

a lysine residue that is essential for the interaction with adapter protein DAP12, and a cytoplasmic tail (Takashi Angata *et al.*, (2007) *Glycobiology* 17(8):838-846).

[0006] Siglec15 is expressed on osteoclasts, and plays a role in osteoclast differentiation and bone remodeling (Hiruma Y *et al.*, (2011) *Biochemical and Biophysical Research Communications* 409(3):424-429; Takashi Angata (2020) *Journal of Biomedical Science* 27:10). The administration of anti-Siglec15 antibodies inhibited osteoclastic bone resorption and increased bone mass in rodent models (Stuible M *et al.*, (2014) *Journal of Biological Chemistry* 289(10):6498-6512; Sato D *et al.*, (2018) *Bone* 116:172-180).

[0007] Siglec15 is also expressed on tumor-associated macrophages and preferentially recognizes the sialyl-Tn antigen, a tumor associated glycan structure. The co-culture of sialyl-Tn/- cancer cell line and M-CSF-induced human macrophages or Siglec15⁺ myeloid cell line induced the production of transforming growth factor- β , which promoted epithelia-mesenchymal transition and metastasis of cancer cells (Takamiya R *et al.*, (2013) *Glycobiology* 23(2):178-187). Lieping Cheng *et al.*, recently discovered that Siglec15 is further expressed on tumor cells and/or tumor-associated stromal cells in clinical non-small cell lung cancer samples. They also found Siglec15 proteins suppressed T cell proliferation and activation, and the anti-Siglec15 antibodies reversed T cell suppression and attenuated cancer growth *in vivo*. As Siglec15 and PD-L1 are mutually exclusive in cancer tissues, Siglec15 may serve as a complementary therapeutic target and offers alternative treatment to patients that are refractory to PD-1/PD-L1 blockade, as mentioned above ((Jun Wang *et al.*, (2019) *supra*). NC318, a humanized anti-Siglec15 antibody, has been clinically tested in patients with advanced solid tumors, including non-small cell lung cancer, ovarian cancer, melanoma, colorectal cancer, and breast cancer, and prolonged stabilization of disease was observed in 54% of the patients and objective response in 5.4% (Sun J *et al.*, (2021) *Clin Cancer Res.* 27(3):680-688).

[0008] In view of Siglec15's engagement in bone remodeling, and tumor development, Siglec15 is definitely an emerging promising therapeutic target. Anti-Siglec15 antibodies with improved pharmaceutical characteristics are needed.

[0009] Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

[0010] The present disclosure provides an isolated mouse, chimeric, human or humanized monoclonal antibody, or an antigen-binding portion thereof, that binds to Siglec15 (e.g., the human Siglec15, and monkey Siglec15) and has comparable, if not higher, binding affinity/capacity to Siglec15 and blocking activity on Siglec15 binding to a ligand such as LRRC4C, as compared to prior art anti-Siglec15 antibodies such as Siglec15-ch5G9 (Nextcure). The antibody or antigen-binding portion thereof is capable of reversing Siglec15 mediated T cell suppression.

[0011] The antibody or antigen-binding portion thereof of the disclosure can be used for a variety of applications, including detection of the Siglec15 protein, and treatment and prevention of Siglec15 associated diseases, such as tumors and osteoporosis.

[0012] Accordingly, in one [aspect/example](#), the disclosure pertains to an isolated monoclonal antibody (e.g., a human, mouse, chimeric or humanized antibody), or an antigen-binding portion thereof, that binds Siglec15, having i) a heavy chain variable region that may comprise a VH CDR1 region, a VH CDR2 region and a VH CDR3 region, wherein the VH CDR1 region, the VH CDR2 region and the VH CDR3 region may comprise amino acid sequences having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to (1) SEQ ID NOs: 1, 2 (X1=D, X2=Q) and 3, respectively; (2) SEQ ID NOs: 1, 2 (X1=E, X2=Q) and 3, respectively; (3) SEQ ID NOs: 1, 2 (X1=D, X2=K) and 3, respectively; (4) SEQ ID NOs: 9, 10 and 11, respectively; or (5) SEQ ID NOs: 33, 34 and 35, respectively; and/or ii) a light chain variable region that may comprise a VL CDR1 region, a VL CDR2 region and a VL CDR3 region, wherein the VL CDR1 region, the VL CDR2 region, and the VL CDR3 region may comprise amino acid sequences having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to (1) SEQ ID NOs: 4, 5 and 6, respectively; (2) SEQ ID NOs: 12, 13 and 14, respectively; or (3) SEQ ID NOs: 36, 37 and 38, respectively. In one aspect, the present disclosure refers to an isolated monoclonal antibody or an antigen-binding portion thereof, binding to Siglec15, comprising i) a heavy chain variable region that comprises a VH CDR1 region, a VH CDR2 region and a VH CDR3 region, wherein the VH CDR1 region, the VH CDR2 region and the VH CDR3 region comprise amino acid sequences having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to (1) SEQ ID NOs: 1, 2 (X1=D, X2=Q) and 3, respectively; (2) SEQ ID NOs: 1, 2 (X1=E, X2=Q) and 3, respectively; (3) SEQ ID NOs: 1, 2 (X1=D, X2=K) and 3, respectively; (4) SEQ ID NOs: 9, 10 and 11, respectively; or (5) SEQ ID NOs: 33, 34 and 35, respectively; and/or ii) a light chain variable region that comprises a VL CDR1 region, a VL CDR2 region and a VL CDR3 region, wherein the VL CDR1 region, the VL CDR2 region, and the VL CDR3 region comprise amino acid sequences having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to (1) SEQ ID NOs: 4, 5 and 6, respectively; (2) SEQ ID NOs: 12, 13 and 14, respectively; or (3) SEQ ID NOs: 36, 37 and 38, respectively.

[0013] The antibody or antigen-binding portion thereof of the disclosure may comprise a heavy chain variable region that may comprise a VH CDR1 region, a VH CDR2 region and a VH CDR3 region, and a light chain variable region that may comprise a VL CDR1 region, a VL CDR2 region and a VL CDR3 region, wherein the VH CDR1 region, the VH CDR2 region, the VH CDR3 region, the VL CDR1 region, the VL CDR2 region, and the VL CDR3 region may comprise amino acid sequences having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to (1) SEQ ID NOs: 1, 2 (X1=D, X2=Q), 3, 4, 5 and 6, respectively; (2) SEQ ID NOs: 1, 2 (X1=E, X2=Q), 3, 4, 5 and 6, respectively; (3) SEQ ID NOs: 1, 2 (X1=D, X2=K), 3, 4, 5 and 6, respectively; (4) SEQ ID NOs: 9, 10, 11, 12, 13 and 14, respectively; or (5) SEQ ID NOs: 33, 34, 35, 36, 37 and 38, respectively, wherein the antibody or antigen-binding fragment thereof binds to Siglec15.

[0014] The heavy chain variable region of the antibody or antigen-binding portion thereof of the disclosure may comprise an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NOs: 7 (X1=D, X2=Q; X1=E, X2=Q; or X1=D, X2=K), 15, 39, 40 or 41, wherein the antibody or antigen-binding fragment thereof binds to Siglec15. The amino acid sequence of SEQ ID NO: 7 (X1=D, X2=Q) may be encoded by the nucleotide sequence of SEQ ID NOs: 27, and the amino acid sequence of SEQ ID NO: 15 may be encoded by the nucleotide sequence of SEQ ID NO: 29.

[0015] The light chain variable region of the antibody or antigen-binding portion thereof of the disclosure may comprise an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NOs: 8, 16, 42 or 43, wherein the antibody or antigen-binding fragment thereof binds to Siglec15. The amino acid sequences of SEQ ID NO: 8 and 16 may be encoded by nucleotide sequences of SEQ ID NOs: 28 and 30.

[0016] The antibody or antigen-binding portion thereof of the disclosure may comprise a heavy chain variable region and a light chain variable region having amino acid sequences having at least 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to (1) SEQ ID Nos: 7 (X1=D, X2=Q) and 8, respectively; (2) SEQ ID Nos: 7 (X1=E, X2=Q) and 8, respectively; (3) SEQ ID Nos: 7 (X1=D, X2=K) and 8, respectively; (4) SEQ ID Nos: 15 and 16, respectively; (5) SEQ ID Nos: 39 and 42, respectively; (6) SEQ ID Nos: 40 and 42, respectively; or (7) SEQ ID Nos: 41 and 43, respectively, wherein the antibody or antigen-binding fragment thereof binds to Siglec15.

[0017] The isolated monoclonal antibody, or the antigen-binding portion thereof, of the present disclosure may comprise a heavy chain and a light chain linked by disulfide bonds, the heavy chain may comprise a heavy chain variable region and a heavy chain constant region, the light chain may comprise a light chain variable region and a light chain constant region, wherein the C terminus of the heavy chain variable region is linked to the N terminus of the heavy chain constant region, and the C terminus of the light chain variable region is linked to the N terminus of the light chain constant region, wherein the heavy chain variable region and the light chain variable region may comprise amino acid sequences described above, and the antibody or antigen-binding portion thereof binds to Siglec15. The heavy chain constant region may be a IgG1, IgG2 or IgG4 heavy chain constant region, e.g., human IgG1, IgG2 or IgG4 heavy chain constant region having the amino acid sequence set forth in e.g., SEQ ID NO.:17. The heavy chain constant region, such as the Fc fragment, may be engineered to have reduced or enhanced FcR binding affinity. The light chain constant region may be kappa constant region, e.g., human kappa constant region having the amino acid sequences set forth in e.g., SEQ ID NO.: 18. The amino acid sequences of SEQ ID Nos: 17 and 18 may be encoded by the nucleotide sequences of SEQ ID Nos: 31 and 32, respectively.

[0018] The antibody of the present disclosure in certain [embodimentexamples](#) may comprise or consist of two heavy chains and two light chains, wherein each heavy chain may comprise the heavy chain constant region, heavy chain variable region or CDR sequences mentioned above, and each light chain may comprise the light chain constant region, light chain variable region or CDR sequences mentioned above, wherein the antibody binds to Siglec15. The antibody or antigen-binding portion thereof of the disclosure can be a full-length antibody, for example, of an IgG1, IgG2 or IgG4 isotype. The antibody or the antigen-binding portion thereof of the present disclosure in other [embodimentexamples](#) may be a single chain variable fragment (scFv) antibody, or an antibody fragment, such as a Fab or F(ab')₂ fragment.

[0019] The disclosure also provides a bispecific molecule that may comprise the antibody, or the antigen-binding portion thereof, of the disclosure, linked to a second functional moiety (e.g., a second antibody) having a different binding specificity than said antibody, or antigen-binding portion thereof. The disclosure also provides an immunoconjugate, such as an antibody-drug conjugate, that may comprise an antibody, or an antigen-binding portion thereof, of the disclosure, linked to a therapeutic agent, such as a cytotoxin. In another [aspectexample](#), the antibody or the antigen binding portion thereof of the present disclosure can be made into part of a chimeric antigen receptor (CAR). Also provided is an immune cell that may comprise the antigen chimeric receptor, such as a T cell and a NK cell. The antibody or the antigen binding portion thereof of the present disclosure can also be encoded by or used in conjunction with an oncolytic virus.

[0020] Nucleic acid molecules encoding the antibody, or the antigen-binding portion thereof, of the disclosure are also encompassed by the disclosure, as well as expression vectors that may comprise such nucleic acids and host cells that may comprise such expression vectors. [In one aspect, the present disclosure refers to a nucleotide encoding any one of the isolated monoclonal antibody or the antigen-binding portion thereof as disclosed herein.](#) [In one aspect, the present disclosure refers to a vector containing the nucleotide as disclosed herein.](#) [In one aspect, the present disclosure refers to a host cell containing the vector as disclosed herein.](#) A method for preparing the anti-Siglec15 antibody or the antigen-binding portion thereof of the disclosure using the host cell is also provided, that may comprise steps of (i) expressing the antibody in the host cell and (ii) isolating the antibody from the host cell or its cell culture.

[0021] Compositions that may comprise the antibody, or the antigen-binding portion thereof, the immunoconjugate, the bispecific molecule, the oncolytic virus, the CAR, the CAR-T cell, the nucleic acid molecule, the expression vector or the host cells of the disclosure, and a pharmaceutically acceptable carrier, are also provided. [In one aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody or antigen-binding portion thereof as disclosed herein, and a pharmaceutically acceptable carrier.](#) In certain [embodimentexamples](#), the pharmaceutical composition may further contain a therapeutic agent such as an anti-cancer agent.

[0021a] [In one aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer in a subject.](#)

[0022] In yet another [aspectexample](#), the disclosure provides a method of modulating an immune response in a subject comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding portion thereof, of the disclosure, or alternatively a nucleic acid molecule capable of expressing the same in the subject, such that the immune response in the subject is modulated. Preferably, the antibody or antigen-binding portion thereof of the disclosure enhances, stimulates or increases the immune response in the subject, e.g., by reversing Siglec15 mediated T cell suppression. In some [embodimentexamples](#), the method comprises administering a bispecific molecule, an immunoconjugate, a CAR-T cell, or an antibody-encoding or antibody-bearing oncolytic virus of the disclosure.

[0023] [In one aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for inhibiting bone loss or increasing bone mass in a subject.](#) In yet another [aspectexample](#), the disclosure provides a method of inhibiting bone loss or increasing bone mass in a subject comprising administering to the subject a therapeutically effective amount of the antibody, or antigen-binding portion thereof, of the disclosure, or alternatively a nucleic acid molecule capable of expressing the same.

[0024] In a further [aspectexample](#), the disclosure provides a method of inhibiting tumor growth in a subject in need thereof, comprising administering to a subject a therapeutically effective amount of the antibody or antigen-binding portion thereof, of the disclosure, or alternatively a nucleic acid molecule capable of expressing the same in the subject. In some [embodimentexamples](#), the method comprises administering a bispecific molecule, an immunoconjugate, a CAR-T cell, or an antibody-encoding or antibody-bearing oncolytic virus of the disclosure. The tumor may be a solid or non-solid tumor. In certain [embodimentexamples](#), the tumor is solid tumor, including, but not limited to, non-small cell lung cancer, ovarian cancer, melanoma, colorectal cancer, breast cancer (including triple-negative breast cancer), head and neck squamous cell carcinoma, endometrial cancer, and squamous cell carcinoma. In some [embodimentexamples](#), at least one additional anti-cancer antibody can be administered with the antibody, or an antigen-binding portion thereof, of the disclosure, such as an anti-VISTA antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-LAG-3 antibody, an anti-CTLA-4 antibody, an anti-TIM-3 antibody, an anti-STAT3 antibody, and/or an anti-ROR1 antibody. In yet another [embodimentexample](#), an antibody, or an antigen-binding portion thereof, of the disclosure is administered with a cytokine (e.g., IL-2, IL-21, GM-CSF and/or IL-4), or a costimulatory antibody (e.g., an anti-CD137 and/or anti-GITR antibody). In another [embodimentexample](#), an antibody, or an antigen-binding portion thereof, of the

disclosure is administered with a chemotherapeutic agent, which may be a cytotoxic agent, such as epirubicin, oxaliplatin, and/or 5-fluorouracil (5-FU). The antibody or antigen-binding portion thereof of the present disclosure may be, for example, mouse, human, chimeric or humanized.

[0025] Other features and advantages of the instant disclosure will be apparent from the following detailed description and examples, which should not be construed as limiting. The contents of all references, Genbank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

[0026] Accordingly, it is an object of the [inventiondisclosure](#) not to encompass within the [inventiondisclosure](#) any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the [inventiondisclosure](#) does not intend to encompass within the scope of the [inventiondisclosure](#) any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product. It may be advantageous in the practice of the [inventiondisclosure](#) to be in compliance with Art. 53(c) EPC and Rule 28(b) and (c) EPC. All rights to explicitly disclaim any [embodimentexamples](#) that are the subject of any granted patent(s) of applicant in the lineage of this application or in any other lineage or in any prior filed application of any third party is explicitly reserved. Nothing herein is to be construed as a promise.

[0027] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the [inventiondisclosure](#).

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings.

[0029] FIGs. 1A-1C show the binding capacities of antibodies A1C8C6H1, A1D1B7H9, A1D5E2H1 and A1D11A7H10 (A), A1E7G5D1, A1E10G7H9, A2A1D2F1 and A2A5C7E8 (B), A2A6B1C2, A2G4C8G7 and A2H5F1A1 (C) to human Siglec15 in a capture ELISA.

[0030] FIGs. 2A-2C show the binding capacities of antibodies A1C8C6H1, A1D1B7H9, A1D5E2H1 and A1D11A7H10 (A), A1E7G5D1, A1E10G7H9, A2A1D2F1 and A2A5C7E8 (B), A2A6B1C2, A2G4C8G7 and A2H5F1A1 (C) to human-siglec15-2D3-1E1 cells expressing human Siglec15 in a cell based binding FACS assay.