

ANTIBODIES SPECIFIC TO SIALIC ACID-BINDING IG-LIKE LECTIN 15 AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of priority to U.S. Provisional Patent Application No. 63/163,680, filed March 19, 2021, which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

10 Sialic acid-binding Ig-like lectin 15 (Siglec15) is a member of the Siglec family of glycan-recognition proteins. Siglec proteins are expressed on various types of leukocytes and play important roles in modulating immune responses via binding to ligands at the extracellular domain and mediating intracellular signaling transduction.

15 Siglec15 was found to be upregulated on human cancer cells and tumor-infiltrating myeloid cells. It has been reported that Siglec15 is an immune suppressor that suppresses antigen-specific T cell responses. Accordingly, Siglec15 is suggested to be a potential therapeutic target for cancer immunotherapy.

SUMMARY

20 The present disclosure is based, at least in part, on the development of antibodies binding to human sialic acid-binding Ig-like lectin 15 (Siglec15). Such anti-Siglec15 antibodies showed high binding affinity and specificity to human Siglec15. Further, certain exemplary antibodies (e.g., clone 2020EP32-H11) are capable of inducing antibody-dependent cytotoxicity (ADCC) against cells expressing Siglec15 and activating
25 immune cells. Further, the exemplary H11 clone (in monoclonal antibody format) showed desirable pharmacokinetic features (e.g., long half-life and little toxicity) as observed in cynomolgus monkeys. Accordingly, the anti-Siglec15 antibodies disclosed herein would be expected to have high therapeutic effects via modulating immune responses and/or neutralizing Siglec15-positive cells or Siglec15 dependent signals.

30 In one aspect, the present disclosure features an isolated antibody that binds sialic acid-binding Ig-like lectin 15 (Siglec15), wherein the antibody binds to the same epitope as a reference antibody or competes against the reference antibody from binding to Siglec-15, and wherein the reference antibody is selected from the group consisting of 2020EP032-H11, 2019EP47-A02, 2019EP47-A05, 2019EP47-A10, 2019EP47-C12,

2020EP032-A08, 2020EP032-A12, 2020EP032-B03, 2020EP032-C09, 2020EP083-G11, 2020EP083-H01, and 2020EP085-G5;

wherein:

(a) 2020EP032-H11 comprises a heavy chain variable region (V_H) set forth as SEQ ID NO: 57 and a light chain variable region (V_L) set forth as SEQ ID NO: 61;

(b) 2019EP47-A02 comprises a V_H set forth as SEQ ID NO: 1 and V_L set forth as SEQ ID NO: 5;

(c) 2019EP47-A05 comprises a V_H set forth as SEQ ID NO: 9 and V_L set forth as SEQ ID NO: 13;

(d) 2019EP47-A10 comprises a V_H set forth as SEQ ID NO: 17 and V_L set forth as SEQ ID NO: 21;

(e) 2019EP47-C12 comprises a V_H set forth as SEQ ID NO: 25 and V_L set forth as SEQ ID NO: 29;

(f) 2020EP032-A08 comprises a V_H set forth as SEQ ID NO: 33 and V_L set forth as SEQ ID NO: 37;

(g) 2020EP032-A12 comprises a V_H set forth as SEQ ID NO: 41 and V_L set forth as SEQ ID NO: 45;

(h) 2020EP032-B03 comprises a V_H set forth as SEQ ID NO: 49 and V_L set forth as SEQ ID NO: 53;

(i) 2020EP032-C09 comprises a V_H set forth as SEQ ID NO: 65 and V_L set forth as SEQ ID NO: 69;

(j) 2020EP083-G11 comprises a V_H set forth as SEQ ID NO: 73 and V_L set forth as SEQ ID NO: 77;

(k) 2020EP083-H01 comprises a V_H set forth as SEQ ID NO: 81 and V_L set forth as SEQ ID NO: 85; and

(l) 2020EP085-G5 comprises a V_H set forth as SEQ ID NO: 89 and V_L set forth as SEQ ID NO: 93.

Accordingly, one example of the present disclosure features an isolated antibody that binds sialic acid-binding Ig-like lectin 15 (Siglec15). Such an antibody binds to the same epitope as a reference antibody or competes against the reference antibody from binding to Siglec15. The reference antibody can be one of the following: 2019EP47-A02,

2019EP47-A05, 2019EP47-A10, 2019EP47-C12, 2020EP032-A08, 2020EP032-A12, 2020EP032-B03, 2020EP032-H11, 2020EP032-C09, 2020EP083-G11, 2020EP083-H01, and 2020EP085-G5.

In some examples, the anti-Siglec15 antibody disclosed herein may comprise: (a) a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3), wherein the HC CDR1, HC CDR2, and HC CDR3 collectively are at least 80% identical to the heavy chain CDRs of the reference antibody; and/or (b) a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3), wherein the LC CDR1, LC CDR2, and LC CDR3 collectively are at least 80% identical to the light chain CDRs of the reference antibody. In some examples, the HC CDRs of the anti-Siglec15 antibody disclosed herein collectively contain no more than 8 amino acid residue variations as compared with the HC CDRs of the reference antibody. Alternatively or in addition, the LC CDRs of the anti-Siglec15 antibody collectively contain no more than 8 amino acid residue variations as compared with the LC CDRs of the reference antibody.

In some instances, the anti-Siglec15 antibody disclosed herein may comprise a V_H that is at least 85% identical to the V_H of the reference antibody, and/or a V_L that is at least 85% identical to the V_L of the reference antibody. In some instances, the anti-Siglec15 antibody has a binding affinity of less than about 50 nM to Siglec-15 expressed on cell surface. In some examples, the anti-Siglec15 antibody has a binding affinity less than 10 nM. In some examples, the anti-Siglec15 antibody has a binding affinity less than 5 nM to Siglec15 expressed on cell surface. In some examples, the anti-Siglec15 antibody has a binding affinity less than 1.5 nM to the Siglec15 expressed on cell surface.

In some examples, the anti-Siglec15 antibody disclosed herein comprises the same heavy chain complementary determining regions (HC CDRs) and the same light chain complementary determining regions (LC CDRs) as the reference antibody. In specific examples, the anti-Siglec15 antibody comprises the same V_H and the same V_L as the reference antibody.

Any of the anti-Siglec15 antibodies disclosed herein may be a human antibody. Alternatively, it may be a humanized antibody. In some instances, the anti-Siglec15

antibody is a full-length antibody. Alternatively, it may be an antigen-binding fragment thereof. In some instances, the anti-Siglec15 antibody may be a single-chain variable fragment (scFv) antibody. In some instances, the antibody is a fusion polypeptide comprising the scFv.

5 In another aspect, the present disclosure features a nucleic acid or a set of nucleic acids, which collectively encodes any of the anti-Siglec15 antibodies disclosed herein. In some instances, a set of nucleic acids disclosed herein refers to two nucleic acid molecules each encoding one chain of the antibody and collectively encoding the heavy and light chain of the antibody. In some examples, the nucleic acid or the set of nucleic acids is a vector or
10 a set of vectors, for example, an expression vector or an expression vector set. In another aspect, the present disclosure features a host cell comprising the nucleic acid or the set of nucleic acids as described herein. Also provided herein is a host cell comprising any of the nucleic acids or the set of nucleic acids coding for any of the anti-Siglec15 antibodies disclosed herein.

15 In yet another aspect, the present disclosure features a pharmaceutical composition comprising any of the anti-Siglec15 antibodies disclosed herein, any of the coding nucleic acids as disclosed herein, the nucleic acid or nucleic acids as disclosed herein, or the host cell as described herein, and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure features a pharmaceutical composition for use as a medicament, wherein
20 the pharmaceutical composition is set forth herein. In yet another aspect, the present disclosure features a use of the pharmaceutical composition as described herein in the manufacture of a medicament for treating a Siglec15+ cancer or an immune disease involving Siglec15+ immune cells in a subject. In yet another aspect, the present disclosure features an *in vitro* method for detecting presence of Siglec-15, comprising: (i) contacting an antibody as
25 described herein with a sample suspected of containing Siglec-15 expressed on cell surface, and (ii) detecting binding of the antibody to Siglec-15.

In addition, the present disclosure provides a method for inhibiting Siglec-15 or Siglec-15⁺ cells in a subject, comprising administering to a subject in need thereof any effective amount of any of the pharmaceutical compositions disclosed herein. In some
30 examples, the pharmaceutical composition comprises an anti-Siglec15 antibody as disclosed herein. In some examples, the subject is a human patient having Siglec15+ disease cells, *e.g.*, tumor cells or immune cells. In some examples, the human patient has a Siglec15+ cancer. Examples include non-small cell lung cancer (NSCLC), ovarian cancer, breast

cancer, head-and-neck cancer, renal carcinoma, pancreatic cancer, endometrial cancer, urothelial cancer, thyroid cancer, colon cancer, colorectal cancer, melanoma, liver cancer, or gastric cancer.

- Moreover, the present disclosure provides a method for detecting presence of
- 5 Siglec-15, comprising: (i) contacting an anti-Siglec15 antibody as disclosed herein with a sample suspected of containing Siglec-15, and (ii) detecting binding of the antibody to Siglec-15. In some instances, the anti-Siglec15 antibody may be conjugated to a detectable label. In some instances, the Siglec-15 is expressed on cell surface. In some examples, the contacting step is performed by administering the antibody to a subject.

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In yet another aspect, the present disclosure also features a method of producing an antibody binding to Siglec-15, comprising (i) culturing the host cell as described herein under conditions allowing for expression of the antibody that binds Siglec-15; and (ii) harvesting the antibody thus produced from the cell culture. The present disclosure also features a method of producing an antibody binding to Siglec-15, comprising: (i) culturing a host cell comprising nucleic acid(s) encoding any of the anti-Siglec15 antibodies disclosed herein under conditions allowing for expression of the antibody that binds Siglec-15; and (ii) harvesting the antibody thus produced from the cell culture.

Also within the present disclosure are anti-Siglec15 antibodies or pharmaceutical compositions comprising such for use in treating a disease or disorder associated with Siglec15, for example, cancer or an immune disorder, or use of such an antibody for manufacturing a medicament for use in treating the target disease.

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 will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

BRIEF DESCRIPTION OF THE 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.

FIG. 1 is a diagram showing anti-Siglec15 IgG antibodies binding to Siglec15/HEK293 by FACS analysis.

FIGs. 2A-2D include diagrams showing epitope binning between 2020EP32-H11 and 5G12 antibodies. **FIG. 2A:** competitive binding of anti-Siglec15 2020EP32-H11 IgG antibody to Siglec15 in the presence of 5G12. **FIG. 2B:** competitive binding of anti-Siglec15 5G12 antibody to Siglec15 in the presence of 2020EP32-H11. **FIG. 2C:** competitive binding of anti-Siglec15 2019EP47-A02 IgG antibody to Siglec15 in the presence of 5G12. **FIG. 2D:** competitive binding of anti-Siglec15 2019EP47-A02 IgG antibody to Siglec15 in the presence of 2020EP32-H11.

FIGs. 3A-3C include diagrams showing binding specificity of anti-Siglec15 antibody 2020EP32-H11. **FIG. 3A:** a diagram showing binding activity of 2020EP32-H11 to various Siglec proteins as determined by surface plasmon resonance (SPR). **FIG. 3B:** a diagram showing binding activity of 2020EP32-H11 to various siglec proteins as measured by ELISA.

What Is Claimed Is:

1. An isolated antibody that binds sialic acid-binding Ig-like lectin 15 (Siglec15), wherein the antibody binds to the same epitope as a reference antibody or competes against the reference antibody from binding to Siglec-15, and wherein the reference antibody is selected from the group consisting of 2020EP032-H11, 2019EP47-A02, 2019EP47-A05, 2019EP47-A10, 2019EP47-C12, 2020EP032-A08, 2020EP032-A12, 2020EP032-B03, 2020EP032-C09, 2020EP083-G11, 2020EP083-H01, and 2020EP085-G5;

wherein:

(a) 2020EP032-H11 comprises a heavy chain variable region (V_H) set forth as SEQ ID NO: 57 and a light chain variable region (V_L) set forth as SEQ ID NO: 61;

(b) 2019EP47-A02 comprises a V_H set forth as SEQ ID NO: 1 and V_L set forth as SEQ ID NO: 5;

(c) 2019EP47-A05 comprises a V_H set forth as SEQ ID NO: 9 and V_L set forth as SEQ ID NO: 13;

(d) 2019EP47-A10 comprises a V_H set forth as SEQ ID NO: 17 and V_L set forth as SEQ ID NO: 21;

(e) 2019EP47-C12 comprises a V_H set forth as SEQ ID NO: 25 and V_L set forth as SEQ ID NO: 29;

(f) 2020EP032-A08 comprises a V_H set forth as SEQ ID NO: 33 and V_L set forth as SEQ ID NO: 37;

(g) 2020EP032-A12 comprises a V_H set forth as SEQ ID NO: 41 and V_L set forth as SEQ ID NO: 45;

(h) 2020EP032-B03 comprises a V_H set forth as SEQ ID NO: 49 and V_L set forth as SEQ ID NO: 53;

(i) 2020EP032-C09 comprises a V_H set forth as SEQ ID NO: 65 and V_L set forth as SEQ ID NO: 69;

(j) 2020EP083-G11 comprises a V_H set forth as SEQ ID NO: 73 and V_L set forth as SEQ ID NO: 77;

(k) 2020EP083-H01 comprises a V_H set forth as SEQ ID NO: 81 and V_L set forth as SEQ ID NO: 85; and

(l) 2020EP085-G5 comprises a V_H set forth as SEQ ID NO: 89 and V_L set forth as SEQ ID NO: 93.

2. The isolated antibody of claim 1, wherein the antibody comprises:

(a) a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3), wherein the HC CDR1, HC CDR2, and HC CDR3 collectively are at least 80% identical to the heavy chain CDRs of the reference antibody; and

(b) a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3), wherein the LC CDR1, LC CDR2, and LC CDR3 collectively are at least 80% identical to the light chain CDRs of the reference antibody.

3. The isolated antibody of claim 1 or claim 2, wherein the HC CDRs of the antibody collectively contain no more than 8 amino acid residue variations as compared with the HC CDRs of the reference antibody; and wherein the LC CDRs of the antibody collectively contain no more than 8 amino acid residue variations as compared with the LC CDRs of the reference antibody; or wherein the antibody comprises a V_H that is at least 85% identical to the V_H of the reference antibody, and/or a V_L that is at least 85% identical to the V_L of the reference antibody.

4. The isolated antibody of any one of claims 1-3, wherein the antibody has a binding affinity of less than about 10 nM to Siglec15 expressed on cell surface.

5. The isolated antibody of claim 1, which comprises the same heavy chain complementary determining regions (HC CDRs) and the same light chain complementary determining regions (LC CDRs) as the reference antibody.

6. The isolated antibody of claim 5, which comprises the same V_H and the same V_L as the reference antibody.

7. The isolated antibody of any one of claims 1-6, wherein the antibody is a human antibody or a humanized antibody.

8. The isolated antibody of any one of claims 1-7, wherein the antibody is a full-length antibody or an antigen-binding fragment thereof; or wherein the antibody is a single-chain antibody (scFv).

9. The isolated antibody of claim 8, wherein the antibody is a fusion polypeptide comprising the scFv.

10. A nucleic acid or a set of nucleic acids, which collectively encodes the antibody of any one of claims 1-9.

11. The nucleic acid or the set of nucleic acids of claim 10, which is a vector or a set of vectors.

12. The nucleic acid or the set of nucleic acids of claim 11, wherein the vector is an expression vector.

13. A host cell comprising the nucleic acid or the set of nucleic acids of any one of claims 10-12.

14. A pharmaceutical composition comprising the antibody of any one of claims 1-9, the nucleic acid or nucleic acids of any one of claims 10-12, or the host cell of claim 13, and a pharmaceutically acceptable carrier.

15. A pharmaceutical composition for use as a medicament, wherein the pharmaceutical composition is set forth in claim 14.

16. Use of the pharmaceutical composition according to claim 14 in the manufacture of a medicament for treating a Siglec15+ cancer or an immune disease involving Siglec15+ immune cells in a subject.

17. The use of claim 16, wherein the a Siglec15+ cancer is selected from the group consisting of non-small cell lung cancer (NSCLC), ovarian cancer, breast cancer, head-and-neck cancer, renal carcinoma, pancreatic cancer, endometrial cancer, urothelial cancer, thyroid cancer, colon cancer, colorectal cancer, melanoma, liver cancer, and gastric cancer.

18. An *in vitro* method for detecting presence of Siglec-15, comprising:
(i) contacting an antibody of any one of claims 1-9 with a sample suspected of containing Siglec-15 expressed on cell surface, and
(ii) detecting binding of the antibody to Siglec-15.

19. The method of claim 18, wherein the antibody is conjugated to a detectable label.

20. A method of producing an antibody binding to Siglec-15, comprising:
(i) culturing the host cell of claim 16 under conditions allowing for expression of the antibody that binds Siglec-15; and
(ii) harvesting the antibody thus produced from the cell culture.