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," are refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (*i.e.*, "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is

not necessarily limited to the order in which the steps or acts of the method are recited.

**WHAT IS CLAIMED IS:**

1. A method for treating a solid tumor, the method comprising administering to a subject in need thereof

(a)an effective amount of an antibody that binds human Galectin-9 (anti-Galectin-9 antibody) and

(b) an effective amount of tislelizumab,

wherein the anti-Galectin-9 antibody comprises:

(i) a light chain variable region ($V_L$) comprising a light chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 1, a light chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 2, and a light chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 3, and

(ii) a heavy chain variable region comprising a heavy chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 4, a heavy chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 5, and a heavy chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 6 and

wherein the anti-Galectin-9 antibody is administered to the subject at a dose of about 0.2 mg/kg to about 18 mg/kg.

2. The method of claim 1, wherein the solid tumor is head and neck cancer, urothelial cancer, gastric esophageal cancer, or non-small cell lung cancer.

3. The method of claim 1 or claim 2, wherein the solid tumor is a metastatic tumor.

4. The method of any one of claims 1-3, wherein the anti-Galectin-9 antibody is administered to the subject at a dose of about 4 mg/kg to about 18 mg/kg.

5. The method of claim 4, wherein the anti-Galectin-9 antibody is administered to the subject at a dose of about 4 mg/kg, about 6.3 mg/kg, about 10 mg/kg, about 12 mg/kg, about 14 mg/kg, about 16 mg/kg, or about 18 mg/kg, optionally wherein the dose of the anti-Galecti-9 antibody is about 6.3 mg/kg, about 10 mg/kg, or about 16 mg/kg.

6. The method of any one of claims 1-5, wherein the anti-Galectin-9 antibody is administered to the subject once a week.

7. The method of claim 6, wherein the anti-Galectin-9 antibody is administered to the subject at a dose of about 6.3 mg/kg, about 10 mg/kg once a week, or about 16 mg/kg.

8. The method of any one of claims 1-7, wherein the anti-Galectin-9 antibody is administered to the subject by intravenous infusion.

9. The method of any one of claims 1-8, wherein the tislelizumab is administered to the subject at a dose of about 200 mg once every 3 weeks, at a dose of about 300 mg every 4 weeks, or at a dose of about 400 mg every six weeks.

10. The method of claim 9, wherein the tislelizumab is administered to the subject at a dose of about 300 mg every 4 weeks.

11. The method of claim 9 or claim 10, wherein the tislelizumab is administered to the subject by intravenous infusion.

12. The method of any one of claims 1-4, wherein the anti-Galectin-9 antibody is administered to the subject at about 6.3 mg/kg once a week by intravenous infusion and the tislelizumab is administered to the subject at a dose of about 300 mg every 4 weeks by intravenous infusion.

13. The method of any one of claims 1-4, wherein the anti-Galectin-9 antibody is administered to the subject at about 10 mg/kg once a week by intravenous infusion and the tislelizumab is administered to the subject at a dose of about 300 mg every 4 weeks by intravenous infusion.

14. The method of any one of claims 1-4, wherein the anti-Galectin-9 antibody is administered to the subject at about 16 mg/kg once a week by intravenous infusion and the tislelizumab is administered to the subject at a dose of about 300 mg every 4 weeks by intravenous infusion.

15. The method of any one of claims 1-14, wherein the tislelizumab is administered to the subject on a day when the subject receives the anti-Galectin 9 antibody or wherein the administration of tislelizumab and the administration of the anti-Galectin 9 antibody are on two consecutive days.

16. The method of any one of claims 1-14, wherein the administration of tislelizumab is performed prior to the administration of the anti-Galectin 9 antibody.

17. The method of any one of claims 1-16, wherein the subject is a human patient having the solid tumor.

18. The method of any one of claims 1-17, wherein the $V_L$ of the anti-Galectin-9 antibody comprises the amino acid sequence of SEQ ID NO: 8, and wherein the $V_H$ of the anti-Galectin-9 antibody comprises the amino acid sequence of SEQ ID NO: 7.

19. The method of claim 18, wherein the anti-Galectin-9 antibody is an IgG1 or IgG4 molecule.

20. The method of claim 19, wherein the anti-Galectin-9 antibody is an IgG4 molecule having a modified Fc region of human IgG4.

21. The method of claim 20, wherein the modified Fc region of human IgG4 comprises the amino acid sequence of SEQ ID NO: 14.

22. The method of any one of claims 1-17, the anti-Galectin-9 antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 19 and a light chain comprising the amino acid sequence of SEQ ID NO: 15.

23. The method of any one of claims 1-22, wherein the subject has undergone one or more prior anti-cancer therapies.

24. The method of claim 23, wherein the one or more prior anti-cancer therapies comprise chemotherapy, immunotherapy, radiation therapy, a therapy involving a biologic agent, or a combination thereof.

25. The method of claim 23 or claim 24, wherein the subject has progressed disease through the one or more prior anti-cancer therapies, or is resistant to the one or more prior therapies.

26. The method of any one of claims 1-25, wherein the subject is a human patient having an elevated level of Galectin-9 relative to a control value.

27. The method of claim 26, wherein the human patient has an elevated serum or plasma level of Galectin-9 relative to the control value.

28. The method of any one of claims 1-27, wherein the human patient has cancer cells expressing Galectin-9, immune cells expressing Galectin-9, or both.

29. The method of any one of claims 1-28, further comprising monitoring occurrence of adverse effects in the subject.

30. The method of claim 29, further comprising reducing the dose of the anti-Galectin-9 antibody, the dose of tislelizumab, or both, when an adverse effect occurs.

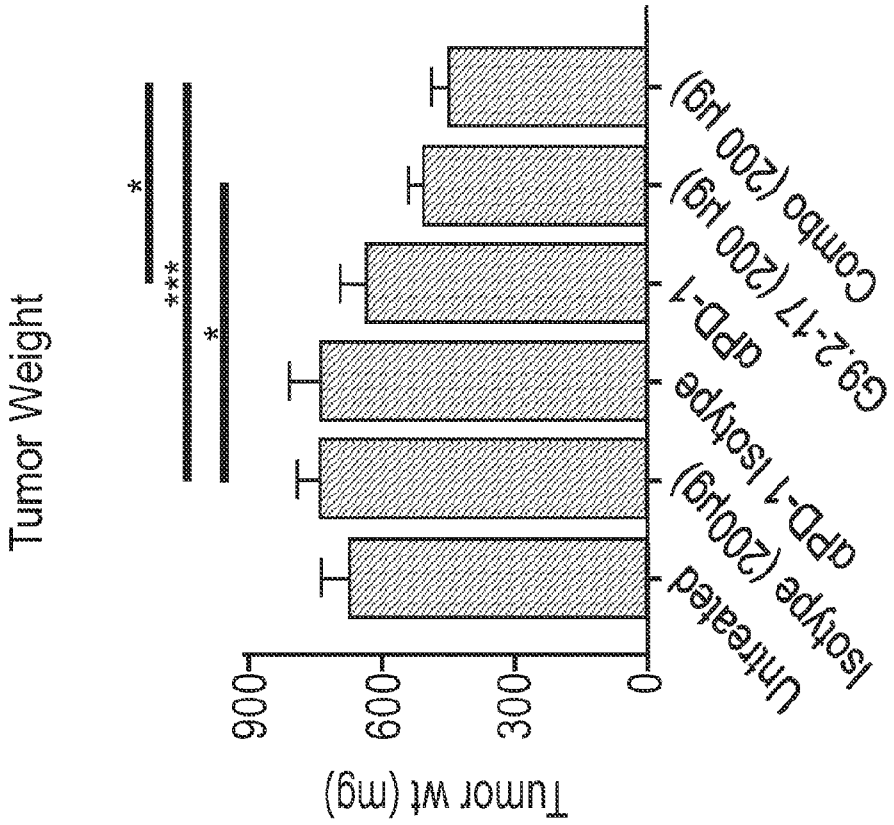

Tumor Weight
Tumor wt (mg)
900
600
300
0
Untreated
Isotype (200µg)
αPD-1 isotype
αPD-1
G9.2-17 (200 µg)
Combo (200 µg)
*
***
*

Figure 1

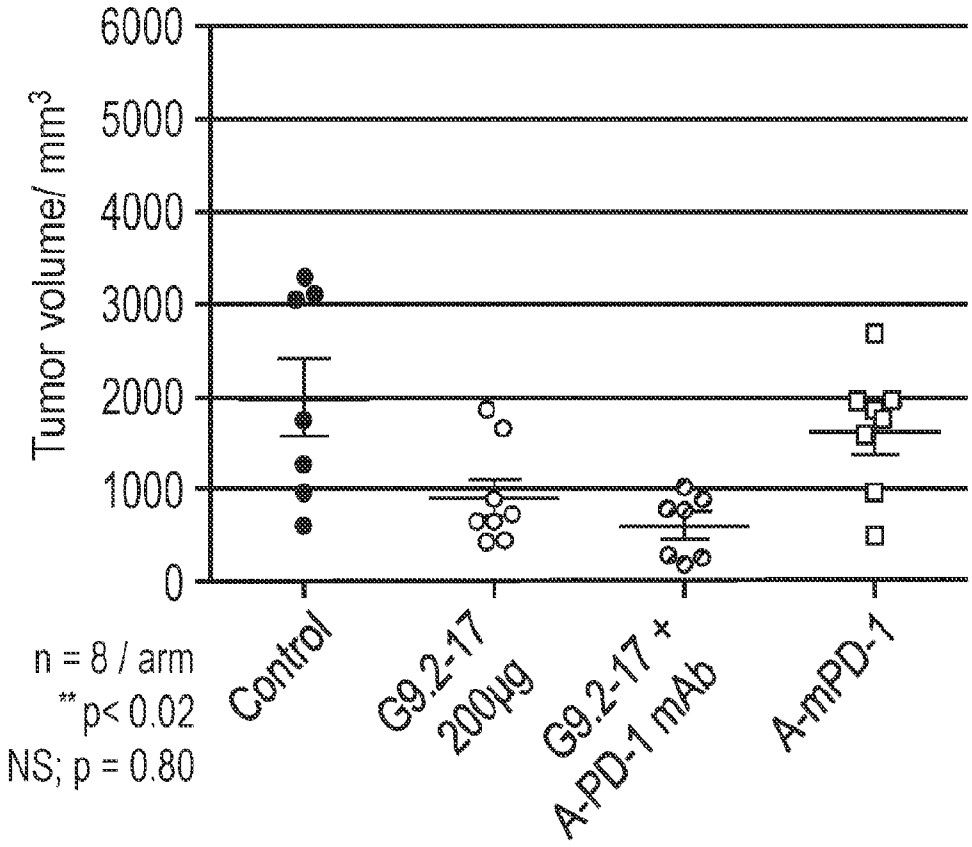



Figure. 2A

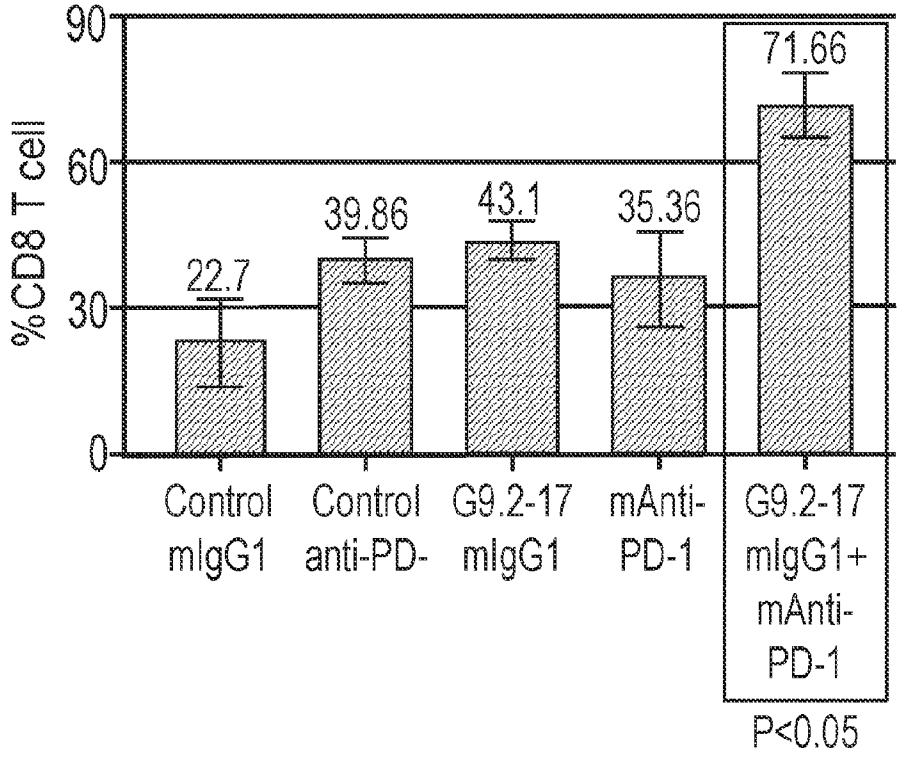



Figure 2B

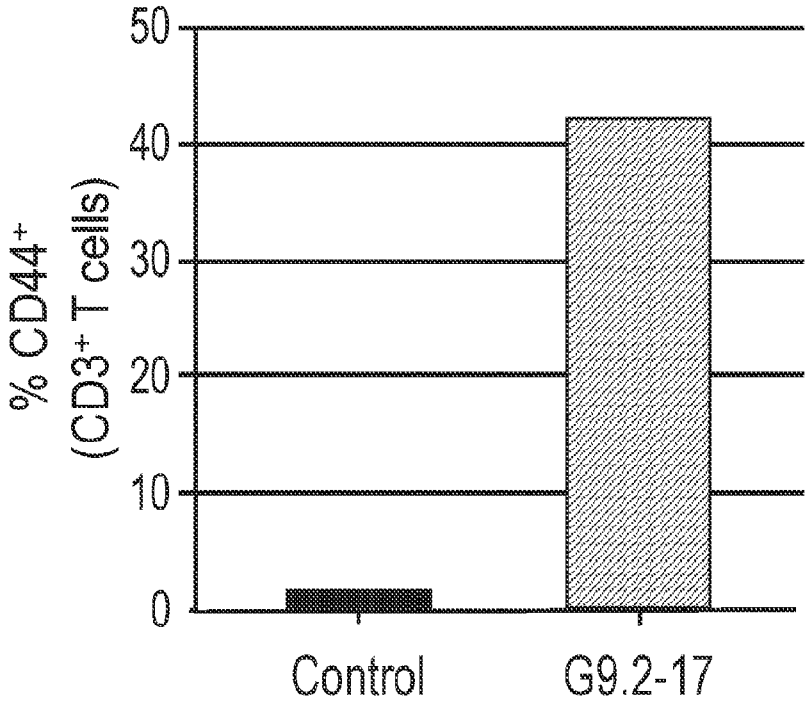



Figure 3A

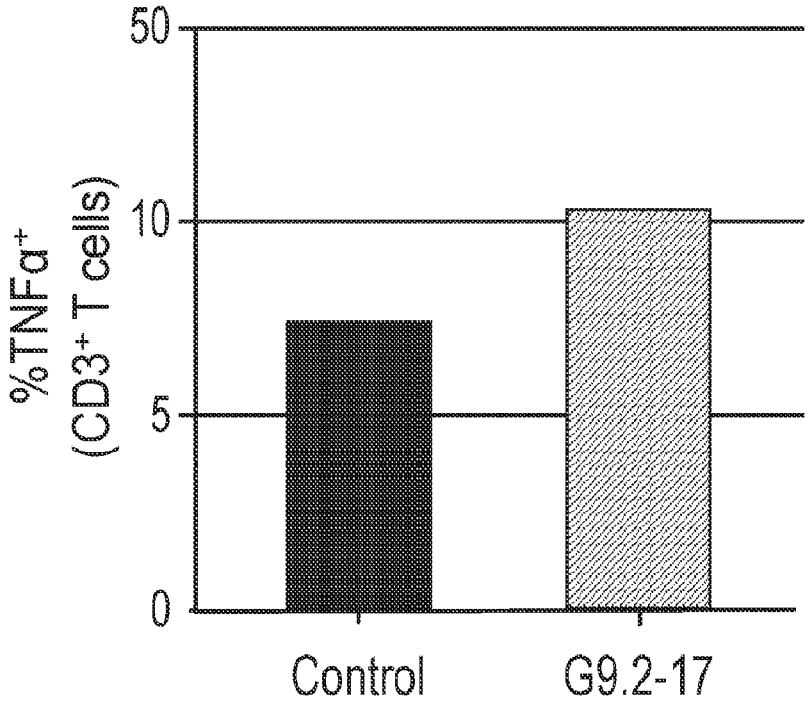



Figure 3B

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/77408

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 39/395, C07K 14/42 (2022.01)
ADD. A61P 35/00, A61P 35/04 (2022.01)

CPC - INV. A61K 2039/505, A61K 2039/507, C07K 16/2851, C07K 2317/565
ADD. A61P 35/00, A61P 35/04, G01N 33/57423

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 2020/223704 A1 (NEW YORK UNIVERSITY) 5 November 2020 (05.11.2020) pg 2 ln 24-25, pg 9 ln 16-17, pg 9 ln 22-23, pg 23 ln 33, pg 25 ln 6-8, SEQ ID NOs: 7, 8. | 1-3 |
| Y | WO 2020/223702 A1 (NEW YORK UNIVERSITY) 5 November 2020 (05.11.2020) claim 5. | 1-3 |

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 23 November 2022 | JAN 03 2023 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer<br>Kari Rodriquez<br>Telephone No. PCT Helpdesk: 571-272-4300 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/77408

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as fi led.

   b. ☐ furnished subsequent to the international fi ling date for the purposes of international search (Rule 13ter.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/77408

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(19)国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 118251234 A
(43)申请公布日 2024.06.25

(21)申请号 202280072581.2

(22)申请日 2022.09.30

(30)优先权数据
63/251,227 2021.10.01 US
63/277,384 2021.11.09 US

(85)PCT国际申请进入国家阶段日
2024.04.28

(86)PCT国际申请的申请数据
PCT/US2022/077408 2022.09.30

(87)PCT国际申请的公布数据
WO2023/056461 EN 2023.04.06

(71)申请人 纯技术LYT股份有限公司
地址 美国
申请人 百济神州瑞士有限公司

(72)发明人 A·菲利波维奇 E·艾伦科
H·帕登 C·科思
S·萨哈斯拉那曼 N·布达

(74)专利代理机构 中国贸促会专利商标事务所有限公司 11038
专利代理师 李程达

(51)Int.Cl.
*A61K 39/395*(2006.01)
*C07K 14/42*(2006.01)
*A61P 35/00*(2006.01)
*A61P 35/04*(2006.01)

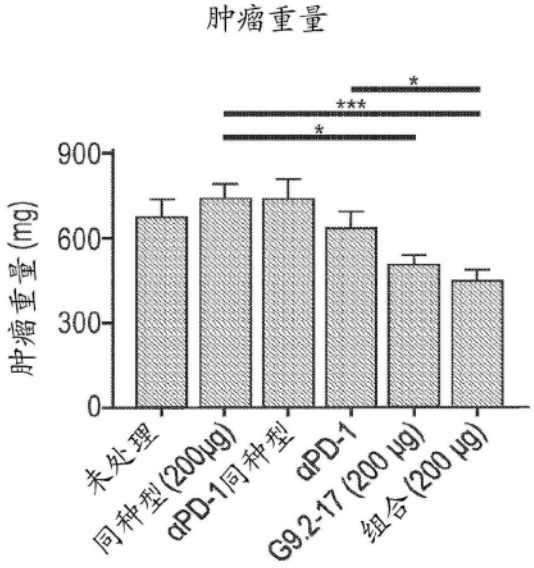
权利要求书2页 说明书73页
序列表（电子公布） 附图3页

(54)发明名称

# 抗半乳糖凝集素-9抗体及其治疗用途

(57)摘要

使用抗半乳糖凝集素-9抗体(例如,G9.2-17(IgG4))与替雷利珠单抗组合来治疗实体瘤(例如,头颈癌、尿道上皮癌等)的方法。




CN 118251234 A

1.一种用于治疗实体瘤的方法,所述方法包括向有需要的受试者施用

(a)有效量的结合人半乳糖凝集素-9的抗体(抗半乳糖凝集素-9抗体),和

(b)有效量的替雷利珠单抗,

其中所述抗半乳糖凝集素-9抗体包含:

(i)轻链可变区($V_L$),其包含如SEQ ID NO:1所示的轻链互补决定区1(CDR1)、如SEQ ID NO:2所示的轻链互补决定区2(CDR2)以及如SEQ ID NO:3所示的轻链互补决定区3(CDR3),和

(ii)重链可变区,其包含如SEQ ID NO:4所示的重链互补决定区1(CDR1)、如SEQ ID NO:5所示的重链互补决定区2(CDR2)以及如SEQ ID NO:6所示的重链互补决定区3(CDR3),并且

其中所述抗半乳糖凝集素-9抗体以约0.2mg/kg至约18mg/kg的剂量施用于所述受试者。

2.如权利要求1所述的方法,其中所述实体瘤是头颈癌、尿道上皮癌、胃食管癌或非小细胞肺癌。

3.如权利要求1或权利要求2所述的方法,其中所述实体瘤是转移性肿瘤。

4.如权利要求1-3中任一项所述的方法,其中所述抗半乳糖凝集素-9抗体以约4mg/kg至约18mg/kg的剂量施用于所述受试者。

5.如权利要求4所述的方法,其中所述抗半乳糖凝集素-9抗体以约4mg/kg、约6.3mg/kg、约10mg/kg、约12mg/kg、约14mg/kg、约16mg/kg、或约18mg/kg的剂量施用于所述受试者,任选地其中所述抗半乳糖凝集素-9抗体的剂量为约6.3mg/kg、约10mg/kg、或约16mg/kg。

6.如权利要求1-5中任一项所述的方法,其中所述抗半乳糖凝集素-9抗体每周一次施用于所述受试者。

7.如权利要求6所述的方法,其中所述抗半乳糖凝集素-9抗体以约6.3mg/kg、约10mg/kg每周一次、或约16mg/kg的剂量施用于所述受试者。

8.如权利要求1-7中任一项所述的方法,其中所述抗半乳糖凝集素-9抗体通过静脉内输注施用于所述受试者。

9.如权利要求1-8中任一项所述的方法,其中所述替雷利珠单抗以约200mg的剂量每3周一次、以约300mg的剂量每4周一次或以约400mg的剂量每六周一次施用于所述受试者。

10.如权利要求9所述的方法,其中所述替雷利珠单抗以约300mg的剂量每4周一次施用于所述受试者。

11.如权利要求9或权利要求10所述的方法,其中所述替雷利珠单抗通过静脉内输注施用于所述受试者。

12.如权利要求1-4中任一项所述的方法,其中所述抗半乳糖凝集素-9抗体通过静脉内输注以约6.3mg/kg每周一次施用于所述受试者,并且所述替雷利珠单抗通过静脉内输注以约300mg的剂量每4周一次施用于所述受试者。

13.如权利要求1-4中任一项所述的方法,其中所述抗半乳糖凝集素-9抗体通过静脉内输注以约10mg/kg每周一次施用于所述受试者,并且所述替雷利珠单抗通过静脉内输注以约300mg的剂量每4周一次施用于所述受试者。

14.如权利要求1-4中任一项所述的方法,其中所述抗半乳糖凝集素-9抗体通过静脉内

输注以约16mg/kg每周一次施用于所述受试者,并且所述替雷利珠单抗通过静脉内输注以约300mg的剂量每4周一次施用于所述受试者。

15.如权利要求1-14中任一项所述的方法,其中所述替雷利珠单抗在所述受试者接受所述抗半乳糖凝集素9抗体当天施用于所述受试者,或者其中所述替雷利珠单抗的施用和所述抗半乳糖凝集素9抗体的施用是在连续两天进行的。

16.如权利要求1-14中任一项所述的方法,其中所述替雷利珠单抗的施用是在所述抗半乳糖凝集素9抗体的施用之前进行的。

17.如权利要求1-16中任一项所述的方法,其中所述受试者是患有所述实体瘤的人类患者。

18.如权利要求1-17中任一项所述的方法,其中所述抗半乳糖凝集素-9抗体的$V_L$包含SEQ ID NO:8的氨基酸序列,并且其中所述抗半乳糖凝集素-9抗体的$V_H$包含SEQ ID NO:7的氨基酸序列。

19.如权利要求18所述的方法,其中所述抗半乳糖凝集素-9抗体是IgG1或IgG4分子。

20.如权利要求19所述的方法,其中所述抗半乳糖凝集素-9抗体是具有人IgG4的经修饰Fc区的IgG4分子。

21.如权利要求20所述的方法,其中所述人IgG4的经修饰Fc区包含SEQ ID NO:14的氨基酸序列。

22.如权利要求1-17中任一项所述的方法,所述抗半乳糖凝集素-9抗体包含含有SEQ ID NO:19的氨基酸序列的重链以及含有SEQ ID NO:15的氨基酸序列的轻链。

23.如权利要求1-22中任一项所述的方法,其中所述受试者已经经历一种或多种先前抗癌疗法。

24.如权利要求23所述的方法,其中所述一种或多种先前抗癌疗法包含化学疗法、免疫疗法、放射疗法、涉及生物剂的疗法或其组合。

25.如权利要求23或权利要求24所述的方法,其中通过所述一种或多种先前抗癌疗法所述受试者的疾病发生进展,或对所述一种或多种先前疗法具有抗性。

26.如权利要求1-25中任一项所述的方法,其中所述受试者是相对于对照值具有升高的半乳糖凝集素-9水平的人类患者。

27.如权利要求26所述的方法,其中所述人类患者具有相对于所述对照值升高的血清或血浆半乳糖凝集素-9水平。

28.如权利要求1-27中任一项所述的方法,其中所述人类患者具有表达半乳糖凝集素-9的癌细胞、表达半乳糖凝集素-9的免疫细胞或两者。

29.如权利要求1-28中任一项所述的方法,所述方法还包括监测所述受试者中副作用的发生。

30.如权利要求29所述的方法,所述方法还包括在发生副作用时降低所述半乳糖凝集素-9抗体的剂量、所述替雷利珠单抗的剂量或两者。

# 抗半乳糖凝集素-9抗体及其治疗用途

[0001] 相关申请的交叉引用

[0002] 本申请根据35U.S.C.§119(e)要求2021年10月1日提交的美国临时申请号63/251,227和2021年11月9日提交的美国临时申请号63/277,384的权益,每篇申请以全文引用的方式并入本文中。

[0003] 序列表

[0004] 本申请含有已经以XML格式以电子方式提交的序列表,并且以全文引用的方式并入本文中。在2022年9月30日创建的所述XML副本的名称为112174-0234-NP003WO01_SEQ.xml,并且大小为25,891字节。

## 背景技术

[0005] 免疫系统具有识别和消灭癌细胞的巨大潜力,但控制肿瘤免疫逃逸的复杂网络是广泛有效的免疫调节的障碍(Martinez-Bosch N等人,Immune Evasion in Pancreatic Cancer:From Mechanisms to Therapy.Cancers(Basel).2018;10(1))。经批准的免疫-肿瘤学(IO)剂向许多肿瘤类型(例如,黑色素瘤、肺癌、肾癌、膀胱癌、一些结肠癌等)递送增加的存活改善,并且除了手术、化疗和放疗之外并且与手术、化疗和放疗结合作为护理标准被快速整合。然而,多种其他侵袭性恶性肿瘤的治疗和存活仍存在重大差距。例如,转移性胰腺导管腺癌(PDAC或PDA)、胆管癌(CCA)和结直肠癌(CRC)的5年存活率仍然分别为<9%、<5%和<15%。这些胃肠肿瘤具有很强的侵袭性,许多患者就诊时已处于晚期疾病阶段,并且已批准的免疫疗法的有效性并不理想(Rizvi等人,Cholangiocarcinoma-evolving concepts and therapeutic strategies;Nat Rev Clin Oncol.2018;15(2):95-111;Kalyan等人,Updates on immunotherapy for colorectal cancer;J Gastrointest Oncol.2018;9(1):160-169)。

[0006] 第一代检查点抑制剂(抗PD-1、抗PD-L1和抗CTLA4)的成功导致新的IO临床试验功效和差异化爆发(Holl等人,Examining Peripheral and Tumor Cellular Immunome in Patients with Cancer;Front Immunol.2019;10:1767)。然而,在成功的同时,也有许多不幸的开发失败案例,因此,仍然需要更加新颖且有效的治疗方法。

[0007] 半乳糖凝集素-9是一种串联重复凝集素,由两个碳水化合物识别结构域(CRD)组成,并且于1997年首次在患有霍奇金淋巴瘤(HL)的患者中发现和描述(Tureci等人,J.Biol.Chem.1997,272,6416-6422)。存在三种同种型,并且其可以位于细胞内或细胞外。已在多种癌症中观察到半乳糖凝集素-9水平升高,包括黑色素瘤、霍奇金淋巴瘤、肝细胞癌、胰腺癌、胃癌、结肠癌和透明细胞肾细胞癌(Wdowiak等人,Int.J.Mol.Sci.2018,19,210)。在肾癌中,具有高半乳糖凝集素-9表达的患者显示出较晚期的疾病进展,其肿瘤大小较大(Kawashima等人;BJU Int.2014;113:320-332)。在黑色素瘤中,半乳糖凝集素-9在57%的肿瘤中表达,并且与健康对照相比,在晚期黑色素瘤患者的血浆中显著增加(Enninga等人,Melanoma Res.2016年10月;26(5):429-441)。许多研究已表明半乳糖凝集素-9作为预后标志物具有实用性,并且最近还表明其可作为潜在的新药物靶标(Enninga等

人,2016;Kawashima等人,BJU Int 2014;113:320-332;Kageshita等人,Int J Cancer.2002年6月20日;99(6):809-16,以及其中的参考文献)。

[0008] 半乳糖凝集素-9已被描述在许多细胞过程(诸如粘附、癌细胞聚集、细胞凋亡和趋化性)中发挥重要作用。最近的研究表明,半乳糖凝集素-9在支持肿瘤的免疫调节中发挥作用,例如,通过负调节Th1型响应、Th2极化和巨噬细胞向M2表型的极化。该工作还包括已经显示半乳糖凝集素-9通过与T细胞免疫球蛋白和粘蛋白蛋白3(TIM-3)受体的相互作用参与T细胞直接失活的研究(Dardalhon等人,J Immunol.,2010,185,1383-1392;Sanchez-Fueyo等人,Nat Immunol.,2003,4,1093-1101)。

[0009] 还发现半乳糖凝集素-9在极化向肿瘤抑制表型的T细胞分化以及促进致耐受性巨噬细胞编程和适应性免疫抑制中发挥作用(Daley等人,Nat Med.,2017,23,556-567)。在胰腺导管腺癌(PDAC)的小鼠模型中,已表明阻断肿瘤微环境(TME)中在先天免疫细胞上发现的半乳糖凝集素-9与受体Dectin-1之间的检查点相互作用可以增加TME中的抗肿瘤免疫响应并减缓肿瘤进展(Daley等人,Nat Med.,2017,23,556-567)。还发现半乳糖凝集素-9与CD206(M2型巨噬细胞的表面标志物)结合,导致CVL22(MDC)(一种巨噬细胞衍生的趋化因子,与肺癌较长的存活期和较低的复发风险相关)的分泌减少(Enninga等人,J Pathol.2018年8月;245(4):468-477)。

## 发明内容

[0010] 本公开至少部分地基于实体瘤(例如,转移性实体瘤),诸如头颈癌或尿道上皮癌的治疗方案的开发,单独或与检查点抑制剂(诸如抗PD-1抗体(例如替雷利珠单抗))组合。

[0011] 因此,在一些方面,本公开提供了一种用于治疗实体瘤的方法,该方法包括向有需要的受试者施用(a)有效量的结合人半乳糖凝集素-9的抗体(抗半乳糖凝集素-9抗体)和(b)有效量的抗PD-1抗体(诸如替雷利珠单抗)。在一些实施方案中,抗半乳糖凝集素-9抗体可包含:(i)轻链可变区($V_L$),其包含如SEQ ID NO:1所示的轻链互补决定区1(CDR1)、如SEQ ID NO:2所示的轻链互补决定区2(CDR2)以及如SEQ ID NO:3所示的轻链互补决定区3(CDR3);和(ii)重链可变区,其包含如SEQ ID NO:4所示的重链互补决定区1(CDR1)、如SEQ ID NO:5所示的重链互补决定区2(CDR2)以及如SEQ ID NO:6所示的重链互补决定区3(CDR3)。在一些情况下,抗半乳糖凝集素-9抗体(例如,如本文所公开的G9.2-17(IgG4))可以以约0.2mg/kg至约18mg/kg的剂量施用给受试者。在一些实例中,抗半乳糖凝集素-9抗体可以每周一次施用于受试者。

[0012] 在一些实施方案中,实体瘤是头颈癌、尿道上皮癌、胃食管癌或非小细胞肺癌。在一些实施方案中,实体瘤是转移性肿瘤(例如,局部晚期或转移性实体瘤)。在一些实施方案中,实体瘤是难治性的和/或复发性的。在一些实施方案中,待通过本文所公开的任何方法治疗的受试者是患有实体瘤的人类患者。

[0013] 在一些实施方案中,抗半乳糖凝集素-9抗体(例如,如本文所公开的G9.2-17(IgG4))可以以约4mg/kg至约18mg/kg的剂量施用于受试者。例如,抗半乳糖凝集素-9抗体以约4mg/kg、约6.3mg/kg、约10mg/kg、约12mg/kg、约14mg/kg、约16mg/kg、或约18mg/kg的剂量施用于受试者。在一个实例中,抗半乳糖凝集素-9抗体的剂量是约6.3mg/kg。在另一实例中,抗半乳糖凝集素-9抗体的剂量是约10mg/kg。在又另一实例中,抗半乳糖凝集素-9抗体

的剂量是约16mg/kg。

[0014] 在一些具体实例中,抗半乳糖凝集素-9抗体(例如,如本文所公开的G9.2-17(IgG4))可以以约6.3mg/kg的剂量每周一次施用于受试者。在一些具体实例中,抗半乳糖凝集素-9抗体(例如,如本文所公开的G9.2-17(IgG4))可以以约10mg/kg的剂量每周一次施用于受试者。在其他具体实例中,抗半乳糖凝集素-9抗体(例如,如本文所公开的G9.2-17(IgG4))可以以约16mg/kg的剂量每周一次施用于受试者。可替代地或此外,抗半乳糖凝集素-9抗体可以通过静脉内输注施用于受试者。

[0015] 在一些实施方案中,替雷利珠单抗以约200mg的剂量每3周一次、以约300mg的剂量每4周一次或以约400mg的剂量每六周一次施用于受试者。在一个实例中,替雷利珠单抗以约300mg的剂量每4周一次施用于受试者。可替代地或此外,替雷利珠单抗通过静脉内输注施用于受试者。

[0016] 在一个实例中,本文所公开的方法包括以约6.3mg/kg的剂量每周一次施用抗半乳糖凝集素-9抗体(例如,G9.2-17(IgG4))以及以约300mg的剂量每4周一次施用替雷利珠单抗。两种抗体都可以经由静脉内输注施用。

[0017] 在一个实例中,本文所公开的方法包括以约10mg/kg的剂量每周一次施用抗半乳糖凝集素-9抗体(例如,G9.2-17(IgG4))以及以约300mg的剂量每4周一次施用替雷利珠单抗。两种抗体都可以经由静脉内输注施用。

[0018] 在一个实例中,本文所公开的方法包括以约16mg/kg的剂量每周一次施用抗半乳糖凝集素-9抗体(例如,G9.2-17(IgG4))以及以约300mg的剂量每4周一次施用替雷利珠单抗。两种抗体都可以经由静脉内输注施用。

[0019] 在一些实例中,替雷利珠单抗在受试者接受抗半乳糖凝集素9抗体当天施用于受试者。可替代地,替雷利珠单抗的施用和抗半乳糖凝集素9抗体的施用在连续两天进行。在一些实例中,替雷利珠单抗的施用是在抗半乳糖凝集素9抗体的施用之前进行。

[0020] 在本文所公开的任何方法中,抗半乳糖凝集素-9抗体可以包含含有SEQ ID NO:8的氨基酸序列的$V_L$链以及含有SEQ ID NO:7的氨基酸序列的$V_H$链。在一些情况下,抗半乳糖凝集素-9抗体是IgG1或IgG4分子。例如,抗半乳糖凝集素-9抗体是具有人IgG4的经修饰Fc区的IgG4分子。在一些实例中,人IgG4的经修饰Fc区包含SEQ ID NO:14的氨基酸序列。在一个实例中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:19的氨基酸序列的重链以及含有SEQ ID NO:15的氨基酸序列的轻链。

[0021] 在本文所公开的任何方法中,受试者已经经历一种或多种先前抗癌疗法。在一些实例中,一种或多种先前抗癌疗法包括化学疗法、免疫疗法、放射疗法、涉及生物剂的疗法或其组合。在一些情况下,通过一种或多种先前抗癌疗法受试者的疾病发生进展,或对一种或多种先前疗法具有抗性。

[0022] 在一些实施方案中,受试者是相对于对照值具有升高的半乳糖凝集素-9水平的人类患者。例如,人类患者具有相对于对照值升高的半乳糖凝集素-9血清或血浆水平。在一些情况下,人类患者具有表达半乳糖凝集素-9的癌细胞、表达半乳糖凝集素-9的免疫细胞、或两者。

[0023] 本文所公开的任何方法中还可包括监测受试者中副作用的发生。可替代地或此外,该方法还可包括在发生副作用时降低抗半乳糖凝集素-9抗体的剂量、替雷利珠单抗的

剂量、或两者。

[0024] 以下各者也在本公开的范围内:用于治疗实体瘤(例如,本文所述的那些并且包括转移性实体瘤)的药物组合物、以及抗半乳糖凝集素-9抗体和抗PD-1抗体(诸如替雷利珠单抗)中的任一者用于制造用于治疗实体瘤的药物的用途,其中在一些实施方案中,本文所公开的用途涉及一种或多种也如本文公开的治疗条件(例如,剂量、给药时间表、施用途径等)。

[0025] 本发明的一个或多个实施方案的细节在下面的描述中阐述。本发明的其他特征或优点从下面的附图和若干实施方案的详述以及所附权利要求中而显而易见。

## 附图说明

[0026] 以下附图构成本说明书的一部分并且被包括以进一步展示本公开的某些方面,通过参考附图并结合本文呈现的具体实施方案的详述可以更好地理解这些方面。

[0027] 图1描绘了显示用单独G9.2-17mIgG2a或与αPD-1mAb组合治疗的小鼠的研究结果的图。每周一次用商业αPD-1(200μg)mAb或G9.2-17mIg2a(200μg)或者G9.2-17和αPD-1的组合或者匹配的同种型处理具有原位植入的KPC肿瘤的小鼠(n=10/组),持续三周。取出肿瘤并称重。每个点代表一只小鼠;*p<0.05;**p<0.01;***p<0.001;****p<0.0001;通过未配对的Student t检验。

[0028] 图2A和图2B描绘了显示G9.2-17在B16F10皮下同源模型中的作用的图。将肿瘤皮下植入并用G9.2-17 IgG1小鼠mAb、抗PD-1抗体或G9.2-17 IgG1小鼠mAb和抗PD-1抗体的组合处理。图9A描绘了显示对肿瘤体积的作用的图。图9B描绘了显示肿瘤内CD8 T细胞浸润的图。结果表明,在组合组中,效应T细胞的肿瘤内存在得到增强。

[0029] 图3A和图3B包括显示用G9.2-17处理的胆管癌患者源性离体肿瘤培养物(类器官)的图。将患者来源的离体肿瘤培养物(类器官)用G9.2-17或同种型对照处理三天。评估CD44(图3A)和TNFα(图3B)在来自PDOT的CD3+T细胞中的表达。

## 具体实施方式

[0030] 本文提供了使用抗半乳糖凝集素-9抗体(例如G9.2-17)与检查点抑制剂(如抗PD-1抗体(例如替雷利珠单抗))组合治疗实体瘤(例如头颈癌、尿道上皮癌和如本文所公开的其他实体瘤)的方法。在一些实施方案中,癌症是转移性的。在一些实施方案中,本文所公开的方法提供了具体剂量和/或给药时间表。在一些情况下,本文所公开的方法靶向特定患者群体,例如,已经历先前治疗并且通过先前治疗显示出疾病进展的患者,或对先前治疗有抗性(从头或获得性)的患者。

[0031] 半乳糖凝集素-9(一种串联重复凝集素)是一种β-半乳糖苷结合蛋白,已被证明在调节细胞-细胞和细胞-基质相互作用中发挥作用。人们发现它在霍奇金病组织和其他病理状态中强烈过表达。在一些情况下,还发现它在肿瘤微环境(TME)中循环。

[0032] 发现半乳糖凝集素-9与Dectin-1(在PDAC中的巨噬细胞上以及在癌细胞上高度表达的先天免疫受体)相互作用(Daley等人,Nat Med.2017;23(5):556-6)。无论半乳糖凝集素-9的来源如何,已经显示其与Dectin-1的相互作用的破坏会导致$CD4^+$和$CD8^+$细胞重编程为抗肿瘤免疫不可或缺的介质。因此,半乳糖凝集素-9充当用于阻断由Dectin-1介导的信

号传导的有价值的治疗靶标。因此,在一些实施方案中,本文所述的抗半乳糖凝集素-9抗体会破坏半乳糖凝集素-9与Dectin-1之间的相互作用。

[0033] 还发现半乳糖凝集素-9可与TIM-3(一种I型细胞表面糖蛋白,在所有种类的急性髓系白血病(除了M3(急性早幼粒细胞白血病))的白血病干细胞的表面上表达,但在正常人造血干细胞(HSC)中不表达)相互作用。已发现由半乳糖凝集素-9连接产生的TIM-3信号传导对免疫细胞具有多效性作用,诱导Th1细胞中的细胞凋亡(Zhu等人,Nat Immunol.,2005,6:1245-1252)并刺激肿瘤坏死因子-α(TNF-α)的分泌,导致单核细胞成熟为树突细胞,导致先天性免疫引起的炎症(Kuchroo等人,Nat Rev Immunol.,2008,8:577-580)。已发现另外的半乳糖凝集素-9/TIM-3信号传导共激活NF-κB和β-连环蛋白信号传导(促进LSC自我更新的两条途径)(Kikushige等人,Cell Stem Cell,2015,17(3):341-352)。干扰半乳糖凝集素-9/TIM-3结合的抗半乳糖凝集素-9抗体可以具有治疗作用,特别是对于白血病和其他血液恶性肿瘤。因此,在一些实施方案中,本文所述的抗半乳糖凝集素-9抗体会破坏半乳糖凝集素-9与TIM-3之间的相互作用。

[0034] 此外,发现半乳糖凝集素-9与CD206(一种甘露糖受体,在M2极化巨噬细胞上高度表达,从而促进肿瘤存活)相互作用(Enninga等人,J Pathol.2018年8月;245(4):468-477)。表达CD206的肿瘤相关巨噬细胞是肿瘤免疫抑制、血管生成、转移和复发的介质(参见例如Scodeller等人,Sci Rep.2017年11月7日;7(1):14655,及其中的参考文献)。具体地,M1(也称为经典活化型巨噬细胞)被Th1相关的细胞因子和细菌产物触发,表达高水平的IL-12,并且具有杀肿瘤作用。相比之下,M2(所谓的替代活化型巨噬细胞)被Th2相关因子活化,表达高水平的抗炎细胞因子诸如IL-10,并且促进肿瘤进展(Biswas和Mantovani;Nat Immunol.2010年10月;11(10):889-96)。M2的促肿瘤作用包括促进血管生成、促进侵袭和转移以及保护肿瘤细胞免受化学疗法诱导的细胞凋亡的影响(Hu等人,Tumour Biol.2015年12月;36(12):9119-9126,以及其中的参考文献)。肿瘤相关巨噬细胞被认为具有M2样表型并且具有促肿瘤作用。已显示半乳糖凝集素-9介导骨髓细胞向M2表型分化(Enninga等人,Melanoma Res.2016年10月;26(5):429-41)。半乳糖凝集素-9结合CD206有可能导致将TAM重新编程为M2表型,类似于之前针对Dectin-1所显示的情况。不希望受理论束缚,阻断半乳糖凝集素-9与CD206的相互作用可以提供一种机制,通过该机制抗半乳糖凝集素-9抗体(例如G9.2-17抗体)可以是治疗上有益的。因此,在一些实施方案中,本文所述的抗半乳糖凝集素-9抗体破坏半乳糖凝集素-9与CD206之间的相互作用。

[0035] 半乳糖凝集素-9还被证明可与蛋白质二硫键异构酶(PDI)和4-1BB相互作用(Bi S等人,Proc Natl Acad Sci U S A.2011;108(26):10650-5;Madireddi等人,J Exp Med.2014;211(7):1433-48)。

[0036] 抗半乳糖凝集素-9抗体可以充当用于治疗与半乳糖凝集素-9相关的疾病(例如,半乳糖凝集素-9信号传导在其中起作用的那些)的治疗剂。不受理论束缚,抗半乳糖凝集素-9抗体可阻断半乳糖凝集素-9介导的信号传导途径。例如,抗体可以干扰半乳糖凝集素-9与其结合配偶体(例如,Dectin-1、TIM-3或CD206)之间的相互作用,从而阻断由半乳糖凝集素-9/配体相互作用触发的信号传导。可替代地,或另外,抗半乳糖凝集素-9抗体还可通过诱导阻断和/或细胞毒性(例如,针对表达半乳糖凝集素-9的病理细胞的ADCC、CDC或ADCP)来发挥其治疗作用。病理细胞是指直接或间接促成疾病的发生和/或发展的细胞。

[0037] 本文所公开的抗半乳糖凝集素-9抗体能够抑制由半乳糖凝集素-9介导的信号传导(例如由半乳糖凝集素-9/Dectin-1或半乳糖凝集素-9/Tim-3介导的信号传导途径)或经由例如ADCC来消除表达半乳糖凝集素-9的病理性细胞。因此,本文所述的抗半乳糖凝集素-9抗体可以用于抑制任何半乳糖凝集素-9信号传导和/或消除半乳糖凝集素-9阳性病理细胞,从而有益于治疗与半乳糖凝集素-9相关的疾病。

[0038] 发现抗半乳糖凝集素-9抗体(诸如G9.2-17)能有效诱导表达半乳糖凝集素-9的细胞的细胞凋亡。此外,抗半乳糖凝集素-9抗体(诸如G9.2-17)自身或与检查点抑制剂(例如,抗PD-1抗体)组合的抗肿瘤作用在小鼠模型中得到证实。如本文所报道,在PDAC和黑色素瘤的小鼠模型以及患者来源的类器官肿瘤模型(PDOT)中测试G9.2-17的功效。所用的原位PDAC KPC小鼠模型(LSL-KrasG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre)概括了人类疾病的许多特征,包括对批准的检查点抑制剂的无反应性(Bisht和Feldmann G;Animal models for modeling pancreatic cancer and novel drug discovery;Expert Opin Drug Discov.2019;14(2):127-142;Weidenhofer等人,Animal models of pancreatic cancer and their application in clinical research;Gastrointestinal Cancer:Targets and Therapy 2016;6)。B16F10黑色素瘤小鼠模型一直是测试免疫疗法的长期标准(Curran等人,PD-1and CTLA-4combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors;Proc Natl Acad Sci U S A.2010;107(9):4275-4280)。

[0039] 从新鲜人肿瘤样品中分离的PDOT保留了自体淋巴样和髓样细胞群体,包括经历抗原的肿瘤浸润CD4和CD8 T淋巴细胞,并且在短期离体培养中对免疫疗法有反应(Jenkins等人,Ex Vivo Profiling of PD-1Blockade Using Organotypic Tumor Spheroids.Cancer Discov.2018;8(2):196-215;Aref等人,3D microfluidic ex vivo culture of organotypic tumor spheroids to model immune checkpoint blockade;Lab Chip.2018;18(20):3129-3143)。如本文所报道,在患者来源的类器官测定中观察到半乳糖凝集素-9在癌细胞上的表达。

[0040] 用G9.2-17小鼠IgG1(G9.2-17mIgG1含有与G9.2-17人IgG4完全相同的结合表位并且具有相同的效应子功能)进行体内研究,其已经作为单一药剂在其中经批准的检查点抑制剂不起作用的原位KPC模型中实现肿瘤生长的显著减少。在B16F10模型中,G9.2-17显著超过抗PD-1的功效。在两种模型中,使用G9.2-17mIgG1通过上调效应T细胞活性和抑制免疫抑制信号来调节肿瘤内免疫微环境以及增强肿瘤内CD8 T细胞浸润都得到证实。

[0041] 这些结果证明,本文公开的抗肿瘤方法(涉及抗半乳糖凝集素-9抗体,任选地与检查点抑制剂组合)将实现针对靶实体瘤的优异治疗功效。

[0042] 因此,本文描述了如本文公开的用于治疗某些癌症的抗半乳糖凝集素-9抗体的治疗用途。

[0043] 与半乳糖凝集素-9结合的抗体

[0044] 本公开提供了用于本文公开的治疗方法中的抗半乳糖凝集素-9抗体G9.2-17及其功能变体。

[0045] 抗体(可以复数形式互换使用)是能够通过位于免疫球蛋白分子的可变区的至少一个抗原识别位点与靶标(诸如碳水化合物、多核苷酸、脂质、多肽等)特异性结合的免疫球

蛋白分子。如本文所用,术语“抗体”(例如抗半乳糖凝集素-9抗体)不仅涵盖完整(例如全长)多克隆或单克隆抗体,而且还涵盖其抗原结合片段(诸如Fab、Fab’、F(ab’)2、Fv)、单链(scFv)、其突变体、包含抗体部分的融合蛋白、人源化抗体、嵌合抗体、双价抗体、纳米抗体、线性抗体、单链抗体、多特异性抗体(例如双特异性抗体)和包含所需特异性的抗原识别位点的免疫球蛋白分子的任何其他修饰的构型,包括抗体的糖基化变体、抗体的氨基酸序列变体以及共价修饰的抗体。抗体(例如,抗半乳糖凝集素-9抗体)包括任何类别的抗体,诸如IgD、IgE、IgG、IgA或IgM(或其亚类),并且抗体不需要属于任何特定类别。取决于抗体重链恒定结构域的氨基酸序列,免疫球蛋白可指定为不同的类别。有五种主要类别的免疫球蛋白:IgA、IgD、IgE、IgG和IgM,并且这些中的几种可以被进一步划分成亚类(同种型),例如IgG1、IgG2、IgG3、IgG4、IgA1和IgA2。对应于不同类别的免疫球蛋白的重链恒定结构域分别称为α、δ、ε、γ和μ。不同类别免疫球蛋白的亚基结构和三维构型是众所周知的。

[0046] 典型的抗体分子包含重链可变区($V_H$)和轻链可变区($V_L$),它们通常参与抗原结合。$V_H$和$V_L$区域可以进一步细分为高变区,也称为“互补决定区”(“CDR”),散布着更保守的区域,称为“框架区”(“FR”)。每个$V_H$和$V_L$通常由三个CDR和四个FR组成,从氨基末端到羧基末端按以下顺序排列:FR1、CDR1、FR2、CDR2、FR3、CDR3、FR4。使用本领域中已知的方法,例如通过Kabat定义、Chothia定义、AbM定义、EU定义、“Contact”编号方案、“IMGT”编号方案、“AHo”编号方案和/或contact定义,可以精确地鉴定框架区和CDR的范围,所有这些方法都是本领域熟知的。参见例如Kabat,E.A.,等人(1991)Sequences of Proteins of Immunological Interest,第五版,U.S.Department of Health and Human Services,NIH公布号91-3242,Chothia等人,(1989)Nature 342:877;Chothia,C.等人(1987)J.Mol.Biol.196:901-917,Al-lazikani等人(1997)J.Molec.Biol.273:927-948;Edelman等人,Proc Natl Acad Sci U S A.1969年5月;63(1):78-85;以及Almagro,J.Mol.Recognit.17:132-143(2004);MacCallum等人,J.Mol.Biol.262:732-745(1996);Lefranc M P等人,Dev Comp Immunol,2003年1月;27(1):55-77;以及Honegger A和Pluckthun A,J Mol Biol,2001年6月8日;309(3):657-70。还可参见hgmp.mrc.ac.uk and bioinf.org.uk/abs)。

[0047] 在一些实施方案中,本文所述的抗半乳糖凝集素-9抗体是全长抗体,其含有两条重链和两条轻链,每条链包括可变结构域和恒定结构域。可替代地,抗半乳糖凝集素-9抗体可以是全长抗体的抗原结合片段。涵盖在全长抗体的术语“抗原结合片段”内的结合片段的实例包括:(i)Fab片段,一种由$V_L$、$V_H$、$C_L$和$C_H1$结构域组成的单价片段;(ii)$F(ab')_2$片段,一种包括通过铰链区的二硫键连接的两个Fab片段的二价片段;(iii)Fd片段,其由$V_H$和$C_H1$结构域组成;(iv)Fv片段,其由抗体的单臂的$V_L$和$V_H$结构域组成,(v)dAb片段(Ward等人,(1989)Nature 341:544-546),其由$V_H$结构域组成;和(vi)保留功能性的分离的互补决定区(CDR)。此外,虽然Fv片段的两个结构域$V_L$和$V_H$由不同的基因编码,但可以使用重组方法通过合成接头将它们连接起来,使它们能够制成单个蛋白质链,其中$V_L$和$V_H$区配对形成单价分子,称为单链Fv(scFv)。参见例如Bird等人(1988)Science242:423-426;和Huston等人(1988)Proc.Natl.Acad.Sci.USA 85:5879-5883。

[0048] 本文所述的任何抗体(例如抗半乳糖凝集素-9抗体)可以是单克隆的或多克隆的。“单克隆抗体”是指均质抗体群体,并且“多克隆抗体”是指异质抗体群体。这两个术语不限制抗体的来源或其制造方式。

[0049] 参考抗体G9.2-17是指能够与人半乳糖凝集素-9结合的抗体,并且包含SEQ ID NO:7的重链可变区和SEQ ID NO:8的轻链可变区,两者在下文提供。在一些实施方案中,用于本文所公开的方法中的抗半乳糖凝集素-9抗体是G9.2-17抗体。在一些实施方案中,用于本文公开的方法中的抗半乳糖凝集素-9抗体是具有与参考抗体G9.2-17相同的重链互补决定区(CDR)和/或与参考抗体G9.2-17相同的轻链互补决定区的抗体。具有相同$V_H$和/或$V_L$ CDR的两种抗体意味着当通过相同方法(例如,本领域已知的Kabat方法、Chothia方法、AbM方法、Contact方法或IMGT方法,参见例如bioinf.org.uk/abs/)确定时,它们的CDR是相同的。

[0050] 参考抗体G9.2-17的重链和轻链CDR在下表1中提供(使用Kabat方法确定):

[0051] 表1.G9.2-17的重链和轻链CDR

[0052]

| | | | |
|---|---|---|---|
| G9.2-17 | $V_L$ CDR1 | RASQSVSSAVA | SEQ ID NO: 1 |
| | $V_L$ CDR2 | SASSLYS | SEQ ID NO: 2 |
| | $V_L$ CDR3 | QQSSTDPIT | SEQ ID NO: 3 |
| | $V_H$ CDR1 | FTVSSSSIH | SEQ ID NO: 4 |
| | $V_H$ CDR2 | YISSSSGYTYYADSVKG | SEQ ID NO: 5 |
| | $V_H$ CDR3 | YWSYPSWWPYRGMDY | SEQ ID NO: 6 |

[0053] 在一些实例中,用于本文所公开的方法中的抗半乳糖凝集素-9抗体可包含(按照Kabat方案)如SEQ ID NO:4所示的重链互补决定区1(CDR1)、如SEQ ID NO:5所示的重链互补决定区2(CDR2)以及如SEQ ID NO:6所示的重链互补决定区3(CDR3),且/或可包含如SEQ ID NO:1所示的轻链互补决定区1(CDR1)、如SEQ ID NO:2所示的轻链互补决定区2(CDR2)以及如SEQ ID NO:3所示的轻链互补决定区3(CDR3)。抗半乳糖凝集素-9抗体(包括参考抗体G9.2-17)可以呈如本文所公开的任何形式,例如全长抗体或Fab。本文所用的术语“G9.2-17(IgG4)”是指G9.2-17抗体,其是IgG4分子(例如,具有包含SEQ ID NO.19的重链和包含SEQ ID NO:15的轻链)。同样,术语“G9.2-17(Fab)”是指G9.2-17抗体,其是Fab分子。

[0054] 在一些实施方案中,抗半乳糖凝集素-9抗体或其结合部分包含重链和轻链可变区,其中轻链可变区CDR1、CDR2和CDR3氨基酸序列分别与SEQ ID NO:1、2和3中所示的轻链可变区CDR1、CDR2和CDR3氨基酸序列具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性。在一些实施方案中,抗半乳糖凝集素-9抗体或其结合部分包含重链和轻链可变区,其中重链可变区CDR1、CDR2和CDR3氨基酸序列分别与SEQ ID NO:4、5和6中所示的重链可变区CDR1、CDR2和CDR3氨基酸序列具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性。

[0055] 另外的半乳糖凝集素-9抗体(例如,与半乳糖凝集素-9的CRD1和/或CRD2区域结合)描述于共同拥有、共同未决的美国专利申请16/173,970以及共同拥有、共同未决的国际专利申请PCT/US18/58028和PCT/US2020/024767,这些专利申请中的每一项的内容通过引用整体并入本文。

[0056] 在一些实施方案中,本文公开的抗半乳糖凝集素-9抗体包含轻链CDR,所述轻链CDR与参考抗体G9.2-17的对应$V_L$ CDR相比单独或共同具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一

性。可替代地或此外,在一些实施方案中,抗半乳糖凝集素-9抗体包含重链CDR,所述重链CDR与参考抗体G9.2-17的对应$V_H$ CDR相比单独或共同具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性。

[0057] 两个氨基酸序列的"同一性百分比"是使用Karlin和Altschul Proc.Natl.Acad.Sci.USA 87:2264-68,1990的算法,按照Karlin和Altschul Proc.Natl.Acad.Sci.USA 90:5873-77,1993中的修改确定的。这样的算法被合并到Altschul等人J.Mol.Biol.215:403-10,1990的NBLAST和XBLAST程序(2.0版)中。可以用XBLAST程序(评分=50,字长=3)进行BLAST蛋白质搜索,以获得与本发明的蛋白质分子同源的氨基酸序列。当两个序列之间存在空位时,可以利用加空位BLAST,如Altschul等人,Nucleic Acids Res.25(17):3389-3402,1997中所述。当使用BLAST和加空位BLAST程序时,可以使用各自程序(例如,XBLAST和NBLAST)的默认参数。

[0058] 在其他实施方案中,本文所述的抗半乳糖凝集素-9抗体包含$V_H$,其包含HC CDR1、HC CDR2和HC CDR3,相对于参考抗体G9.2-17的HC CDR1、HC CDR2和HC CDR3,它们总共包含至多8个氨基酸残基变化(8、7、6、5、4、3、2或1个变化,包括添加、缺失和/或取代)。可替代地或此外,在一些实施方案中,本文所述的抗半乳糖凝集素-9抗体包含$V_H$,其包含LC CDR1、LC CDR2和LC CDR3,相对于参考抗体G9.2-17的LC CDR1、LC CDR2和LC CDR3,它们总共包含至多8个氨基酸残基变化(8、7、6、5、4、3、2或1个变化,包括添加、缺失和/或取代)。

[0059] 在一个实例中,氨基酸残基变异是保守氨基酸残基取代。如本文所用,"保守氨基酸取代"是指不改变进行氨基酸取代的蛋白质的相对电荷或大小特征的氨基酸取代。变体可以根据本领域普通技术人员已知的用于改变多肽序列的方法来制备,如在编辑此类方法的参考文献中发现的方法,例如Molecular Cloning:A Laboratory Manual,J.Sambrook等人编,第二版,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,New York,1989,或Current Protocols in Molecular Biology,F.M.Ausubel等人编,John Wiley& Sons,Inc.,New York。氨基酸的保守取代包括在以下组内的氨基酸之间进行的取代:(a)M、I、L、V;(b)F、Y、W;(c)K、R、H;(d)A、G;(e)S、T;(f)Q、N;和(g)E、D。

[0060] 在一些实施方案中,本文公开的具有本文公开的重链CDR的抗半乳糖凝集素-9抗体含有来源于种系$V_H$片段亚类的框架区。此类种系$V_H$区是本领域熟知的。参见例如IMGT数据库(www.imgt.org)或www.vbase2.org/vbstat.php。实例包括IGHV1亚家族(例如,IGHV1-2、IGHV1-3、IGHV1-8、IGHV1-18、IGHV1-24、IGHV1-45、IGHV1-46、IGHV1-58和IGHV1-69)、IGHV2亚家族(例如,IGHV2-5、IGHV2-26和IGHV2-70)、IGHV3亚家族(例如,IGHV3-7、IGHV3-9、IGHV3-11、IGHV3-13、IGHV3-15、IGHV3-20、IGHV3-21、IGHV3-23、IGHV3-30、IGHV3-33、IGHV3-43、IGHV3-48、IGHV3-49、IGHV3-53、IGHV3-64、IGHV3-66、IGHV3-72和IGHV3-73、IGHV3-74)、IGHV4亚家族(例如,IGHV4-4、IGHV4-28、IGHV4-31、IGHV4-34、IGHV4-39、IGHV4-59、IGHV4-61和IGHV4-B)、IGHV亚家族(例如,IGHV5-51或IGHV6-1)和IGHV7亚家族(例如,IGHV7-4-1)。

[0061] 可替代地或此外,在一些实施方案中,具有本文公开的轻链CDR的抗半乳糖凝集素-9抗体含有来源于种系Vκ片段的框架区。实例包括IGKV1框架(例如,IGKV1-05、IGKV1-12、IGKV1-27、IGKV1-33或IGKV1-39)、IGKV2框架(例如,IGKV2-28)、IGKV3框架(例如,

IGKV3-11、IGKV3-15或IGKV3-20)和IGKV4框架(例如,IGKV4-1)。在其他情况下,抗半乳糖凝集素-9抗体包含含有来源于种系Vλ片段的框架的轻链可变区。实例包括IGλ1框架(例如,IGλV1-36、IGλV1-40、IGλV1-44、IGλV1-47、IGλV1-51)、IGλ2框架(例如,IGλV2-8、IGλV2-11、IGλV2-14、IGλV2-18、IGλV2-23)、IGλ3框架(例如,IGλV3-1、IGλV3-9、IGλV3-10、IGλV3-12、IGλV3-16、IGλV3-19、IGλV3-21、IGλV3-25、IGλV3-27)、IGλ4框架(例如,IGλV4-3、IGλV4-60、IGλV4-69)、IGλ5框架(例如,IGλV5-39、IGλV5-45)、IGλ6框架(例如,IGλV6-57)、IGλ7框架(例如,IGλV7-43、IGλV7-46)、IGλ8框架(例如,IGλV8-61)、IGλ9框架(例如,IGλV9-49)或IGλ10框架(例如,IGλV10-54)。

[0062] 在一些实施方案中,用于本文公开的方法中的抗半乳糖凝集素-9抗体可以是具有与参考抗体G9.2-17相同的重链可变区($V_H$)和/或相同的轻链可变区($V_L$)的抗体,所述$V_H$和$V_L$区氨基酸序列在以下提供:

[0063] $V_H$:

[0064] EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPG KGLEWVAYISSSSGYTYYADSVKGR FTISADTSKNTAYLQMNSL RAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSS(SEQ ID NO:7)

[0065] $V_L$:

[0066] DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGK APKLLIYSASSLYSGVPSRFSGSRS GTDFTLTISSLQPEDFATYYCQ QSSTDPITFGQGTKVEIKR(SEQ ID NO:8)

[0067] 在一些实施方案中,抗半乳糖凝集素-9抗体与SEQ ID NO:7的重链可变区具有至少80%序列同一性(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%同一性)。可替代地或此外,抗半乳糖凝集素-9抗体与SEQ ID NO:8的轻链可变区具有至少80%序列同一性(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%同一性)。

[0068] 在一些情况下,本文公开的抗半乳糖凝集素-9抗体是参考抗体G9.2-17的功能变体。功能性变体可在结构上类似于参考抗体(例如,在如本文所公开的G9.2-17的重链和/或轻链CDR中的一个或多个中包含有限数目的氨基酸残基变化,或相对于如本文所公开的G9.2-17的重链和/或轻链CDR、或G9.2-17的VH和/或VL的序列同一性),具有与人半乳糖凝集素-9基本上类似的结合亲和力(例如,具有相同阶的$K_D$值)。

[0069] 在一些实施方案中,如本文所述的抗半乳糖凝集素-9抗体可以结合半乳糖凝集素-9并将其活性抑制至少20%(例如,31%、35%、40%、45%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。提供抑制剂效力量度的表观抑制常数($Ki^{app}$或$K_{i,app}$)与降低酶活性所需的抑制剂浓度有关,并且不依赖于酶浓度。本文所述的抗半乳糖凝集素-9抗体的抑制活性可以通过本领域已知的常规方法来确定。

[0070] 抗体的$K_{i,}^{app}$值可通过以下过程来确定:测量不同浓度的抗体对反应程度(例如,酶活性)的抑制作用;将作为抑制剂浓度的函数的伪一阶速率常数(v)的变化拟合到修正的Morrison方程(方程1)中,得到表观Ki值的估值。对于竞争性抑制剂,$Ki^{app}$可以从提取自$K_{i,}^{app}$与底物浓度关系图的线性回归分析的y截距获得。

[0071] $$v = A \cdot \frac{([E]-[I]-K_i^{app}) + \sqrt{([E]-[I]-K_i^{app})^2 + 4[E]\cdot K_i^{app}}}{2} \qquad \text{(方程 1)}$$

[0072] 其中A等于$v_o$/E,在不存在抑制剂(I)的情况下酶促反应的初始速度($v_o$)除以总酶浓度(E)。在一些实施方案中,本文所述的抗半乳糖凝集素-9抗体对于靶抗原或抗原表位具有1000、900、800、700、600、500、400、300、200、100、50、40、30、20、19、18、17、16、15、14、13、12、11、10、9、8、7、6、5pM或更小的$Ki^{app}$值。在一些实施方案中,相对于第二靶标(例如,半乳糖凝集素-9的CRD1),抗半乳糖凝集素-9抗体对于第一靶标(例如,半乳糖凝集素-9的CRD2)具有较低的$Ki^{app}$。$Ki^{app}$的差异(例如,特异性或其他比较)可以为至少1.5、2、3、4、5、10、15、20、37.5、50、70、80、91、100、500、1000、10,000或$10^5$倍。在一些实例中,相对于第二抗原(例如,处于第二构象的相同第一蛋白质或其模拟物;或第二蛋白质),抗半乳糖凝集素-9抗体更大地抑制第一抗原(例如,处于第一构象的第一蛋白质或其模拟物)。在一些实施方案中,使抗半乳糖凝集素-9抗体中的任一种进一步亲和力成熟以降低抗体对靶抗原或其抗原表位的$Ki^{app}$。

[0073] 在一些实施方案中,抗半乳糖凝集素-9抗体抑制例如肿瘤浸润免疫细胞诸如巨噬细胞中的Dectin-1信号传导。在一些实施方案中,抗半乳糖凝集素-9抗体将由半乳糖凝集素-9触发的Dectin-1信号传导抑制至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。这种抑制活性可以通过常规方法测定诸如常规测定来确定。可替代地或此外,抗半乳糖凝集素-9抗体抑制由半乳糖凝集素-9引发的T细胞免疫球蛋白粘蛋白-3(TIM-3)信号传导。在一些实施方案中,抗半乳糖凝集素-9抗体抑制T细胞免疫球蛋白粘蛋白-3(TIM-3)信号传导,例如在肿瘤浸润性免疫细胞中,例如在一些实施方案中,抑制至少30%(例如31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。这种抑制活性可以通过常规方法测定诸如常规测定来确定。

[0074] 在一些实施方案中,抗半乳糖凝集素-9抗体抑制例如肿瘤浸润免疫细胞中的CD206信号传导。在一些实施方案中,抗半乳糖凝集素-9抗体将由半乳糖凝集素-9触发的CD206信号传导抑制至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。这种抑制活性可以通过常规方法测定诸如常规测定来确定。在一些实施方案中,抗半乳糖凝集素-9抗体将半乳糖凝集素-9与CD206的结合阻断或阻止至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。这种抑制活性可以通过常规方法测定诸如常规测定来确定。

[0075] 在一些实施方案中,抗半乳糖凝集素-9抗体诱导表达半乳糖凝集素-9的靶细胞中的细胞毒性,诸如ADCC,例如,其中靶细胞是癌细胞或免疫抑制性免疫细胞。在一些实施方案中,抗半乳糖凝集素-9抗体将免疫细胞诸如T细胞或癌细胞中的细胞凋亡诱导至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。这种抑制活性可以通过常规方法测定诸如常规测定来确定。在一些实施方案中,本文所述的抗半乳糖凝集素-9抗体中的任一种诱导针对表达半乳糖凝集素-9的靶细胞的细胞毒性,诸如补体依赖性细胞毒性(CDC)。

[0076] 抗体依赖性细胞介导的吞噬作用(ADCP)是抗体通过吞噬作用介导其部分或全部作用的重要作用机制。在这种情况下,抗体介导抗原呈递细胞对特异性抗原的摄取。ADCP可以由单核细胞、巨噬细胞、嗜中性粒细胞和树突状细胞通过FcγRIIa、FcγRI和FcγRIIIa介导,其中巨噬细胞上的FcγRIIa(CD32a)代表主要途径。

[0077] 在一些实施方案中,抗半乳糖凝集素-9抗体诱导靶细胞(例如,表达半乳糖凝集

素-9的癌细胞或免疫抑制性免疫细胞)的细胞吞噬作用(ADCP)。在一些实施方案中,抗半乳糖凝集素-9抗体将靶细胞例如癌细胞或免疫抑制性免疫细胞的吞噬作用增加至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。

[0078] 在一些实施方案中,本文所述的抗半乳糖凝集素-9抗体诱导针对靶细胞例如癌细胞或免疫抑制性免疫细胞的细胞毒性,诸如补体依赖性细胞毒性(CDC)。在一些实施方案中,抗半乳糖凝集素-9抗体将针对靶细胞的CDC增加至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。

[0079] 在一些实施方案中,抗半乳糖凝集素-9抗体诱导例如肿瘤浸润T细胞中的T细胞活化,即直接或间接抑制半乳糖凝集素-9介导的T细胞活化的抑制。在一些实施方案中,抗半乳糖凝集素-9抗体将T细胞活化提高至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。T细胞活化可通过常规方法测定,诸如如使用用于测量细胞因子和检查点抑制剂的熟知测定(例如,测量CD44、TNFα、IFNγ和/或PD-1)。在一些实施方案中,抗半乳糖凝集素-9抗体促进CD4+细胞活化至少30%(例如31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。在非限制性实例中,抗半乳糖凝集素抗体诱导CD4+细胞中的CD44表达。在一些实施方案中,抗半乳糖凝集素-9抗体将CD4+细胞中的CD44表达增加至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。在非限制性实例中,抗半乳糖凝集素抗体诱导CD4+细胞中的IFNγ表达。在一些实施方案中,抗半乳糖凝集素-9抗体将CD4+细胞中的IFNγ表达增加至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。在非限制性实例中,抗半乳糖凝集素抗体诱导CD4+细胞中的TNFα表达。在一些实施方案中,抗半乳糖凝集素-9抗体将CD4+细胞中的TNFα表达增加至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。

[0080] 在一些实施方案中,抗半乳糖凝集素-9抗体将CD8+细胞活化提高至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。在非限制性实例中,抗半乳糖凝集素抗体诱导CD8+细胞中的CD44表达。在一些实施方案中,抗半乳糖凝集素-9抗体将CD8+细胞中的CD44表达增加至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。在非限制性实例中,抗半乳糖凝集素抗体诱导CD8+细胞中的IFNγ表达。在一些实施方案中,抗半乳糖凝集素-9抗体将CD8+细胞中的IFNγ表达增加至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。在非限制性实例中,抗半乳糖凝集素抗体诱导CD8+细胞中的TNFα表达。在一些实施方案中,抗半乳糖凝集素-9抗体将CD8+细胞中的TNFα表达增加至少30%(例如,31%、35%、40%、50%、60%、70%、80%、90%、95%或更大,包括其中的任何增量)。

[0081] 在一些实施方案中,如本文所述的抗半乳糖凝集素-9抗体对靶抗原(例如,半乳糖凝集素-9)或其抗原表位具有合适的结合亲和力。如本文所用,“结合亲和力”是指表观缔合常数或$K_A$。$K_A$是解离常数($K_D$)的倒数。本文所述的抗半乳糖凝集素-9抗体对靶抗原或抗原表位可具有至少$10^{-5}$、$10^{-6}$、$10^{-7}$、$10^{-8}$、$10^{-9}$、$10^{-10}$M或更低的结合亲和力($K_D$)。结合亲和力增加对应于$K_D$降低。结合亲和力(或结合特异性)可以通过多种方法来确定,包括平衡透析、平衡结

合、凝胶过滤、ELISA、表面等离振子共振或光谱法(例如,使用荧光测定)。用于评价结合亲和力的示例性条件是在HBS-P缓冲液(10mM HEPES pH7.4、150mM NaCl、0.005％(v/v)表面活性剂P20)中。

[0082] 这些技术可以用于测量作为靶蛋白浓度的函数的结合的结合蛋白的浓度。在某些条件下,结合的结合蛋白的分数浓度([结合]/[总])一般通过以下方程与总靶蛋白的浓度([靶])相关:

[0083] [结合]/[总]＝[靶]/(Kd+[靶])

[0084] 然而,不总是需要精确确定$K_A$,因为有时获得亲和力的定量测量结果(例如,使用诸如ELISA或FACS分析的方法确定,与$K_A$成比例,因此可以用于比较,诸如确定较高亲和力是否高例如2倍)、获得亲和力的定性测量结果或获得亲和力的推断结果(例如,通过功能测定(例如,体外或体内测定)中的活性)就足够。在一些情况下,体外结合测定指示体内活性。在其他情况下,体外结合测定不一定指示体内活性。在一些情况下,紧密结合是有益的,但在其他情况下,紧密结合在体内并不理想,并且具有较低结合亲和力的抗体是更理想的。

[0085] 在一些实施方案中,如本文所述的抗半乳糖凝集素-9抗体中的任一种的重链还包含重链恒定区(CH)或其部分(例如,CH1、CH2、CH3或其组合)。重链恒定区可以是任何合适的来源,例如人、小鼠、大鼠或兔。在一个具体实例中,重链恒定区来自如本文所述的任何IgG亚家族的人IgG(γ重链)。

[0086] 在一些实施方案中,本文所述的抗体的重链恒定区包含恒定区(例如,SEQ ID NO:4、5、6)的单一结构域(例如,CH1、CH2或CH3)或任何单一结构域的组合。在一些实施方案中,本文所述的抗体的轻链恒定区包含恒定区的单一结构域(例如,CL)。下文列出了示例性的轻链和重链序列。下文列出了示例性的轻链和重链序列。hIgG1 LALA序列包括两个突变(L234A和L235A(EU编号),它们抑制FcgR结合)以及P329G突变(EU编号)以消除补体C1q结合,从而消除所有免疫效应子功能。hIgG4 Fab臂交换突变序列包括抑制Fab臂交换的突变(S228P;EU编号)。IL2信号序列(MYRMQLLSCIALSLALVTNS;SEQ ID NO:9)可以位于可变区的N末端。它用于表达载体中,其在分泌过程中被切割,因此不在成熟的抗体分子中。成熟蛋白(分泌后)的重链以“EVQ”开头,轻链以“DIM”开头。示例性重链恒定区的氨基酸序列提供如下:

[0087] hIgG1重链恒定区(SEQ ID NO:10)

[0088] ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*

[0089] hIgG1 LALA重链恒定区(SEQ ID NO:12)

[0090] ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*

[0091] hIgG4重链恒定区(SEQ ID NO:13)

[0092] ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSPGK*

[0093] hIgG4重链恒定区(SEQ ID NO:20)

[0094] ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGK*

[0095] hIgG4突变重链恒定区(SEQ ID NO:14)

[0096] ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSPGK*

[0097] hIgG4突变重链恒定区(SEQ ID NO:21)

[0098] ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGK*

[0099] 在一些实施方案中,具有任何上述重链恒定区的抗半乳糖凝集素-9抗体与具有以下轻链恒定区的轻链配对:

[0100] 轻链恒定区(SEQ ID NO:11)

[0101] TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0102] 示例性全长抗半乳糖凝集素-9抗体提供如下:

[0103] G9.2-17hIgG1重链(SEQ ID NO:16)

[0104] EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK*

[0105] G9.2-17hIgG1 LALA重链(SEQ ID NO:17)

[0106] EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK*

[0107] G9.2-17hIgG4重链(SEQ ID NO:18)

[0108] EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDK RVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLS PGK*

[0109] G9.2-17hIgG4重链(SEQ ID NO:22)

[0110] EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDK RVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLS LGK*

[0111] G9.2-17hIgG4 Fab臂交换突变重链(SEQ ID NO:19)

[0112] EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDK RVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLS PGK*

[0113] G9.2-17hIgG4 Fab臂交换突变重链(SEQ ID NO:23)

[0114] EVQLVESGGGLVQPGGSLRLSCAASGFTVSSSSIHWVRQAPGKGLEWVAYISSSSGYTYYADSVKGRF TISADTSKNTAYLQMNSLRAEDTAVYYCARYWSYPSWWPYRGMDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSE STAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDK RVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLS LGK*

[0115] 任何上述重链可以与下文所示的(SEQ ID NO:15)的轻链配对：

[0116] DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQSSTDPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC*

[0117] 在一些实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:10具有至少80％(例如,80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％及其中的任何增量)序列同一性的重链IgG1恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体的恒定区包含含有SEQ ID NO:10的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体的恒定区包含由SEQ ID NO:10组成的重链IgG1恒定区。

[0118] 在一些实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:20具有至少80％(例如,80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％及其中的任何增量)序列同一性的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体的恒定区包含含有SEQ ID NO:20的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体的恒定区包含由SEQ ID NO:20组成的重链IgG4恒定区。

[0119] 在一些实施方案中,恒定区来自人IgG4。在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:13具有至少80％(例如,80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％及其中的任何增量)序列同一性的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:13的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:13组成的重链IgG4恒定区。

[0120] 在一些实施方案中,恒定区来自人IgG4。在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:20具有至少80％(例如,80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％及其中的任何增量)序列同一性的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:20的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:20组成的重链IgG4恒定区。

[0121] 在这些实施方案中的任一个中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:11具有至少80％(例如,80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％及其中的任何增量)序列同一性的轻链恒定区。在一些实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:11的轻链恒定区。在一些实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:11组成的轻链恒定区。

[0122] 在一些实施方案中,IgG是具有最小Fc受体接合的突变体。在一个实例中,恒定区来自人IgG1 LALA。在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:12具有至少80％(例如,80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％及其中的任何增量)序列同一性的重链IgG1恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:12的重链IgG1恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:12组成的重链IgG1恒定区。

[0123] 在一些实施方案中,抗半乳糖凝集素-9抗体包含修饰的恒定区。在一些实施方案中,抗半乳糖凝集素-9抗体包含免疫惰性的修饰的恒定区,例如,不触发补体介导的裂解,或不刺激抗体依赖性细胞介导的细胞毒性(ADCC)。可以使用美国专利号5,500,362中公开的方法来评估ADCC活性。在其他实施方案中,如Eur.J.Immunol.(1999)29:2613-2624、PCT

申请号PCT/GB99/01441和/或英国专利申请号9809951.8中所述修饰恒定区。在一些实施方案中,IgG4恒定区是重链交换减少的突变体。在一些实施方案中,恒定区来自人IgG4 Fab臂交换突变体S228P。

[0124] 在一个实施方案中,抗半乳糖凝集素-9抗体的恒定区包含与SEQ ID NO:14具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体的恒定区包含含有SEQ ID NO:14的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体的恒定区包含由SEQ ID NO:14组成的重链IgG4恒定区。

[0125] 在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:21具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:21的重链IgG4恒定区。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:21组成的重链IgG4恒定区。

[0126] 在一些实施方案中,抗半乳糖凝集素-9抗体的轻链具有对应于SEQ ID NO:15的链;并且示例性重链的氨基酸序列对应于SEQ ID NO:10(hIgG1);12(hIgG1 LALA);13(hIgG4);20(hIgG4);14(hIgG4mut);以及21(hIgG4 mut)。

[0127] 在一些实施方案中,抗半乳糖凝集素-9抗体具有包含SEQ ID NO:15、基本上由其组成或由其组成的轻链。在一些实施方案中,抗半乳糖凝集素-9抗体具有包含选自由SEQ ID NO:16-19、22和23组成的组的序列中的任一个、基本上由其组成或由其组成的重链。在一些实施方案中,抗半乳糖凝集素-9抗体具有包含SEQ ID NO:15、基本上由其组成或由其组成的轻链以及包含选自由SEQ ID NO:16-19组成的组的序列中的任一个、基本上由其组成或由其组成的重链。在一些实施方案中,抗半乳糖凝集素-9抗体具有包含SEQ ID NO:15的轻链以及包含选自由SEQ ID NO:16-19、22和23组成的组的序列中的任一个的重链。在一些实施方案中,抗半乳糖凝集素-9抗体具有基本上由SEQ ID NO:15组成的轻链以及基本上由选自由SEQ ID NO:16-19、22和23组成的组的序列中的任一个组成的重链。在一些实施方案中,抗半乳糖凝集素-9抗体具有由SEQ ID NO:15组成的轻链以及由选自由SEQ ID NO:16-19、22和23组成的组的序列中的任一个组成的重链。在一个具体实施方案中,抗半乳糖凝集素-9抗体具有基本上由SEQ ID NO:15组成的轻链以及基本上由SEQ ID NO:19组成的重链。在另一个具体实施方案中,抗半乳糖凝集素-9抗体具有基本上由SEQ ID NO:15组成的轻链以及基本上由SEQ ID NO:20组成的重链。

[0128] 在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:16具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:16的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:16组成的重链序列。

[0129] 在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:17具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:17的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:17组

成的重链序列。

[0130] 在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:18具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:18的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:18组成的重链序列。

[0131] 在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:22具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:22的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:22组成的重链序列。

[0132] 在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:19具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:19的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:19组成的重链序列。

[0133] 在一个实施方案中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:23具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:23的重链序列。在一个实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:23组成的重链序列。

[0134] 在这些实施方案中的任一个中,抗半乳糖凝集素-9抗体包含与SEQ ID NO:15具有至少80%(例如,80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%及其中的任何增量)序列同一性的轻链序列。在一些实施方案中,抗半乳糖凝集素-9抗体包含含有SEQ ID NO:15的轻链序列。在一些实施方案中,抗半乳糖凝集素-9抗体包含由SEQ ID NO:15组成的轻链序列。

[0135] 在具体实例中,在本文所公开的治疗方法中使用的抗半乳糖凝集素-9抗体具有SEQ ID NO:19的重链和SEQ ID NO:15的轻链。在一些实施方案中,在本文所公开的治疗方法中使用的抗半乳糖凝集素-9抗体是G9.2-17 IgG4。

[0136] 在一些实施方案中,本文所公开的任何抗半乳糖凝集素-9抗体(例如,G9.2-17(IgG4))可以具有缺失的重链的C端赖氨酸残基。

[0137] 抗半乳糖凝集素-9抗体的制备

[0138] 如本文所述的能够结合半乳糖凝集素-9的抗体可以通过本领域已知的任何方法制备,包括但不限于重组技术。下面提供了一个实例。

[0139] 可以将编码如本文所述的抗半乳糖凝集素-9抗体的重链和轻链的核酸克隆到一个表达载体中,每个核苷酸序列与合适的启动子可操作地连接。在一个实例中,编码重链和轻链的核苷酸序列中的每一个与不同的启动子可操作地连接。可替代地,编码重链和轻链的核苷酸序列可以与单个启动子可操作地连接,使得重链和轻链均由同一启动子表达。必要时,可以在重链与轻链编码序列之间插入内部核糖体进入位点(IRES)。

[0140] 在一些实例中,将编码抗体的两条链的核苷酸序列克隆到两个载体中,可以将所述载体引入相同或不同的细胞中。当两条链在不同的细胞中表达时,可以将它们中的每一者从表达其的宿主细胞中分离出来,并且可以将分离的重链和轻链混合并在允许形成抗体的合适条件下孵育。

[0141] 一般来讲,可以使用本领域已知的方法将编码抗体的一条或所有链的核酸序列克隆到与合适的启动子可操作地连接的合适的表达载体中。例如,可以使核苷酸序列和载体在合适的条件下与限制酶接触,以在每个分子上产生可以彼此配对并用连接酶连接在一起的互补端。可替代地,可以将合成的核酸接头连接到基因的末端。这些合成接头含有对应于载体中特定限制性位点的核酸序列。表达载体/启动子的选择将取决于用于产生抗体的宿主细胞的类型。

[0142] 多种启动子可以用于表达本文所述的抗体,包括但不限于巨细胞病毒(CMV)中间早期启动子、病毒LTR诸如劳斯肉瘤(Rous sarcoma)病毒LTR、HIV-LTR、HTLV-1LTR、猿猴病毒40(SV40)早期启动子、大肠埃希氏菌(E.coli)lac UV5启动子和单纯疱疹tk病毒启动子。

[0143] 也可以使用可调节启动子。此类可调节启动子包括使用来自大肠埃希氏菌的lac阻遏物作为转录调节剂来调节带有lac操纵子的哺乳动物细胞启动子的转录的那些[Brown,M.等人,Cell,49:603-612(1987)]、使用四环素阻遏物(tetR)的那些[Gossen,M.和Bujard,H.,Proc.Natl.Acad.Sci.USA 89:5547-5551(1992);Yao,F.等人,Human Gene Therapy,9:1939-1950(1998);Shockelt,P.等人,Proc.Natl.Acad.Sci.USA,92:6522-6526(1995)]。其他系统包括使用雌二醇、RU486、二酚米乐甾酮(diphenol murislerone)或雷帕霉素的FK506二聚体、VP16或p65。可从Invitrogen、Clontech和Ariad获得诱导型系统。

[0144] 可以使用包括具有操纵子的阻遏物的可调节启动子。在一个实施方案中,来自大肠埃希氏菌的lac阻遏物可以作为转录调节剂起作用以调节带有lac操纵子的哺乳动物细胞启动子的转录(M.Brown等人,Cell,49:603-612(1987);Gossen和Bujard(1992);M.Gossen等人,Natl.Acad.Sci.USA,89:5547-5551(1992)),将四环素阻遏物(tetR)与转录激活因子(VP 16)组合以产生tetR-哺乳动物细胞转录激活因子融合蛋白tTa(tetR-VP16),其中带有tetO的最小启动子来源于人巨细胞病毒(hCMV)主要立即早期启动子以产生tetR-tet操纵子基因系统,从而控制哺乳动物细胞中的基因表达。在一个实施方案中,使用四环素诱导型开关。当四环素操纵子正确地定位在CMVIE启动子的TATA元件的下游时,四环素阻遏物(tetR)单独而不是tetR-哺乳动物细胞转录因子融合衍生物可以作为强效的反式调节剂起作用以调节哺乳动物细胞中的基因表达(Yao等人,Human Gene Therapy,10(16):1392-1399(2003))。这种四环素诱导型开关的一个特别优势是它不需要使用四环素阻抑物-哺乳动物细胞反式激活因子或阻抑物融合蛋白(在一些情况下,它们可能对细胞有毒性)(Gossen等人,Natl.Acad.Sci.USA,89:5547-5551(1992);Shockett等人,Proc.Natl.Acad.Sci.USA,92:6522-6526(1995))就能实现其可调节效果。

[0145] 另外,载体可以含有例如以下中的一些或全部:选择标记基因,诸如用于在哺乳动物细胞中选择稳定或瞬时转染子的新霉素基因;来自人CMV的立即早期基因的用于高水平转录的增强子/启动子序列;来自SV40的用于mRNA稳定性的转录终止和RNA加工信号;SV40多瘤病毒复制起点和用于适当游离基因复制的ColE1;内部核糖体结合位点(IRESe);多功能多克隆位点;以及用于有义和反义RNA的体外转录的T7和SP6 RNA启动子。用于产生含有

转基因的载体的合适载体和方法是本领域熟知和可获得的。

[0146] 可用于实践本文所述的方法的聚腺苷酸化信号的实例包括但不限于人胶原I聚腺苷酸化信号、人胶原II聚腺苷酸化信号和SV40聚腺苷酸化信号。

[0147] 可将包含编码抗体中的任一种的核酸的一种或多种载体(例如,表达载体)引入合适的宿主细胞中以产生抗体。可以在用于表达抗体或其任何多肽链的合适条件下培养宿主细胞。可以经由常规方法(例如,亲和纯化)由培养的细胞(例如,从细胞或培养上清液中)回收此类抗体或其多肽链。如果需要,可以将抗体的多肽链在合适的条件下孵育允许产生抗体的合适的时间段。

[0148] 在一些实施方案中,用于制备本文所述的抗体的方法涉及编码也如本文所述的抗半乳糖凝集素-9抗体的重链和轻链的重组表达载体。可以通过常规方法(例如,磷酸钙介导的转染)将重组表达载体引入合适的宿主细胞(例如,dhfr-CHO细胞)中。可以选择阳性转化体宿主细胞并在允许形成抗体的两条多肽链表达的合适条件下培养,可以从细胞或培养基中回收该抗体。必要时,可以在允许抗体形成的合适条件下孵育从宿主细胞回收的两条链。

[0149] 在一个实例中,提供了两种重组表达载体,一种编码抗半乳糖凝集素-9抗体的重链,而另一种编码抗半乳糖凝集素-9抗体的轻链。可通过常规方法,例如磷酸钙介导的转染,将两种重组表达载体均引入合适的宿主细胞(例如,dhfr-CHO细胞)中。可替代地,可以将表达载体中的每一种引入合适的宿主细胞中。可以选择阳性转化体并在允许抗体的多肽链表达的合适条件下培养。当将两种表达载体引入同一宿主细胞中时,可以从宿主细胞或培养基中回收其中产生的抗体。如果需要,可以从宿主细胞或培养基中回收多肽链,然后在允许抗体形成的合适条件下培养。当将两种表达载体引入不同宿主细胞时,可以从对应的宿主细胞或从对应的培养基中回收它们中的每一种。然后可以在用于抗体形成的合适条件下孵育两条多肽链。

[0150] 使用标准分子生物学技术来制备重组表达载体、转染宿主细胞、选择转化体、培养宿主细胞并从培养基中回收抗体。例如,可以用蛋白A或蛋白G偶联基质通过亲和层析来分离一些抗体。

[0151] 编码如本文所述的抗半乳糖凝集素-9抗体的重链、轻链或两者的核酸、含有所述核酸的载体(例如,表达载体)以及包含所述载体的宿主细胞中的任一中在本公开的范围内。

[0152] 可以使用本领域已知的方法来表征如此制备的抗半乳糖凝集素-9抗体,由此检测和/或测量半乳糖凝集素-9生物活性的降低、改善或中和。例如,在一些实施方案中,ELISA型测定适用于Dectin-1或TIM-3信号传导的半乳糖凝集素-9抑制的定性或定量测量。

[0153] 可以通过将候选抗体与Dectin-1和半乳糖凝集素-9一起孵育并监测以下特征中的任何一个或多个来验证抗半乳糖凝集素-9抗体的生物活性:(a)Dectin-1与半乳糖凝集素-9之间的结合和由所述结合介导的信号转导的抑制;(b)预防、改善或治疗实体瘤的任何方面;(c)阻断或减少Dectin-1活化;(d)抑制(减少)半乳糖凝集素-9的合成、产生或释放。可替代地,TIM-3可用于使用上述方案验证抗半乳糖凝集素-9抗体的生物活性。可替代地,CD206可以用于使用上述方案来验证抗半乳糖凝集素-9抗体的生物活性。

[0154] 在一些实施方案中,在受试者中评估生物活性或功效,例如通过测量外周和肿瘤内T细胞比率、T细胞活化或通过巨噬细胞分型。

[0155] 确定抗半乳糖凝集素-9抗体的生物活性的其他测定包括测量CD8+和CD4+(常规)T细胞活化(在体外或体内测定中,例如通过测量炎性细胞因子水平,例如IFNγ、TNFα、CD44、ICOS颗粒酶B、穿孔素、IL2(上调);CD26L和IL-10(下调));测量巨噬细胞例如从M2到M1表型的重新编程(体外或体内)(例如,增加的MHCII、减少的CD206、增加的TNFα和iNOS)。可替代地,可以如本文所述例如在体外测定中评估ADCC的水平。

[0156] 治疗方法

[0157] 本公开提供了用于治疗实体瘤的方法,所述实体瘤包括但不限于头颈癌、尿道上皮癌、胃食管癌或非小细胞肺癌,所述方法使用单独的或与检查点抑制剂(诸如抗PD-1抗体,例如替雷利珠单抗)组合的任何抗半乳糖凝集素抗体(例如G9.2-17,例如G9.2-17 IgG4)。通过本文所公开的方法治疗的其他靶标实体瘤可包括胰腺导管腺癌(PDAC)、结肠直肠癌(CRC)、肝细胞癌(HCC)、胆管癌、肾细胞癌和乳腺癌。

[0158] 根据待治疗的疾病类型或疾病部位,可以使用医学领域普通技术人员已知的常规方法将药物组合物施用于受试者。在一些实施方案中,抗半乳糖凝集素-9抗体和/或抗PD-1抗体可以通过静脉内输注施用于受试者。

[0159] 可注射组合物可含有各种载剂,诸如植物油、二甲基乙酰胺、二甲基甲酰胺、乳酸乙酯、碳酸乙酯、肉豆蔻酸异丙酯、乙醇和多元醇(甘油、丙二醇、液体聚乙二醇等)。对于静脉内输注,可以通过滴注方法施用水溶性抗体,由此输注含有抗体和生理学上可接受的赋形剂的药物制剂。生理学上可接受的赋形剂可包括例如5%右旋糖、0.9%盐水、林格氏溶液或其他合适的赋形剂。肌内制剂(例如,抗体的合适可溶性盐形式的无菌制剂)可以溶解在药物赋形剂(如注射用水,0.9%盐水或5%葡萄糖溶液)中并施用。

[0160] 如本文所用,术语“治疗”是指将包含一种或多种活性剂的组合物应用或施用至患有靶标疾病或病症、疾病/病症的症状或对疾病/病症的倾向的受试者,目的是治愈、愈合、缓解、缓和、改变、补救、减轻、改善或影响病症、疾病或病症的症状或对疾病或病症的倾向。

[0161] 缓解目标疾病/障碍包括延迟疾病的发展或进展或者降低疾病严重性或者延长存活。缓解疾病或延长存活不一定需要治愈性结果。如本文所用,“延迟”靶标疾病或病症的发展是指推迟、阻碍、减缓、延迟、稳定和/或延缓疾病的进展。这种延迟可以是不同的时间长度,这取决于疾病的历史和/或被治疗的个体。“延迟”或减轻疾病的发展或延迟疾病发作的方法是与不使用该方法相比,降低在给定时间范围内出现疾病的一种或多种症状的可能性和/或减轻在给定的时间范围内症状的程度的方法。此类比较通常基于临床研究,使用的受试者数量足以得出具有统计学意义的结果。

[0162] 疾病的“发展”或“进展”是指疾病的初始表现和/或随后的进展。可以使用本领域众所周知的标准临床技术检测和评估疾病的发展。然而,发展也指可能无法检测的进展。出于本公开的目的,发展或进展是指症状的生物学过程。“发展”包括发生、复发和发作。如本文所用,靶疾病或病症的“发作”或“发生”包括初次发作和/或复发。

[0163] (i)用抗半乳糖凝集素9抗体治疗

[0164] 本文所述的抗半乳糖凝集素-9抗体中的任一种可以用于本文所述的方法中的任一种中。在一些实施方案中,抗半乳糖凝集素-9抗体是G9.2-17,例如G9.2-17(IgG4)。此类抗体可以用于治疗与半乳糖凝集素-9相关的疾病。在一些方面,本发明提供治疗癌症的方法。在一些实施方案中,本公开提供了用于减少、改善或消除与癌症相关的一种或多种症状

的方法。

[0165] 在一些实施方案中,抗半乳糖凝集素9抗体是具有与参考抗体G9.2-17相同的重链CDR序列和/或相同的轻链CDR序列的抗体。在一些实施方案中,抗半乳糖凝集素9抗体是具有与参考抗体G9.2-17相同的VH和VL序列的抗体。在一些实施方案中,此类抗体是IgG1分子(例如,具有如本文所公开的那些的野生型IgG1恒定区或其突变体)。可替代地,抗体是IgG4分子(例如,具有如本文所述的那些的野生型IgG4恒定区或其突变体)。在一些实施方案中,抗体包含如SEQ ID NO:1所示的轻链互补决定区1(CDR1)、如SEQ ID NO:2所示的轻链互补决定区2(CDR2)以及如SEQ ID NO:3所示的轻链互补决定区3(CDR3),且/或包含如SEQ ID NO:4所示的重链互补决定区1(CDR1)、如SEQ ID NO:5所示的重链互补决定区2(CDR2)以及如SEQ ID NO:6所示的重链互补决定区3(CDR3)。在一些实施方案中,抗体包含含有SEQ ID NO:7的重链可变区。在一些实施方案中,抗体包含含有SEQ ID NO:8的轻链可变区。在一些实施方案中,抗体包含含有SEQ ID NO:7的重链可变区以及含有SEQ ID NO:8的轻链可变区。在一些实施方案中,抗体包含含有SEQ ID NO:19的重链。在一些实施方案中,抗体包含含有SEQ ID NO:15的轻链。在具体实例中,本文所用的抗半乳糖凝集素-9抗体(G9.2-17(IgG4))具有SEQ ID NO:19的重链和SEQ ID NO:15的轻链。

[0166] 有效量的本文所述的抗半乳糖凝集素-9抗体(例如,G9.2-17(IgG4))可以经由合适的途径,全身或局部施用于需要治疗的受试者(例如,人)。在一些实施方案中,抗半乳糖凝集素-9抗体通过静脉内施用来施用,例如作为团注或通过一段时间内的连续输注,通过肌内、腹膜内、脑脊髓内、皮下、动脉内、关节内、滑膜内、鞘内、肿瘤内、尿道下、口服、吸入或局部途径施用。在一个实施方案中,抗半乳糖凝集素9抗体通过静脉内输注施用至受试者。在一个实施方案中,抗半乳糖凝集素-9抗体以腹膜内方式施用于受试者。

[0167] 如本文所用,“有效量”是指单独或与一种或多种其他活性剂组合赋予受试者治疗效果所需的每种活性剂的量。在一些实施方案中,治疗效果是在肿瘤微环境中降低半乳糖凝集素-9活性和/或量/表达、降低Dectin-1信号传导、降低TIM-3信号传导、降低CD206信号传导或增加抗肿瘤免疫响应。增加抗肿瘤响应的非限制性实例包括增加效应T细胞的活化水平或将TAM从M2表型转换为M1表型。在一些情况下,抗肿瘤响应包括增加ADCC响应。确定一定量的抗体是否达到治疗效果对于本领域技术人员来说是显而易见的。如本领域技术人员所认识到的,有效量根据所治疗的特定疾患、疾患的严重程度、个体患者参数(包括年龄、身体状况、体型、性别和体重)、治疗的持续时间、同步治疗的性质(如果有的话)、具体的施用途径以及健康从业者知识和专业知识范围内的类似因素而变化。这些因素是本领域普通技术人员所熟知的,并且仅需常规实验即可解决。通常优选使用个别组分或其组合的最大剂量,即根据合理的医学判断的最高安全剂量。

[0168] 经验考虑(诸如半衰期)通常有助于确定剂量。例如,与人免疫系统相容的抗体(诸如人源化抗体或全人抗体)在一些情况下用于延长抗体的半衰期和防止抗体受到宿主免疫系统的攻击。施用频率可以在治疗过程中确定和调整,并且通常但不一定基于靶标疾病/病症的治疗和/或抑制和/或改善和/或延迟。可替代地,抗体的持续连续释放制剂可能是合适的。用于实现持续释放的各种制剂和装置是本领域已知的。

[0169] 在一个实例中,如本文所述的抗体的剂量在已经给予一次或多次抗体施用的个体中凭经验确定。向个体给予递增剂量的拮抗剂。为了评估拮抗剂的功效,可以遵循疾病/病

症的指标。

[0170] 在一些实施方案中,本文所述的抗体(例如,G9.2-17,诸如G9.2-17(IgG4))以足以在体内将肿瘤中免疫抑制性免疫细胞中的半乳糖凝集素-9(和/或Dectin-1或TIM-3或CD206)的活性抑制至少20%(例如,30%、40%、50%、60%、70%、80%、90%或更大)的量施用于需要治疗的受试者。在其他实施方案中,本文所述的抗体(例如,G9.2-17)以有效将肿瘤中免疫抑制性免疫细胞中的半乳糖凝集素-9(和/或Dectin-1或TIM-3或CD206)的活性水平降低至少20%(例如,30%、40%、50%、60%、70%、80%、90%或更大)(与治疗前或对照受试者中的水平相比)的量施用。在一些实施方案中,本文所述的抗体(例如,G9.2-17)以足以在体内促进TAM中M1样编程至少20%(例如,30%、40%、50%、60%、70%、80%、90%或更大)(与治疗前或对照受试者中的水平相比)的量施用于需要治疗的受试者。

[0171] 术语“约”或“大约”意指在如本领域普通技术人员所确定的特定值的可接受误差范围内,其部分取决于如何测量或确定该值,即测量系统的限制。例如,根据本领域的实践,“约”可以意指在可接受的标准偏差内。可替代地,“约”可以意指给定值的最多±20%、优选最多±10%、更优选最多±5%且更优选最多±1%的范围。可替代地,特别是对于生物系统或过程,该术语可以意指在值的一个数量级内,优选在2倍内。在本申请和权利要求中描述特定值的情况下,除非另有说明,否则术语“约”是隐含的,并且在该上下文中表示在特定值可接受的误差范围内。

[0172] 在一些实施方案中,抗体是G9.2-17 IgG4。在一些实施方案中,以约0.2mg/kg至约32mg/kg,例如0.2mg/kg、0.63mg/kg、2mg/kg、4mg/kg、6mg/kg、6.3mg/kg、8mg/kg、10mg/kg、12mg/kg和16mg/kg或更高剂量水平的剂量将抗半乳糖凝集素-9抗体施用于受试者。在一些实施方案中,以约1mg/kg至约32mg/kg的剂量将抗半乳糖凝集素-9抗体施用于受试者,例如剂量可选自2mg/kg、4mg/kg、8mg/kg、12mg/kg和16mg/kg或更高剂量水平。在一些实施方案中,以约0.2mg/kg至约32mg/kg的剂量将抗半乳糖凝集素-9抗体施用于受试者,例如剂量可选自0.2mg/kg、0.63mg/kg、2mg/kg、4mg/kg、6mg/kg、6.3mg/kg、10mg/kg和16mg/kg或更高剂量水平。在一些实施方案中,例如经由静脉内输注每两周一次施用抗体。

[0173] 在一些实施方案中,本文所公开的抗半乳糖凝集素9抗体(例如,G9.2-17 IgG4)经由静脉内输注30分钟至6小时来施用。在一些实例中,抗半乳糖凝集素-9抗体的静脉内输注可进行30分钟至2小时。在其他实例中,抗半乳糖凝集素9抗体可以通过长输注时间段(例如约2-6小时,例如约2-4小时或约4-6小时)来施用。在具体实例中,可在约3小时、约4小时、约5小时或约6小时的时段内静脉内输注示例性抗半乳糖凝集素-9抗体。

[0174] 在一些实施方案中,可以以约0.2mg/kg至约32mg/kg的剂量将用于本文所公开的任何方法的抗半乳糖凝集素-9抗体(例如,如本文所公开的G9.2-17(IgG4))施用于受试者,例如剂量可选自0.2mg/kg、0.63mg/kg、2mg/kg、4mg/kg、6mg/kg、6.3mg/kg、8mg/kg、10mg/kg、12mg/kg和16mg/kg或更高剂量水平。在一些实施方案中,以约1mg/kg至约32mg/kg的剂量将抗半乳糖凝集素-9抗体施用于受试者,例如剂量可选自2mg/kg、4mg/kg、8mg/kg、12mg/kg和16mg/kg或更高剂量水平。在一些实施方案中,以约0.2mg/kg至约32mg/kg的剂量将抗半乳糖凝集素-9抗体施用于受试者,例如剂量可选自0.2mg/kg、0.63mg/kg、2mg/kg、4mg/kg、6mg/kg、6.3mg/kg、10mg/kg或16mg/kg或更高剂量水平。

[0175] 在一些实施方案中,用于本文所公开的任何方法的抗半乳糖凝集素-9抗体(例如,

如本文所公开的G9.2-17(IgG4))可以例如经由静脉内输注每周一次施用于需要治疗的患者。可替代地,抗半乳糖凝集素-9抗体可以每两周一次(例如通过静脉内输注)施用于患者。在一些实施方案中,抗半乳糖凝集素-9抗体每周一次施用持续一个周期,每周一次施用持续两个周期,每周一次施用持续3个周期,每周一次施用持续4个周期,或每周一次施用持续超过4个周期。在其他实施方案中,抗半乳糖凝集素-9抗体每2周一次施用持续一个周期,每2周一次施用持续两个周期,每2周一次施用持续3个周期,每2周一次施用持续4个周期,或每2周一次施用持续超过4个周期。

[0176] 在一些实施方案中,治疗的持续时间为12-24个月或更长。在一些实施方案中,周期延续3个月至6个月或6个月至12个月或12个月至24个月或更长的持续时间。在一些实施方案中,将周期长度例如暂时或永久调节为更长的持续时间,例如3周或4周。

[0177] 鉴于半乳糖凝集素-9的促肿瘤作用是通过与免疫细胞的相互作用(例如,经由TIM-3、CD44和41BB与淋巴样细胞的相互作用,以及经由dectin-1和CD206与巨噬细胞的相互作用)介导的,并且鉴于半乳糖凝集素-9在大量肿瘤中表达,靶向半乳糖凝集素-9(例如,使用半乳糖凝集素-9结合抗体来抑制与其受体的相互作用)提供了可以应用于多种不同肿瘤类型的治疗方法。

[0178] (ii)用抗PD-1抗体的组合治疗

[0179] 本文所公开的任何方法还可以包括将有效量的抗PD-1抗体(例如替雷利珠单抗)施用于患者。PD-1抑制剂的实例包括抗PD-1抗体,诸如派姆单抗、纳武单抗、替雷利珠单抗、多塔利单抗和西米普利单抗。此类检查点抑制剂可以与根据本公开的抗半乳糖凝集素9抗体同时或依序(以任何顺序)施用。在一些实施方案中,检查点分子是PD-L1。PD-L1抑制剂的实例包括抗PD-L1抗体,诸如德瓦鲁单抗、阿维鲁单抗和阿替雷利珠单抗。

[0180] 在一些实施方案中,结合PD-1的抗体是替雷利珠单抗。在一些实施方案中,本文所述的方法包括以约200mg的剂量每3周一次将替雷利珠单抗静脉内施用于受试者。在一些实施方案中,本文所述的方法包括以约400mg的剂量每6周一次将替雷利珠单抗静脉内施用于受试者。在一些实施方案中,本文所述的方法包括以约300mg的剂量每4周一次将替雷利珠单抗施用于受试者。在一些实施方案中,在28天周期中每4周以约300mg静脉内施用替雷利珠单抗。可替代地或此外,例如在约30分钟内,以静脉内输注施用替雷利珠单抗。在一些实施方案中,结合PD-1的抗体是多塔利单抗。在一些实施方案中,本文所述的方法包括以约500mg的剂量每三周静脉内或以约1000mg的剂量每六周静脉内将多塔利单抗施用于受试者。

[0181] 在一些情况下,检查点抑制剂(诸如本文所公开的任何抗PD-1抗体(例如,替雷利珠单抗)和本文所公开的任何抗半乳糖凝集素9抗体诸如(G9.2-17(IgG4)))可在同一天施用。在一些实例中,检查点抑制剂可以在施用抗半乳糖凝集素-9抗体之前施用于受试者。在其他情况下,连续两天进行检查点抑制剂(例如,抗PD-1抗体)的施用和抗半乳糖凝集素-9抗体的施用。检查点抑制剂(例如,抗PD-1抗体)可在给药的第一天施用于受试者,并且抗半乳糖凝集素-9抗体可以在第二天施用于受试者。

[0182] 在其他情况下,检查点抑制剂(诸如本文所公开的任何抗PD-1抗体)可以在施用本文所公开的抗半乳糖凝集素9抗体(诸如G9.2-17)之前约1-7天(例如,1天、2天、3天、4天、5天、6天或7天)施用。

[0183] 在一些实例中,抗半乳糖凝集素9抗体可以在施用检查点抑制剂(例如,抗PD-1抗体)之前施用给受试者。在其他情况下,连续两天进行抗半乳糖凝集素-9抗体的施用和检查点抑制剂(例如,抗PD-1抗体)的施用。抗半乳糖凝集素-9抗体可以在给药的第一天施用于受试者,并且检查点抑制剂(例如,抗PD-1抗体)可以在第二天施用于受试者。

[0184] 在其他情况下,本文所公开的抗半乳糖凝集素-9抗体(诸如G9.2-17)可以在施用检查点抑制剂(诸如本文所公开的任何抗PD-1抗体)之前约1-7天(例如,1天、2天、3天、4天、5天、6天或7天)施用。

[0185] 在本文所述的任何方法实施方案中,抗半乳糖凝集素-9抗体(单独或与抗PD-1抗体(诸如替雷利珠单抗)组合)可以每2周一次施用持续一个周期,每2周一次施用持续两个周期,每2周一次施用持续三个周期,每2周一次施用持续四个周期,或每2周一次施用持续超过四个周期。在一些实施方案中,治疗为1至3个月、3至6个月、6至12个月、12至24个月或更久。在一些实施方案中,治疗为每2周一次持续1至3个月、每2周一次持续3至6个月、每2周一次持续6至12个月或每2周一次持续12至24个月或更长时间。

[0186] 在一些实例中,本文提供的方法包括向需要治疗的受试者(例如,患有头颈癌、尿道上皮癌或如本文所公开的其他实体瘤的人类患者)每周一次施用2mg/kg至20mg/kg的剂量的抗半乳糖凝集素-9抗体(如G9.2-17(IgG4))和每4周一次施用例如300mg的剂量的抗PD-1抗体(如替雷利珠单抗)。在一个实例中,对患者每周一次给予4mg/kg的剂量的G9.2-17(IgG4)且每4周一次给予300mg剂量的替雷利珠单抗。在另一实例中,对患者每周一次给予6.3mg/kg的剂量的G9.2-17(IgG4)且每4周一次给予300mg剂量的替雷利珠单抗。在又一实例中,对患者每周一次给予10mg/kg的剂量的G9.2-17(IgG4)且每4周一次给予300mg剂量的替雷利珠单抗。可替代地,对患者每周一次给予16mg/kg的剂量的G9.2-17(IgG4)且每4周一次给予300mg剂量的替雷利珠单抗。

[0187] (iii)用于治疗的患者

[0188] 患有任何上述癌症的受试者可以通过常规医学检查来鉴定,例如实验室测试、器官功能测试、基因测试、介入操作(活检、手术)任何和所有相关的成像方式。在一些实施方案中,待通过本文所述的方法治疗的受试者是已经历或经受以概述的治疗方式的任何组合或顺序全身和/或局部递送的抗癌疗法方案(例如,化学疗法、放射疗法、肿瘤治疗电场(TTFields)、免疫疗法、生物疗法、小分子抑制剂、抗激素疗法、基于细胞的疗法和/或手术)的人类癌症患者。在一些实施方案中,受试者已接受先前免疫调节剂或上文列出的任何其他抗肿瘤剂或治疗方式。此类免疫调节剂的非限制性实例包括但不限于抗PD-1、抗PD-L1、抗CTLA-4、抗TIGIT、抗PVRIG、抗LAG-3、抗CD47、抗CD40、抗CSFR1、抗CD73、抗SIRP、抗A2AR、抗OX40、抗CD137等。在一些实施方案中,受试者通过治疗显示出疾病进展。在其他实施方案中,受试者对治疗有抗性(从头或获得性)。在一些实施方案中,这样的受试者被证明患有晚期恶性肿瘤(例如,无法手术或为转移性的)。可替代地或此外,在一些实施方案中,受试者不具有可用的标准治疗选项或不符合标准治疗选项,该标准治疗选项是指在临床环境中常用于治疗对应实体瘤的疗法。

[0189] 肿瘤治疗场(Tumor-treating field;TTField)是使用中频(~100-500kHz)和低强度(1-3V/cm)的交变电场来破坏细胞分裂的癌症治疗方式。在本文所述的实施方案中的任一个中,可在肿瘤治疗电场(TTFields)方案之前、与其同时或在其之后单独或与检查点

抑制剂诸如抗PD-1抗体组合施用抗半乳糖凝集素-9抗体。

[0190] 在一些情况下,受试者可以是患有难治性疾病(例如难治性头颈癌或难治性尿道上皮癌)的人类患者。如本文所用,“难治性”是指对治疗没有响应或变得有抗性的肿瘤。在一些情况下,受试者可以是患有复发性疾病(例如复发性头颈癌或复发性尿道上皮癌)的人类患者。如本文所用,“复发性”或“复发”是指在治疗改善(例如,部分或完全响应)一段时间后重新出现或进展的肿瘤。

[0191] 在一些实施方案中,待通过本文所公开的方法治疗的人类患者满足以下实施例3中公开的一个或多个纳入和排除标准。例如,人类患者可以是18岁或更大;患有组织学确认的不可切除的转移性或不可手术的癌症(例如,没有标准治疗选项),具有>3个月的预期寿命,具有可用于生物标志物分析的最近存档的肿瘤样品(例如,通过IHC评估的半乳糖凝集素-9肿瘤组织表达水平的存档物种);根据RECIST v1.1具有可测量的疾病,具有东部肿瘤协作组(ECOG)体力状态0-1或Karnofsky评分>70;没有可用的护理标准选项,具有高MSI-H(微卫星不稳定性高和MSS(微卫星稳定);在晚期/转移性环境中接受至少一种线的全身治疗;具有足够的血液学和终末器官功能(在以下实施例1中定义;例如,例如,嗜嗜中性粒细胞计数≥1x $10^9$/L,血小板计数≥100x $10^9$/L(对于第1部分中的HCC≥50x $10^9$/L);前一周未输注的情况下血红蛋白≥9.0g/dL,肌酐≤1.5x ULN,AST(SGOT)≤3x ULN(当存在HCC或肝转移时≤5x ULN),ALT(SGPT)≤3x ULN(当存在HCC或肝转移时≤5x ULN),胆红素≤1.5x ULN(患有已知吉尔伯特氏病(Gilbert's disease)的患者的胆红素可≤3.0x ULN),白蛋白≥3.0g/dL,INR和PTT≤1.5x ULN;和/或淀粉酶和脂肪酶≤1.5x ULN));已经完成脑转移的治疗(如果有的话)(参见以下实施例1);在过去一个月内没有活动性感染并且没有严重感染的证据;自在第一次抗Gal-9抗体施用之前的最后一次剂量的抗癌治疗起具有至少四(4)周或5个半衰期(以较短者为准)。

[0192] 可替代地或此外,适合于本文所公开的治疗的受试者可以不具有以下各者中的一者或多者:诊断患有未知原发性的转移性癌症;任何活动性不受控出血和任何有出血体质的患者(例如活动性消化性溃疡病);在抗半乳糖凝集素-9抗体施用的4周或5个半衰期内接受任何其他研究药剂;在第一剂抗半乳糖凝集素-9抗体的4周内接受放射疗法,除了有限范围的姑息性放射疗法,如用于治疗骨痛或局灶性疼痛肿瘤块;具有真菌性肿瘤块;具有活动性临床严重感染>2级NCI-CTCAE 5.0版;具有症状性或活动性脑转移;具有≥CTCAE 3级毒性(参见实施例1中的细节和例外);具有第二恶性肿瘤史(参见实施例1中的例外);具有严重或不受控制的全身性疾病、充血性心力衰竭的证据;具有严重的非愈合伤口、活动性溃疡或未治疗的骨折;具有需要反复引流程序的不受控的胸腔积液、心包积液或腹水;具有未明确接受手术和/或放射治疗的脊髓压迫。活动性或先前治疗的软脑膜疾病;具有显著的血管疾病;具有活动性自身免疫病症(参见实施例1中的例外);需要全身免疫抑制治疗;肿瘤相关疼痛(>3级)对广泛镇痛干预(口服和/或贴剂)无响应;尽管使用双膦酸盐,仍具有不受控制的高钙血症;具有归因于先前检查点抑制剂疗法(CIT)的免疫相关4级不良事件的任何历史;接受器官移植;和/或正在进行透析;和/或Child-Pugh评分≥7。在一些情况下,人类患者可能不患有在接受至少一个先前线数的系统疗法时进展的转移性肝细胞癌;已经拒绝或不耐受索拉非尼;或者已经将标准疗法视为无效、不可耐受或不适当,或者没有有效标准疗法可用。

[0193] 可替代地或此外,经受本文所公开的任何治疗的人类患者可以不具有:(i)未知原发性的转移性癌症;(ii)临床上显著的、活动性不受控制的出血,任何出血体质(例如活动性消化性溃疡病);(iii)在治疗的第一剂量的4周内的放射疗法;(iv)具有真菌性肿瘤块;(v)由于先前的癌症治疗所致的≥CTCAE 3级毒性(脱发和白癜风除外);(v)第二恶性肿瘤史;(vi)严重或不受控制的全身性疾病,充血性心力衰竭>纽约心脏协会(NYHA)2级或6个月内心肌梗塞(MI)的证据;(vii)严重未愈合伤口,活动性溃疡或未经治疗的骨折;(viii)需要反复引流程序的不受控制的胸腔积液、心包积液或腹水;(ix)对嵌合或人源化抗体或融合蛋白的严重过敏、过敏性或其他超敏反应的病史;(x)治疗后6个月内的显著血管疾病(例如,需要手术修复的主动脉瘤或近期动脉血栓形成),治疗前3个月内的肺栓塞、中风或短暂性脑缺血发作史,和/或治疗前6个月内的腹部瘘或胃肠穿孔史;(xi)活动性自身免疫病症(I型糖尿病、仅需要激素替代的甲状腺功能减退、白癜风、银屑病或脱发除外);(xii)需要全身性免疫抑制治疗;(xii)肿瘤相关疼痛(>3级)对广泛的镇痛干预(口服和/或贴剂)无响应;(xiii)尽管使用双膦酸盐,仍具有不受控制的高钙血症;(xiv)接受器官移植。

[0194] 在一些情况下,受试者是具有如相对于对照水平升高的半乳糖凝集素-9水平的人类患者。半乳糖凝集素-9的水平可以是人类患者中半乳糖凝集素-9的血浆或血清水平。在其他实例中,半乳糖凝集素-9的水平是肿瘤内癌细胞的半乳糖凝集素-9的水平。在其他实例中,半乳糖凝集素-9的水平是肿瘤内免疫细胞的半乳糖凝集素-9的水平。在其他实例中,半乳糖凝集素-9的水平可以是细胞表面半乳糖凝集素-9的水平,例如癌细胞上半乳糖凝集素-9的水平。在一个实例中,半乳糖凝集素-9的水平可以是例如在癌细胞表面上的表达半乳糖凝集素-9的癌细胞的水平、或在免疫细胞中表达的半乳糖凝集素-9的水平,此水平是在患者来源的器官型肿瘤球状体(PDOT)中所测量,其可以通过例如以下实施例中所公开的方法制备。对照水平可指没有实体瘤的相同物种(例如,人)的受试者的匹配样品中半乳糖凝集素-9的水平。在一些实例中,对照水平代表健康受试者中半乳糖凝集素-9的水平。在一些实施方案中,对照水平可以是治疗前的基线水平。

[0195] 为了鉴定这样的受试者,可以从疑似患有实体瘤的受试者获得合适的生物样品,并且可以使用常规方法(例如,ELISA或FACS)来分析生物样品以确定其中所含的半乳糖凝集素-9(例如,游离、细胞表面表达或总体)的水平。在一些实施方案中,例如,如本文所述制备类器官培养物,并用于评估受试者中的半乳糖凝集素-9水平。从作为类器官制备过程的一部分获得的某些级分中得到的单细胞也适用于评估受试者中的半乳糖凝集素-9水平。在一些情况下,用于测量游离形式或在细胞表面表达的半乳糖凝集素-9水平的测定涉及使用特异性结合半乳糖凝集素-9(例如,特异性结合人半乳糖凝集素-9)的抗体。本领域已知的任何抗半乳糖凝集素-9抗体可以在上述任何测定中试验适用性,然后以常规方式用于此类测定中。在一些实施方案中,本文所述的抗体(例如,G9.2-17抗体)可用于如测定中。在一些实施方案中,抗体描述于美国专利号10,344,091和WO2019/084553中,所述专利各自的相关公开内容为了本文引用的目的和主题以引用的方式并入。在一些实例中,抗半乳糖凝集素-9抗体是Fab分子。如本文所公开的用于确定半乳糖凝集素-9水平的测定方法也在本公开的范围内。

[0196] (iv)对治疗的响应

[0197] 本文所公开的治疗的功效可通过常规实践评估。在一些实施方案中,与治疗前或

对照受试者中的水平相比,本文所公开的任何方法可将抗肿瘤活性(例如,随时间推移减少细胞增殖、肿瘤生长、肿瘤体积和/或肿瘤负担或负荷或减少转移性病灶的数目)增加至少约10%、20%、25%、30%、40%、50%、60%、70%、75%、80%、85%、90%、95%或更多。在一些实施方案中,通过比较受试者在施用药物组合物之前和之后的细胞增殖、肿瘤生长和/或肿瘤体积来测量减少。在一些实施方案中,本文所公开的方法可以将癌症的一种或多种症状改善至少约10%、20%、30%、40%、50%、60%、70%、80%、90%、95%或更多。在一些实施方案中,在药物组合物施用之前、期间和之后,在生物样品(如血液、血清、血浆、尿液、腹膜液和/或来自组织或器官的活检)中测量受试者中的癌细胞和/或生物标志物。在一些实施方案中,所述方法包括施用本发明的组合物以将受试者的肿瘤体积、大小、负荷或负担减小至不可检测的大小或者减小至小于治疗前受试者的肿瘤体积、大小、负荷或负担的约1%、2%、5%、10%、20%、25%、30%、40%、50%、60%、70%、75%、80%或90%。在其他实施方案中,提供了用于将受试者中的细胞增殖速率或肿瘤生长速率降低至不可检测的速率或者降低至小于治疗前的速率的约1%、2%、5%、10%、20%、25%、30%、40%、50%、60%、70%、75%、80%或90%的方法。在其他实施方案中,方法包括施用本发明的组合物以将受试者中转移性病灶的发展或数量或大小降低至不可检测的比率或者降低至小于治疗前的比率的约1%、2%、5%、10%、20%、25%、30%、40%、50%、60%、70%、75%、80%或90%。

[0198] 可根据RECIST或RECIST 1.1标准和/或irRC、irRECIST、iRECIST、imRECISTPDAC来评估对治疗(例如,如本文所述的实体瘤的治疗)的响应,如以下实施例1中和Eisenhower等人,New response evaluation criteria in solid tumours:Revised RECIST guideline (version 1.1);European Journal Of Cancer 45(2009)228-247;或Borcoman等人,Annals of Oncology 30:385-396,2019;Nishino等人,Clin Cancer Res 2013;19(14):3936-3943中所述,其各自的内容以全文引用的方式并入本文中。

[0199] 在一些实施方案中,提供了用于例如与在G9.2-17 IgG4治疗方案开始之前获得的基线水平相比改善和或控制总体响应/肿瘤负担/肿瘤大小(例如,在大约2、3、6或12个月时或者在更晚的时间)的方法,所述方法包括施用本文所述的抗半乳糖凝集素-9抗体。在一些实施方案中,所述方法用于在大约2个月时改善和或控制总体响应/肿瘤负担/肿瘤大小。在一些实施方案中,当抗-半乳糖凝集素-9抗体(例如,G9.2-17(IgG4))在与检查点抑制剂(例如,抗-PD-1抗体,如替雷利珠单抗)的组合方案施用时,可以改善或控制总体响应/肿瘤负担/肿瘤大小(例如,在大约2、3、6或12个月或更晚的时间),例如,与治疗开始之前获得的基线水平相比。在一些实施方案中,提供了产生完全响应、部分响应或稳定疾病(例如,如在大约2个月、3个月、6个月或12个月时或在更晚的时间或在任何其他临床指示的时间点所测量)的方法,所述方法包括施用本文所述的抗半乳糖凝集素-9抗体。这种响应可以在一定时间段内是暂时的,或是永久的。

[0200] 在一些实施方案中,本文所公开的方法可改善完全响应,部分响应或稳定疾病的可能性(例如,在约2个月、3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点测量),例如与在开始G9.2-17 IgG4治疗方案前获得的基线水平相比。这种响应可以在一定时间段内是暂时的,或是永久的。在一些实施方案中,治疗可以产生例如与在G9.2-17 IgG4治疗方案开始之前获得的基线水平相比减轻或减弱的进展疾病(例如,如在大约2个月、3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点

所测量)。这种减弱可以是暂时的或永久的。在这些实施方案的任一个中,抗半乳糖凝集素-9抗体可以与检查点抑制剂(例如,抗PD-1抗体)组合施用。

[0201] 在一些实施方案中,本文所公开的方法可减弱疾病进展或减少进展性疾病(例如,如在约3个月、6个月或12个月,或在稍后时间或在任何其他临床指示的时间点测量)。方法包括将治疗有效量的如本文所公开的抗半乳糖凝集素-9抗体施用于受试者。在这些实施方案中的任一个中,可与检查点抑制剂(例如,抗PD-1抗体)组合施用抗半乳糖凝集素-9抗体。

[0202] 在本文所述的任何方法中,可以根据irC标准、RECIST标准、RECIST1.1.、irRECIST或iRECIST、或imRECIST标准、或本领域已知的其他标准来评估部分响应、稳定疾病、完全响应、部分响应、稳定疾病、进展性疾病、疾病进展(例如,在约2个月、3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点测量)(参见例如Borcoman等人,Annals of Oncology 30:385-396,2019’iRC:Hoos等人,J.Immunother.30(1):1-15)。

[0203] 与治疗开始前的基线水平相比,部分响应是响应于治疗的肿瘤大小或体内癌症程度(即肿瘤负担)的减小。例如,根据RECIST响应标准,部分响应被定义为,以基线总直径为参考,靶病灶的直径总和减少至少30%。进展性疾病是一种正在生长、扩散或恶化的疾病。例如,根据RECIST响应标准,进展性疾病包括其中观察到靶病灶直径总和至少增加20%并且总和还必须显示至少5mm的绝对增加的疾病。此外,一个或多个新病灶的出现也被认为是进展。与治疗开始前的基线水平相比,在程度或严重度上既没有减少也没有增加的肿瘤被认为是稳定的疾病。例如,根据RECIST响应标准,以研究时的最小总直径作为参考,当既没有足够的收缩满足部分响应的条件,也没有足够的增加满足进展性疾病的条件时,出现稳定的疾病。

[0204] 在一些实施方案中,本公开提供用于相对于受试者中治疗开始前的基线肿瘤大小,永久地或在最小时间段内减小或维持受试者(包括人类受试者)中的肿瘤大小(例如,在大约2个月、3个月、6个月或12个月,或在稍后时间或在任何其他临床指示的时间点测量)的方法,所述方法包括将治疗有效量的单独抗半乳糖凝集素-9抗体或与检查点抑制剂(例如抗PD-1抗体)组合施用于受试者。可以根据本领域已知的方法测量肿瘤大小,例如肿瘤直径,所述方法包括根据特定的测量方案,例如如上文引用的Eisenhower等人中所述,结合各种软件工具从CT和MRI图像进行测量。因此,在一些实施方案中,在定期安排的再分期扫描(例如,有/没有造影剂的CT、有/没有造影剂的MRI、PET-CT(诊断CT)和/或X射线、超声和/或其他相关成像方式)中测量肿瘤大小。在一些实施方案中,肿瘤大小减小、维持肿瘤大小是指靶病灶的大小。在一些实施方案中,肿瘤大小减小、肿瘤大小维持是指非靶病灶的大小。根据RECIST 1.1标准,当在基线存在超过一个的可测量病灶时,代表所有相关器官的最多总共五个病灶(且每个器官最多两个病灶)的所有病灶应鉴定为靶病灶。包括病理性淋巴结的所有其他病灶(或疾病部位)均应鉴定为非靶病灶。

[0205] 在一些实施方案中,本公开提供了用于增加减少或维持肿瘤负担的可能性(例如,如在大约2个月、3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点测量)的方法,所述方法包括将治疗有效量的单独的如本文所公开的抗半乳糖凝集素-9抗体或与检查点抑制剂(例如,抗PD-1抗体,如替雷利珠单抗)组合施用于受试者。在一些实施方案中,治疗可以导致减小肿瘤负担或维持肿瘤负担的更大可能性(例如,如在大约2个月、3个月、6个月或12个月时,或在稍后的时间或在任何其他临床指示的时间点所测量)。如本

文所用,肿瘤负担是指受试者体内癌症的数量、肿瘤的大小或体积,考虑所有疾病部位。可以使用本领域已知的方法来测量肿瘤负担,包括但不限于FDG正电子发射断层摄影术(FDG-PET)、磁共振成像(MRI)和光学成像,包括生物发光成像(BLI)和荧光成像(FLI)。

[0206] 在一些实施方案中,本文所述的方法增加疾病进展或无进展存活的时间(例如,如大约2个月、3个月、6个月或12个月,或在稍后时间或在在治疗开始后的任何其他临床指示的时间点所测量)。无进展存活期可以是永久的或是一定时间段内的无进展存活期。在一些实施方案中,所述方法提供无进展存活的更大可能性(永久无进展存活或在某一量的时间内的无进展存活,例如3、6或12个月或例如在大约2个月、3个月、6个月或12个月,或在稍后的时间或在治疗开始后的任何其他临床指示的时间点所测量)。无进展存活(PFS)定义为在临床试验中从随机分配(例如,从治疗开始)到疾病进展或任何原因导致的死亡的时间。在一些实施方案中,所述方法实现更长的存活期或更大的存活可能性,例如在一定时间,例如在6或12个月时。

[0207] 对治疗(例如,如本文所述的实体瘤的治疗)的响应可以根据iRECIST标准进行评估,如Seymour等人,iRECIST:guidelines for response criteria for use in trials; The Lancet,第18卷,2017年3月(其内容以全文引用的方式并入本文中)中所述。iRECIST被开发用于特别地在癌症免疫治疗试验中使用修改的RECIST1.1标准,以确保一致的设计和数据收集,并可用作实体瘤测量的标准方法的指南,以及用于在使用免疫疗法的试验中使用的肿瘤大小客观变化的定义。iRECIST基于RECIST 1.1。使用iRECIST分配的响应具有前缀“i”(即,免疫)—例如,“免疫”完全响应(iCR)或部分响应(iPR)、以及未确认的进展性疾病(iUPD)或确认的进展性疾病(iCPD)或稳定疾病(iSD),以将它们与使用RECIST 1.1分配的响应区分开,并且所有这些均在Seymour等人RECIST 1.1中所定义。在一些实施方案中,可以将标准与治疗开始前的基线水平进行比较。在这些实施方案中的任一个中,可单独或与检查点抑制剂(例如抗PD-1抗体,诸如本所公开的那些)组合施用抗半乳糖凝集素-9抗体。

[0208] 因此,在一些实施方案中,本公开提供了用于与治疗开始之前疾病的基线水平相比改善总体响应(iOR)或实现“免疫”完全响应(iCR)、部分响应(iPR)或稳定疾病(iSD)(例如,如在大约2个月、3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)的方法。“免疫”响应(例如,iCR、iPR或iSD)的减少可以在一定时间段内是暂时的,或是永久的。在一些实施方案中,治疗可以提高完全响应(iCR)、部分响应(iPR)或稳定疾病(iSD)(例如,如在大约2个月、3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)的可能性。例如,在一些实施方案中,本公开提供了用于减弱疾病进展或减轻进展性疾病(例如,减轻未证实的进展性疾病(iUPD)或减轻经证实的进展性疾病(iCPD))(例如,如在大约2个月、3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)的方法,所述方法包括向受试者施用治疗有效量的如本文公开的抗半乳糖凝集素-9抗体。可以将这些上面提及的iRECIST标准中的任一种与治疗开始前的基线水平进行比较。在这些方法中的任一种中,抗半乳糖凝集素-9抗体可以单独或与检查点抑制剂(例如,抗PD-1抗体)组合施用。

[0209] iUPD或iCPD的减少可以在一定时间段内是暂时的,或是永久的。在一些实施方案中,治疗可导致未确认的进展性疾病(iUPD)或确认的进展性疾病(iCPD)的总体减少的更大

可能性(例如,如在大约2个月、3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点所测量)。在一些实施方案中,本公开提供用于根据iRECIST标准减少受试者(包括人类受试者)中的新病灶的数量的方法(例如,如在大约2个月、3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点所测量),所述方法包括将治疗有效量的如本文所公开的抗半乳糖凝集素-9抗体施用于受试者。病灶的数量减少可以相对于治疗开始之前的基线水平,并且减少可以在一定时间段内是暂时的,或是永久的。在这些实施方案中的任一个中,可与检查点抑制剂(例如,抗PD-1抗体)组合施用抗半乳糖凝集素-9抗体。

[0210] 另外的标准可用于测量治疗响应。例如,可以根据irRC标准测量肿瘤负担(Hoos等人,2007)。在irRC中,通过将"指标"病灶与新病灶组合来测量肿瘤负担,即新病灶被认为是肿瘤负担变化。在irRC中,免疫相关的完全响应(irCR)是测量或未测量的所有病灶消失并且没有新病灶;免疫相关的部分响应(irPR)是如由irRC所定义的肿瘤负担相对于基线下降50%;并且免疫相关的进展性疾病(irPD)是肿瘤负担相对于记录的最低水平增加25%。其他一切都被认为是免疫相关的稳定疾病(irSD)。

[0211] 免疫相关的RECIST(irRECIST)基于RECIST的一维测量,并且在irRECIST中进一步重新定义特定的免疫相关标准。最近,基于NSCLC(免疫修饰的RECIST(imRECIST))中的阿特珠单抗数据评价了新标准,其需要在初始评估后至少4周确认疾病进展(Hodi等人,JCO 2018;36(9):850-858)。对于RECIST 1.1.、irRC、irRECIST、iRECIST和imRECIST的比较,参见例如Borcoman等人,Annals of Oncology 30:385-396,2019中的图4;Nishino等人,Clin Cancer Res2013;19(14):3936-3943,这些文献的内容通过引用整体并入本文。这些标准中的任一种都适合确定本文所述的方法中的任一种的响应率。

[0212] (v)监测不良事件和调节治疗条件

[0213] 此外,可以监测用单独的本文所公开的任何抗-半乳糖凝集素-9抗体(例如,G9.2-17)或与本文所公开的检查点抑制剂(例如,抗-PD-1,如替雷利珠单抗)组合治疗的受试者的副作用(例如,严重的副作用)的出现。在以下实施例3中提供了监测的示例性副作用。如果观察到出现副作用,则可以改变那名受试者的治疗条件。例如,可减少抗半乳糖凝集素-9抗体的剂量和/或可延长给药间隔。可由合格的临床医生评估减少的合适性和程度。在一个实施方案中,实施前一剂量水平的30%或50%的减少水平。在一个具体实例中,实施按照临床医生的评估或至少30%的减少水平(减少至剂量水平1,第一次剂量减少时的水平)。如果需要,实施再一次剂量减少剂量水平-1的30%(剂量水平-2,第二次剂量减少时的水平)。在另一个实例中,实施再一次剂量减少剂量水平-1的50%(剂量水平-2)。在一些实施方案中,实施一次或多次剂量减少前一剂量水平的约10%至约80%。在一些实施方案中,实施一次或多次剂量减少前一剂量水平的约10%至约20%、约20%至约30%、约30%至约40%、约40%至约50%、约50%至约60%或约70%至约80%。在一些实施方案中,实施一次或多次剂量减少前一剂量水平的10%至20%、20%至30%、30%至40%、40%至50%、50%至60%或70%至80%。在一些实施方案中,实施一次或多次剂量减少前一剂量水平的约10%、约20%、约30%、约40%、约50%、约60%、约70%或约80%。在一些实施方案中,实施一次或多次剂量减少前一剂量水平的10%、20%、30%、40%、50%、60%、70%或80%。可替代地或此外,可以减少检查点抑制剂的剂量和/或可以延长检查点抑制剂的给药间隔。在一些情况下(例如,出现危及生命的副作用),可以终止治疗。

[0214] 在一些情况下,如果在患者中观察到副作用,则可以降低抗半乳糖凝集素-9抗体(诸如G9.2-17(IgG4))的剂量和/或抗PD-1抗体(如替雷利珠单抗)的剂量。在一些情况下,剂量可降低50%。当需要时,剂量可进一步降低50%。参见例如以下实施例3。

[0215] (vi)用于评估对治疗的响应的生物标志物

[0216] 对治疗的响应还可以通过以下中的一种或多种来表征:血液和肿瘤免疫表型、细胞因子谱(血清)、血液(血清或血浆)中的可溶性半乳糖凝集素-9水平、半乳糖凝集素-9肿瘤组织表达水平和表达模式(通过免疫组织化学)(肿瘤、基质、免疫细胞)、肿瘤突变负担(TMB)、PD-L1表达(例如,通过免疫组织化学)、错配修复状态或与疾病相关的肿瘤标志物(例如,如在大约3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点所测量)。此类肿瘤标志物的实例包括但不限于CA15-3、CA-125、CEA、CA19-9、α-胎儿蛋白。可以将这些参数与治疗开始前的基线水平进行比较。在这些实施方案中的任一个中,可单独或与检查点抑制剂(例如,抗PD-1抗体)组合施用抗半乳糖凝集素-9抗体。

[0217] 在本文所公开的任何方法中,受试者可以在治疗之前、期间和/或之后检查以下特征中的一者或多者:(a)来自受试者的血液样品中的一种或多种肿瘤标志物,任选地其中一种或多种肿瘤标志物包括CA15-3、CA-125、CEA、CA19-9和/或α-胎儿蛋白,以及任何其他肿瘤类型特异性肿瘤标志物;(b)细胞因子概况;和(c)半乳糖凝集素9血清/血浆水平;d)外周血单核细胞免疫分型;e)肿瘤组织活检/切除标本多重免疫分型;f)肿瘤组织活检/切除标本半乳糖凝集素9表达水平和模式;g)任何其他免疫评分测试,如:PD-L1免疫组织化学、肿瘤突变负担(TMB)、肿瘤微卫星不稳定性状态、以及如以下的组:Immunoscore®-HalioDx、ImmunoSeq-Adaptive Biotechnologies、在NanoString nCounter®基因表达系统,18-基因特征,PanCancer IO 360™测定(NanoString Technologies)上开发的TIS等。也可以使用对靶标肿瘤(如PDAC)具有特异性的其他合适的生物标志物。在一个非限制性实例中,PD-L1(SP263)(Roche,Ventana)可用于使用免疫组织化学来检测癌症组织中的PD-L1。

[0218] 在一些实施方案中,本文描述了用于改变血液中或肿瘤中的免疫细胞和免疫细胞标志物的水平(例如,免疫活化)的方法,其包括施用单独的抗Gal-9抗体或与检查点抑制剂(例如,抗PD-1抗体)的组合。可以使用本领域已知的方法,诸如多重流式细胞术和多重免疫组织化学,在患者血液和组织样品中测量这种变化。例如,可以在治疗开始之前的基线处和在治疗期间的不同时间点评估一组表型和功能性PBMC免疫标志物。表2列出了可用于这些评估方法的标志物的非限制性实例。流式细胞术(FC)是分析细胞表型和功能的一种快速且信息量大的首选技术方法,在免疫表型监测方面已占据突出地位。它允许表征复杂混合物(诸如血液)中的许多细胞亚群,包括稀有亚群,并代表了一种获取大量数据的快速方法。FC的优点是速度快、灵敏度高且特异性强。标准化抗体组和程序可以用于分析和分类免疫细胞亚型。多重IHC是一种强大的研究工具,它提供在免疫子集数量和位置方面描述肿瘤免疫背景的客观定量数据,并允许在单个组织切片上评估多个标志物。计算机算法可以用于量化患者活检的整个切片图像中基于IHC的生物标志物含量,从而将显色IHC方法和染色与数字病理学方法相结合。

[0219] 因此,在一些实施方案中,本文描述了用于调节免疫响应,例如调节如表2中的那些免疫活化标志物的方法,所述方法包括施用单独的抗-gal9抗体或与检查点抑制剂疗法的组合。在一些实施方案中,调节包括以下中的一者或多者:(1)血浆或肿瘤组织中更多CD8

细胞的增加,(2)血浆或肿瘤组织中T调节细胞(Treg)的减少,(3)血浆或肿瘤组织中M1巨噬细胞的增加,和(4)血浆或肿瘤组织中MDSC的减少,和(5)血浆或肿瘤组织中M2巨噬细胞的减少(例如,如在大约2个月、3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点所测量)。在一些实施方案中,使用上述技术评估的或本领域已知的标志物选自CD4、CD8、CD14、CD11b/c和CD25。可以将这些参数与治疗开始前的基线水平进行比较。

[0220] 表2.PBMC分型标志物

[0221]

| PBMC分型标志物 | | PBMC分型标志物 | |
|---|---|---|---|
| CD3 | 总T细胞 | CD16 | NK细胞 |
| CD4 | CD4+T细胞 | CD11b | 单核细胞/巨噬细胞 |
| CD8 | CD8+T细胞 | CD11c | 单核细胞/巨噬细胞,DCs |
| CD25 | Treg激活 | CD14 | 单核细胞亚群,巨噬细胞 |
| CD27 | T细胞成熟;初始/记忆B细胞 | CD33 | 总骨髓细胞 |
| CD38 | T细胞成熟;初始/记忆B细胞 | FceR1a | 抗原呈递DC细胞 |
| CD45RA | 初始/记忆细胞 | CD19 | 总B细胞 |
| CD45RO | 初始/记忆细胞 | T-bet | T细胞亚群 |
| CD56 | NKT/NK细胞{T细胞亚群} | gdTCR | γδT细胞 |
| CD127 | T细胞亚群 | CD274(PDL-1) | 检查点 |
| CD152(CLTA-4) | 检查点 | Tim-3 | 检查点 |
| CD279(PD-1) | 检查点 | TCRVa24-Ja18 | iNKT细胞 |
| FoxP3 | Treg细胞 | 活/死 | 通用 |
| HLA-DR | 活化/抗原呈递 | CD45 | 通用 |

[0222] (vii)调节免疫响应

[0223] 在一些实施方案中,本文描述了用于调节促炎性和抗炎性细胞因子的方法,所述方法包括单独或与检查点抑制剂疗法组合施用抗ga19。在一些实施方案中,提供了用于以下中的一项或多项的方法:(1)增加血浆或肿瘤组织中IFNγ的水平;(2)增加血浆或肿瘤组织中TNFα的水平;(3)降低血浆或肿瘤组织中IL-10的水平(例如,如在大约3个月、6个月或12个月或者在稍后时间或在任何其他临床指示的时间点所测量)。可以将这些参数与治疗开始前的基线水平进行比较。

[0224] 在一些实施方案中,可在给药前1次肿瘤活检与在可行时间进行的重复活检之间评估细胞因子水平或免疫细胞水平。在一些实施方案中,可在2次重复活检之间评估细胞因子水平或免疫细胞水平。在一些实施方案中,提供了用于调节血液(血清或血浆)中的可溶性半乳糖凝集素-9水平或半乳糖凝集素-9肿瘤组织表达水平和通过免疫组织化学得到的表达模式(肿瘤、基质、免疫细胞)中的一种或多种(例如,如在大约3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)的方法。在一些实施方案中,所述方法降低血液(血清或血浆)中的可溶性半乳糖凝集素-9水平或半乳糖凝集素-9肿瘤组织表达水平或通过免疫组织化学得到的表达模式(肿瘤、基质、免疫细胞)(例如,如在大约3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)。可以将半乳糖凝集素-9水平与治疗开始前的基线水平进行比较。在一些实施方案中,可将半乳糖凝集素-9水平与未接受治疗的对照组或健康受试者进行比较。在这些实施方案中的任一个中,可单独或与检查点抑制剂(例如,抗PD-1抗体)组合施用抗半乳糖凝集素-9抗体。在一些实施方案中,提供了用于调节PD-L1表达(例如,如通过免疫组织化学所评估)的方

法,所述方法包括单独或与检查点抑制剂(例如,抗半乳糖凝集素-9抗体)组合施用抗半乳糖凝集素-9抗体。在一些实施方案中,所述方法调节与疾病相关的一种或多种肿瘤标志物(增加或减少)(例如,如在大约2个月、3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)。此类肿瘤标志物的实例包括但不限于CA15-3、CA-125、CEA、CA19-9、α-胎儿蛋白。可以将这些参数与治疗开始前的基线水平进行比较。在这些实施方案中的任一个中,可单独或与检查点抑制剂(例如,抗PD-1抗体)组合施用抗半乳糖凝集素-9抗体。

[0225] 在一些实施方案中,本公开提供了调节受试者中的免疫响应的方法。如本文所用,术语“免疫响应”包括受免疫细胞活性调节例如T细胞激活影响的T细胞介导的和/或B细胞介导的免疫响应。在本公开的一个实施方案中,免疫响应是T细胞介导的。如本文所用,术语“调节”意指改变或更改,并且包括上调和下调。例如,“调节免疫响应”意指改变或更改一个或多个免疫响应参数的状态。T细胞介导的免疫响应的示例性参数包括T细胞的水平(例如,效应T细胞的增加或减少)和T细胞激活的水平(例如,某些细胞因子产生的增加或减少)。B细胞介导的免疫响应的示例性参数包括B细胞水平的增加、B细胞激活和B细胞介导的抗体产生。

[0226] 当免疫响应受到调节时,一些免疫响应参数可能会降低,而另一些可能会增加。例如,在一些情况下,调节免疫响应引起一个或多个免疫响应参数的增加(或上调)和一个或多个其他免疫响应参数的降低(或下调),并且结果是免疫响应的总体增加,例如炎性免疫响应的总体增加。在另一个实例中,调节免疫响应引起一个或多个免疫响应参数的增加(或上调)和一个或多个其他免疫响应参数的降低(或下调),并且结果是免疫响应的总体降低,例如炎性响应的总体降低。在一些实施方案中,总体免疫响应的增加即总体炎性免疫响应的增加由肿瘤重量、肿瘤大小或肿瘤负担的减少或本文所述的任何RECIST或iRECIST标准确定。在一些实施方案中,总体免疫响应的增加由一种或多种促炎细胞因子(例如,包括两种或更多种、三种或更多种等)或大部分促炎细胞因子的水平增加来确定(一种或多种、两种或更多种等或大部分抗炎和/或免疫抑制性细胞因子和/或最强效的抗炎或免疫抑制性细胞因子中的一种或多种降低或保持恒定)。在一些实施方案中,总体免疫响应的增加由一种或多种最强效的促炎细胞因子的增加的水平(一种或多种抗炎和/或免疫抑制细胞因子包括一种或多种最强效的细胞因子减少或保持不变)确定。在一些实施方案中,总体免疫响应的增加由一种或多种包括大多数免疫抑制和/或抗炎细胞因子的降低的水平(一种或多种或大多数促炎细胞因子包括例如最强效的促炎细胞因子的水平增加或保持不变)确定。在一些实施方案中,总体免疫响应的增加由一种或多种最强效的抗炎和/或免疫抑制细胞因子的增加的水平(一种或多种或大多数促炎细胞因子包括例如最强效的促炎细胞因子增加或保持不变)确定。在一些实施方案中,总体免疫响应的增加由上述任一项的组合确定。此外,一种类型的免疫响应参数的增加(或上调)可以导致另一种类型的免疫响应参数的相应减少(或下调)。例如,某些促炎细胞因子的产生的增加可以导致某些抗炎和/或免疫抑制细胞因子的下调,反之亦然。

[0227] 在一些实施方案中,本公开提供了用于调节受试者(包括人类受试者)的免疫响应(例如,如在大约2个月、3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)的方法,所述方法包括向受试者施用治疗有效量的如本文公开的抗半

乳糖凝集素-9抗体。在一些实施方案中,本公开提供了用于例如与治疗开始之前的基线水平相比(例如,与在抗Gal9抗体治疗方案开始之前获得的基线水平相比)调节受试者(包括人类受试者)的血液中或肿瘤中的免疫细胞和免疫细胞标志物(包括但不限于本文在表2中所述的那些)的水平的方法,所述包括向受试者施用治疗有效量的如本文公开的抗半乳糖凝集素-9抗体。在一些实施方案中,调节的总体结果是促炎免疫细胞的上调和/或免疫抑制性免疫细胞的下调。在一些实施方案中,本公开提供了用于调节免疫细胞的水平的方法,其中所述调节包括以下一项或多项:(1)增加血浆或肿瘤组织中的CD8细胞,(2)减少血浆或肿瘤组织中的Treg,(3)增加血浆或肿瘤组织中的M1巨噬细胞和(4)减少血浆或肿瘤组织中的MDSC,以及(5)减少血浆或肿瘤组织中的M2巨噬细胞,并且其中所述方法包括向受试者施用治疗有效量的如本文所公开的抗半乳糖凝集素-9抗体。在一些实施方案中,评估此类免疫细胞的水平的标志物包括但不限于CD4、CD8、CD14、CD11b/c和CD25。在一些实施方案中,本公开提供了用于调节受试者(包括人类受试者)的血液或肿瘤中的促炎性和免疫抑制性细胞因子的水平(例如,如在大约2个月、3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点所测量)(例如,与治疗开始前的基线水平相比)的方法,所述方法包括将治疗有效量的如本文所公开的抗半乳糖凝集素-9抗体施用于受试者。在一些实施方案中,调节的总体结果是促炎细胞因子的上调和/或免疫抑制细胞因子的下调。在一些实施方案中,本公开提供了用于调节细胞因子细胞的水平的方法,其中调节涵盖以下项中的一种或多种:(1)增加血浆或肿瘤组织中IFNγ的水平;(2)增加血浆或肿瘤组织中TNFα的水平;(3)降低血浆或肿瘤组织中IL-10的水平。

[0228] 在一些实施方案中,本公开提供了用于改变血液(血清或血浆)中的可溶性半乳糖凝集素-9水平或半乳糖凝集素-9肿瘤组织表达水平和通过免疫组织化学得到的表达模式(肿瘤、基质、免疫细胞)中的一种或多种(例如,如在2周、4周、1个月、2个月、3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)的方法,所述方法包括向受试者施用治疗有效量的如本文公开的抗半乳糖凝集素-9抗体。在所述方法的一些实施方案中,血液(血清或血浆)中的可溶性半乳糖凝集素-9水平或半乳糖凝集素-9肿瘤组织表达水平和通过免疫组织化学得到的表达模式(肿瘤、基质、免疫细胞)中的一种或多种保持不变。在一些实施方案中,本文提供的方法降低以下中的一者或多者:血液(血清或血浆)中的可溶性半乳糖凝集素-9水平、或半乳糖凝集素-9肿瘤组织表达水平和表达模式(通过免疫组织化学)(肿瘤、基质、免疫细胞)(例如,如在2周、4周、1个月、2个月、3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点所测量)。可以将半乳糖凝集素-9水平与治疗开始前的基线水平进行比较。在一些实施方案中,可以将半乳糖凝集素-9水平与健康受试者进行比较。在一些实施方案中,治疗产生PD-L1表达(例如,通过免疫组织化学)的改变。16mg/kg或更高剂量水平、16mg/kg或更高剂量水平、16mg/kg或更高剂量水平。

[0229] 在一些实施方案中,本公开提供了用于改变PD-L1表达(例如,如通过免疫组织化学所评估)(例如,如在2周、4周、1个月、2个月、3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)的方法,所述方法包括向受试者施用治疗有效量的如本文公开的抗半乳糖凝集素-9抗体。在方法的一些实施方案中,PD-L1表达(例如通过免疫组织化学所评估)保持不变。可以将PD-L1水平与治疗开始前的基线水平进行比较。

在一些实施方案中,本文提供的方法降低PD-L1表达,例如如通过免疫组织化学所评估。可以使用本领域已知的常规方法来测量PD-L1水平。在一个非限制性实例中,PD-L1(SP263)(Roche,Ventana)可用于使用免疫组织化学来检测癌症组织中的PD-L1。16mg/kg或更高剂量水平16mg/kg或更高剂量水平16mg/kg或更高剂量水平。

[0230] 在一些实施方案中,本公开提供了用于改变(增加或减少)与疾病相关的一种或多种肿瘤标志物的方法(例如,如在2周、4周、1个月、3个月、6个月或12个月,或在稍后的时间或在任何其他临床指示的时间点所测量),所述方法包括将治疗有效量的如本文所公开的抗半乳糖凝集素-9抗体施用于受试者。在所述方法的一些实施方案中,与疾病相关的一种或多种肿瘤标志物(增加或减少)保持不变。此类肿瘤标志物的实例包括但不限于CA15-3、CA-125、CEA、CA19-9、α-胎儿蛋白。可以将肿瘤标志物的水平与治疗开始前的基线水平进行比较。在一些实施方案中,本文提供的方法减少与疾病相关的一种或多种肿瘤标志物的发生。

[0231] 在一些实施方案中,本公开提供了用于改变与疾病相关的一种或多种生物标志物(增加或减少)(例如,如在2周、4周、1个月、2个月、3个月、6个月或12个月时或者在更晚的时间或者在任何其他临床指示的时间点所测量)的方法,所述方法包括向受试者施用治疗有效量的如本文公开的抗半乳糖凝集素-9抗体。可以使用常规方法来测量来自患者的临床组织中生物标志物的水平,诸如多重免疫荧光(mIF)技术,如本文在实施例中所述。生物标志物的示例性组可包括CD3、CD4、CD8、CD45RO、FoxP3、CD11b、CD14、CD15、CD16、CD33、CD68、CD163、HLA-DR、精氨酸酶1、颗粒酶B、Ki67、PD-1、PD-L1和PanCK。

[0232] 用于治疗实体瘤的试剂盒

[0233] 本公开还提供了用于治疗或减轻实体瘤(诸如本文所公开的那些)(例如,头颈癌或尿道上皮癌)的试剂盒。这种试剂盒可以包括一个或多个容器,其包含抗半乳糖凝集素-9抗体(例如,本文所述的那些中的任一者)(例如G9.2-17(IgG4))和本文也描述的与抗半乳糖凝集素-9抗体共同使用的检查点抑制剂(如本文所公开的抗PD-1抗体,例如替雷利珠单抗)。

[0234] 在一些实施方案中,试剂盒可以包括根据本文所述的方法中的任一种使用的说明书。所包括的说明书可包括施用抗半乳糖凝集素-9抗体和抗PD-1抗体以治疗靶疾病(如本文所述的那些)、延迟靶疾病(如本文所述的那些)发作或缓解靶疾病(如本文所述的那些)的说明书。在一些实施方案中,试剂盒还包括基于例如应用如本文所述的诊断方法鉴定个体是否患有靶疾病来选择适合于治疗的个体的描述。在又其他实施方案中,说明书包括将抗体施用于有患靶标疾病风险的个体的描述。

[0235] 涉及使用如本文所公开的抗半乳糖凝集素-9抗体和抗PD-1抗体的说明书一般包括关于预期治疗的剂量、给药时间表和施用途径的信息。容器可以是单位剂量、散装包装(例如,多剂量包装)或亚单位剂量。本发明的试剂盒中提供的说明书通常是在标签或包装插页(例如,包括在试剂盒中的纸片)上的书面说明书,但机器可读说明书(例如,在磁盘或光存储盘上携带的说明书)也是可接受的。

[0236] 本发明的试剂盒在合适的包装中。合适的包装包括但不限于小瓶、瓶、罐、柔性包装(例如密封的聚酯薄膜或塑料袋)等。还涵盖与特定设备(诸如吸入器、鼻施用设备(例如雾化器))或输注设备(诸如微型泵)组合使用的包装。在一些实施方案中,试剂盒具有无菌

进入端口(例如容器可以是静脉内溶液袋或具有可被皮下注射针刺穿的塞子的小瓶)。在一些实施方案中,容器还具有无菌进入端口(例如容器是静脉内溶液袋或具有可被皮下注射针刺穿的塞子的小瓶)。组合物中的至少一种活性剂是本文所述的抗半乳糖凝集素-9抗体。

[0237] 试剂盒可任选地提供附加组分,诸如缓冲液和解释性信息。通常,试剂盒包括容器和在容器上或与容器相关的标签或包装插页。在一些实施方案中,本发明提供包含上述试剂盒的内容物的制品。

[0238] 一般技术

[0239] 除非另有指示,否则本发明的实践采用分子生物学(包括重组技术)、微生物学、细胞生物学、生物化学和免疫学的常规技术,这些技术在本领域的技术范围内。此类技术在文献中充分解释,诸如Molecular Cloning:A Laboratory Manual,第二版(Sambrook等人,1989)Cold Spring Harbor Press;Oligonucleotide Synthesis(M.J.Gait编辑,1984);Methods in Molecular Biology,Humana Press;Cell Biology:A Laboratory Notebook(J.E.Cellis编辑,1998)Academic Press;Animal Cell Culture(R.I.Freshney编辑,1987);Introduction to Cell and Tissue Culture(J.P.Mather和P.E.Roberts,1998)Plenum Press;Cell and Tissue Culture:Laboratory Procedures(A.Doyle,J.B.Griffiths和D.G.Newell编辑,1993-8)J.Wiley and Sons;Methods in Enzymology(Academic Press,Inc.);Handbook of Experimental Immunology(D.M.Weir和C.C.Blackwell编辑);Gene Transfer Vectors for Mammalian Cells(J.M.Miller和M.P.Calos编辑,1987);Current Protocols in Molecular Biology(F.M.Ausubel等人编辑,1987);PCR:The Polymerase Chain Reaction(Mullis等人编辑,1994);Current Protocols in Immunology(J.E.Coligan等人编辑,1991);Short Protocols in Molecular Biology(Wiley和Sons,1999);Immunobiology(C.A.Janeway和P.Travers,1997);Antibodies(P.Finch,1997);Antibodies:a practical approach(D.Catty.编辑,IRL Press,1988-1989);Monoclonal antibodies:a practical approach(P.Shepherd和C.Dean编辑,Oxford University Press,2000);Using antibodies:a laboratory manual(E.Harlow和D.Lane(Cold Spring Harbor Laboratory Press,1999);The Antibodies(M.Zanetti和J.D.Capra编辑,Harwood Academic Publishers,1995)。

[0240] 无需进一步阐述,相信本领域技术人员基于以上描述可以最大限度地利用本发明。因此,以下具体实施方案应被解释为仅是说明性的,而不以任何方式限制本公开的其余部分。为了本文引用的目的或主题,本文引用的所有出版物均以引用的方式并入。

[0241] 实施例

[0242] 实施例1:在胰腺癌小鼠模型中单独的或与检查点抑制组合的抗Gal-9抗体评价,以及用G9.2-17mIgG1处理的小鼠的肿瘤质量和免疫特征

[0243] 在胰腺癌小鼠模型中评估G9.2-17mIgG1对肿瘤重量和免疫特征的影响。对8周龄C57BL/6雄性(Jackson Laboratory,Bar Harbor,ME)小鼠施用胰腺内注射的FC1242 PDAC细胞,所述细胞源自Pdx1Cre;KrasG12D;Trp53R172H(KPC)小鼠(Zambirinis CP等人,TLR9 ligation in pancreatic stellate cells promotes tumorigenesis.J Exp Med.2015;212:2077-94)。将肿瘤细胞悬浮在含有50% Matrigel(BD Biosciences,Franklin Lakes,NJ)的PBS中,并经由剖腹术将$1x10^5$个肿瘤细胞注射到胰腺体中。小鼠(n=10/组)i.p.接受

一个预处理剂量,然后接受3个剂量(q.w.)的商业α半乳糖凝集素-9mAb(RG9-1,200ug,BioXcell,Lebanon,NH)或G9.2-17mIgG1(200μg)或者配对同种型G9.2-Iso或大鼠IgG2a(LTF-2,BioXcell,Lebanon,NH)(200μg)(每周一个剂量,持续三周)。3周后处死小鼠,且收获肿瘤用于通过流式细胞术进行分析。按照常规实践处理和准备组织并进行流式细胞术分析。参见例如美国专利号10,450,374。

[0244] 用单独或与αPD-1mAb组合的G9.2-17mIgG2a处理的小鼠的肿瘤质量和免疫特征

[0245] 在胰腺癌的小鼠模型中单独或与免疫疗法组合评估G9.2-17mIgG2a对肿瘤重量和免疫特征的影响。向8周龄的C57BL/6雄性小鼠(Jackson Laboratory,Bar Harbor,ME)的胰内注射来源于Pdx1Cre、KrasG12D、Trp53R172H(KPC)小鼠的FC1242 PDAC细胞。将肿瘤细胞悬浮在含有50% Matrigel(BD Biosciences,Franklin Lakes,NJ)的PBS中,并将1x105个肿瘤细胞经由剖腹术注射到胰腺体中。小鼠i.p.接受一个预处理剂量,然后单独或组合接受3个剂量(q.w.)的G9.2-17mIgG2a(200μg)或中和αPD-1mAb(29F.1A12,200μg,BioXcell,Lebanon,NH)或者如所指示的配对同种型(LTF-2和C1.18.4,BioXcell,Lebanon,NH)。在第26天处死小鼠,并收获肿瘤用于分析。按照常规实践处理和准备组织并进行流式细胞术分析。参见例如US 10,450,374。每个点代表一只小鼠;*p<0.05;**p<0.01;***p<0.001;****p<0.0001;通过未配对Student t检验进行。这些结果显示用两个剂量水平的G9.2-17mIgG2a单药处理都降低了肿瘤生长,而单独的抗PD-1对肿瘤大小没有影响。图1.

[0246] 相较于抗半乳糖凝集素-9mIgG1 200μg,在使用抗半乳糖凝集素-9mIgG1 200μg+抗PD-1的治疗中观察到细胞毒性T细胞(CD8)水平显著提高(p<0.01),并且相较于单独的抗PD-1,在抗半乳糖凝集素-9IgG1 200μg+抗PD-1之间观察到水平显著提高(p<0.001)。此类结果表明,预期抗Gal9抗体和抗PD-1抗体组合将实现优异的治疗效果。

[0247] 此外,在本文所述的B16F10皮下同源模型中研究对用G9.2-17IgG1小鼠mAb(又名G9.2-17mIgG)、抗PD-1抗体、或G9.2-17 IgG1小鼠mAb与抗PD-1抗体的组合处理的肿瘤免疫响应。如图2A和图2B所示,在小鼠模型中,G9.2-17和抗PD-1组合在减少肿瘤体积和增加CD8+细胞方面显示出协同作用。图3A和图3B显示了G9.2-17抗体增加了肿瘤内T细胞中的CD44和TNFa表达。

[0248] 实施例2:G9.2-17(IgG4)在人体研究中的药代动力学

[0249] 对患有转移性实体瘤的患者进行单独的或与化学疗法或免疫检查点抑制剂(例如,PD1拮抗剂)组合的G9.2-17(IgG4)的安全性、药代动力学和抗肿瘤活性的1/2期、开放标签的多中心研究。本研究评价以0.2、0.63、2.0、6.3、10或16mg/kg的剂量水平每2周(Q2W)以及10和16mg/kg的剂量水平每周(QW)施用的G9.2-17(IgG4)。参见WO2020/223702、WO2022/109302、国际专利申请号PCT/US2022/027127和国际专利申请号PCT/US2022/027142,其各自的相关公开内容出于所引用的主题和目的以引用的方式并入本文中。

[0250] 本研究的13名受试者的初步PK数据可用于分析。基于初步数据,发现G9.2-17(IgG4)的β半衰期(即,2-区室模型的消除半衰期)长于非区室分析(NCA)来源的半衰期。这些结果表明,在0.2mg/kg至16mg/kg剂量范围内,G9.2-17(IgG4)的消除半衰期在102至224小时(即,4.3至9.3天)范围内,表明G9.2-17(IgG4)的每周给药可能是该抗体在人类治疗中的适当给药时间表。

[0251] 实施例3:G9.2-17(IgG4)作为单一药剂以及与替雷利珠单抗组合在局部晚期或转

移性实体瘤患者中的安全性、药代动力学和抗肿瘤活性的1/2期、开放标签的多中心研究

[0252] 这是一项患有复发性和/或难治性的不可切除的局部晚期或转移性实体瘤的患者中的开放标签、非随机、多中心的1/2期研究,其中具有剂量递增期(第1部分)和群组扩展期(第2部分)。本研究将在美国多达20个地点进行。研究持续时间估计为12-24个月。存活随访将持续长达2年。

[0253] 治疗持续时间

[0254] 计划继续施用研究药物,直到疾病进展、不可接受的毒性或退出研究。在疾病进展之前停止研究药物并且没有用其他抗癌疗法治疗的患者将继续研究,直到疾病进展的时间。

[0255] 治疗期

[0256] 本研究由第1部分和第2部分的以下时间段组成(参见以下公开内容):

[0257] 筛选期:第一次剂量前长达4周(第-28天至第-1天)

[0258] 治疗期:28天治疗周期

[0259] 治疗后时间段:最后一次治疗后30天(治疗访视结束/提前终止访视)

[0260] 免疫介导的不良反应(IMAR)随访期:所有用G9.2-17 IgG4+替雷利珠单抗治疗的患者必须在最后一剂的研究药物后90天+/-7天返回,以评估潜在的IMAR。

[0261] 随访期:长达2年的长期随访(每3个月随访一次)。

[0262] 研究设计

[0263] 第1部分:单一药剂:剂量递增期

[0264] 将使用连续再评估方法(CRM)进行剂量发现研究,以确立剂量限制性毒性(DLT)并帮助评价可能的推荐2期剂量(RP2D)。每个治疗群组1-6中有两至6名患者被分配从0.2mg/kg的剂量开始,在每个28天周期的第1天和第15天每2周(Q2W)依次接受更高的G9.2-17 IgG4静脉内(IV)输注。分配到特定剂量递增群组的患者将接受所述群组的对应研究剂量。他们接受研究药物,直到疾病进展、不可接受的毒性或因其他原因退出研究。

[0265] 第1部分,群组1-8纳入最多约44名患者。评价总共6个剂量水平:

[0266] • 剂量递增群组1=0.2mg/kg Q2W

[0267] • 剂量递增群组2=0.63mg/kg Q2W

[0268] • 剂量递增群组3=2mg/kg Q2W

[0269] • 剂量递增群组4=6.3mg/kg Q2W

[0270] • 剂量递增群组5=10mg/kg Q2W

[0271] • 剂量递增群组6=16mg/kg Q2W

[0272] 如果在群组1-6中未达到RP2D,则考虑到RP2D,可包括额外2个剂量水平:

[0273] • 剂量递增群组7=10mg/kg QW

[0274] • 剂量递增群组8=16mg/kg QW

[0275] 在第1部分中,通过IV输注Q2W(群组1-6)或QW(群组7-8)依序施用增加剂量的G9.2-17(IgG4)。

[0276] 对于群组1-6,每次给药2名患者。可以基于对集中于在先前剂量水平下DLT的出现的患者安全性数据以及来自先前群组的其他相关安全性和剂量数据的分析起始剂量递增。可在最少28天(1个周期)后进行剂量递增。

[0277] 在群组6完成后,评价每周一次(QW)G9.2-17 IgG4给药时间表,前提是尚未达到RP2D。当分析了群组6的安全性数据并且未鉴别出DLT时,允许患者进入群组7。

[0278] 群组7和8中每剂量水平四名患者被分配在每个28天周期的第1、8、15和22天接受IV输注的G9.2-17 IgG4 QW。从群组7中的前4名患者开始,如果没有鉴定出DLT,则剂量递增至下一群组。

[0279] 允许在鉴定RP2D之前在早期群组中治疗的那些患者剂量递增至RP2D。他们可以继续服用RP2D,直到它们因毒性、疾病进展或其他原因而停止治疗。

[0280] 剂量递增基于在之前剂量水平下治疗的患者中DLT的产生。对于每个剂量群组,先前DLT概率将从符合GLP的毒性研究以及临床前模型指定。对于指定的靶标DLT率和剂量水平的总数量来说,幂模型d^exp(a)的骨架将根据Lee和Cheung的方法,使用位于中值剂量水平和间距测量δ=0.05的通过PK/PD数据调整的先前MTD或OBD生成(Lee和Cheung,2011)。参数"a"的先验分布具有均值为零的正态分布,先验方差信息最少。RP2D是来源于第1部分的OBD/MTD剂量。

[0281] 对于在DLT窗口外经历毒性(包括IMAR)的患者来说,仅当获得临床益处,并且可以继续使用较低剂量的G9.2-17 IgG4获得时,允许剂量减少。G9.2-17 IgG4的剂量最初将降低50%,并且可能进一步降低50%,如方案中提供的剂量调节指南所定义。不允许进一步剂量减少,剂量调节的详细说明见附录8。

[0282] 第1部分组合治疗:剂量递增

[0283] 对于第1部分组合治疗(下面列出的群组9-14)来说,使用4+2设计算法对患者给药。各疾病指示(群组9和10:胰腺管腺癌[PDAC];群组11和12:头颈[H/N];群组13和14-尿道上皮癌)如下在独立群组中进行:

[0284] • 组合群组9=G9.2-17 IgG4 6.3mg/kg QW+吉西他滨/nab-紫杉醇(PDAC)(至多n=10)

[0285] • 组合群组10=G9.2-17 IgG4 16.0mg/kg QW+吉西他滨/nab-紫杉醇(PDAC)(至多n=10)

[0286] • 组合群组11=G9.2-17 IgG4 6.3mg/kg QW+替雷利珠单抗300mg每4周(Q4W)(H/N)(至多n=6)

[0287] • 组合群组12=G9.2-17 IgG4 16.0mg/kg QW+替雷利珠单抗300mg Q4W(H/N)(至多n=6)

[0288] • 组合群组13=G9.2-17 IgG4 6.3mg/kg QW+替雷利珠单抗300mg Q4W(尿道上皮)(至多n=6)

[0289] • 组合群组14=G9.2-17 IgG4 16.0mg/kg QW+替雷利珠单抗300mg Q4W(尿道上皮)(至多n=6)

[0290] 群组9、11和13(对于PDAC、H/N和尿道上皮,分别6.3mg/kg较低剂量)可以并行进行。这些群组中每一个的较高剂量(16.0mg/kg)对纳入DLT出现开放,并且评价其他安全性参数。

[0291] 剂量递减

[0292] 在周期1期间的DLT事件中,发生以下。如果在群组9的第1周期中,4名患者中有1名达到DLT,则将另外2名患者添加到同一群组中。如果群组9中4-6名患者中有2名或更多患者

达到DLT,则第10群组在较低剂量的G9.2-17 IgG4下开放。群组11-12和13-14分别使用相同程序。

[0293] 对于在DLT窗口外经历毒性(包括IMAR)的患者来说,仅当获得临床益处,并且可以继续使用较低剂量的G9.2-17 IgG4获得时,允许剂量减少。G9.2-17 IgG4的剂量最初可降低50%,并且可能进一步降低50%,如方案中提供的剂量调节指南所定义。不允许进一步减少剂量。

[0294] 第2部分:扩展

[0295] 一旦在第1部分群组的任一个中建立了安全性,并且鉴定了初步功效和或PD信号,则可以启动1个或多个扩展群组以进一步评价在所述特定肿瘤类型中的安全性和功效。每个扩展群组的样品大小将基于(1)护理标准[零假设]可用与(2)第1部分的每种肿瘤类型的建议组合疗法[备择假设]预期的点估计值确定。在起始第2部分之前,可提交具有关于扩展群体、治疗方案和统计方法的细节的方案修正案。

[0296] 在第2部分中,患者接受RP2D的作为单一药剂的G9.2-17 IgG4(如第1部分中所测定),或RP2D-1与PD-1替雷利珠单抗的组合,在患有头颈癌、尿道上皮癌或其他实体瘤的患者中。

[0297] G9.2-17 IgG4。如果由于任何原因不能完成同一天施用,则可在第一天施用替雷利珠单抗,并在第二天施用G9.2-17 IgG4。

[0298] 研究目标和终点

[0299] 第1部分-剂量递增

[0300]

| 目标 | 终点 |
|---|---|
| **主要** | |
| 确立作为单一药剂治疗的 G9.2-17 IgG4 以及与吉西他滨/nab-紫杉醇或替雷利珠单抗组合的安全性和耐受性<br>确定 G9.2-17 IgG4 的推荐 2 期剂量(RP2D) | ● 安全性参数评价，包括不良事件、生命体征测量、临床安全性实验室测试、12 导联心电图(ECG)、超声心动图/心脏超声(ECHO)、体检<br>● 评价 DLT<br>● 确定 RP2D |
| **次要** | |
| 表征 G9.2-17 IgG4 的药代动力学(PK)特征 | 评价 G9.2-17 IgG4 的 PK 参数(包括但不限于从时间零到 336 h 的曲线下面积[$AUC_{0-336h}$]、观察到的最大血清浓度[$C_{max}$]、达到 $C_{max}$ 的时间[$T_{max}$]、估计的半衰期[$t_{1/2}$]) |
| 评估 G9.2-17 IgG4 的药效学(PD) | ● 通过流式细胞术得到外周血单核细胞(PBMC)免疫分型<br>● 通过酶联免疫吸附测定(ELISA)得到的预先指定的细胞因子谱(血清)<br>● 通过 ELISA 得到血液(血清)中的可溶性 gal-9 水平<br>● Gal-9 肿瘤组织表达水平和表达模式(通过免疫组织化学(IHC)) (在肿瘤细胞和免疫细胞上的表达以及组合评分)<br>● 肿瘤组织多重免疫分型<br>● 通过 IHC 得到程序性死亡配体-1(PD-L1)表达<br>● 通过 IHC 得到错配修复(MMR)状态 |
| 评估 G9.2-17 IgG4 的免疫原性 | 抗药物抗体(ADA)的评价 |
| **探索性** | |
| 评价作为单一药剂治疗的 G9.2-17 IgG4 以及与吉西他滨/nab-紫杉醇或替雷利珠单抗组合的初步功效 | ● 评价确认的客观响应率(ORR)<br>● 评价疾病控制率(DCR)<br>● 评价响应持续时间(DoR)<br>● 评价无进展存活期(PFS)<br>● 评价总存活期(OS) |

[0301] 第2部分-群组扩展

[0302]

| 目标 | 终点 |
|---|---|
| **主要** | |
| 确立作为单一药剂治疗以及与替雷利珠单抗组合的G9.2-17 IgG4安全性和耐受性。 | ● 评价安全性参数，包括不良事件、生命体征测量、临床安全性实验室测试、12导联ECG、ECHO、体检<br>● 评价DLT |
| 评估用单独G9.2-17 IgG4或与替雷利珠单抗组合治疗的CRC或CCA患者中的ORR | 评价CRC或CCA患者中确认的ORR-3 |
| 评估用G9.2-17 IgG4与组合治疗的PDAC患者中的PFS | 评价PDAC患者的PFS-6 |
| **次要** | |
| 表征G9.2-17 IgG4的药代动力学(PK)特征 | 评价G9.2-17 IgG4的PK参数 |
| 评估G9.2-17 IgG4的药效学(PD) | ● 通过流式细胞术得到PBMC免疫分型<br>● 通过ELISA得到细胞因子谱(血清)<br>● 通过ELISA得到血液(血清)中的可溶性gal-9水平<br>● Gal-9肿瘤组织表达水平和通过IHC得到表达模式(在肿瘤细胞和免疫细胞上表达以及组合评分)<br>● 肿瘤组织多重免疫分型<br>● 组织中的PD-L1表达(通过IHC)<br>● 组织中的MMR状态(通过IHC)<br>● 组织中的TMB |
| 评估G9.2-17 IgG4的免疫原性 | 评价ADA |
| 评价G9.2-17 IgG4的功效 | 确认的ORR<br>PFS<br>DCR<br>DoR<br>OS |

[0303] 单一药剂以及组合治疗

[0304] 用于实体瘤患者(例如,头颈癌或尿道上皮癌)的单一药剂群组或组合药剂群组的治疗可平行进行。

[0305] G9.2-17 IgG4单一治疗

[0306] 单一治疗中G9.2-17 IgG4的起始剂量可以是第1部分中鉴定的RP2D。在阶段I对23名患者测试研究药物后,如果≤1名患者有响应,则可终止该试验群组。如果试验进行到西蒙最佳设计(Simon's optimal design)的阶段II,则在每个单一药剂群组中额外治疗大约33名患者。如果响应患者的总数≤5,则所述组中的研究药物将被拒绝。如果≥6名患者具有确认的ORR-3,则将激活所述群组的第3部分扩展群组,并在方案修正案中进行描述。

[0307] 当获得临床益处并可继续以较低剂量的G9.2-17 IgG4获得时,则可允许剂量减少。G9.2-17 IgG4的剂量最初可降低50%,并且可能进一步降低50%,如本文中提供的剂量

调节指南所定义。

[0308] G9.2-17 IgG4+替雷利珠单抗组合治疗

[0309] G9.2-17 IgG4在与替雷利珠单抗组合治疗中的剂量可以是RP2D-1,其是紧接在第1部分中鉴定的RP2D剂量之前的剂量。最佳两阶段设计还可用于测试ORR-3≤10%的零假设与ORR-3≥25%的备择假设。

[0310] 为确保患者安全性,将进行安全行导入,其中对前8名患者给药。仅当≤2名患者发展DLT(其将低于25%的靶标毒性水平(TTL))时,本组才可继续纳入。如果3名或更多患者发展DLT,则可终止所治疗的癌症类型的本组合组。如果在治疗的第一个28天期间,在患者中的8个安全性导入中的任一个中发生DLT,则所述患者可以永久地停止研究药物施用。

[0311] 对于在DLT窗口外经历毒性的患者来说,仅当获得临床益处并且可以继续使用较低剂量的G9.2-17 IgG4获得时,可允许剂量减少。G9.2-17 IgG4的剂量最初可降低50%,并且可能进一步降低50%,如方案中提供的剂量调节指南所定义。不可允许进一步剂量减少。如本文和下表7的指南所定义,也可以允许替雷利珠单抗的剂量调节。

[0312] 如果IMAR发生/复发不是通过任一药剂的剂量减少来管理,则可以停止两种研究药物。

[0313] 剂量限制性毒性(DLT)标准

[0314] 在本试验中评估的剂量限制性毒性被定义为临床上显著的血液学和/或非血液学AE或评估为与转移性肿瘤疾病进展、并发疾病或伴随药物无关的异常实验室值,并且可能与研究药物有关或与研究药物有关,并且在进行研究的第一个周期(28天)期间发生。在治疗的第一个28天期间的第1部分或第2部分经历DLT的任何患者将永久停止施用研究药物。

[0315] DLT是满足以下标准中的任一个的毒性:

[0316] •任何并非明确由于潜在疾病或外部原因导致的死亡

[0317] •潜在药物诱导肝损伤(Hy法则病例)的指示,如下:

[0318] ○ALT或AST>3x正常上限(ULN),其中在24小时后通过重复测试确认,以及

[0319] ○血清总胆红素(TBL)>2x ULN,其中在24小时后通过重复测试确认

[0320] ○对于升高的TBL和/或AT不能发现其他解释,诸如病毒性肝炎(A、B或C)、酒精性或自身免疫性肝炎、预先存在的或急性肝病、胆囊阻塞或胆管疾病、吉尔伯特氏综合征(Gilbert syndrome)、疾病进展或能够引起观察到的作用的其他药物。

[0321] •任何持续时间的所有4级非血液学和血液学毒性

[0322] •所有3级非血液学和血液学毒性。例外情况如下:

[0323] ○3级恶心、呕吐和腹泻,无需住院治疗或全胃肠外营养支持,且可在48小时内通过支持性护理管理至≤2级。

[0324] ○3级电解质异常,其在24h内纠正为≤2级。

[0325] ○3级电解质异常,其持续<24-72小时,在临床上不复杂,并且自发消退或对常规医疗干预有响应。

[0326] ○≥与胰腺炎症状或临床表现无关的3级淀粉酶或脂肪酶。

[0327] 统计方法:

[0328] 样品大小

[0329] 剂量递增将基于在先前剂量水平治疗的患者中存在或不存在DLT。对于每个剂量

群组来说,先前DLT概率将从符合良好实验室规范(GLP)的毒性研究以及临床前模型指定。对于指定的靶DLT率和剂量水平总数来说,幂模型d^exp(a)的骨架将根据Lee和Cheung(2011)的方法,使用位于中值剂量水平和间距测量δ=0.05的通过药代动力学(PK)/药效学(PD)数据调整的先前MTD生成。参数"a"的先验分布具有均值为零的正态分布,先验方差信息最少。如果最低研究剂量水平的Agresti和Coull二项式CI的下限超过靶标DLT率,则为了安全性将停止试验。

[0330] CRM试验模拟分析(具有1000次迭代)表明需要平均大约20名患者来告知RP2D的选择,RP2D是估计DLT概率小于或等于25% TTL的最大剂量。

[0331] 所使用的CRM基于前6个群组,但其本身不必确定RP2D,因为来自群组7至14的数据也用于确定RP2D。

[0332] 预计研究的第1部分的总样品大小大约为80名患者。如果认为有必要,则回填将提供额外的患者纳入。

[0333] 本研究的第2部分(群组扩展期)可采用西蒙二阶段最佳设计(Simon's two-stage optimal design),以确立LYT-200在第1部分中证明安全性和初步功效的肿瘤类型患者中的安全性和功效。在第2部分中,总样品大小可能取决于由于第1部分单一药物和组合群组中的安全性和功效发现而选择的扩展群组的数量。

[0334] 随机化分层:

[0335] 这是一项开放标签研究。在第1部分,患者将根据研究的CRM设计分配治疗。在第2部分中,将患者分配到治疗组,例如根据纳入和排除标准。

[0336] 分析群体

[0337] 除非另有说明,否则意向治疗(ITT)群体可定义为接受至少一剂研究药物的患者。可对ITT进行初步功效分析。可对ITT进行患者处置。

[0338] 功效群体可定义为ITT中且具有至少一个可测量的ORR 3或PFS 6评估的所有患者。该群体可用于敏感性分析。

[0339] 符合方案(PP)群体可定义为接受至少一个完整周期的G9.2-17(IgG4)且无重大方案偏离的任何患者。

[0340] 安全性群体(SAF)可定义为接受至少一剂研究药物的所有患者。可对SAF进行安全性分析。

[0341] PK/PD群体可定义为已经接受至少一个完整周期的G9.2-17(IgG4)的那些患者。

[0342] 一般统计计划

[0343] 在最后一位患者发生了其主要终点事件后,可进行数据库锁定和初步分析。可在研究完成后进行最终研究分析。所有分析可能均是描述性的。

[0344] 安全性分析

[0345] 除非另有说明,否则所有安全性分析均可对SAF进行,并可使用描述性统计进行分析

[0346] 功效分析

[0347] 对于ITT和PP,可描述性总结根据RECIST v1.1评估的疾病响应。可以对功效群体进行敏感性分析。

[0348] 药代动力学、药效动力学和免疫原性

[0349] 可描述性总结对PK/PD群体的PK、PD和免疫原性。

[0350] 评估时间表

[0351] 表3提供了评估时间表,除了研究药物施用如下:可以在每个周期的C1D1和C1D15施用G9.2-17 IgG4治疗。在第2部分中,可以在G9.2-17 IgG4组合方案的每个周期的第1天施用替雷利珠单抗。可从C2开始在第1、8和15+/-3天施用研究药物。所有用G9.2-17 IgG4+替雷利珠单抗治疗的患者必须在最后一次剂量的研究药物后90天+/-7天返回,以评估潜在的免疫介导的不良反应(IMAR)。

[0352] 研究群体

[0353] 符合以下纳入标准且不符合任何排除标准后的患者符合研究条件。

[0354] 纳入标准

[0355] 部分I

[0356] 1.书面知情同意书(患者精神正常,能够理解并愿意签署知情同意书)

[0357] 2.年龄≥18岁的男性或非妊娠女性

[0358] 3.根据研究者的判断,能够遵守研究方案

[0359] 4.组织学确认的不可切除的局部晚期或转移性癌症。对于纳入本研究的患者的癌症疾患所接受的先前疗法线没有限制。

[0360] a.对于第1部分组合尿道上皮癌群组:组织学或细胞学确诊诊断为肾盂、输尿管、膀胱或尿道的不可切除的局部晚期或转移性尿道上皮癌(即,移行细胞癌)。

[0361] b.对于第1部分组合头颈癌群组:组织学确认的局部晚期或转移性SCCHN(口腔、口咽、下咽或喉)。

[0362]

表 3：评估时间表

| | 给药前 | 治疗阶段 | | | | | | | | | | | | | | 治疗后 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 第 1 周期(28 天) | | | | 第 2 周期(28 天) | | | 第 3 周期(28 天) | | | 第 4 周期(28 天)及以后 | | | | 治疗结束或提前终止 | IMAR 随访 T | 长期随访 U 每 3 个月 |
| 研究日 | 筛选 -28 至 -1 | 1 | 2 | 8 | 15 | 1 | 8 | 15 | 1 | 2 | 8 | 15 | 1 | 8 | 15 | 最后一次剂量后 30 天 | 最后一次剂量后 90 天 | |
| 周期日 | | C1 D1 | C1 D2 | C1 D8 | C1 D15 | C2 D1 | C2 D8 | C2 D15 | C3 D1 | C3 D2 | C3 D8 | C3 D15 | CX D1 | CX D8 | CX D15 | -- | -- | -- |
| +/-允许的天数 | | | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 3 | 7 | n/a |
| 研究药物施用 A | | X | | X | X | X | X | X | X | | X | X | X | X | X | | | |
| **研究程序和检查** | | | | | | | | | | | | | | | | | | |
| 资格评估和书面知情同意书 | X | | | | | | | | | | | | | | | | | |
| 人口统计资料 B | X | | | | | | | | | | | | | | | | | |
| 病史 C | X | | | | | | | | | | | | | | | | | |
| 既往和伴随用药 D | X | X | X | X | X | X | | X | X | X | X | X | X | | X | X | | |
| 不良事件 E | | X | X | X | X | X | | X | X | X | X | X | X | | X | X | X | |
| ECHO/MUGA F | X | | | | | | | | | | | | X | | | | | |
| 12 导联 ECG (QTcF) | X | X | | | | X | | | X | | | | X | | | | | |
| 体检 G | X | X | | | X | X | | X | X | | | X | X | | X | X | X | |
| ECOG | X | X | | | X | X | | X | X | | | X | X | | X | X | | |
| 生命体征 H | X | X | | | X | X | | X | X | | | X | X | | X | X | | |

[0363]

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 肿瘤成像评估(具有对比的 CT 优选) N | X | | | | | | | X | | | | | | X 如果在上次扫描>8周研究结束，则重复 | | X U |
| **临床实验室** | | | | | | | | | | | | | | | | |
| 妊娠测试 I | X | | | | X | | | X | | | X | | | X | | |
| 血液学 J | X | X | | X | X | | X | X | | | X | X | | X | X | |
| 血清化学 K | X | X | | X | X | | X | X | | | X | X | | X | X | |
| TSH、游离 T4、血清脂肪酶、淀粉酶、PTH、FSH、LH、游离皮质醇 | X | X | | | X | | | X | | | X | | | X | X | |
| 凝血 L | X | X | | X | X | | X | X | | | X | X | | X | X | |
| 尿液分析 M | X | X | | X | X | | X | X | | | X | X | | X | X | |
| **药效动力学和药代动力学** | | | | | | | | | | | | | | | | |
| 肿瘤活检 O | X | | | | | | | | | | X ± 7 d | | | | | |
| 肿瘤类型相关生物标志物 P | X | X | | | X | X | X | | | | X | X | | X | X | |
| 用于生物标志物 PD 分析的血液采样 Q | | X | X | X | X | X | | | X | X | X | X | | | X | |
| PK 血液采样 R | | X | X | X | X | X | | | X | X | X | X | | | | |
| 免疫原性(ADA) S | | X | | | | X | | | X | | | X | | | X | |
| 存活期随访 | | | | | | | | | | | | | | | | X U |

ADA：抗药物抗体；AE：不良事件；ALT：丙氨酸转氨酶；APTT：活化部分凝血致活酶时间；AST：

[0364]

天冬氨酸转氨酶；C：周期；CPK：肌酸磷酸激酶；COVID19：冠状病毒 SARS-CoV-2；CRP：C 反应蛋白；CT：计算机断层扫描；D 或 d：天；ECG：心电图；ECOG：东部肿瘤协作组；ECHO：超声心动图/心脏超声；FSH：促卵泡激素；IMAR：免疫介导的不良反应；INR：国际标准化比率；LDH：乳酸脱氢酶；LH：黄体生成素；min：分钟；MUGA：多门采集扫描；PD：药效学；PK：药代动力学；PT：凝血酶原时间；PTH：甲状旁腺激素；PTT：部分凝血致活酶时间；QTcF：QT 间期，Fridericia 校正公式；RBC：红细胞计数；SGOT：血清谷氨酸-草酰乙酸转氨酶；SGPT：血清谷丙转氨酶；TSH：促甲状腺激素；WBC：白细胞计数。

A) 研究药物施用：将在每个周期的 C1D1 和 C1D15 施用 G9.2-17 IgG4 治疗。

B) 人口统计数据：数据包括年龄、性别、种族和民族。

C) 病史：除一般病史以外，数据收集还包括肿瘤史、手术/移植和放射疗法史以及 COVID-19 史和检查。

D) 既往和伴随用药(包括疫苗和补充治疗/补充剂)：数据包括每种药物的名称、适应症、剂量、途径、开始和结束日期。还应获得过敏和不耐受、研究期间的剂量调节、剂量更改时间表及其原因。

E) 不良事件：将记录研究药物施用后开始或恶化的任何 AE。应跟踪 AE，直至消退至以下之一：基线、稳定或被视为不可逆。收集所有 SAE，直至研究药物最后一次给药后 30 天。必须从患者书面同意之日起收集所有与研究程序相关的 SAE。

F) ECHO/MUGA：这种心脏功能评估是在筛选时进行，并在第 4 周期的第 1 天重复；评估窗口为+/- 5 天。

[0365]

当有临床指征时，应更频繁地进行，每 3 个月一次。

G) 体格检查：包括在筛选时检查身高以确定体表面积。在所有预定检查时间包括体重。仅对具有稳定且/或预先治疗过的脑转移患者进行神经系统检查。

H) 生命体征：体温、心率、血压、呼吸频率。

I) 妊娠测试(血液或尿液)：仅针对具有生育能力且子宫在原位的妇女。试验结果必须在预定给药前可用。

J) 血液学：分析包括差分全血细胞计数、血小板、血红蛋白。在给药前收集血液样品。

K) 血清化学：分析包括白蛋白、碱性磷酸酶、胆红素(总胆红素、直接胆红素)、血尿素氮、钙、CPK、肌酐、电解质(钠、钾、氯化物、镁、磷)、γ谷氨酰转移酶(γ GT)、葡萄糖、血红蛋白 A1c (HgbA1c)(仅当有 1 型或 2 型糖尿病史时)、LDH、SGPT (ALT)或 SGOT (AST)、总蛋白。仅在有临床指征时，才评估空腹血糖。在给药前收集血液样品。

L) 凝血：在给药前收集血液样品。分析包括 APTT、PT、PTT 和 INR (如果使用允许的抗凝剂)、CRP 和肌钙蛋白。

M) 尿液分析：分析包括颜色、外观、比重试纸、蛋白质、白细胞酯酶、葡萄糖、酮、尿胆素原、亚硝酸盐、WBC、RBC、pH 值。(仅当患者出现临床症状时才进行尿液培养和敏感性检测。)

[0366]

N) 肿瘤成像评估：对于筛选，必须在 28 天的筛选期内进行评估。在研究中，每 8 周±7 天(即，C3D1、C5D1、C7D1、C9D1 等)进行评估，如果在之前 4-6 周内未进行评估，则在治疗结束时进行评估。如果有临床指征，可更频繁地进行评估。如果在扫描中看到客观响应，将在 4 周(+7 d)后进行确认扫描。在此确认扫描后，将从确认扫描之日起以每 8 周±7 天的频率恢复预定扫描。

O) 肿瘤活检：如果筛选时患者的 MMR/MSI 状态未知，则应在当地实验室进行检查。在第 2 部分中，将执行 TMB 组织分析。研究中的活检安排在 C3D15 ± 7 天，且仅在第 3 周期中肿瘤成像扫描后进行。已经认识到，多种临床因素可能使得难以获得足够样本。决定在治疗中不进行活检应与医学监查员讨论。

P) 肿瘤类型相关生物标志物：将在筛选时和每个周期剂量施用前收集血液样品，视肿瘤类型而定。治疗 6 个月后，血液采样可减少至每 3 个周期一次。

Q) PD 血液采样：将在给药日在给药前收集血液样品。治疗 6 个月后可减少至每 3 个周期一次。

R) PK 血液采样：第 1 周期和第 3 周期第 1 天：将在给药前和研究药物输注结束(EOI)时、研究药物施用后 2 小时和 4 小时(±30 分钟)收集血液样品。第 1 周期和第 3 周期第 15 天，将仅在给药前和 EOI 时收集血液样品。第 1 周期和第 3 周期第 2 天和第 8 天(非给药日)，将仅在一个时间点收集 PK 血液样品。第 2 周期和第 4 周期：仅在第 1 天收集血液样品，且应在给药前和 EOI 时进行。此后每 2 个周期(即，C6D1、C8D1 等)在给药前和 EOI 时收集 PK 血液样品。

S) ADA 血液采样：将在第 1-4 周期的第 1 天，在给药前收集血液样品。此后，每 2 个周期在第 1 天在给

[0367]

药前(即，C6D1、C8D1等)收集血液样品。

T) 所有用G9.2-17 IgG4 +替雷利珠单抗治疗的患者必须在最后一次剂量的研究药物后90天+/-7天返回，以评估潜在的免疫介导的不良反应(IMAR)。

U) 长期随访：对于由于疾病进展以外的原因而停止治疗且未接受另外的全身抗癌治疗的患者，应尽可能继续进行肿瘤成像。将至少每3个月收集一次存活数据。可以更频繁地收集存活数据，以支持数据清理或监管提交工作。随访可以通过电话、电子信息或图表审查进行，并将在患者进行治疗结束/提前终止访视后持续长达2年。

[0368] 5.对于尿道上皮癌和头颈癌组合癌症群组,允许预先暴露于免疫疗法,具有标准护理治疗选项和/或在临床试验背景内。如果患者在任何时间点接受抗PD-1和/或含有抗

PD-L1的方案,则根据这些治疗方案之一的RECIST 1.1或iRECIST标准(如果这些测量可用的话),他们必须表现出至少稳定的疾病。如果RECIST或iRECIST测量不可用,则需要在先前的抗PD-1和/或含有抗PD-L1的方案中的任一者上实现至少4个月的临床PFS。

[0369] 6.第1部分组合尿道上皮和头颈群组不需要PD-L1表达,然而需要新鲜活检或存档组织用于通过IHC评估PD-L1,或历史PD-L1表达(通过IHC)必须可用。如果PD-L1表达数据已经可用,则只要可行,这就不推翻用于获得新鲜活检的方案偏好。

[0370] 7.对于口咽起源的第1部分组合群组群头颈癌患者来说:人乳头瘤病毒(HPV)状态需要在筛选期或患者服用研究药物时的任何时间点确立,除非历史上已知。作为HPV+的替代物的p16+、HPV RNA ISH或DNA PCR都是可接受的。本研究接受HPV+和HPV-患者两者。

[0371] 8.根据研究者的判断,预期寿命>3个月。

[0372] 9.ECOG体力状态0-1。

[0373] 10.患者能够并愿意接受治疗前和治疗中/治疗后活检。根据研究者的判断,计划的活检不应使患者出现显著增加的并发症风险。将尽一切努力在重复活检时对同一病灶进行活检。如果患者根据所有其他标准合格,但拒绝同意活检或有其他医学原因排除活检,将与申办者讨论。

[0374] 11.根据RECIST v1.1,可测量的疾病。注意,预期活检的病灶不应是靶病灶。

[0375] 12.充分的血液学和终末器官功能,由在第一剂研究药物治疗之前获得的以下实验室结果定义,条件是在最后7天内不施用抗癌治疗:

[0376] a.嗜中性粒细胞计数≥1x $10^9$/L

[0377] b.血小板计数≥100x $10^9$/L;第1部分的肝细胞癌(HCC)≥50x$10^9$/L

[0378] c.前一周未输注的情况下血红蛋白≥9.0g/dL

[0379] d.肌酐≤1.5x ULN;或eGFR>50mg/mmol

[0380] e.天冬氨酸转氨酶AST(SGOT)≤3x ULN(当存在HCC或肝转移时,≤5x ULN)

[0381] f.丙氨酸转氨酶(ALT[SGPT])≤3x ULN(当存在HCC或肝转移时,≤5xULN)

[0382] g.胆红素≤1.5xULN(患有已知吉尔伯特氏病的患者的胆红素可≤3.0xULN)

[0383] h.白蛋白≥3.0g/dL

[0384] i.国际标准化比率(INR)和部分凝血致活酶时间(PTT)≤1.5xULN,除非患者接受抗凝疗法。

[0385] 13.无活动性严重感染或需要胃肠外抗生素的感染的证据。

[0386] 14.具有生育能力的妇女必须在开始治疗前72h内具有阴性妊娠测试。对于有生育能力的妇女:同意在治疗期间以及最后一次研究治疗后至少180天内保持禁欲(避免异性性交)或使用每年失败率<1%的避孕方法。

[0387] 如果妇女是月经初潮后的,没有达到绝经后状态(≥连续12个月的闭经,除了绝经外没有确定的原因),并且没有经历手术绝育(切除卵巢和/或子宫),则她具有生育能力。

[0388] 每年失败率<1%的避孕方法的实例包括双侧输卵管结扎、男性绝育、抑制排卵的激素避孕药、激素释放宫内节育器和铜宫内节育器。应根据临床试验的持续时间以及患者的偏好和惯常生活方式来评价性禁欲的可靠性。定期禁欲(例如,日历(calendar)、排卵、症状热(symptom-thermal)或排卵后方法)和体外排精(withdrawal)是不可接受的避孕方法。有生育能力的男性在研究期间必须采用有效的避孕方法,除非存在不孕症的记录。

[0389] 15.自第一次G9.2-17(IgG4)施用前最后一次抗癌疗法起四(4)周或5个半衰期(以较短者为准)。

[0390] 16.如果先前在临床试验开始之前使用,则允许双膦酸盐治疗(例如,唑来膦酸)或地诺单抗。

[0391] 17.患者:

[0392] a.已经接受过针对转移性或局部晚期疾病的至少一种先前全身疗法线的患者,和/或

[0393] b.患有没有可用的标准护理选项的肿瘤类型。

[0394] 18.先前未接受过含吉西他滨方案的患者。

[0395] 排除标准

[0396] 1.不愿意或不能遵守方案要求的患者

[0397] 2.被诊断患有原发不明的转移性癌症的患者

[0398] 3.当前非法药物成瘾

[0399] 4.临床显著的、活动性不受控的出血,以及具有出血体质(例如,活动性消化性溃疡疾病)的任何患者。允许防治性或治疗性使用抗凝剂。

[0400] 5.怀孕和/或哺乳期女性

[0401] 6.在第一剂研究药物前所施用药物的3周内或5个半衰期内(以较短者为准)接受任何其他研究药物或参与涉及另一种研究药剂治疗实体瘤的任何其他临床试验,或在第一剂研究药物的4周内进行大手术或计划手术(其包括牙科手术)。

[0402] 7.在第一剂量的研究药物的4周内接受放射疗法,但有限场的姑息性放射疗法除外,诸如用于治疗骨痛或局灶性疼痛肿瘤块,并且不会危及响应评估所需的可测量病灶(RECIST v1.1)。

[0403] 8.具有真菌性肿瘤块的患者

[0404] 9.可能混淆试验结果、干扰患者

[0405] 在整个试验期间参与试验或在治疗研究者看来参与不符合患者的最佳利益的任何疾患、治疗、任何活动性感染或实验室异常的历史或当前证据

[0406] 10.用先前的检查点抑制剂产生4级免疫介导的毒性。2级或3级肺炎或者导致免疫疗法治疗停止的任何其他3级检查点抑制剂相关毒性。允许低级别(<3级)毒性,诸如由先前治疗产生的神经病变、可管理的电解质异常和淋巴细胞减少、脱发和白癜风。

[0407] 11.需要其他积极治疗的其他先前或其他伴随恶性肿瘤病史。

[0408] 12.活动性脑、癌性脑膜炎或柔脑膜转移的患者。患有脑转移的患者是符合条件的,条件是他们在确定性疗法后至少4周显示临床和放射学上稳定的疾病,并且在第一剂研究药物前至少4周未使用类固醇(>10mg/天的泼尼松或等效物)。

[0409] 13.严重或不受控制的全身性疾病、充血性心力衰竭>纽约心脏协会(NYHA)2级、6个月内心肌梗死(MI)或研究者认为不希望患者参与试验的实验室结果的证据

[0410] 14.研究者认为严重损害患者安全性或损害LYT-200毒性评估解释的任何医学疾患

[0411] 15.严重未愈合伤口、活动性溃疡或未经治疗的骨折,除非例如是肋骨骨折(其不引起治疗)

[0412] 16.需要反复引流程序的不受控制的胸腔积液、心包积液或腹水。出于本研究的目的,“复发”定义为在最后30天内的$^{3}$ 3引流。

[0413] 17.对嵌合或人源化抗体或融合蛋白的严重过敏、过敏性或其他超敏反应的病史

[0414] 18.第1周期第1天的6个月内的显著血管疾病(例如,需要手术修复的主动脉瘤或近期动脉血栓形成)

[0415] 19.第1周期第1天前3个月内的肺栓塞、中风或短暂性脑缺血发作史

[0416] 20.活动性自身免疫病症(I/II型糖尿病、仅需要激素替代的甲状腺功能减退、白癜风、银屑病或斑秃除外)。

[0417] 21.需要全身免疫抑制治疗,包括但不限于环磷酰胺、硫唑嘌呤、甲氨蝶呤、沙利度胺和抗肿瘤坏死因子(抗TNF)药剂。已经接受或正在接受急性、低剂量全身免疫抑制药物(例如,≤10mg/天的泼尼松或等效物)的患者可入组。替代疗法(例如,甲状腺素、胰岛素、生理皮质类固醇替代疗法[例如,用于肾上腺或垂体机能不全的≤10mg/天泼尼松等效物])不被认为是一种全身性治疗形式。允许使用吸入性皮质类固醇和盐皮质激素(例如,氟氢可的松)、局部类固醇、鼻内类固醇、关节内类固醇和眼科类固醇。

[0418] 22.严重肿瘤相关疼痛(3级,CTCAE]v.5.0对广泛镇痛干预(口服和/或贴剂)无响应

[0419] 23.尽管使用双膦酸盐,仍具有高钙血症(根据CTCAE v 5.0,定义为$^{3}$ 3级)

[0420] 24.如下的任何其他疾病、代谢功能障碍、体检发现或临床实验室发现:合理怀疑某一疾病或病症是使用研究药物的禁忌,或可能影响结果解释或使患者面临治疗并发症的高风险

[0421] 25.接受器官移植

[0422] 26.进行透析的患者

[0423] 27.对于第1部分,对于患有转移性去势抵抗性前列腺癌的患者,允许继续进行激素雄激素剥夺疗法

[0424] 28.进入试验前<6周内HCC的任何消融疗法(射频消融或经皮乙醇注射)

[0425] 29.肝性脑病或严重肝腺瘤

[0426] 30.Child-Pugh评分≥7

[0427] 研究药物和其他干预

[0428] 研究干预被定义为意图根据研究方案施用于/用于研究参与者的任何研究性剂、市场产品、安慰剂或医疗器械。

[0429] 与G9.2-17 IgG4组合施用的药剂

[0430] 替雷利珠单抗

[0431] 替雷利珠单抗是一种抑制PD-1的mAb药物,正在开发用于治疗癌症。替雷利珠单抗配制在带橡胶塞的一次性玻璃小瓶(20R玻璃,USPI型)中,用于IV注射,在10mL缓冲等渗溶液中总共含有100mg替雷利珠单抗mAb。替雷利珠单抗在28天的周期中每4周以300mg在大约30分钟内作为静脉内输注施用(除非另有指导)。

[0432] 替雷利珠单抗的活性成分是一种针对PD-1的人源化IgG4变体mAb,以高特异性和亲和力(KD=0.15nM)与人PD-1的ECD结合。替雷利珠单抗的赋形剂包括:柠檬酸钠二水合物、柠檬酸一水合物、L-组氨酸盐酸盐一水合物、L-组氨酸、海藻糖二水合物、聚山梨酯-20

和WFI。替雷利珠单抗竞争性地阻断PD-L1和PD-L2的结合,抑制PD-1介导的负信号传导,并在基于细胞的体外测定中增强T细胞中的功能活性。另外,替雷利珠单抗在几种人癌症同种异体异种移植模型和人PD-1转基因小鼠模型中显示出抗肿瘤活性。

[0433] 通过体外测定,IgG4变体抗体对Fc γ RIIIA和C1q具有非常低的结合亲和力,表明在人体内具有较低或不具有ADCC和CDC效应。与天然IgG4抗体不同,通过体外测定替雷利珠单抗没有可观察到的Fab臂交换活性,预测该抗体在体内是稳定的,不太可能形成双特异性抗体。

[0434] 在各种晚期实体瘤中,替雷利珠单抗暴露与功效之间的暴露-反应(E-R)关系支持300mg Q4W方案。预计300mg Q4W方案在安全性或功效结果方面与200mg Q3W方案没有临床差异。

[0435] 替雷利珠单抗的安全特性与治疗相关的3级或以上毒性发生率相对较低的药物的治疗类别一致。

[0436] 替雷利珠单抗AE根据其发生频率列于下表4中。报告的可能与IMAR相关的AE总结在表5中。

[0437] 表4.根据频率报告的替雷利珠单抗的不良事件(非IMAR相关)

[0438]

| 器官部位 | 不良事件 |
|---|---|
| 血液和淋巴系统/ | 贫血(8%-21%，重度 1%-8%) |

[0439]

| | |
|---|---|
| 发热 | 白细胞减少症(5%-12%，重度 2%) |
| 嗜中性粒细胞减少症 | 淋巴细胞减少症(<1%) |
| | 嗜中性粒细胞减少症(3%-9%，重度 1%-3%) |
| | 血小板减少症(1%-9%，重度 1%) |
| 胃肠 | *致吐可能性：低* [3] |
| | 便秘(13%，重度<1%) |
| | 腹泻(5%-13%，重度 1%) |
| | 恶心(4%-13%，重度<1%) |
| | 口腔炎、口疮性溃疡(3%，重度<1%) |
| | 呕吐(10%，重度 1%) |
| 全身性病症及给药部位各种反应 | *外渗危险：无* [4] |
| | 发冷(1%，重度 0%) |
| | 疲劳、乏力(1%-23%，重度 2%) |
| | 热病(5%-33%，重度 1%) |
| 感染及侵染类疾病 | 肺炎(3%) |
| 各类损伤、中毒及手术并发症 | ***输注相关反应***(2%-29%，重度<2%) |
| 调查 | 碱性磷酸酶升高(6%，重度 1%) |
| | ALT 升高(1%-16%，重度 2%) |
| | AST 升高(1%-17%，重度 3%) |
| | 胆红素升高(5%-12%，重度 2%) |
| | 肌酸磷酸激酶升高(1%-6%，重度 1%) |
| | 高血糖症(2%-5%，重度 1%) |
| | 低钠血症(1%，重度 1%) |
| | 脂肪酶升高(1%，重度 1%) |
| 临床上重要的副作用以***粗体、斜体***表示 | |
| | 蛋白尿(1%-6%) |
| | 甲状腺激素(游离三碘甲腺原氨酸和甲状腺素)下降(1%) |
| | 促甲状腺激素(TSH)升高(1%-7%) |
| 代谢及营养类疾病 | 食欲减退(1%-15%，重度<1%) |
| | 1 型糖尿病(1%，重度 1%) |
| | 糖尿病酮症酸中毒(<1%) |
| 各种肌肉骨骼及结缔组织疾病 | 关节痛(2%-6%) |
| 呼吸系统、胸及纵隔疾病 | 咳嗽(13%) |
| | 呼吸困难(6%，重度 1%) |
| | 瘙痒(8%-14%，重度<1%) |
| | 皮疹(6%-18%，重度 1%) |
| | 皮肤反应，不明确(1%，重度<1%) |

[0440] 通过破坏PD-1介导的信号传导,替雷利珠单抗可恢复抗肿瘤免疫并阻止肿瘤生长的进展。这种免疫系统活性的恢复可能导致涉及1个或多个身体系统的免疫相关不良反应,

在极少数情况下可能危及生命或致命。虽然这些事件通常在使用替雷利珠单抗治疗期间变得明显,但它们也可能在停止替雷利珠单抗治疗后发生。

[0441] 表5.报告的视为IMAR的替雷利珠单抗的不良事件*

| 器官部位 | 不良反应 |
|---|---|
| 心脏 | 免疫介导的心肌炎(1%，重度<1%) |
| 内分泌 | 免疫介导的肾上腺功能不全(<1%) |
| | 免疫介导的甲状腺功能亢进症(3%-4%，重度<1%) |
| | 免疫介导的甲状腺功能减退症(6%-21%，重度 1%) |
| | 免疫介导的甲状腺炎(1%) |
| 眼 | 免疫介导的葡萄膜炎(1%) |
| 胃肠 | 免疫介导的结肠炎(1%，重度 1%) |
| | 免疫介导的胰腺炎(1%，重度<1%) |
| | 免疫介导的肝炎(1%-3%，重度 1%-2%) |
| 各种肌肉骨骼及结缔组织疾病 | 关节痛(2%-6%) |
| | 免疫介导的肌炎/横纹肌溶解症(1%，重度 1%) |
| | 免疫介导的肾炎、肾功能障碍(1%，重度 1%) |
| 免疫系统 | 免疫介导的不良事件(21%-22%，重度 5%-11%) |
| 呼吸系统、胸及纵隔疾病 | 咳嗽(13%) |
| | 呼吸困难(6%，重度 1%) |
| | 免疫介导的肺炎、间质性肺病(2%-7%，重度 1%-6%) |
| 皮肤和皮下组织 | 免疫介导的皮炎(1%，重度<1%) |
| | 免疫介导的瘙痒(2%-4%) |
| | 免疫介导的皮疹(3%，重度<1%) |
| | 免疫介导的白癜风(1%) |

[0442]

[0443] 关于G9.2-17(IgG4)和替雷利珠单抗的组合引起的IMAR的管理也参见下表6。

[0444] 表6:G9.2-17 IgG4+替雷利珠单抗组合治疗引起的免疫介导的不良反应(IMAR)的管理

| IMAR | 管理指南 |
|---|---|
| 免疫介导的肺炎 | • 监测患者肺炎的放射影像学成像体征和症状。<br>• 1级，考虑暂停替雷利珠单抗，直至患者体征和症 |

[0445]

| IMAR | 管理指南 |
| --- | --- |
| | 状有显著改善<br>• 对于中度(2 级)肺炎暂停替雷利珠单抗和 G9.2-17 IgG4，且对于重度(3 级)或危及生命(4 级)的肺炎永久停用替雷利珠单抗和 G9.2-17 IgG4。对于中度(2 级)，施用剂量为 1 至 2 mg/kg/天泼尼松当量的皮质类固醇。对于更严重(3-4 级)肺炎，永久停用 G9.2-17 IgG4 和替雷利珠单抗，并建议在医院环境中使用 IV 甲泼尼龙 2 至 4 mg/kg/天进行治疗，随后皮质类固醇逐渐减量。<br>• 对于中度(2 级)肺炎，暂停替雷利珠单抗和 G9.2-17 IgG4，直至消退至<2 级。恢复 G9.2-17 IgG4，剂量减少 50%。<br>• 仅当症状完全消退或用泼尼松龙≤ 10 mg/天控制时，才可重新开始替雷利珠单抗。对于 2 级肺炎，暂停替雷利珠单抗，且仅当症状完全消退或用泼尼松龙≤ 10 mg/天控制时才重新开始。如果使用皮质类固醇后症状持续存在，则永久停用替雷利珠单抗治疗。<br>• ≥2 级肺炎复发后，永久停用研究药物和替雷利珠单抗的治疗。 |
| [0446] 免疫介导的结肠炎和腹泻 | • 监测患者结肠炎的体征和症状。<br>• 对于 2 级腹泻或结肠炎，暂停替雷利珠单抗和 G9.2-17 IgG4 的治疗，并开始口服泼尼松龙 0.5 mg/kg/天(非包肠溶衣的)。如果开始使用皮质类固醇后情况恶化或无改善，则将剂量增加至 1 至 2 mg/kg/天泼尼松当量。<br>• 随着体征和症状消退，在 2 至 4 周内类固醇逐渐减量。当体征和症状改善或消退至基线时，恢复替雷利珠单抗治疗。对于 3 级或 4 级结肠炎，施用泼尼松龙 1 至 2 mg/kg/天，转为口服泼尼松龙，并在改善明显时在至少 4 周内逐渐减少该剂量。对于复发性重度(3 级)或危及生命的 4 级腹泻或结肠炎，永久停用替雷利珠单抗和 G9.2-17 IgG4。<br>• 当体征和症状改善或消退至基线时，恢复 G9.2-17 IgG4，剂量减少 50%。替雷利珠单抗的任何剂量调节均由研究者自行决定。<br>• 如果≥3 级复发，则永久停用研究药物和替雷利珠单抗的治疗。<br>• 如果≥2 级结肠炎复发，暂停 G9.2-17 IgG4 和替雷利珠单抗，直至结肠炎消退至基线。应用上述治疗方案。恢复 G9.2-17 IgG4，剂量再减少 50%。不允许对 G9.2-17 IgG4 进一步减少剂量。 |

| IMAR | 管理指南 |
|---|---|
| | • 如果≥2 级结肠炎复发，永久停用这两种研究药物的治疗。<br>• 对于危及生命的(4 级)，永久停用 G9.2-17 IgG4 和替雷利珠单抗。 |
| 免疫介导的肝炎 | • 监测肝功能的变化。<br>• 对于中度(2 级)转氨酶升高，施用剂量为 0.5 至 1 mg/kg/天泼尼松当量的皮质类固醇。<br>• 对于中度(2 级)免疫介导的肝炎，暂停替雷利珠单抗和 G9.2-17 IgG4。当消退至< 2 级且泼尼松龙逐渐减量至≤ 10 mg 时，恢复替雷利珠单抗和 G9.2-17 IgG4。将恢复 G9.2-17 IgG4，剂量减少 50%。<br>• 如果≥2 级肝炎复发，永久停用 G9.2-17 IgG4 和替雷利珠单抗的治疗。<br>• 对于中度(2 级)、重度(3 级)或危及生命的(4 级)转氨酶升高，伴有或不伴有总胆红素升高，施用剂量为 0.5 至 2 mg/kg/天泼尼松当量的皮质类固醇，随后皮质类固醇逐渐减量。<br>• 对于重度(3 级)或危及生命的(4 级)免疫介导的肝炎，永久停用 G9.2-17 IgG4 和替雷利珠单抗的治疗。 |
| 免疫介导的内分泌病：垂体炎 | • 按照临床指示施用激素替代物和皮质类固醇。对于中度(2 级)垂体炎，暂停 G9.2-17 IgG4 和替雷利珠单抗。按照临床指示首先施用口服泼尼松龙 0.5 至 1 mg/kg/天并添加激素替代物，直至消退/改善至≤ 2 级。在至少 1 个月内皮质类固醇逐渐减量。恢复 G9.2-17 IgG4，恢复时剂量减少 50%。对于由内分泌医师治疗的无症状且可控制的患者，通常不需要停药。<br>• 如果≥2 级毒性复发，永久停用 G9.2-17 IgG4 和替雷利珠单抗的治疗。<br>• 对于重度(3 级)和危及生命的(4 级)垂体炎，永久停用 G9.2-17 IgG4 和替雷利珠单抗的治疗。 |
| 肾上腺功能不全 | • 监测患者肾上腺功能不全的体征和症状。<br>• 对于中度(2 级)肾上腺功能不全，暂停 G9.2-17 IgG4 和替雷利珠单抗。按照临床指示首先施用口服泼尼松龙 0.5 至 1 mg/kg/天，直至消退/改善至≤ 2 级。在至少 1 个月内皮质类固醇逐渐减量。恢复 G9.2-17 IgG4，恢复时剂量减少 50%。对于由内分泌医师治疗的无症状且可控制的患者，通常不需要停药。<br>• 如果≥2 级毒性复发，永久停用 G9.2-17 IgG4 和替雷利珠单抗的治疗。<br>• 对于重度(3 级)和危及生命的(4 级)肾上腺功能不全，永久停用 G9.2-17 IgG4 和替雷利珠单抗的治疗。 |

[0447]

[0448]

| IMAR | 管理指南 |
| --- | --- |
| 甲状腺功能减退症和甲状腺功能亢进症 | • 在替雷利珠单抗治疗之前和期间定期监测甲状腺功能。<br>• 对甲状腺功能减退症施用激素替代疗法。<br>• 开始医疗管理以控制甲状腺功能亢进症。对甲状腺疼痛患者施用口服泼尼松龙 0.5 mg/kg/天。<br>• 甲状腺毒症患者应使用 β 受体阻断剂进行治疗，并可能需要使用卡比马唑进行额外治疗。<br>• 对于 3 级或 4 级甲状腺炎，暂停替雷利珠单抗，直至这种情况消退至 0-1 级，然后考虑恢复治疗。<br>• 对于≥3 级和 4 级甲状腺功能减退症或甲状腺功能亢进症，停用 G9.2-17 IgG4 和替雷利珠单抗 |
| 1 型糖尿病 | • 监测患者的高血糖症或其他糖尿病体征和症状。<br>• 就重度(3 级)高血糖症而言，暂停 G9.2-17 IgG4 和替雷利珠单抗，直至实现代谢控制，直至血糖已稳定至基线或 0 至 1 级且患者无高血糖症症状。对于 1 型糖尿病，按照临床指示施用胰岛素。<br>• 当消退至≤2 级时，遵循 1 型糖尿病的 G9.2-17 IgG4 IMAR 指南。对于危及生命的(4 级)高血糖症，永久停用这两种研究药物的治疗。 |
| 免疫介导的肾炎和肾功能障碍<br>(定义为肾功能障碍或≥2 级肌酐升高，需要皮质类固醇，且没有明确的替代病因) | • 在治疗之前和期间定期监测患者的肾功能。如果发生免疫介导的肾炎，应增加肾功能检查的频率。<br>• 对于中度(2 级)或重度(3 级)加重的肾炎，暂停 G9.2-17 IgG4 和替雷利珠单抗。当消退至<2 级时，恢复 G9.2-17 IgG4，剂量减少 50%。如果≥2 级毒性复发，永久停用 G9.2-17 IgG4 和替雷利珠单抗的治疗。<br>• 确保水合作用，中度(2 级)肾炎使用 0.5 至 1 mg/kg/天泼尼松当量的皮质类固醇，或者重度(3 级)肾炎使用 1 至 2 mg/kg/天泼尼松当量的皮质类固醇。<br>• 对于危及生命的(4 级)肾炎以及复发的 3 级肾炎，永久停用 G9.2-17 IgG4 和替雷利珠单抗的治疗。 |
| 免疫介导的皮肤不良反应<br>(替雷利珠单抗可引起免疫介导的皮疹，包括史蒂文斯-约翰逊综合征(SJS)和中毒性表皮坏死松解症(TEN)，一些病例带来致命后果。) | • 对于≤2 级皮疹，治疗可以使用中等强度的局部或口服类固醇加以控制或消退，同时继续使用替雷利珠单抗和 G9.2-17 IgG4。<br>• 监测患者的疑似重度皮肤反应并排除其他原因。<br>• 对于重度(3 级)，暂停这两种药物，直至体征和症状变得轻微；对于中度(2 级)至重度(3 级)皮疹，在口服或 IV 泼尼松龙治疗时没有改善或恶化，永久停用；对于危及生命的(4 级)皮疹，永久停用。<br>• 对于 SJS 或 TEN 的症状或体征，永久停用替雷利珠单抗和 G9.2-17 IgG4，并将患者转到专业护理以进行评估和治疗。 |

[0449]

| IMAR | 管理指南 |
| --- | --- |
| | • 口服泼尼松龙的建议剂量为 0.5 至 1 mg/kg/天，持续 3 天，并在 2 至 4 周内逐渐减量(中度皮疹)；使用甲基泼尼松龙 0.5 至 1 mg/kg/天，转为口服泼尼松龙并在至少 4 周内逐渐减量(重度皮疹)。<br>• 当消退至<2 级时，恢复 G9.2-17 IgG4，剂量减少 50%。 |
| 免疫介导的脑炎 | • 监测神经功能的变化。<br>• 对有神经症状的患者的评价可包括但不限于咨询神经科医生、脑部 MRI 和腰椎穿刺。<br>• 在有新发中度至重度神经体征或症状的患者中暂停 G9.2-17 IgG4 和替雷利珠单抗，并评价以排除感染性或其他中度至重度神经恶化的原因。<br>• 如果排除了其他病因，对患有免疫介导的脑炎的患者施用剂量为 1 至 2 mg/kg/天泼尼松当量的皮质类固醇，随后皮质类固醇逐渐减量。<br>• 对于免疫介导的脑炎，永久停用 G9.2-17 IgG4 和替雷利珠单抗的治疗。 |
| 其他免疫介导的不良反应<br>以下免疫介导的不良反应在不到 1%的接受替雷利珠单抗治疗的患者中发生：关节炎、脑炎、横纹肌溶解症、肌炎、心肌炎、胰腺炎和葡萄膜炎。 | • 对于任何疑似免疫介导的不良反应，排除其他原因。<br>• 基于不良反应的严重程度，永久停止或停用 G9.2-17 IgG4 和替雷利珠单抗、施用高剂量皮质类固醇，且(如果合适)起始激素替代疗法。<br>• 改善至 1 级或以下后，开始皮质类固醇逐渐减量并持续逐渐减量至少 1 个月。<br>• 考虑在基于事件的严重程度完成皮质类固醇减量后重新开始 G9.2-17 IgG4 和替雷利珠单抗。恢复 G9.2-17 IgG4，剂量减少 50%。在停止替雷利珠单抗疗法后可能发生免疫介导的不良反应。 |
| 输注反应<br>(临床试验中小于 1.0%的患者中报告。) | • 使用替雷利珠单抗可能观察到的输注相关反应的症状包括发烧、发冷/寒战、恶心、瘙痒、血管性水肿、低血压、头痛、支气管痉挛、荨麻疹、皮疹、呕吐、肌痛、头晕或高血压。<br>• 输注期间应密切监测患者的这些体征和症状。<br>• 对于 1 级反应，将输注速率降低 50%；对于 2 级反应，停止输注，如果输注相关反应已消退或严重度降低至 1 级，则仅以先前速率的 50%谨慎恢复。对于 3 级或 4 级输注相关反应，停止输注并永久停用替雷利珠单抗。后续输注应在术前用药后以降低的输注速率进行。 |
| 同种异体造血干细胞移植(HSCT)的并发症<br>(用 PD-1 受体阻断抗体 | • 移植相关并发症包括超急性移植物抗宿主病(GVHD)、急性 GVHD、慢性 GVHD、降低强度调节后的肝静脉闭塞病(VOD)和需要类固醇的发热综 |

[0450]

| IMAR | 管理指南 |
| --- | --- |
| 治疗之前或之后接受同种异体 HSCT 的患者中可能发生致命的和其他严重的并发症。) | 合征(没有确定的感染原因)。<br>• 尽管在 PD-1 阻断与同种异体 HSCT 之间进行了干预治疗，但这些并发症仍可能发生。<br>• 密切跟踪患者以寻找移植相关并发症的证据并及时干预。<br>• 考虑在同种异体 HSCT 之前或之后使用 PD-1 受体阻断抗体治疗的益处与风险。 |
| 胚胎-胎儿毒性<br>(基于其作用机制和来自动物研究的数据，当施用于孕妇时，替雷利珠单抗可能引起对胎儿的损害。) | • 告知孕妇对胎儿的潜在风险。<br>• 告知有生育能力的女性在用含替雷利珠单抗方案治疗期间和最后一剂替雷利珠单抗后至少 5 个月使用有效避孕。 |

[0451] 对于联合臂中发生的非IMAR、血液学和非血液学AE,根据因果关系评估:

[0452] ○如果是G9.2-17 IgG4相关,则遵循对于G9.2-17 IgG4的AE管理说明

[0453] ○如果组合药剂相关(替雷利珠单抗),则遵循对于替雷利珠单抗的表7中的管理说明。

[0454] 剂量调节

[0455] 可基于在先前剂量水平的至少2名患者中所获得的安全性、耐受性和初步PK数据,决定进入第1部分中的G9.2-17 IgG4的下一剂量水平。

[0456] 还可以基于获得的PK数据调整给药时间表。详细的剂量调节说明可如表7-9所述获得:

[0457] 表7:针对AE(除IMAR外)的替雷利珠单抗的推荐剂量调节

[0458]

| 不良反应 | 严重度* | 剂量调节 |
| --- | --- | --- |
| AE | 可能相关的 3 级不良事件 | 永久停用 |

[0459]

| 不良反应 | 严重度* | 剂量调节 |
| --- | --- | --- |
| | 危及生命的或 4 级不良反应 | |

[0460] *根据NCI CTCAE V5对毒性进行分级。

[0461] 表8.G9.2-17 IgG4引起的免疫介导的不良反应(IMAR)的管理

[0462]

| IMAR | 管理指南 |
| --- | --- |
| 免疫介导的肺炎 | • 监测患者肺炎的放射影像学成像体征和临床症状。<br>• 对于中度肺炎暂停 G9.2-17 IGG4，且对于重度或危及生命的肺炎永久停用 G9.2-17 IGG4。<br>• 对于中度(2 级)或更严重(3-4 级)肺炎，施用剂量为 1 至 2 mg/kg/天泼尼松当量的皮质类固醇，随后皮质类固醇逐渐减量。<br>• 暂停 G9.2-17 IGG4，直至消退至<2 级。对于中度(2 级)肺炎。恢复治疗，剂量减少 50%。<br>• 在 G9.2-17 IGG4 减少剂量下 2 级肺炎复发后，永久停用 G9.2-17 IGG4。 |
| 免疫介导的结肠炎 | • 监测患者结肠炎的体征和症状。<br>• 对于持续时间超过 5 天的中度(2 级)结肠炎，以 0.5 至 1 mg/kg/天泼尼松等效物的剂量施用皮质类固醇，随后皮质类固醇逐渐减量。<br>• 如果开始使用皮质类固醇后情况恶化或无改善，则将剂量增加至 1 至 2 mg/kg/天泼尼松当量。<br>• 当初始 2 级结肠炎消退至<2 级时，恢复 G9.2-17 IGG4，剂量减少 50%。<br>• 如果≥2 级结肠炎复发，暂停 G9.2-17 IGG4，直至结肠炎消退至<2 级。恢复 G9.2-17 IGG4，剂量再减少 50%。不允许对 G9.2-17 IGG4 进一步减少剂量。<br>• 对于重度(3 级)或危及生命的(4 级)结肠炎，施用剂量为 1 至 2 mg/kg/天泼尼松当量的皮质类固醇，随后皮质类固醇逐渐减量。<br>• 当 3 级结肠炎消退至≤2 级时，恢复 G9.2-17 IGG4，剂量减少 50%。<br>• 在重新开始使用 G9.2-17 IGG4 后，对于危及生命的(4 级)或对于≥2 级的复发性结肠炎，永久停用 G9.2-17 IGG4。 |
| 免疫介导的肝炎 | • 监测肝功能的变化。<br>• 对于中度(2 级)转氨酶升高，施用剂量为 0.5 至 1 mg/kg/天泼尼松当量的皮质类固醇。<br>• 对于中度(2 级)免疫介导的肝炎，暂停 G9.2-17 IGG4。当消退至<2 级时，恢复 G9.2-17 IGG4，剂量 |

[0463]

| IMAR | 管理指南 |
| --- | --- |
| | 减少 50%。<br>• 如果≥2 级肝炎复发，永久停用 G9.2-17 IGG4。<br>• 对于重度(3 级)或危及生命的(4 级)转氨酶升高，伴有或不伴有总胆红素升高，施用剂量为 1 至 2 mg/kg/天泼尼松当量的皮质类固醇，随后皮质类固醇逐渐减量。<br>• 对于重度(3 级)或危及生命的(4 级)免疫介导的肝炎，永久停用 G9.2-17 IGG4。 |
| 免疫介导的内分泌病:<br>垂体炎 | • 对于中度(2 级)或更严重的垂体炎，按照临床指示施用激素替代并且以 1 mg/kg/天泼尼松等效物的剂量施用皮质类固醇，随后皮质类固醇逐渐减量。<br>• 对于中等(2 级)或严重的(3 级)停用 G9.2-17 IG4，且当消退至<2 级时，恢复 G9.2-17 IGG4，剂量减少 50%。<br>• 如果≥2 级毒性复发，则永久停用 G9.2-17 IGG4。<br>• 对于危及生命的(4 级)垂体炎，永久停用 G9.2-17 IGG4。 |
| 肾上腺功能不全 | • 监测患者肾上腺功能不全的体征和症状。<br>• 对于中度(2 级)，暂停 G9.2-17 IGG4，且消退至<2 级后，恢复 G9.2-17 IGG4，剂量减少 50%。<br>• 如果≥2 级毒性复发，则永久停用 G9.2-17 IGG4。<br>• 对于重度(3 级)或危及生命的(4 级)肾上腺功能不全，施用剂量为 1 至 2 mg/kg/天泼尼松当量的皮质类固醇，随后皮质类固醇逐渐减量。<br>• 在重新开始使用 G9.2-17 IGG4 后，对于重度(3 级)或危及生命的(4 级)肾上腺功能不全，或≥2 级的复发性结肠炎，永久停用 G9.2-17 IGG4。 |
| 甲状腺功能减退症和甲状腺功能亢进症 | • 在纳武单抗治疗之前和期间定期监测甲状腺功能。<br>• 对甲状腺功能减退症施用激素替代疗法。<br>• 开始医疗管理以控制甲状腺功能亢进症。<br>• 对于<3 级甲状腺功能减退症或甲状腺功能亢进症，没有 G9.2-17 IGG4 建议剂量调节。<br>• 对于≥ 3 级甲状腺功能减退症或甲状腺功能亢进症，停用 G9.2-17 IGG4 |
| 1 型糖尿病 | • 就重度(3 级)高血糖症而言，暂停 G9.2-17 IGG4，直至达到代谢控制。<br>• 消退至≤2 级后，恢复 G9.2-17 IGG4，剂量减少 50%。<br>• 如果≥2 级毒性复发，则永久停用 G9.2-17 IGG4。<br>• 对于危及生命的(4 级)高血糖症，永久停用 G9.2-17 IGG4。 |
| 免疫介导的肾炎和肾功能障碍 | • 在治疗前和治疗期间定期监测患者的血清肌酐升高。 |

[0464]

| IMAR | 管理指南 |
| --- | --- |
| (定义为肾功能障碍或≥2级肌酐升高，需要皮质类固醇，且没有明确的替代病因) | • 对于中度(2级)或重度(3级)血清肌酐升高，施用剂量为0.5至1 mg/kg/天泼尼松当量的皮质类固醇，如果情况恶化或无改善，则将皮质类固醇的剂量增加至1至2 mg/kg/天泼尼松当量。<br>• 对于中度(2级)或重度(3级)血清肌酐升高，暂停G9.2-17 IGG4。当消退至<2级时，恢复G9.2-17 IGG4，剂量减少50%。<br>• 如果≥2级毒性复发，永久停用G9.2-17 IGG4。<br>• 对于危及生命的(4级)血清肌酐升高，施用剂量为1至2 mg/kg/天泼尼松当量的皮质类固醇，随后皮质类固醇逐渐减量。<br>• 对于危及生命的(4级)血清肌酐升高，永久停用G9.2-17 IGG4。 |
| 免疫介导的皮肤不良反应<br>(免疫介导的皮疹，包括史蒂文斯-约翰逊综合征(SJS)和中毒性表皮坏死松解症(TEN)) | • 对于重度皮疹，暂停，对于危及生命的皮疹，永久停用。<br>• 对于SJS或TEN的症状或体征，暂停G9.2-17 IGG4，并将患者转到专业护理以进行评估和治疗。<br>• 如果确认SJS或TEN，永久停用G9.2-17 IGG4。<br>• 对于≥2级免疫介导的皮疹，施用剂量为1至2 mg/kg/天泼尼松当量的皮质类固醇，随后皮质类固醇逐渐减量，适用于重度(3级)或危及生命的(4级)皮疹。<br>• 对于中度(2级)和重度(3级)皮疹，暂停G9.2-17 IGG4，且当消退至<2级时，恢复G9.2-17 IGG4，剂量减少50%。<br>• 如果≥2级皮疹复发，暂停G9.2-17 IGG4，直至消退至<2级，恢复G9.2-17 IGG4，剂量再减少50%。不允许进一步减少剂量。<br>• 对于危及生命的(4级)皮疹，永久停用G9.2-17 IGG4。 |
| 免疫介导的脑炎 | • 监测神经功能的变化。<br>• 对有神经症状的患者的评价可包括但不限于咨询神经科医生、脑部MRI和腰椎穿刺。<br>• 在有新发中度至重度神经体征或症状的患者中暂停G9.2-17 IGG4，并评价以排除感染性或其他中度至重度神经恶化的原因。<br>• 如果排除了其他病因，对患有免疫介导的脑炎的患者施用剂量为1至2 mg/kg/天泼尼松当量的皮质类固醇，随后皮质类固醇逐渐减量。<br>• 对于免疫介导的脑炎，永久停用G9.2-17 IGG4。 |
| 其他免疫介导的不良反应<br>(可包括：心肌炎、横 | • 如果葡萄膜炎与其他免疫介导的不良反应同时发生，应考虑为伏格特-小柳-原田(Vogt-Koyanagi-Harada)样综合征，并且可能需要全身 |

| IMAR | 管理指南 |
| --- | --- |
| 纹肌溶解症、肌炎、葡萄膜炎、虹膜炎、胰腺炎、面神经和外展神经麻痹、脱髓鞘、风湿性多肌痛、自身免疫性神经病变、格-巴二氏综合征、垂体机能减退症、全身性炎症反应综合征、胃炎、十二指肠炎、结节病、组织细胞坏死性淋巴结炎(菊池淋巴结炎)、运动功能障碍、血管炎、再生障碍性贫血、心包炎和肌无力综合征。) | 性类固醇治疗以降低永久性视力丧失的风险。<br>• 对于任何疑似免疫介导的不良反应，排除其他原因。<br>• 基于不良反应的严重度(等级)，永久停用或暂停G9.2-17 IGG4，施用高剂量皮质类固醇，并在适当时开始激素替代疗法。<br>• 改善至 1 级或以下后，开始皮质类固醇逐渐减量并持续逐渐减量至少 1 个月。<br>• 基于事件的严重度，考虑在皮质类固醇逐渐减量完成后重新开始 G9.2-17 IGG4，以 50%的剂量减少恢复。 |

[0465]

[0466] 表9:G9.2-17 IgG4的推荐剂量调节(DLT窗口外的AE且除IMAR外)

| 不良反应 | 严重程度 | G9.2-17 IgG4 剂量调节 |
| --- | --- | --- |
| 非血液学和血液学毒性 | ≥4 级 | • 永久停止用 G9.2-17 IgG4 治疗。 |
| 血液学毒性 | 3 级 | • 暂停治疗，直至毒性消退至≤2 级。<br>• 恢复 G9.2-17 IgG4，比前一剂量减少 50%。<br>• 如果再次复发，严重程度≥3 级，则保持直至消退至≤2 级，并进一步实施50%剂量减少和医疗监测。<br>• 不允许进一步减少剂量。 |
| 非血液学毒性(**表 8** 所列的那些除外，G9.2-17 IgG4 引起的免疫介导的不良反应(IMAR)的管理) | 3 级 | • 暂停治疗，直至毒性消退至≤2 级。<br>• 恢复 G9.2-17 IgG4，比前一剂量减少 50%。<br>• 如果再次复发，严重程度≥3 级，则保持直至消退至≤2 级，并进一步实施50%剂量减少和医疗监测。<br>• 不允许进一步减少剂量。 |
| 胃肠(恶心、呕吐) | 3 级<br>不需要住院或肠外营养支持；通过支持性护理进行管理 | • 暂停 G9.2-17 IgG4 直到毒性消退至≤2 级<br>• 恢复 G9.2-17 IgG4，且无剂量减少 |

[0467]

[0468]

| 不良反应 | 严重程度 | G9.2-17 IgG4 剂量调节 |
|---|---|---|
| 电解质异常 | 3 级，在 24 小时内纠正为≤2 级 | • 如果复发，则暂停 G9.2-17 IgG4 直到毒性消退至≤2 级<br>• 随后恢复 G9.2-17 IgG4，比前一剂量减少 50%<br>• 如果复发，则暂停 G9.2-17 IgG4 直到毒性消退至≤2 级<br>• 恢复 G9.2-17 IgG4，剂量再降低 50%<br>• 不允许进一步减少剂量 |
| 电解质异常 | ≥3 级持续<24-72 小时临床上不复杂；自发消退或对常规医疗干预有响应 | |
| 淀粉酶或脂肪酶升高 | ≥3 级<br>与胰腺炎的症状或临床表现无关 | |

[0469] 剂量施用和剂量延迟

[0470] 如遇到输注相关反应,中断输注,且如有临床指征,施用相关药物(例如,抗组胺药、止吐药、类固醇、退热药、β受体阻断剂等)。如果认为恢复输注是适当的,则以较慢的输注速率恢复。

[0471] 对于同一患者的后续周期,应用适当的术前药物(按照临床指示需要,抗组胺药、止吐药、类固醇、退热药、β受体阻断剂等)并考虑采用较慢的输注速率。

[0472] 如果发生任何可能与一种或多种研究药物相关或与一种或多种研究药物相关的临床意义AE≥3级,则将在继续给药前与医学监查员讨论。对于≥3级AE,可能必需剂量延迟。

[0473] 剂量减少

[0474] 对于正在评价DLT(在28天DLT窗口内)的任何患者,不可允许剂量减少。如果必需剂量减少,则研究干预将按如下方式施用:

[0475] 对于第1部分和第2部分,单独的G9.2-17 IgG4患者:当评估得出临床受益并可继续在剂量减少条件下获得时,可允许剂量减少,参见表8(对于IMAR)或表9(对于其他AE)。

[0476] 对于G9.2-17 IgG4或G9.2-17 IgG4替雷利珠单抗的第2部分组合治疗群组,源自经批准药物的临床试验的经验(如经批准的产品标签中所总结)将告知不良事件管理,包括延迟、降低剂量和/或完全停用替雷利珠单抗的指南,参见表6-7和9。

[0477] 与替雷利珠单抗的施用相关的特定AE的剂量调节

[0478] 表6(对于IMAR)和表7(对于其他AE)中提供了基于特定AE的替雷利珠单抗调节的建议。

[0479] 针对IMAR的剂量调节

[0480] 如果发生IMAR,则对G9.2-17 IgG4和/或替雷利珠单抗的剂量管理指南参见表8和表6。进行所有相关医学检查/测试以便确认不良事件为IMAR。

[0481] 研究干预的停止

[0482] 在极少数情况下,患者可能有必要永久停止研究干预。如果因疾病进展以外的原因永久停止研究干预,且患者未接受其他抗癌疗法治疗,则患者将继续评估疾病进展长达2年。有关停止研究干预和随访时收集的数据以及需要完成的任何进一步评价,参见评估时间表。

[0483] 研究人员必须尽一切努力保持患者接受研究治疗,直到满足研究治疗终止的原因

之一(疾病进展、与研究药物相关的毒性、撤回同意书)。如果患者有影像学进展但无明确临床进展,且未开始替代治疗,则患者可继续研究治疗。然而,如果患者具有明确的临床进展而无影像学进展,则应停止研究治疗,并为患者建议可用的治疗选项。

[0484] 患者可因以下原因之一在疾病进展前停止:

[0485] • 根据本文提供的定义的DLT

[0486] • 需要停止一种或多种研究治疗的AE在DLT窗口外发生/复发

[0487] • 需要停止一种或多种研究治疗的IMAR发生/复发

[0488] • 妨碍进一步施用治疗或如果继续接受研究治疗可能危及患者安全的并发疾病或医学疾患

[0489] • 怀孕

[0490] • 使用非方案抗癌疗法

[0491] 患者也可因以下原因之一在疾病进展前停止:

[0492] • 患者部分显著偏离方案(包括缺乏依从性)

[0493] 伴随疗法

[0494] 参与者在纳入时正在接受的或在研究期间接受的任何药物或疫苗(包括非处方药或处方药、消遣性药物、维生素和/或草药补充剂)必须连同以下信息一起记录:

[0495] • 使用原因

[0496] • 施用日期,包括开始和结束日期

[0497] • 剂量信息,包括剂量和频率

[0498] 准许药物

[0499] 允许使用以下伴随用药:

[0500] • 接受联合治疗方案的患者的任何护理标准术前用药。

[0501] • 对于在治疗(C1D1)之前已经稳定至少6个月的骨转移,继续使用双膦酸盐治疗(例如,唑仑膦酸)或狄诺塞麦,

[0502] • 吸入性皮质类固醇和盐皮质激素(例如,氟氢可的松)、局部类固醇、鼻内类固醇、关节内类固醇和眼科类固醇的用途

[0503] • 抗凝剂的预防或治疗用途\

[0504] • 允许在研究期之前或期间接种COVID-19、普通流感和/或其他常见临床所需适应症(例如破伤风、肺炎球菌、HBV等)的疫苗。必须记录疫苗接种的时间和类型。

[0505] 违禁药物

[0506] 在本研究中不允许使用以下药物:

[0507] • 为任何适应症伴随施用其他研究剂,G9.2-17 IGG4除外

[0508] • 全身性免疫抑制治疗,包括但不限于环磷酰胺、硫唑嘌呤、甲氨蝶呤、沙利度胺和抗TNF剂。但是,允许患者服用急性、低剂量的全身性免疫抑制药物(例如,≤10mg/天的泼尼松或等效物)。

[0509] • 替代疗法(例如,用于肾上腺或垂体功能不全的甲状腺素、胰岛素、生理性皮质类固醇替代疗法[例如,≤10mg/天的泼尼松等效物])不被视为全身性治疗的形式。

[0510] 功效评估

[0511] 评估时间表的表中提供了所有功效评估的计划时间点。

[0512] RECIST v1.1肿瘤评估标准

[0513] 在筛选肿瘤评估时,将肿瘤病灶/淋巴结分类为可测量或不可测量的,其中根据测量平面中的最长直径记录可测量肿瘤病灶(病理淋巴结除外,其在最短轴测量)。当在筛选时存在多于一个可测量的病灶时,代表所有受累器官的最多总共五个病灶(以及每个器官最多两个病灶)的所有病灶都应被鉴定为靶病灶。应基于其大小来选择靶病灶(具有最长直径的病灶)。可以计算所有靶病灶的直径总和,并报告为基线直径总和。

[0514] 包括病理性淋巴结的所有其他病灶(或疾病部位)均应被鉴定为非靶病灶,并且也应在筛选时进行记录。不需要测量,并且这些病灶应作为“存在”、“不存在”或“明确进展”跟踪。

[0515] 根据RECIST v1.1指南(Eisenhauer等人,2009),使用以下疾病响应测量来评估肿瘤靶病灶。

[0516] • 完全响应(CR):所有靶病灶消失。任何病理性淋巴结(无论是靶标还是非靶标)的短轴必须减小到<10mm。

[0517] • 部分响应(PR):以基线直径总和为参考,靶病灶直径总和至少减少30%。

[0518] • 稳定疾病(SD):在进行研究中,将最小直径总和作为参考,既没有足够的收缩来符合PR,也没有足够的增加来符合PD。

[0519] • 进展性疾病:以研究中最小总和作为参考(如果在研究中最小,则包括基线总和),靶病灶直径总和至少增加20%。除20%的相对增加以外,总和还必须证明至少5mm的绝对增加。(注释:一个或多个新病灶的出现也被认为是进展)。

[0520] 以下指南可用于评价非靶病灶。也参见下表10。

[0521] • 完全响应(CR):所有非靶标病灶消失且肿瘤标志物水平正常化。所有淋巴结的大小必须是非病理性的(<10mm短轴)。

[0522] • 非CR/非PD:一个或多个非靶标病灶持续存在和/或肿瘤标志物水平维持在正常范围之上。

[0523] • 进展性疾病(PD):现有非靶标病灶的明确进展。(注释:一个或多个新病灶的出现也被认为是进展)。

[0524] 不同时间点的疾病响应量度将允许计算以下内容:

[0525] • 疾病控制率(DCR),定义为达到CR、PR和SD的患者的百分比。

[0526] • 客观响应率(ORR),定义为肿瘤大小缩小到预定量(肿瘤收缩≥30%)的患者的比例。

[0527] • 无进展存活期(PFS),定义为从研究药物治疗开始到疾病进展(肿瘤生长≥30%)的时间。

[0528] • 响应持续时间(DoR),定义为肿瘤继续对治疗有响应而不发生癌症生长或扩散的时间长度。

[0529] • 总存活期(OS)被定义为从研究药物治疗开始到因任何原因死亡的时间。

[0530] 表10.基线时患有可测量疾病的患者的总时间点响应的评价

[0531]

| 靶标病灶 | 非靶病灶 | 新病灶 | 总体响应 |
|---|---|---|---|
| CR | CR | 否 | CR |
| CR | 非CR/非PD | 否 | PR |

| | | | |
|---|---|---|---|
| CR | NE | 否 | PR |
| PR | 非PD或NE | 否 | PR |
| SD | 非PD或NE | 否 | SD |
| 未全部评价 | 非PD | 否 | NE |
| 进展性疾病 | 任何 | 是或否 | 进展性疾病 |
| 任何 | 进展性疾病 | 是或否 | 进展性疾病 |
| 任何 | 任何 | 是 | 进展性疾病 |

[0532] CR:完全响应,非PD:非进展性疾病,PR:部分响应,SD:稳定疾病,NE:不可评价

[0533] *当靶标病灶显示SD/PR而非靶标病灶的某些子集不可评价时,必须谨慎决定是将此时间点的总体响应称为SD/PR还是NE。这是基于不可评价的病灶(如果它们显示生长)是否会在所见的其他病灶响应的背景下引起进展性疾病的总体响应。如果不可评价的非靶病灶占总体疾病负担的很大比例,则适当的时间点响应是NE。

[0534] 不良事件管理

[0535] AE可能在第一剂研究药物施用前未记录。将记录在研究药物施用后开始的AE或在研究药物施用后恶化的与医学史相关的症状。应跟踪AE,直至它们消退、恢复至基线或被确定为稳定或慢性疾患。收集所有SAE,直到最后一剂研究药物后30天

[0536] 免疫介导的不良反应

[0537] 鉴定替雷利珠单抗的免疫介导的不良反应(IMAR)。

[0538] 所指出的具体IMAR是:

[0539] • 免疫介导的肝炎

[0540] • 免疫介导的肾炎

[0541] • 免疫介导的肺炎

[0542] • 免疫介导的肺炎

[0543] • 免疫介导的结肠炎和腹泻免疫介导的内分泌病

[0544] • 免疫介导的皮肤反应

[0545] • 其他免疫介导的不良反应:关节炎、脑炎、横纹肌溶解、肌炎、心肌炎、胰腺炎和葡萄膜炎。

[0546] 监测计划旨在限制联合药物开发期间发生的IMAR的严重程度和持续时间,并包括:在预定访视中进行体检、生命体征、安全性实验室评估(包括血液学、生物化学),在新的给药周期的每个第1天(给药前)评估内分泌功能,评估凝血状态和尿液分析。评估时间表(参见实施例1)还涵盖每三个月一次评估射血分数并进行定期ECG。

[0547] 将进行所有相关医学检查/测试以便确认不良事件为IMAR。

[0548] 这些IMAR的管理的说明包括在表8App4(对于单独G9.2-17IgG4)和表6(对于G9.2-17 IgG4+替雷利珠单抗组合治疗)中。

[0549] 等效方案

[0550] 从以上描述中,本领域技术人员可容易地确定本发明的基本特征,并且在不背离本发明精神和范围的前提下,可对本发明做各种变化和修改以使其适应于各种用途和条件。因此,其他实施方案也在权利要求范围内。

[0551] 虽然本文已描述和说明了若干发明实施方案,但是本领域普通技术人员将容易想

到用于执行本文功能和/或获得结果和/或本文所述的一个或多个优点的多种其他手段和/或结构,并且每个此类变化和/或修改被认为是在本文所述的本发明实施方案的范围内。更一般地,本领域技术人员将容易地理解,本文所述的所有参数、尺寸、材料和构造都意味着是示例性的,并且实际的参数、尺寸、材料和/或构造取决于使用本发明的教导的一种或多种具体应用。本领域技术人员认识到或能够仅使用常规实验来确定本文所述的具体发明实施方案的许多等效方案。因此,应当理解,前述实施方案仅以示例的方式呈现,并且在所附权利要求及其等效物的范围内,可以不同于具体描述和要求保护的方式实施发明实施方案。本公开的发明实施方案针对本文所述的每个单独的特征、系统、物品、材料、试剂盒和/或方法。另外,如果此类特征、系统、物品、材料、试剂盒和/或方法不相互矛盾,则两个或更多个此类特征、系统、物品、材料、试剂盒和/或方法的任何组合包括在本公开的发明范围内。

[0552] 如本文所定义和使用的所有定义,应理解为优先于字典定义、以引用的方式并入的文件中的定义和/或所定义术语的普通含义。

[0553] 本文公开的所有参考文献、专利和专利申请均以引用的方式并入每一个所引用的主题,在某些情况下,可涵盖整个文件。

[0554] 除非有相反的明确指示,否则如本文在说明书和权利要求中所用,不定冠词“一种/一个(a)”和“一种/一个(an)”,应当被理解为意指“至少一个/一种”。

[0555] 如本文在说明书和权利要求中所用,短语“和/或”应当被理解为意指所连接的元素的“任一个或两者”,即元素在一些情况下结合地存在而在其他情况下分离地存在。用“和/或”列出的多个要素应以相同的方式解释,即如此结合的“一个或多个”要素。除通过“和/或”从句具体标识的元素外,还可任选地存在其他元素,无论与具体标识的那些元素相关还是无关。因此,作为非限定性实例,对“A和/或B”的提及,当与开放性措辞诸如“包含/包括”结合使用时,在一个实施方案中可仅指A(任选地包括除B外的元素);在另一个实施方案中可仅指B(任选地包括除A外的元素);在另一个实施方案中,可指A和B(任选地包括其他元素);等等。

[0556] 如本文在说明书和权利要求中所用,“或”应当被理解为具有与如上定义的“和/或”相同的含义。例如,当分隔列表中的项目时,“或”或“和/或”应被解释为包含性的,即,包含至少一个,但也包含超过一个、多个或一系列要素,以及任选地另外的未列出的项目。只有明确地指明相反的术语,诸如“……中的仅一个”或“……中的恰好一个”或当用于权利要求中时的“由……组成”将指包含多个元素或元素列表中的恰好一个元素。一般而言,如本文所用的术语“或”当前面是排他性术语(如“任一个”、“……中的一个”、“……中的仅一个”或“……中的恰好一个”)时,仅应被解释为表示排他性的替代方案(即,“一个或另一个,但不是两个”)。“基本上由……组成”,当用于权利要求中时,应当具有其在专利法领域中使用的普通含义。

[0557] 如本文在说明书和权利要求中所用,关于一个或多个元素的列表的短语“至少一个”应当被理解为意指选自元素列表中的任一个或多个元素的至少一个元素,但不一定包括元素列表内具体列出的每一和每个元素的至少一个并且不排除元素列表中的元素的任何组合。该定义还允许可任选地存在除短语“至少一个”所指的元素列表内具体标识的元素外的元素,无论与那些具体标识的元素相关还是不相关。因此,作为非限定性实例,“A和B的

至少一个”(或,等同地,“A或B的至少一个”,或等同地,“A和/或B的至少一个”)可在一个实施方案中指至少一个(任选地包括多于一个)A而无B存在(且任选地包括除B外的元素);在另一个实施方案中指至少一个(任选地包括多于一个)B而无A存在(且任选地包括除A外的元素);在另一个实施方案中指至少一个(任选地包括多于一个)A和至少一个(任选地包括多于一个)B(且任选地包括其他元素);等等。

[0558] 还应当理解,除非有相反的明确指示,否则在包括多于一个步骤或行为的本文要求保护的任何方法的步骤或行为的顺序不一定限定于叙述所述方法的步骤或行为的顺序。

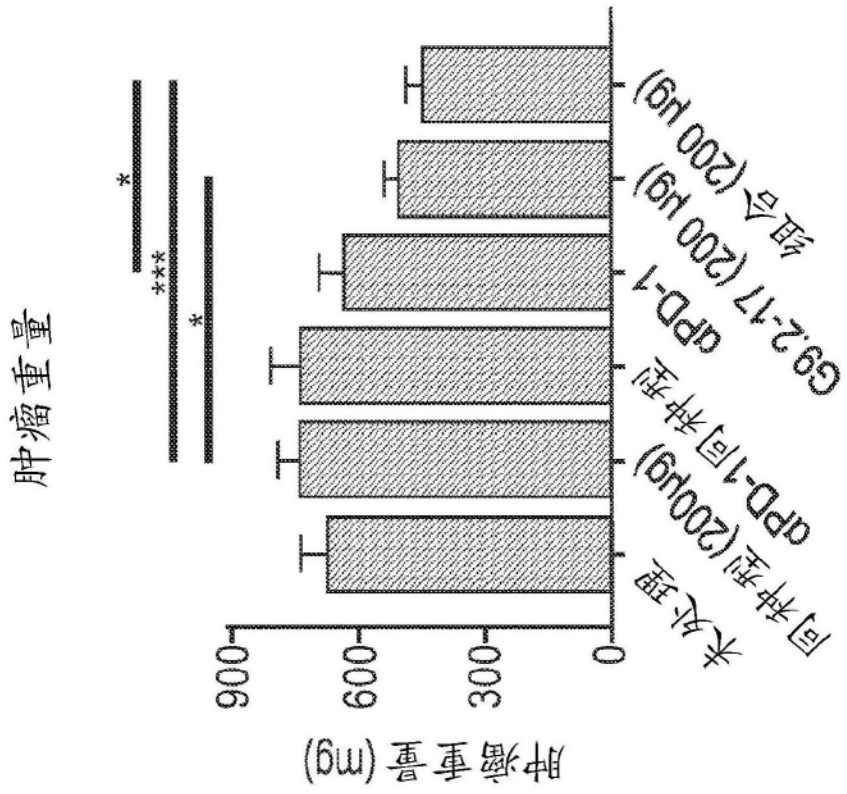

肿瘤重量
*
***
*
未处理
同种型(200μg)
αPD-1同种型
αPD-1
G9.2-17 (200 μg)
组合 (200 μg)
0
300
600
900
肿瘤重量 (mg)

图1

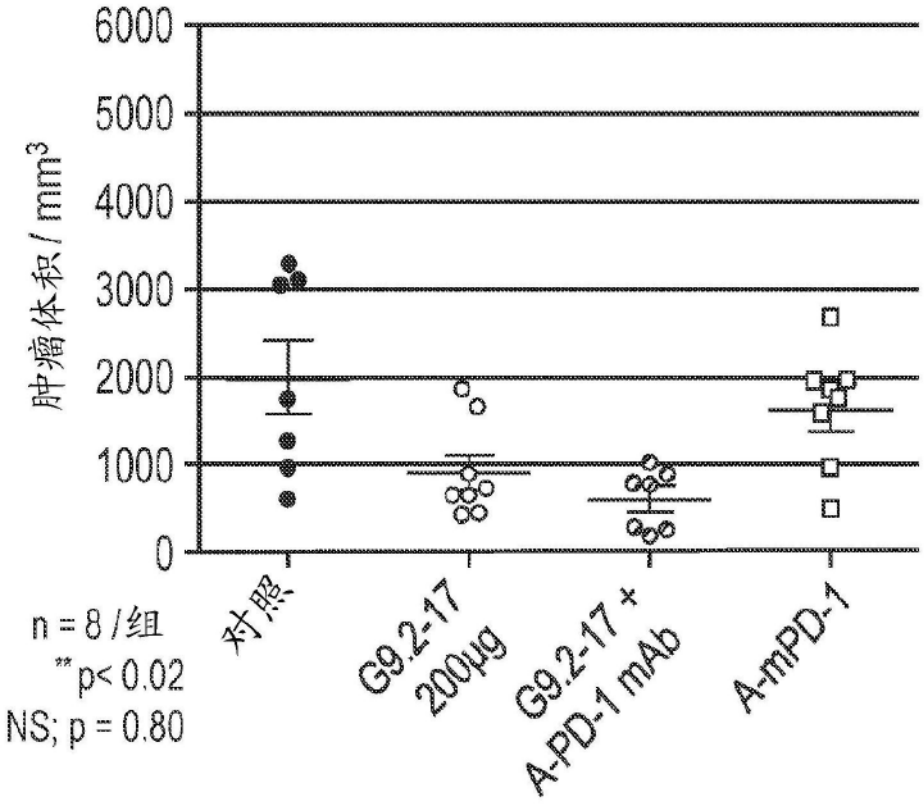

肿瘤体积 / mm3
6000
5000
4000
3000
2000
1000
0
n = 8 /组
** p< 0.02
NS; p = 0.80
对照
G9.2-17 200μg
G9.2-17 + A-PD-1 mAb
A-mPD-1

图2A

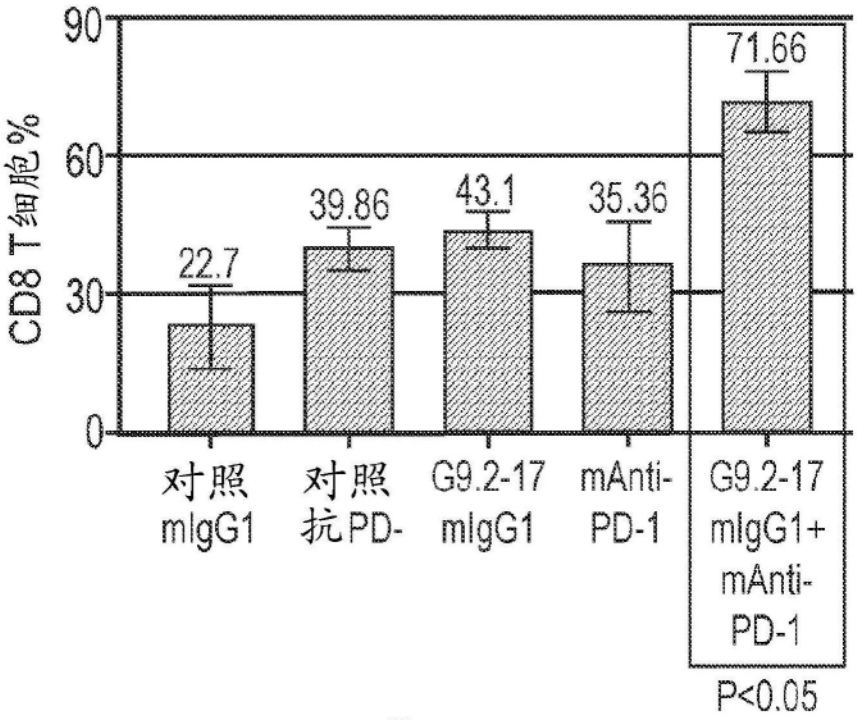

CD8 T细胞%
90
60
30
0
22.7
39.86
43.1
35.36
71.66
对照 mIgG1
对照 抗PD-
G9.2-17 mIgG1
mAnti-PD-1
G9.2-17 mIgG1+ mAnti-PD-1
P<0.05

图2B

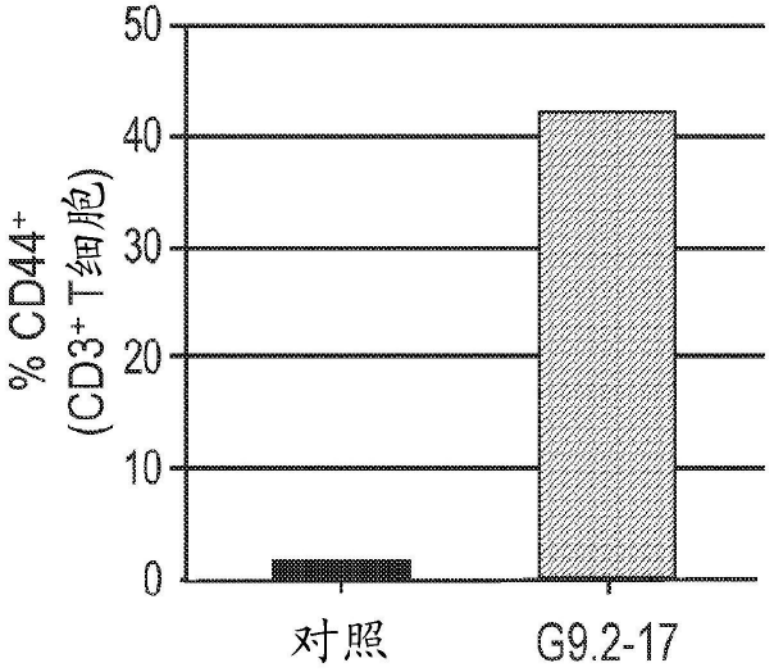

% CD44+ (CD3+ T细胞)
50
40
30
20
10
0
对照
G9.2-17

图3A

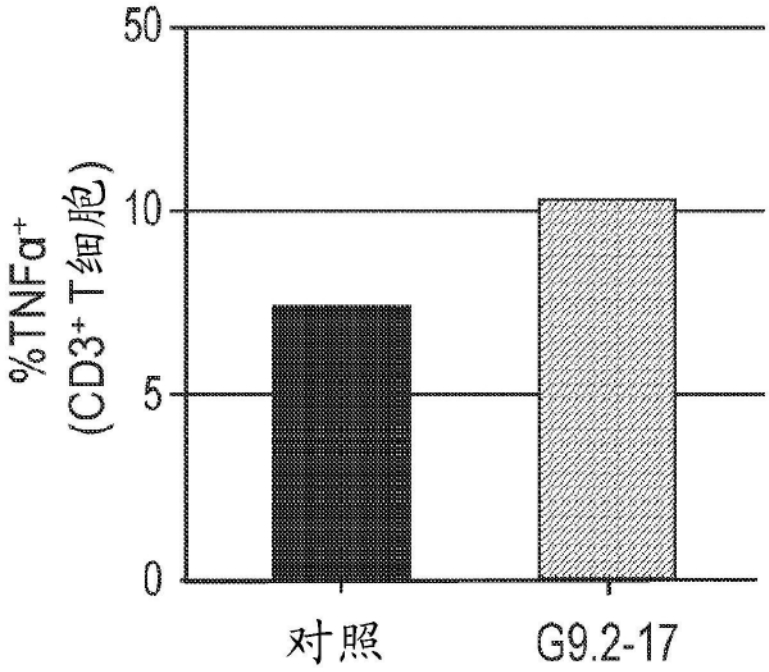



图3B