

decreased overall survival in patients with breast cancer. See, *e.g.*, Plitas *et al.*, *Immunity* (2016) 45:1122-1134. CCR8 therefore represents a promising and safer target for Treg cell depletion in cancer treatment. Therefore, agents that recognize CCR8, and methods of using such agents, are desired.

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SUMMARY

The present disclosure provides anti-CCR8 antibodies, composition, and methods of preparation and using the same.

Example 1. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to C-C motif chemokine receptor 8 (CCR8), wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 32, and a light chain variable domain (VL) comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 26, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 27, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 28.

Example 2. The foregoing antibody of Example 1, which binds to CCR8 independent of sulfation of CCR8.

Example 3. The foregoing antibody of Example 1 or 2, wherein the antibody binds to an epitope comprised of one or more of amino acid residues 2-6 of SEQ ID NO: 106.

Example 4. The foregoing antibody of any one of Examples 1-3, comprising a sequence selected from the group consisting of: (a) a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 35-47; (b) a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 48-52; and (c) a VH sequence as defined in (a) and a VL sequence as defined in (b).

Example 5. The foregoing antibody of any one of Examples 1-4, comprising a VH sequence selected from the group consisting of SEQ ID NOs: 35-47 and a VL sequence selected from the group consisting of SEQ ID NOs: 48-52.

Example 6. The foregoing antibody of any one of Examples 1-5, comprising a sequence selected from the group consisting of: (a) a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO: 47; (b) a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO: 48; and (c) a VH sequence as defined in (a) and a VL sequence as defined in (b).

Example 7. The foregoing antibody of any one of Examples 1-6, comprising a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to an amino acid sequence of SEQ ID NO: 47 and a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to an amino acid sequence of SEQ ID NO: 48.

Example 8. The foregoing antibody of any one of Examples 1-7, wherein the VL comprises a V4M mutation, a P43A mutation, a F46L mutation, a C90Q mutation, or a combination thereof.

Example 9. The foregoing antibody of any one of Examples 1-8, wherein the VH comprises a G49S mutation, a K71R mutation, a S73N mutation, or a combination thereof.

Example 10. The foregoing antibody of any one of Examples 1-9, comprising the heavy chain amino acid sequence of SEQ ID NO: 55, and the light chain amino acid sequence of SEQ ID NO: 56.

Example 11. The foregoing antibody of any one of Examples 1-9, comprises the heavy chain amino acid sequence of SEQ ID NO: 60, and the light chain amino acid sequence of SEQ ID NO: 56.

Example 12. The foregoing antibody of any one of Examples 1-9, comprises the heavy chain amino acid sequence of SEQ ID NO: 111, and the light chain amino acid sequence of SEQ ID NO: 56.

Example 13. The foregoing antibody of any one of Examples 1-9, comprises the heavy chain amino acid sequence of SEQ ID NO: 113, and the light chain amino acid sequence of SEQ ID NO: 56.

Example 14. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to CCR8 comprising a VH sequence selected from the group consisting of SEQ ID NOs: 35-47 and a VL sequence selected from the group consisting of SEQ ID NOs: 48-52.

Example 15. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to CCR8 comprising a VH sequence of SEQ ID NO: 47 and a VL sequence of SEQ ID NO: 48.

Example 16. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 6, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 7, and a light chain variable domain (VL) comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 1, (e) CDR-L2

comprising the amino acid sequence of SEQ ID NO: 2, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 3.

Example 17. The foregoing antibody of Example 16, which binds to CCR8 independent of sulfation of CCR8.

5 Example 18. The foregoing antibody of Example 16 or 17, wherein the antibody binds to an epitope comprised of one or more of amino acid residues 91-104 and 172-193 of SEQ ID NO: 106.

Example 19. The foregoing antibody of any one of Examples 16-18, comprising a sequence selected from the group consisting of: (a) a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to an amino acid sequence
10 selected from the group consisting of SEQ ID NOs: 10-21; (b) a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25; and (c) a VH sequence as defined in (a) and a VL sequence as defined in (b).

Example 20. The foregoing antibody of any one of Examples 16-19, comprising a VH
15 sequence selected from the group consisting of SEQ ID NOs: 10-21 and a VL sequence selected from the group consisting of SEQ ID NOs: 22-25.

Example 21. The foregoing antibody of any one of Examples 16-20, comprising a sequence selected from the group consisting of: (a) a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence
20 of SEQ ID NO: 21; (b) a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO: 24; and (c) a VH sequence as defined in (a) and a VL sequence as defined in (b).

Example 22. The foregoing antibody of any one of Examples 16-21, comprising a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at
25 least about 99% identity to an amino acid sequence of SEQ ID NO: 21 and a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to an amino acid sequence of SEQ ID NO: 24.

Example 23. The foregoing antibody of any one of Examples 16-22, wherein the VL comprises a Y2I mutation.

30 Example 24. The foregoing antibody of any one of Examples 16-23, wherein the VH comprises a S73N mutation, a V78L mutation, a T76N mutation, a F91Y mutation, and a P105Q mutation, or a combination thereof.

Example 25. The foregoing antibody of any one of Examples 16-24, comprising the heavy chain amino acid sequence of SEQ ID NO: 57, and the light chain amino acid sequence of SEQ ID
35 NO: 58.

Example 26. The foregoing antibody of any one of Examples 16-24, comprises the heavy chain amino acid sequence of SEQ ID NO: 61, and the light chain amino acid sequence of SEQ ID NO: 58.

5 Example 27. The foregoing antibody of any one of Examples 16-24, comprises the heavy chain amino acid sequence of SEQ ID NO: 112, and the light chain amino acid sequence of SEQ ID NO: 58.

Example 28. The foregoing antibody of any one of Examples 16-24, comprises the heavy chain amino acid sequence of SEQ ID NO: 114, and the light chain amino acid sequence of SEQ ID NO: 58.

10 Example 29. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to CCR8 comprising a VH sequence selected from the group consisting of SEQ ID NOs: 10-21 and a VL sequence selected from the group consisting of SEQ ID NOs: 22-25.

15 Example 30. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to CCR8 comprising a VH sequence of SEQ ID NO: 21 and a VL sequence of SEQ ID NO: 24.

20 Example 31. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 82 or SEQ ID NO: 83, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 84, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 85, and a light chain variable domain (VL) comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 73, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 74, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 75.

25 Example 32. The foregoing antibody of Example 31, comprising a sequence selected from the group consisting of: (a) a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO: 95; (b) a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO: 94; and (c) a
30 VH sequence as defined in (a) and a VL sequence as defined in (b).

Example 33. The foregoing antibody of Example 31 or 32, comprising a VH sequence of SEQ ID NO: 95 and a VL sequence of SEQ ID NO: 94.

35 Example 34. The foregoing antibody of any one of Examples 31-33, comprising the heavy chain amino acid sequence of SEQ ID NO: 101, and the light chain amino acid sequence of SEQ ID NO: 100.

Example 35. The foregoing antibody of any one of Examples 31-33, comprising the heavy chain amino acid sequence of SEQ ID NO: 115, and the light chain amino acid sequence of SEQ ID NO: 100.

5 Example 36. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 86 or SEQ ID NO: 87, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 88, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 89, and a light chain variable domain (VL) comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 76, (e) CDR-L2
10 comprising the amino acid sequence of SEQ ID NO: 77, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 78.

Example 37. The foregoing antibody of Example 36, comprising a sequence selected from the group consisting of: (a) a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO:
15 97; (b) a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO: 96; and (c) a VH sequence as defined in (a) and a VL sequence as defined in (b).

Example 38. The foregoing antibody of Example 36 or 37, comprising a VH sequence of SEQ ID NO: 97 and a VL sequence of SEQ ID NO: 96.

20 Example 39. The foregoing antibody of any one of Examples 36-38, comprising the heavy chain amino acid sequence of SEQ ID NO: 103, and the light chain amino acid sequence of SEQ ID NO: 102.

Example 40. The foregoing antibody of any one of Examples 36-38, comprising the heavy chain amino acid sequence of SEQ ID NO: 116, and the light chain amino acid sequence of SEQ ID
25 NO: 102.

Example 41. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 90 or SEQ ID NO: 91, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 92, and (c) CDR-
30 H3 comprising the amino acid sequence of SEQ ID NO: 93, and a light chain variable domain (VL) comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 79, (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 80, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 81.

Example 42. The foregoing antibody of Example 41, comprising a sequence selected from the
35 group consisting of: (a) a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ

ID NO: 99; (b) a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO: 98; and (c) a VH sequence as defined in (a) and a VL sequence as defined in (b).

5 Example 43. The foregoing antibody of Example 41 or 42, comprising a VH sequence of SEQ ID NO: 99 and a VL sequence of SEQ ID NO: 98.

Example 44. The foregoing antibody of any one of Examples 41-43, comprising the heavy chain amino acid sequence of SEQ ID NO: 105, and the light chain amino acid sequence of SEQ ID NO: 104.

10 Example 45. The foregoing antibody of any one of Examples 41-44, comprising the heavy chain amino acid sequence of SEQ ID NO: 117, and the light chain amino acid sequence of SEQ ID NO: 104.

Example 46. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to CCR8, wherein the antibody binds to CCR8 independent of sulfation of CCR8.

15 Example 47. The foregoing antibody of Example 46, wherein the antibody binds to an epitope comprised of one or more of amino acid residues 2-6 of SEQ ID NO: 106.

Example 48. The foregoing antibody of Example 46, wherein the antibody binds to binds to an epitope comprised of one or more of amino acid residues 91-104 and 172-193 of SEQ ID NO: 106.

20 Example 49. In certain non-limiting examples, the presently disclosed subject matter provides for a monoclonal antibody that binds to mouse CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 65 or SEQ ID NO: 66, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 67 and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 68, and a light chain variable domain (VL) comprising (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 62, (e) CDR-L2
25 comprising the amino acid sequence of SEQ ID NO: 63, and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 64.

Example 50. The foregoing antibody of Example 49, comprising a sequence selected from the group consisting of: (a) a VH sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO: 70; (b) a VL sequence having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to the amino acid sequence of SEQ ID NO: 69; and (c) a VH
30 sequence as defined in (a) and a VL sequence as defined in (b).

Example 51. The foregoing antibody of Example 49 or 50, comprising a VH sequence of SEQ ID NO: 70 and a VL sequence of SEQ ID NO: 69.

Example 52. The foregoing antibody of any one of Examples 49-51, comprising the heavy chain amino acid sequence of SEQ ID NO: 72, and the light chain amino acid sequence of SEQ ID NO: 71.

5 Example 53. The foregoing antibody of any one of Examples 1 to 48, which is a human antibody.

Example 54. The foregoing antibody of any one of Examples 1 to 48, which is a humanized antibody.

Example 55. The foregoing antibody of any one of Examples 1 to 52, which is a chimeric antibody.

10 Example 56. The foregoing antibody of any of Examples 1 to 55, which is an antibody fragment that binds to CCR8.

Example 57. The foregoing antibody of any of Examples 1 to 56, which is a full-length antibody.

Example 58. The foregoing antibody of Example 57, which is a full-length IgG1 antibody.

15 Example 59. The foregoing antibody of any of Examples 1 to 58, comprising a IgG1 constant domain comprising the amino acid sequence of SEQ ID NO: 53 or SEQ ID NO: 59.

Example 60. The foregoing antibody of any of Examples 1 to 59, comprising a kappa constant domain comprising the amino acid sequence of SEQ ID NO: 54.

20 Example 61. The foregoing antibody of any of Examples 1 to 60, wherein the antibody binds to CCR8 with a binding affinity (Kd) of from about 1×10^{-12} M to about 1×10^{-11} M.

Example 62. The foregoing antibody of any of Examples 1 to 48, wherein the CCR8 is a human CCR8.

Example 63. The foregoing antibody of any of Examples 1 to 62, wherein the antibody is afucosylated.

25 Example 64. In certain non-limiting examples, the presently disclosed subject matter provides for an isolated nucleic acid encoding the foregoing antibody of any of Examples 1 to 63.

Example 65. In certain non-limiting examples, the presently disclosed subject matter provides for a host cell comprising the foregoing nucleic acid of Example 64.

30 Example 66. In certain non-limiting examples, the presently disclosed subject matter provides for a method of producing an antibody that binds to CCR8 comprising culturing the foregoing host cell of Example 65 under conditions suitable for the expression of the antibody.

Example 67. The foregoing method of Example 66, further comprising recovering the antibody from the host cell.

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Example 68. In certain non-limiting examples, the presently disclosed subject matter provides for an antibody produced by the foregoing method of Example 67.

Example 69. In certain non-limiting examples, the presently disclosed subject matter provides for a pharmaceutical composition comprising the foregoing antibody of any of Examples 1 to 63 and a
5 pharmaceutically acceptable carrier.

Example 70. The foregoing pharmaceutical composition of Example 69, further comprising an additional therapeutic agent.

Example 71. The foregoing antibody of any one of Examples 1 to 63 or the foregoing pharmaceutical composition of any one of Examples 69 to 70 for use as a medicament.

10 Example 72. The foregoing antibody of any one of Examples 1 to 63 or the foregoing pharmaceutical composition of any one of Examples 69 to 70 for use in treating cancer.

Example 73. In certain non-limiting examples, the presently disclosed subject matter provides for use of the foregoing antibody of any one of Examples 1 to 63 or the foregoing pharmaceutical composition of any of Examples 69 to 70 in the manufacture of a medicament for treating cancer.

15 Example 74. In certain non-limiting examples, the presently disclosed subject matter provides for use of the foregoing antibody of any one of Examples 1 to 63 or the foregoing pharmaceutical composition of any of Examples 69 to 70 in the manufacture of a medicament for depleting regulatory T cells.

Example 75. In certain non-limiting examples, the presently disclosed subject matter provides
20 for a method of treating cancer in a subject in need thereof comprising administering to the subject an effective amount of the foregoing antibody of any one of Examples 1 to 63 or the foregoing pharmaceutical composition of any of Examples 69 to 70 .

Example 76. In certain non-limiting examples, the presently disclosed subject matter provides for a method of depleting regulatory T cells in a tumor microenvironment in a subject having cancer
25 comprising administering to the subject an effective amount of the foregoing antibody of any of Examples 1 to 63 or the foregoing pharmaceutical composition of any of Examples 69 to 70 sufficient to deplete the regulatory T cells in the tumor microenvironment.

Example 77. In certain non-limiting examples, the presently disclosed subject matter provides for a method of depleting regulatory T cells outside of a tumor microenvironment in a subject having
30 cancer comprising administering to the subject an effective amount of the foregoing antibody of any of Examples 1 to 63 or the foregoing pharmaceutical composition of any of Examples 69 to 70 sufficient to deplete the regulatory T cells outside of the tumor microenvironment.

Example 78. In certain non-limiting examples, the presently disclosed subject matter provides for an in vitro method of depleting regulatory T cells from a cancer cell population,

comprising contacting the cell population with the foregoing antibody of any of Examples 1 to 63 or the foregoing pharmaceutical composition of any of Examples 69 to 70 in an amount sufficient to deplete the regulatory T cells from the cell population.

Example 79. The foregoing use or method of any one of Examples 73-78, wherein the cancer is selected from the group consisting of bladder cancer, blastoma, blood cancer, bone cancer, brain cancer, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, gastric cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, sarcoma, skin cancer, testicular cancer, and uterine cancer.

Example 80. The foregoing use or method of any one of Examples 74, 76, 78, and 79, wherein the regulatory T cells present in the tumor microenvironment of the cancer are depleted.

Example 81. The foregoing use or method of any one of Examples 74, 77, 78, and 79, wherein the regulatory T cells outside of the tumor microenvironment of the cancer are depleted.

Example 82. The foregoing use or method of any one of Examples 73-81, further comprising administering an additional therapeutic agent.

Example 83. The foregoing use or method of Example 82, wherein the additional therapeutic agent is an anti-cancer agent.

Example 84. The foregoing use or method of Example 83, wherein the anti-cancer agent is selected from the group consisting of a microtubule disruptor, an antimetabolite, a topoisomerase inhibitor, a DNA intercalator, an alkylating agent, a hormonal therapy, a kinase inhibitor, a receptor antagonist, an activator of tumor cell apoptosis, antiangiogenic agent, an immunomodulatory agent, an inhibitor of cell adhesion, a cytotoxic or cytostatic agent, an activator of cell apoptosis, an agent that increases the sensitivity of cells to apoptotic inducers, a cytokine, an anti-cancer vaccine or oncolytic virus, a toll-like receptor (TLR) agent, a bispecific antibody, a cellular therapy, and immune cell engager.

Example 85. The foregoing use or method of Example 83 or 84, wherein the anti-cancer agent is a PD-L1 binding antagonist.

Example 86. The foregoing use or method of Example 85, wherein the PD-L1 binding antagonist is atezolizumab.

Example 87. The foregoing use or method of any one of Examples 73-85, wherein the subject is a human.

Example 88. The foregoing use or method of any one of Examples 73-85, wherein the subject is a mouse.

Example 89. In certain non-limiting examples, the presently disclosed subject matter provides for a method of treating a disease in a mouse comprising administering an effective amount of a monoclonal antibody of any one of foregoing Examples 49-52 to the mouse to treat the disease.

Example 90. The forgoing method of Example 89, wherein the mouse comprises a xenograft.

Example 91. The forgoing antibody of Example 63, wherein the proportion of afucosylation is between about 80% to about 95%.

Example 92. The forgoing antibody of any one of Examples 1-15 and 46-48, wherein the
5 mean clearance after a single 10 mg/kg dose administered intravenously on day 1 is between about 3 to about 5 mL/day/kg over a 35 day period.

In one aspect, the present disclosure refers to a monoclonal antibody that binds to C-C motif chemokine receptor 8 (CCR8), wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30,
10 (b) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and (c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 32, and a light chain variable domain (VL) comprising (d) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 26, (e) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 27, and (f) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 28.

15 In one aspect, the present disclosure refers to a monoclonal antibody that binds to CCR8, wherein the antibody comprises a VH comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 35-47 and a VL comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48-52.

In one aspect, the present disclosure refers to a monoclonal antibody that binds to CCR8,
20 wherein the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 47 and a VL comprising the amino acid sequence of SEQ ID NO: 48.

In one aspect, the present disclosure refers to a monoclonal antibody that binds to CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 5, (b) a CDR-H2 comprising
25 the amino acid sequence of SEQ ID NO: 6, and (c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 7, and a light chain variable domain (VL) comprising (d) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 1, (e) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 2, and (f) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 3.

In one aspect, the present disclosure refers to a monoclonal antibody that binds to CCR8,
30 wherein the antibody comprises a VH comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 10-21 and a VL comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25.

In one aspect, the present disclosure refers to a monoclonal antibody that binds to CCR8, wherein the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 21 and a VL comprising the amino acid sequence of SEQ ID NO: 24.

5 In one aspect, the present disclosure refers to a monoclonal antibody that binds to CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 82 or SEQ ID NO: 83, (b) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 84, and (c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 85, and a light chain variable domain (VL) comprising (d) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 73, (e) a CDR-L2 comprising the amino acid sequence of SEQ
10 ID NO: 74, and (f) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 75.

In one aspect, the present disclosure refers to a monoclonal antibody that binds to CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 86 or SEQ ID NO: 87, (b) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 88, and (c) a CDR-H3 comprising the amino acid sequence
15 of SEQ ID NO: 89, and a light chain variable domain (VL) comprising (d) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 76, (e) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 77, and (f) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 78.

In one aspect, the present disclosure refers to a monoclonal antibody that binds to CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) a CDR-H1
20 comprising the amino acid sequence of SEQ ID NO: 90 or SEQ ID NO: 91, (b) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 92, and (c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 93, and a light chain variable domain (VL) comprising (d) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 79, (e) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 80, and (f) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 81.

25 In one aspect, the present disclosure refers to a monoclonal antibody that binds to CCR8, wherein the antibody binds to CCR8 independent of sulfation of CCR8.

In one aspect, the present disclosure refers to a monoclonal antibody that binds to mouse CCR8, wherein the antibody comprises a heavy chain variable domain (VH) comprising (a) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 65 or SEQ ID NO: 66, (b) a CDR-H2
30 comprising the amino acid sequence of SEQ ID NO: 67 and (c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 68, and a light chain variable domain (VL) comprising (d) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 62, (e) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 63, and (f) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 64.

35 In one aspect, the present disclosure refers to an isolated nucleic acid encoding the antibody disclosed herein.

In one aspect, the present disclosure refers to a host cell comprising the nucleic acid disclosed herein.

In one aspect, the present disclosure refers to a method of producing an antibody that binds to CCR8, wherein the method comprises culturing the host cell disclosed herein under conditions
5 suitable for the expression of the antibody, optionally wherein the method further comprises recovering the antibody from the host cell.

In one aspect, the present disclosure refers to an antibody produced by the method disclosed herein.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising the
10 antibody disclosed herein and a pharmaceutically acceptable carrier, optionally wherein the pharmaceutical composition further comprises an additional therapeutic agent.

In one aspect, the present disclosure refers to the antibody disclosed herein or the pharmaceutical composition disclosed herein for use as a medicament.

In one aspect, the present disclosure refers to the antibody disclosed herein or the
15 pharmaceutical composition disclosed herein for use in treating cancer.

In one aspect, the present disclosure refers to use of the antibody disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating cancer.

In one aspect, the present disclosure refers to the antibody disclosed herein or the pharmaceutical composition disclosed herein for use in depleting regulatory T cells.

20 In one aspect, the present disclosure refers to use of the antibody disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for depleting regulatory T cells.

In one aspect, the present disclosure refers to the antibody disclosed herein or the pharmaceutical composition disclosed herein for use in depleting regulatory T cells in the tumor
25 microenvironment in a subject having cancer.

In one aspect, the present disclosure refers to use of the antibody disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for depleting regulatory T cells in the tumor microenvironment in a subject having cancer.

30 In one aspect, the present disclosure refers to the antibody disclosed herein or the pharmaceutical composition disclosed herein for use in depleting regulatory T cells outside of the tumor microenvironment in a subject having cancer.

In one aspect, the present disclosure refers to use of the antibody disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for depleting regulatory T cells outside of the tumor microenvironment in a subject having cancer.

35 In one aspect, the present disclosure refers to an *in vitro* method of depleting regulatory T cells from a cancer cell population, wherein the method comprises contacting the cell population with the antibody disclosed herein or the pharmaceutical composition disclosed herein in an amount sufficient to deplete the regulatory T cells from the cell population.

40 In one aspect, the present disclosure refers to the monoclonal antibody disclosed herein for use in treating a disease in a mouse, optionally wherein the mouse comprises a xenograft.

In one aspect, the present disclosure refers to use of the monoclonal antibody disclosed herein in the manufacture of a medicament for treating a disease in a mouse, optionally wherein the mouse comprises a xenograft.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the results of a screening of anti-CCR8 monoclonal antibodies (mAbs) selectively binding to Treg cells (Tregs) from human colorectal cancer dissociated tumor cells (DTC) (obtained from Discovery Life Sciences). Shown are mean fluorescent intensity (MFI) values for CD8 T cells (defined as CD45+ CD14- CD3+ CD8+ CD4-) (circles, ○), conventional CD4 T cells (defined as CD45+ CD14- CD3+ CD8- CD4+ FOXP3-) (squares, □), and Treg cells (defined as CD45+ CD14- CD3+ CD8- CD4+ FOXP3+) (triangles, ▲). Three of the five anti-CCR8 mAb clones Ab1-Ab5 specifically stained intratumoral Treg cells and not conventional CD4 or CD8 T cells, and were ranked based on CCR8 MFI: **hu.Ab4.H1L1** > **hu.Ab5.H1L1** > **hu.Ab3.H1L1**.

FIGS. 2A-2B depicts the proposed mechanism of action of natural killer (NK) cell-mediated antibody-dependent cellular cytotoxicity (ADCC), resulting in depletion of tumor-infiltrating CCR8-expressing Tregs (**FIG. 2A**) and the ADCC activities of human/cyno cross-reactive anti-CCR8 mAbs brought forward for further study (**FIG. 2B**). EC₅₀ values were determined as 0.02 nM, 0.02 nM, and 0.08 nM for anti-CCR8 mAbs **hu.Ab3.H1L1**, **hu.Ab5.H1L1**, and **hu.Ab4.H1L1**, respectively.

FIGS. 3A-3D depict the agonist and antagonist activities of the human/cyno cross-reactive anti-CCR8 mAbs **hu.Ab4.H1L1**, **hu.Ab5.H1L1**, and **hu.Ab3.H1L1**, as well as comparator anti-CCR8 mAbs (the humanized anti-human Yoshida anti-CCR8 antibody, murine anti-human CCR8 mAb 433H (BD Biosciences), and murine anti-human CCR8 mAb L263G8 (Biolegend)). As shown in **FIG. 3A**, CCL1, a known ligand for CCR8, shows agonist activity, but none of the anti-CCR8 test mAbs show agonistic effects. The data in **FIG. 3B** shows anti-CCR8 mAb **hu.Ab4.H1L1** demonstrates antagonistic (neutralizing) activity against the CCR8 ligand CCL1 (20 nM of ligand), whereas anti-CCR8 mAbs **hu.Ab5.H1L1** and **hu.Ab3.H1L1** demonstrate no ligand blocking (non-neutralizing) activity at the concentration studied. The data in **FIG. 3C** shows that comparator anti-CCR8 mAbs (the humanized anti-human Yoshida anti-CCR8 antibody, murine anti-human CCR8 mAb 433H (BD Biosciences), and murine anti-human CCR8 mAb L263G8 (Biolegend)) do not show agonistic effects, whereas the CCR8 ligand CCL1 does. The data in **FIG. 3D** shows comparator anti-CCR8 mAbs (the humanized anti-human Yoshida anti-CCR8 antibody, murine anti-human CCR8 mAb 433H (BD Biosciences), and murine anti-human Biolegend L263G8 (Biolegend)) demonstrate