

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 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 (PDA), 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 CCL22 (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).

Accordingly, modulating the activity of Galectin-9 and/or one or more of its receptors may provide a novel cancer therapy approach, alone or in combination with existing therapies. Described herein are novel human antibodies which bind to human Galectin-9 and their therapeutic use in the treatment of cancer.

SUMMARY

The present disclosure is based, at least in part, on the development of anti-Galectin-9 antibodies that potently suppress signaling triggered by Galectin-9. Such antibodies are capable of suppressing Galectin-9 signaling and/or eliminating Galectin-9 positive pathologic cells, thereby benefiting treatment of diseases associated with Galectin-9.

In one aspect, the present disclosure refers to an isolated antibody, which binds human galectin-9, wherein the antibody comprises a heavy chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 361, a heavy chain complementary determining region 2

(CDR2) set forth as SEQ ID NO: 388, and a heavy chain complementary determining region 3 (CDR3) set forth as SEQ ID NO: 406 and/or comprises a light chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 328, a light chain complementary determining region 2 (CDR2) set forth as SEQ ID NO: 329, and a light chain complementary determining region 3 (CDR3) set forth as SEQ ID NO: 352.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to the use of the isolated antibody as described herein in the manufacture of a pharmaceutical composition for inhibiting the activity of galectin-9 in a solid tumor in a subject. In still another aspect, the present disclosure refers to the use of the isolated antibody as described herein in the manufacture of a pharmaceutical composition for treating a solid tumor that expresses galectin-9 in a human subject. In yet another aspect, the present disclosure refers to the use of the isolated antibody as described herein and a checkpoint inhibitor in the manufacture of a pharmaceutical composition for combination therapy for inhibiting the activity of galectin-9 or for treating a solid tumor in a subject.

Accordingly, one example of the present disclosure provides an isolated anti-Galectin-9 antibody, which binds to an epitope in a carbohydrate recognition domain (CRD) of a Galectin-9 polypeptide, for example, a human Galectin-9 polypeptide. In some examples, the anti-Galectin-9 antibody described herein may bind to both a human Galectin-9 polypeptide and a non-human Galectin-9 polypeptide (e.g., a mouse Galectin-9, a rat Galectin-9, or a primate Galectin-9). In some examples, the anti-Galectin-9 antibody binds exclusively to one of the Galectin-9 CRDs. In some examples, the anti-Galectin-9 antibody binds to both of the Galectin-9 CRDs, e.g., with similar or different affinities. In some examples, the anti-Galectin-9 antibody disclosed herein binds an epitope within the CRD1 region. In some examples, the anti-Galectin-9 antibody disclosed herein binds an epitope within the CRD1 region, which CRD1 region may have the amino acid sequence of SEQ ID NO: 3. In some examples, the anti-Galectin-9 antibody disclosed herein binds an epitope within the CRD1 region having the amino acid sequence of SEQ ID NO: 3. In some examples, the anti-Galectin-9 antibody binds to the same epitope as a reference antibody selected from the group consisting of G9.1-1, G9.1-2, G9.1-3, G9.1-4, G9.1-5, G9.1-6, G9.1-7, G9.1-8, G9.1-8m1, G9.1-8m2, G9.1-8m3, G9.1-8m4, G9.1-8m5, G9.1-8m6, G9.1-8m7, G9.1-8m8, G9.1-8m9, G9.1-8m10, G9.1-8m11, G9.1-8m12, G9.1-8m13, G9.1-8m14, G9.1-9, G9.1-10, and G9.1-11 antibodies, and/or competes against the reference antibody from binding to the CRD1 region. In some examples, the anti-Galectin-9

antibody, and harvesting the antibody thus produced from the cell culture (*e.g.*, from the culture medium).

Further, the present disclosure provides a pharmaceutical composition, comprising any of the anti-Galectin-9 antibodies or a nucleic acid(s) encoding such, and a pharmaceutically acceptable carrier.

In yet another example, the present disclosure features a method of inhibiting Galectin-9-mediated cell signaling in a subject, the method comprising administering to a subject in need thereof an effective amount of an anti-Galectin-9 antibody or a pharmaceutical composition comprising an anti-Galectin-9 antibody. In some examples, the anti-Galectin-9 antibody is any of the anti-Galectin-9 antibodies disclosed herein or a pharmaceutical composition comprising such. In some examples, the subject in need thereof is a human patient having, suspected of having, or at risk for having, an autoimmune disease, a solid cancer, a microbial disease, a hematological malignancy, or an allergic disorder. Exemplary autoimmune diseases include, but are not limited to, a rheumatoid condition (*e.g.*, rheumatoid arthritis), an autoimmune respiratory disease, an autoimmune metabolic and/or endocrine disorder (*e.g.*, type I diabetes), or a fibrotic condition. Exemplary solid tumors include, but are not limited to, pancreatic ductal adenocarcinoma (PDA), colorectal cancer (CRC), melanoma, cholangiocarcinoma, breast cancer, small cell and non small cell lung cancer, upper and lower gastrointestinal malignancies, gastric cancer, squamous cell head and neck cancer, genitourinary cancer, hepatocellular carcinoma, ovarian cancer, sarcomas, mesothelioma, glioblastoma, esophageal cancer, bladder cancer, urothelial cancer, renal cancer, cervical and endometrial cancer. Exemplary hematological malignancies include, but are not limited to, acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphomas, multiple myeloma, and acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndromes, or myeloproliferative neoplasms and other myeloproliferative and myelodysplastic disorders. In some examples, the effective amount of the pharmaceutical composition is sufficient to block interaction between Galectin-9 and Dectin-1. In some examples, the effective amount of the pharmaceutical composition is sufficient to block interaction between Galectin-9 and CD206. Alternatively, or in addition, but not limited to, the effective amount of the pharmaceutical composition is sufficient to block interaction between Galectin-9 and Tim-3.

Further, the present disclosure provides a method for modifying, eliminating and/or reducing pathologic cells expressing Galectin-9 (*e.g.*, via antibody-dependent cell cytotoxicity or ADCC), the method comprising administering to a subject having pathologic cells expressing Galectin-9 an effective amount of an anti-Galectin-9 antibody, such as any of the anti-Galectin-9

SEQ ID NO:2, and the CRD2 domain (SEQ ID NO: 6) spans residues 226-353 of SEQ ID NO: 2.

Galectin-9 polypeptides from other species are known in the art and can be obtained from publicly available gene database, for example, GenBank, using either the human sequence or the mouse sequence as a query. The CRD1 and CRD2 domains of a Galectin-9 polypeptide can be identified by aligning the sequence of that Galectin-9 polypeptide with that of the human or mouse Galectin-9 as described herein.

The antibodies described herein bind Galectin-9 or a fragment thereof (*e.g.*, CRD1 or CRD2). As used herein, the term “anti-Galectin-9 antibody” refers to any antibody capable of binding to a Galectin-9 polypeptide, which can be of a suitable source, for example, human or a non-human mammal (*e.g.*, mouse, rat, rabbit, primate such as monkey, etc.). In some embodiments, the anti-Galectin-9 antibody can be used therapeutically to suppress the bioactivity of Galectin-9. In some embodiments, the anti-Galectin-9 antibody may be used in research or may be used in diagnostic/prognostic methods, *e.g.*, for the detection of cells expressing Galectin-9 in an assessment of treatment eligibility and/or efficacy. Alternatively, or in addition, an anti-Galectin-9 antibody may block the interaction between Galectin-9 and its ligand (*e.g.*, Dectin-1, TIM-3), thereby suppressing the signaling pathway triggered by, for example, the Galectin-9/Dectin-1 or Galectin-9/TIM-3 interaction. An anti-Galectin-9 antibody may also elicit the death of cells expressing Galectin-9, for example, through an antibody-dependent cellular cytotoxicity (ADCC) mechanism.

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

What Is Claimed Is:

1. An isolated antibody, which binds human galectin-9, wherein the antibody comprises a heavy chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 361, a heavy chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 388, and a heavy chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 406 and/or comprises a light chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 328, a light chain complementarity determining region 2 (CDR2) set forth as SEQ ID NO: 329, and a light chain complementarity determining region 3 (CDR3) set forth as SEQ ID NO: 352.
2. The isolated antibody of claim 1, wherein the antibody binds to an epitope in a carbohydrate recognition domain (CRD) of galectin-9.
3. The isolated antibody of claim 2, wherein the CRD is CRD2.
4. The isolated antibody of claim 3, wherein the CRD2 consists of the amino acid sequence of SEQ ID NO: 4.
5. The isolated antibody of claim 1, which is a full-length antibody or an antigen-binding fragment thereof.
6. The isolated antibody of claim 1, which is a single chain antibody.
7. The isolated antibody of claim 1, which is a human antibody or a humanized antibody.
8. The isolated antibody of claim 1, wherein the antibody comprises a VH set forth as SEQ ID NO: 55 and a VL set forth as SEQ ID NO: 54.

9. The isolated antibody of claim 8, wherein the antibody binds to an epitope in a carbohydrate recognition domain (CRD) of galectin-9, wherein the CRD is CRD2.
10. The isolated antibody of claim 8, which is a full-length antibody or an antigen-binding fragment thereof.
11. The isolated antibody of claim 8, which is a single chain antibody.
12. The isolated antibody of claim 8, which is a human antibody or a humanized antibody.
13. The isolated antibody of claim 8, which is an IgG molecule.
14. The isolated antibody of claim 13, wherein the antibody is an IgG1 or IgG4 molecule.
15. The isolated antibody of claim 14, wherein the antibody is an IgG4 molecule, wherein the IgG4 has a S228P mutation.
16. The isolated antibody of claim 14, wherein the antibody comprises a heavy chain constant region set forth as SEQ ID NO: 422, and/or a light chain constant region set forth as SEQ ID NO: 418.
17. The isolated antibody of claim 8, wherein the antibody comprises a V_H set forth as SEQ ID NO: 55 and a V_L set forth as SEQ ID NO: 54, and has a heavy chain constant region set forth as SEQ ID NO: 422 and a light chain constant region set forth as SEQ ID NO: 418.
18. The isolated antibody of claim 17, wherein the antibody comprises a heavy chain set forth as SEQ ID NO: 316 and a light chain set forth as SEQ ID NO: 108.
19. The isolated antibody of claim 18, wherein the antibody is G9.2-17.

20. The isolated antibody of claim 19, wherein the antibody binds to an epitope in a carbohydrate recognition domain (CRD) of galectin-9, wherein the CRD is CRD2.

21. A pharmaceutical composition comprising the antibody of claim 19 and a pharmaceutically acceptable carrier.

22. A pharmaceutical composition comprising the antibody of claim 17 and a pharmaceutically acceptable carrier.

23. A pharmaceutical composition comprising the antibody of claim 8 and a pharmaceutically acceptable carrier.

24. The isolated antibody of claim 1, which is an IgG molecule.

25. The isolated antibody of claim 24, wherein the antibody is an IgG1 or IgG4 molecule.

26. The isolated antibody of claim 25, wherein the antibody is an IgG4 molecule, wherein the IgG4 has a S228P mutation.

27. The isolated antibody of claim 25, wherein the antibody comprises a heavy chain constant region set forth as SEQ ID NO: 422, and/or a light chain constant region set forth as SEQ ID NO: 418.

28. The isolated antibody of claim 1, wherein the antibody has a V_L CDR1 set forth as SEQ ID NO: 328, a V_L CDR2 set forth as SEQ ID NO: 329, a V_L CDR3 set forth as SEQ ID NO: 352, a V_H CDR1 set forth as SEQ ID NO: 361, a V_H CDR2 set forth as SEQ ID NO: 388, a V_H CDR3 set forth as SEQ ID NO: 406, and has a heavy chain constant region set forth as SEQ ID NO: 422 and a light chain constant region set forth as SEQ ID NO: 418.

29. The isolated antibody of claim 1, wherein the antibody comprises a heavy chain set forth as SEQ ID NO: 316 and/or a light chain set forth as SEQ ID NO:108.

30. A pharmaceutical composition comprising the antibody of claim 1 and a pharmaceutically acceptable carrier.

31. Use of the isolated antibody of any one of claims 1 to 20 and 24 to 29 in the manufacture of a pharmaceutical composition for inhibiting the activity of galectin-9 in a solid tumor in a subject.

32. Use of the isolated antibody of any one of claims 1 to 20 and 24 to 29 in the manufacture of a pharmaceutical composition for treating a solid tumor that expresses galectin-9 in a human subject.

33. The use according to claim 31 or 32, wherein the antibody is a full-length antibody or an antigen-binding fragment thereof.

34. The use according to claim 31 or 32, wherein the antibody is single chain variable fragment or an IgG molecule.

35. The use according to claim 34, wherein the antibody is an IgG1 or an IgG4 antibody.

36. The use according to claim 31 or 32, wherein the antibody comprises a V_H set forth as SEQ ID NO: 55 and/or a V_L set forth as SEQ ID NO: 54.

37. The use according to claim 31 or 32, wherein the antibody comprises a heavy chain constant region set forth as SEQ ID NO: 422, and/or a light chain constant region set forth as SEQ ID NO: 418.

38. The use according to claim 31, wherein the subject is suspected of having, or is at risk for a solid tumor that expresses gal-9.

39. The use according to claim 37 or claim 38, wherein the solid tumor is selected from the group consisting of glioblastoma, glioma, melanoma, skin squamous cell carcinoma, sarcoma, upper and lower gastrointestinal cancers, carcinoid tumors, neuroendocrine tumors, breast cancers, lung cancers, head and neck cancers, and genitourinary cancers.

40. The use according to claim 39, wherein the upper and lower gastrointestinal cancers are selected from the group consisting of esophageal cancer, gastric cancer, pancreatic cancer, colorectal cancer, biliary tract cancers, cholangiocarcinoma, gall bladder cancer, liver cancer, and duodenal cancer.

41. Use of the isolated antibody of any one of claims 1 to 20 and 24 to 29 and a checkpoint inhibitor in the manufacture of a pharmaceutical composition for combination therapy for inhibiting the activity of galectin-9 in a solid tumor or for treating a solid tumor that expresses gal-9 in a subject.

42. The use according to claim 41, wherein the checkpoint inhibitor is an anti-PD-1 antibody.