

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 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).

## SUMMARY

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 pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), hepatocellular carcinoma (HCC), and

cholangiocarcinoma (CAA), involving an antibody capable of binding to human Galectin-9, either alone or in combination with a checkpoint inhibitor such as an anti-PD-1 antibody.

In one aspect, the present disclosure describes a use of an antibody that binds human Galectin-9 (anti-Galectin-9 antibody) in the manufacture of a medicament for treating cancer in a subject in need thereof, wherein the anti-Galectin-9 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 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 and wherein the anti-Galectin-9 antibody is to be administered to the subject at a dose of about 1 mg/kg to about 32 mg/kg.

Accordingly, one example of the present disclosure describes a method for treating a solid tumor in a subject by administering an antibody that binds human Galectin-9. In some examples, the solid tumor is pancreatic adenocarcinoma (PDA), colorectal cancer (CRC), or hepatocellular carcinoma (HCC), or cholangiocarcinoma. In some examples, the method comprises administering to a subject having a solid tumor, *e.g.*, PDA, CRC, HCC, or CCA an effective amount of an antibody that binds human Galectin-9 (referred to herein as an anti-Gal 9 antibody or anti-Galectin-9 antibody).

In some examples, the anti-Galectin-9 antibody is antibody G9.2-17, the structure of which is described herein. In some examples, the anti-Galectin-9 antibody comprises the same heavy chain complementarity determining regions (CDRs) and/or the same light chain CDRs as reference antibody G9.2-17, the sequences of which are described herein. In some examples, the anti-Galectin-9 antibody comprises the heavy chain variable domain of antibody G9.2-17, and/or a light chain variable domain of antibody G9.2-17.

In some examples, 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 examples, the antibody comprises a heavy chain variable region comprising SEQ ID NO: 7. In some examples, the antibody comprises a light chain variable region comprising SEQ ID NO: 8.

In some examples, the antibody comprises a heavy chain comprising SEQ ID NO: 19. In some examples, the antibody comprises a light chain comprising SEQ ID NO: 15. In some examples, the antibody is G9.2-17 IgG4.

5 In some examples, the anti-Galectin-9 antibody is administered to the subject at a dose of about 1 mg/kg to about 30 mg/kg (*e.g.*, about 3 mg/kg to about 15 mg/kg or about 2 mg/kg to about 16 mg/kg) once every 2-3 weeks. In some examples, the anti-Galectin-9 antibody is administered to the subject at a dose selected from 2 mg/kg, 4 mg/kg, 8 mg/kg, 12 mg/kg, or 16 mg/kg. In some examples, the antibody is administered once every 2 weeks. In some examples, the anti-Galectin-9 antibody is administered to the subject at a dose selected  
10 from 2 mg/kg, 4 mg/kg, 8 mg/kg, 12 mg/kg, or 16 mg/kg once every 2 weeks. In some examples, 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 examples, the duration of treatment is 0-3 months, 3-6 months, 12-24 months or longer. In some examples, the duration  
15 of treatment is 12-24 months or longer. In some examples, 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 examples, the cycle length is modified, *e.g.*, temporarily or permanently to a longer duration, *e.g.*, 3 weeks or 4 weeks. In some examples, the anti-Galectin-9 antibody is administered to the subject by intravenous infusion. In some examples, the cancer is  
20 metastatic cancer, including a metastatic cancer of any of the above mentioned cancers. In some examples, the method of treatment comprising administering the anti-Galectin-9 antibody does not include any other concurrent anti-cancer therapy.

In some examples, the method of treatment employing the anti-Galectin-9 antibody includes another concurrent anti-cancer therapy. Thus, in some examples, the method of  
25 treatment employing the anti-Galectin-9 antibody further comprises administering to the subject an immune checkpoint inhibitor. In some examples, the immune checkpoint inhibitor is an antibody that binds PD-1, for example, pembrolizumab, nivolumab, tislelizumab, or cemiplimab. In some examples, the antibody that binds PD-1 is nivolumab, which is administered to the subject at a dose of 240 mg once every two weeks. In some examples,  
30 the antibody that binds PD-1 is nivolumab, which is administered to the subject at a dose of 480 mg once every 4 weeks. In some examples, the antibody that binds PD-1 is pembrolizumab, which is administered at a dose of 200 mg once every 3 weeks. In some examples, the antibody that binds PD-1 is cemiplimab, which is administered at a dose of 350 mg once every 3 weeks. In some examples, the antibody that binds PD-1 is Tislelizumab,

which is administered at a dose of 200 mg once every 3 weeks. In some examples, the immune checkpoint inhibitor is administered by intravenous infusion.

In some examples, the anti-Galectin-9 antibody comprises a heavy chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 4, a heavy chain  
5 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 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. In some  
10 examples, the anti-Galectin-9 antibody comprises a heavy chain variable domain of SEQ ID NO: 7, and/or a light chain variable domain of SEQ ID NO: 8. In some examples, the anti-Galectin-9 antibody is a full-length antibody. In some examples, the anti-Galectin-9 antibody is an IgG1 or IgG4 molecule. In some examples, the anti-Galectin-9 antibody is a human IgG4 molecule having a modified Fc region of human IgG4. In some examples, the modified  
15 Fc region of human IgG4 comprises the amino acid sequence of SEQ ID NO: 14. In some examples, the modified Fc region of human IgG4 comprises the amino acid sequence of SEQ ID NO: 21. In some examples, 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 some examples, the anti-Galectin-9 antibody  
20 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23 and a light chain comprising the amino acid sequence of SEQ ID NO: 15.

In any of the methods disclosed herein, the subject (e.g., a human patient) may have undergone one or more prior anti-cancer therapies, *e.g.*, surgery, chemotherapy, immunotherapy, radiation therapy, a therapy involving a biologic, targeted small molecule,  
25 hormonal agent, or a combination thereof. In some examples, the subject has progressed disease through the one or more prior anti-cancer therapies. In other examples, the subject is resistant (*e.g.*, *de novo*, or acquired) to the one or more prior therapies. In other examples, the subject has relapsed after one or more prior therapies.

In any of the treatment methods disclosed herein, the subject can be a human patient  
30 having an elevated level of Galectin-9 relative to a control value. In some examples, the human patient has an elevated serum or plasma level of Galectin-9 relative to the control value. In some examples, the human patient has an elevated level of Galectin-9 expressed on the surface of cells derived from the human patient as relative to the control value. Such cells can be cancer cells and/or immune cells in the tumor and/or in the blood of a cancer patient.

In some examples, the cancer cells are in tumor organoids derived from the human patient. In some examples, the control value is based on a value obtained from a healthy human subject.

Any of the treatment methods disclosed herein may further comprise monitoring occurrence of adverse effects in the subject. In case adverse effects (e.g., one or more severe adverse effects occur), either the dose of the anti-Galectin-9 antibody (e.g., G9.2-17), or the dose of the checkpoint inhibitor if co-used (e.g., the anti-PD-1 antibody such as nivolumab), or both may be reduced.

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 for manufacturing a medicament for treating the solid tumor, wherein the uses disclosed herein, in some examples, 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 examples of the disclosure are set forth in the description below. Other features or advantages of the present disclosure are apparent from the following drawing and detailed description of several examples, 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** is a graph showing a representative size exclusion chromatography (SEC) profile for the anti-Galectin-9 antibody. The high molecular weight peaks are labeled.

**FIGURES 2A-2F** include bar graphs showing levels of Galectin-9 expression as measured in T cells (CD3<sup>+</sup>), macrophages (CD11b<sup>+</sup>), and tumor cells (Epcam<sup>+</sup>) in S2 and S3 organoid fractions derived from a pancreatic adenocarcinoma biopsy using anti-Galectin-9 G9.2-17 Fab fragment and a commercially available anti-Galectin-9 antibody (9M1-3). S2 fraction: organoids. S3 fraction: single cells. Corresponding isotype for G9.2-17 Fab (“Fab isotype”) and “fluorescence minus one” (FMO) 9M1-3 (“Gal9 FMO”) were used as controls for specificity, background staining and fluorescence bleed through from other channels.

*Figure 2A* shows levels of Galectin-9 in CD3<sup>+</sup> cells as measured in the S3 fraction. *Figure*

2B shows levels of Galectin-9 in CD11b<sup>+</sup> cells as measured in the S3 fraction. *Figure 2C* shows levels of Galectin-9 in Epcam<sup>+</sup> cells as measured in the S3 fraction. *Figure 2D* shows levels of Galectin-9 in CD3<sup>+</sup> cells as measured in the S2 fraction. *Figure 2E* shows levels of Galectin-9 in CD11b<sup>+</sup> cells as measured in the S2 fraction. *Figure 2F* shows levels of

5 Galectin-9 in Epcam<sup>+</sup> cells as measured in the S2 fraction.

**FIGURES 3A-3F** include bar graphs showing levels of Galectin-9 expression as measured in T cells (CD3<sup>+</sup>), macrophages (CD11b<sup>+</sup>,) and tumor cells (Epcam<sup>+</sup>) in S2 and S3 organoid fractions derived from a colorectal carcinoma biopsy using anti-Galectin-9 G9.2-17

WHAT IS CLAIMED IS:

1. Use of an antibody that binds human Galectin-9 (anti-Galectin-9 antibody) in the manufacture of a medicament for treating cancer in a subject in need thereof, wherein the anti-Galectin-9 antibody comprises a light chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 1, a light chain complementary determining region 2 (CDR2) set forth as SEQ ID NO: 2, and a light chain complementary determining region 3 (CDR3) set forth as SEQ ID NO: 3 and comprises a heavy chain complementarity determining region 1 (CDR1) set forth as SEQ ID NO: 4, a heavy chain complementary determining region 2 (CDR2) set forth as SEQ ID NO: 5, and a heavy chain complementary determining region 3 (CDR3) set forth as SEQ ID NO: 6 and wherein the anti-Galectin-9 antibody is to be administered to the subject at a dose of about 1 mg/kg to about 32 mg/kg.
2. The use of claim 1, wherein the cancer is a solid tumor.
3. The use of claim 2, wherein the solid tumor is pancreatic cancer, colorectal cancer (CRC), hepatocellular carcinoma (HCC), or cholangiocarcinoma (CCA).
4. The use of claim 2 or claim 3, wherein the solid tumor is a metastatic tumor.
5. The use of any one of claims 1-4, wherein the anti-Galectin-9 antibody is to be administered to the subject once every two weeks.
6. The use of claim 4, wherein the anti-Galectin-9 antibody is to be administered to the subject at a dose of about 1 mg/kg to about 16 mg/kg.
7. The use of any one of claims 1-6, wherein the anti-Galectin-9 antibody is to be administered to the subject at a dose of about 1 mg/kg, about 2 mg/kg, about 4 mg/kg, about 6 mg/kg, about 8 mg/kg, about 10 mg/kg, about 12 mg/kg, or about 16 mg/kg.
8. The use of any one of claims 1-7, wherein the anti-Galectin-9 antibody is to be administered to the subject by intravenous infusion.
9. The use of any one of claims 1-8, wherein the subject is free of other anti-cancer therapy concurrently with the treatment involving the anti-Galectin-9 antibody.

10. The use of any one of claims 1-9, wherein the method further comprises that an immune checkpoint inhibitor is to be administered to the subject.

11. The use of claim 10, wherein the immune checkpoint inhibitor is an antibody that binds PD-1.

5 12. The use of claim 11, wherein the antibody that binds PD-1 is selected from the group consisting of pembrolizumab, nivolumab, tislelizumab or cemiplimab.

13. The use of claim 11 or claim 12, wherein the antibody that binds PD-1 is nivolumab, which is to be administered to the subject at a dose of 240 mg once every two weeks.

10 14. The use of claim 11 or 12, wherein the antibody that binds PD-1 is tislelizumab, which is to be administered at a dose of 200 mg once every 3 weeks.

15. The use of any one of claims 10-14, wherein the immune checkpoint inhibitor is to be administered by intravenous infusion.

15 16. The use of any one of claims 1-15, wherein the anti-Galectin-9 antibody comprises a light chain variable domain of SEQ ID NO: 8, and a heavy chain variable domain of SEQ ID NO: 7.

17. The use of any one of claims 1-16, wherein the anti-Galectin-9 antibody is a full-length antibody.

20 18. The use of any one of claims 1-17, wherein the anti-Galectin-9 antibody is an IgG1 or IgG4 molecule.

19. The use of claim 18, wherein the anti-Galectin-9 antibody is an IgG4 molecule having a modified Fc region of human IgG4.

20. The use of claim 19, wherein the modified Fc region of human IgG4 comprises the amino acid sequence of SEQ ID NO: 14.

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21. The use of any one of claims 1-20, wherein 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.

22. The use of any one of claims 1-21, wherein the subject has undergone one or more prior anti-cancer therapies.

23. The use of claim 22, wherein the one or more prior anti-cancer therapies comprise chemotherapy, immunotherapy, radiation therapy, a therapy involving a biologic agent, or a combination thereof.

24. The use of claim 22 or claim 23, 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.

25. The use of any one of claims 1-22, wherein the subject is a human patient having an elevated level of Galectin-9 relative to a control value.

26. The use of claim 25, wherein the human patient has an elevated serum or plasma level of Galectin-9 relative to the control value.

27. The use of claim 25, wherein the human patient has cancer cells expressing Galectin-9.

28. The use of claim 25, wherein the human patient has immune cells expressing Galectin-9.

29. The use of claim 27, wherein the cancer cells are in tumor organoids derived from the human patient.

30. The use of claim 28, wherein the immune cells are in tumor organoids derived from the human patient.

31. The use of any one of claims 1-30, further comprising monitoring occurrence of adverse effects in the subject.

32. The use of claim 31, further comprising reducing the dose of the anti-Galectin-9 antibody, optionally the dose of the checkpoint inhibitor, or both.