

ANTI-CCR8 ANTIBODIES FOR TREATING CANCER

Throughout this application, various publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. Full citations for these publications may be found at the end of the specification immediately preceding the

5 claims. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, these disclosures are incorporated into the present application only to the extent that no conflict exists between the information incorporated by reference and the

10 information provided by explicit disclosure in the present application. Moreover, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Nos.

15 63/157,618, filed March 5, 2021; 63/041,992, filed June 21, 2020; and 62/993,570, filed March 23, 2020, the content of each of which is hereby incorporated herein by reference in its entirety.

SEQUENCE LISTING

The present application contains a Sequence Listing which has been submitted

20 electronically in ASCII format and is hereby incorporated herein by reference in its entirety. The ASCII copy was created on March 19, 2021, is named 20210319_SEQ1_13358WOPCT.txt, and is 81,920 bytes in size.

FIELD OF THE INVENTION

The disclosed invention relates to isolated antibodies (Abs), *e.g.*, monoclonal

25 antibodies (mAbs), that bind specifically to C-C Motif Chemokine Receptor 8 (CCR8), and methods for treating a cancer in a subject comprising administering to the subject an anti-CCR8 Ab as monotherapy or in combination with an anticancer agent such as an immune checkpoint inhibitor.

BACKGROUND OF THE INVENTION

30 Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Chakravarthi *et al.*, 2016).

Harnessing the attributes of the adaptive immune system to treat cancer makes immunotherapy unique among all cancer treatment modalities in its applicability to a broad range of cancers and in inducing durable anti-tumor effects.

5 Considerable success has already been achieved in treating diverse solid tumors and hematological malignancies by stimulating the activity of cytotoxic T cells using checkpoint inhibitors such as the anti-PD-1 Ab, nivolumab (OPDIVO®) and the anti-CTLA-4 Ab, ipilimumab (YERVOY®). However, typically less than around 15% of patients benefit long-term from treatment with a checkpoint inhibitor in cancers amenable to this treatment (Haslam and Prasad, 2019), and checkpoint inhibitors have proven to be
10 less effective in certain cancers, including breast and prostate cancers. The persistence of immunosuppressive mechanisms, especially those mediated by regulatory T cells (Tregs), may contribute to the observed resistance of certain cancers or certain patients to treatment with checkpoint inhibitors (Fares *et al.*, 2019; Han *et al.*, 2019). As one means of overcoming this resistance, the present application discloses methods of stimulating the
15 immune system by reducing the immunosuppressive effects of Tregs.

 Tumor-infiltrating CD4⁺ CD25⁺ FOXP3⁺ Tregs, a subset of CD4⁺ T cells, are mediators of immunological self-tolerance. Deficiency of genes involved in Treg development and function result in systemic autoimmunity in both mice (Fontenot *et al.*, 2003; Khattri *et al.*, 2003; Tivol *et al.*, 1995) and humans (Yagi *et al.*, 2004; Kuehn *et al.*,
20 2014), revealing an important non-redundant role for Tregs in maintaining immune homeostasis. Tregs suppress the immune system via multiple mechanisms including downregulating the induction and proliferation of effector T cells, secretion of chemokines and inhibitory cytokines, and suppression of dendritic cell maturation and function (Shitara and Nishikawa, 2018; Han *et al.*, 2019). Mechanisms utilized by Tregs
25 to promote self-tolerance may be co-opted in the tumor microenvironment to suppress the anti-tumor immune response. Indeed, systemic depletion of Tregs in mice is sufficient to enable immune-mediated tumor regression (Teng *et al.*, 2010). Thus, Tregs are thought to play a role in mediating peripheral tolerance to self-antigens, preventing autoimmune disease, and suppressing anti-tumor immune responses.

30 Consequently, reducing the activity or numbers of tumor-infiltrating Tregs has been identified as an attractive approach to reversing immunosuppressive activity in the tumor microenvironment and augmenting anti-tumor immunity (Finotello and Trajanoski, 2017; Han *et al.*, 2019). A variety of methods have been and are being pursued to target

Tregs in cancer immunotherapy, including ADCC-mediated depletion of Tregs using antibodies (Abs) to antigens expressed on Tregs such as CD25 (Arce Vargas *et al.*, 2017), CCR4 (Ishida *et al.*, 2012; Hagemann *et al.*, 2014) and CTLA-4 (Korman *et al.*, 2017), and inhibition of E3 ubiquitin ligase Siah2 (Scortegagna *et al.*, 2020) and Yes-associated protein (YAP; Ni *et al.*, 2018). However, clinical attempts to specifically target or deplete
5 Treg from the tumor microenvironment have been unsuccessful. Diphtheria toxin fused to IL-2 (denileukin diftitox) failed to effectively reduce Treg numbers in melanoma patients (Luke *et al.*, (2016), and despite documented anti-CTLA-4 mediated Treg depletion in mouse tumor models (Selby *et al.*, 2013; Simpson *et al.*, 2013), clear evidence for Treg
10 depletion by ipilimumab or tremelimumab (anti-human CTLA-4 Abs) in human cancer is unclear (Sharma *et al.*, 2019a; Sharma *et al.*, 2019b). Treg depletion was achieved with the nonfucosylated (nf) anti-CCR4 Ab mogamulizumab, but significant depletion of conventional CD4⁺ T cells and modest reductions in CD8⁺ T cell numbers were also observed (Kurose *et al.*, 2015), limiting its utility in the treatment of solid tumors. Thus,
15 there remains a need for a safe and effective Treg depleting agent that also spares T effector cells (Teffs) for optimal anti-tumor responses.

CCR8 is a chemokine receptor that has recently been identified as a potential specific marker for tumor-infiltrating Tregs, as CCR8 expression is selectively upregulated in these Tregs in multiple cancers, including breast, colorectal, and lung
20 (Plitas *et al.*, 2016; De Simone *et al.*, 2016; Wang *et al.*, 2019), and as a core member of the IRF4-dependent 'effector' Treg gene program (Alvisi *et al.*, 2020). These CCR8⁺ Tregs represent a highly activated and suppressive subpopulation of Tregs, and high abundance of CCR8⁺ Tregs in these tumor types is associated with poor prognosis (Wang *et al.*, 2019; De Simone *et al.*, 2016). Therefore, CCR8 may be a promising therapeutic
25 target to effect the depletion of tumor-resident Tregs in order to augment anti-tumor immunity. Targeting CCR8, while depleting suppressive Tregs, may also have the advantage of not depleting cytolytic effector cells that drive anti-tumor immune responses. Moreover, because CCR8 is rarely expressed on Tregs and Teffs in peripheral blood or in other tissues, targeting CCR8⁺ tumor Tregs may pose minimal toxicity risks.

30 CCR8 is a seven-transmembrane G-protein-coupled chemokine receptor (GPCR) expressed primarily on intratumoral FOXP3^{hi} Tregs (Wang *et al.*, 2019; Plitas *et al.*, 2016; De Simone *et al.*, 2016). The N-terminus and ECL2 regions of CCR8 are important for binding to its functional ligand C-C Motif Chemokine Ligand 1 (CCL1), which is

produced by intratumoral myeloid cells and T cells. In addition to affecting the migration of Tregs to the tumor microenvironment (TME), the CCL1:CCR8 interaction also potentiates the immunosuppressive capacity of Tregs through upregulation of CCR8, FOXP3, IL-10, and other suppressive factors (Vila-Caballer *et al.*, 2019).

5 Recently issued U.S. Patent No. 10,087,259 claims a method of treating cancer by administering to a cancer patient an Ab agent that binds specifically to CCR8 so that tumor-infiltrating Treg cells are specifically depleted in the patient to a greater extent than are normal-tissue infiltrating T cells. However, this patent does not exemplify any Ab agent that binds specifically to CCR8 nor does it demonstrate any method of treating any
10 cancer by administering an anti-CCR8 Ab to a subject.

 U.S. Patent No. 10,550,191 claims a method for treating a cancer comprising administering an Ab against CCR8. The Examples demonstrate that a single Ab, a commercially available rat IgG2b anti-mouse CCR8 (anti-mCCR8) Ab (Clone SA214G2; BioLegend, San Diego, CA), reduces the volume of a variety of tumors in mouse tumor
15 models. No anti-human CCR8 (anti-hCCR8) Ab, or any chimeric, humanized or human Ab suitable for use in human therapy is disclosed.

 PCT Publication No. WO 2018/112033 relates to methods for treating cancer by administering to a subject an agent that induces cytotoxicity in tumor-infiltrating Treg cells that express a specified gene product included in Table 1 or 2 and thereby decreases
20 the number or activity of tumor-infiltrating Treg cells in the subject. CCR8 is not a gene product included in Table 1 or 2, but Example 8 demonstrates a moderate level of anti-tumor activity of an anti-mCCR8 Ab in a mouse MC38 colon adenocarcinoma model.

 PCT Publication No. WO 2019/157098 relates to an immunogenic composition comprising a recombinant *Listeria* strain and an anti-CCR8 Ab, and a method of treating
25 a tumor in a subject comprising administering this immunogenic composition to the subject. Similar to U.S. Patent No. 10,550,191, WO 2019/157098 does not report generating any anti-CCR8 Ab but instead demonstrates the use of the commercial SA214G2 anti-mCCR8 Ab, in combination with *Listeria*-based immunotherapy, to treat implanted colon carcinoma tumors in a mouse model.

30 More recently, various groups have reported the generation of humanized anti-CCR8 Abs for use in depleting tumor-associated Tregs and treating cancer (*see, e.g.*, Dépis *et al.*, 2020; WO 2020/138489; Harbour BioMed, 2020).

 The invention disclosed herein demonstrates that CCR8 expression is highly

restricted to tumor Tregs from diverse tumor types. The invention comprises the production of anti-CCR8 Abs, specifically anti-CCR8 mAbs, including human, humanized and chimeric anti-hCCR8 mAbs, and demonstrates that anti-CCR8-mediated Treg depletion in mouse tumor models requires Fc engagement. The present disclosure
5 also describes the development of nf anti-hCCR8 Abs that mediate tumor-specific Treg depletion in *ex vivo* human tumor culture systems. Anti-CCR8 Ab treatment as monotherapy or in combination with checkpoint blockade, for example, inhibition of the PD-1/PD-L1 signaling pathway, induces potent anti-tumor responses and may provide clinical benefit to patients who do not respond to anti-PD-1 monotherapy. The
10 combination of the mechanisms of action of anti-CCR8-mediated Treg depletion and checkpoint blockade offers a unique opportunity to increase the killing of tumor cells and thereby treat a wide range of cancers.

SUMMARY OF THE INVENTION

The present invention provides isolated Abs, preferably mAbs, that specifically
15 bind to CCR8, such as human CCR8 (hCCR8), expressed on the surface of a cell and exhibit various functional properties, including properties that are desirable in a therapeutic Ab. These properties include binding with high affinity to CCR8-expressing cells, such as tumor-infiltrating, activated CD4⁺FOXP3^{high} Tregs; other than Tregs, binding only to rare and scattered immune cells in the medulla of the thymus and dermis
20 of the skin but not binding to many other tissues; mediating depletion of the CCR8-expressing cells, such as tumor-infiltrating, activated CD4⁺FOXP3^{high} Tregs, by ADCC; mediating depletion specifically of tumor-infiltrating Tregs but not of CCR8⁺ T cells in normal tissues; inhibiting binding of CCL1 to CCR8 and inhibiting CCR8/CCL1 signaling; not causing internalization of CCR8 when bound to CCR8 on the surface of a
25 cell either in the presence or absence of a cross-linking Ab; mediating specific depletion of CCR8⁺ Tregs *in vitro* and in human tumor *ex-vivo* tissue samples; and inhibiting growth of tumor cells in a subject, preferably a human subject, when administered to the subject as monotherapy or in combination with another anti-cancer agent. In preferred embodiments, the anti-CCR8 Ab is a modified mAb comprising a modified heavy chain
30 constant region, such as a hypofucosylated or nonfucosylated (nf) heavy chain constant region, that binds with higher affinity to an Fcγ receptor (FcγR) and mediates enhanced ADCC compared to an unmodified mAb.

Specifically, this disclosure provides an isolated Ab, preferably a mAb, or an

antigen-binding portion thereof, that specifically binds to CCR8 expressed on the surface of a cell and mediates depletion of the CCR8-expressing cell by ADCC. In certain embodiments, the CCR8 is hCCR8 having the amino acid sequence set forth in SEQ ID NO: 1. In certain other embodiments, the Ab, *e.g.*, the mAb, or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG3 isotype.

The disclosure also provides a modified anti-hCCR8 mAb, or an antigen-binding portion thereof, which comprises a modified heavy chain constant region that binds with higher affinity to an Fc γ receptor (Fc γ R) and mediates enhanced ADCC compared to an unmodified mAb or antigen-binding portion thereof. In preferred embodiments, the modified mAb or antigen-binding portion thereof comprises a modified IgG1 heavy chain constant region which exhibits reduced fucosylation. In certain embodiments, the mAb or antigen-binding portion thereof binds to a N-terminal peptide of hCCR8, wherein the epitope comprises at least one amino acid within a peptide having the sequence Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁ (SEQ ID NO: 2) and further comprises sulfated tyr-15 and/or sulfated tyr-17 residues. In certain preferred embodiments, the mAb or antigen-binding portion thereof binds to a N-terminal peptide of hCCR8, wherein the epitope comprises at least one amino acid within a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109) and further comprises sulfated tyr-15 and sulfated tyr-17 residues.

In certain embodiments, the anti-CCR8 mAb or antigen-binding portion thereof exhibits at least one, *e.g.*, at least 2, 3, 4, 5, 6, 7 or all of the following properties: (a) specifically binds to CCR8 expressed on the surface of a cell with an EC₅₀ of about 1 nM or lower or an EC₅₀ of about 2 nM or lower; (b) binds to rare and scattered immune cells in the medulla of the thymus and dermis of the skin but does not bind to human cerebrum, cerebellum, heart, liver, lung, kidney, tonsil, spleen, thymus, colon, stomach, pancreas, adrenal, pituitary, skin, peripheral nerve, testis or uterus tissue, or peripheral blood mononuclear cells (PBMCs); (c) inhibits binding of CCL1 to CCR8 and inhibits CCR8/CCL1 signaling with an IC₅₀ of about 5 nM or lower; (d) when bound to CCR8 on the surface of a cell mediates depletion of the cell with an EC₅₀ of about 10 pM or lower or an EC₅₀ of about 60 pM or lower; (e) when administered to a subject mediates depletion of tumor-infiltrating Tregs but substantially spares CCR8⁺ T cells in the spleen, blood, skin or thymus; (f) when bound to CCR8 on the surface of a cell does not cause

internalization of CCR8 either in the presence or absence of a cross-linking Ab; (g) inhibits growth of tumor cells in a subject when administered as monotherapy to the subject; and (h) inhibits growth of tumor cells in a subject when administered to the subject in combination with an additional therapeutic agent, such as an immune
5 checkpoint inhibitor, for treating a cancer.

In particular, in certain preferred embodiments, the anti-CCR8 mAb or antigen-binding portion thereof exhibits at least the following properties: (a) specifically binds to CCR8 expressed on the surface of a cell with an EC_{50} of about 1 nM or lower or an EC_{50} of about 2 nM or lower; (b) inhibits binding of CCL1 to CCR8 and inhibits CCR8/CCL1
10 signaling with an IC_{50} of about 5 nM or lower; (c) when bound to CCR8 on the surface of a cell mediates depletion of the cell with an EC_{50} of about 10 pM or lower or an EC_{50} of about 60 pM or lower; (d) when administered to a subject mediates depletion of tumor-infiltrating Tregs but substantially spares CCR8⁺ T cells in the spleen, blood, skin or thymus; (e) inhibits growth of tumor cells in a subject when administered as monotherapy
15 to the subject; and (f) inhibits growth of tumor cells in a subject when administered to the subject in combination with an additional therapeutic agent, such as an immune checkpoint inhibitor, for treating a cancer.

In other embodiments, the anti-CCR8 mAb or antigen-binding portion thereof exhibits at least the following properties: (a) specifically binds to CCR8 expressed on the
20 surface of a cell with an EC_{50} of about 1 nM or lower or an EC_{50} of about 2 nM or lower; (b) when bound to CCR8 on the surface of a cell mediates depletion of the cell with an EC_{50} of about 10 pM or lower or an EC_{50} of about 60 pM or lower; (c) when administered to a subject mediates depletion of tumor-infiltrating Tregs but substantially spares CCR8⁺ T cells in the spleen, blood, skin or thymus; and (d) inhibits growth of tumor cells in a
25 subject when administered to the subject in combination with an additional therapeutic agent, such as an immune checkpoint inhibitor, for treating a cancer.

These properties have been examined in detail for certain Abs of the invention including, for example, those designated herein as 14S15 and 4A19. MAb 4A19 is a nf humanized Ab whereas mAb 14S15 is a nf chimeric Ab comprising a mouse Fab
30 fragment grafted onto a human Fc region, and several of the assays described herein were performed with mAbs including 14S15 and 4A19. The 14S15 mAb was subsequently humanized to generate the Ab designated 14S15h by modifying the framework sequences to correspond to the closest human germline sequences (*see* Example 10). The final

iteration of the humanized mAb, produced after affinity maturation of the heavy chain variable domain, exhibited a binding affinity for hCCR8 comparable to, and in fact slightly higher, than that of the original mouse or chimeric versions of this Ab (K_D of 0.64 nM for 14S15h Fab fragment vs. 1.4 nM for the starting mouse mAb). Thus, 14S15h is expected to exhibit the same or very similar functional properties as those demonstrated with the chimeric 14S15 mAb. The functional properties noted above, alone or in combination, may therefore be present in combination with the structural features of mAbs 14S15, 14S15h and 4A19.

For example, in certain embodiments of the invention, an Ab or antigen-binding portion thereof may comprise one or more of the above properties (*e.g.* at least 2, 3, 4, 5 or 6 of the above properties) and comprise the CDR1, CDR2 and CDR3 domains in each of a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16. As another example, such an Ab or antigen-binding portion thereof may comprise the following CDR domains as defined by the Kabat method: a V_H CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 33; a V_H CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 34; a V_H CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 35; a V_L CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 36; a V_L CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 37; and a V_L CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 38. As a further example, such an Ab or antigen-binding portion thereof may comprise a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16. As another example, such an Ab may comprise a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 100 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 112. Optionally, the Ab has reduced fucosylation of its heavy chain, or has a hypofucosylated or nonfucosylated heavy chain constant region as described elsewhere herein.

For example, in certain other embodiments of the invention, an Ab or antigen-

binding portion thereof may comprise one or more of the above properties (*e.g.* at least 2, 3, 4, 5 or 6 of the above properties) and comprise the CDR1, CDR2 and CDR3 domains in each of a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116. As another example, such an Ab or antigen-binding portion thereof may comprise the following CDR domains as defined by the Kabat method: a V_H CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 103; a V_H CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 104; a V_H CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 105; a V_L CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 106; a V_L CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 107; and a V_L CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 108. As a further example, such an Ab or antigen-binding portion thereof may comprise a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116. As another example, such an Ab may comprise a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 117 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 118. Optionally, the Ab has reduced fucosylation of its heavy chain, or has a hypofucosylated or nonfucosylated heavy chain constant region as described elsewhere herein.

For example, in certain other embodiments of the invention, an Ab or antigen-binding portion thereof may comprise one or more of the above properties (*e.g.* at least 2, 3, 4, 5 or 6 of the above properties) and comprise the CDR1, CDR2 and CDR3 domains in each of a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18. As another example, such an Ab or antigen-binding portion thereof may comprise the following CDR domains as defined by the Kabat method: a V_H CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 45; a V_H CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 46; a V_H CDR3 comprising

consecutively linked amino acids having the sequence set forth as SEQ ID NO: 47; a V_L CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 48; a V_L CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 49; and a V_L CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 50. As a further example, such an Ab or antigen-binding portion thereof may comprise a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18. As another example, such an Ab may comprise a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114. Optionally, the Ab has reduced fucosylation of its heavy chain, or has a hypofucosylated or nonfucosylated heavy chain constant region as described elsewhere herein.

The disclosure further provides an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which specifically binds to hCCR8 expressed on the surface of a cell and comprises the CDR1, CDR2 and CDR3 domains in each of the following pairs of heavy and light chain variable regions:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 3 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 15;

(b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;

(c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 5 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 17;

(d) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;

(e) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 7 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 19;

(f) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 8 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 20;

5 (g) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 9 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 21;

(h) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 10 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 22;

10 (i) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 11 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 23;

(j) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 12 and a V_L comprising consecutively linked amino acids having
15 the sequence set forth as SEQ ID NO: 24;

(k) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 13 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 25;

(l) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 14 and a V_L comprising consecutively linked amino acids having
20 the sequence set forth as SEQ ID NO: 26; or

(m) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116.

25 The isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which specifically binds to hCCR8 expressed on the surface of a cell and comprising the CDR1, CDR2 and CDR3 domains in each of the following pairs of heavy and light chain variable regions are particular examples:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the
30 sequence set forth as SEQ ID NO: 16;

(b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having

the sequence set forth as SEQ ID NO: 116; and

(c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18,

5 An isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which specifically binds to hCCR8 expressed on the surface of a cell and comprising the CDR1, CDR2 and CDR3 domains in the following pair of heavy and light chain variable regions is a particular example:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18.

Sequences of the CDRs may be defined by a variety of methods, including the Kabat, Chothia, AbM, contact and IMGT definitions. Unless explicitly indicated otherwise, CDRs in the present disclosure have been identified by the Kabat definitions.

15 The disclosure also provides an isolated nucleic acid encoding any of the anti-CCR8 mAbs or antigen-binding portions thereof described herein. The disclosure provides an expression vector comprising said isolated nucleic acid, and a host cell comprising said expression vector. This host cell may be used in a method for preparing an anti-CCR8 mAb or an antigen-binding portion thereof, which method comprises
20 expressing the mAb or antigen-binding portion thereof in said host cell and isolating the mAb or antigen-binding portion thereof from the host cell.

In certain embodiments, the present disclosure provides a method for treating a subject afflicted with a cancer comprising administering to the subject a therapeutically effective amount of an anti-CCR8 mAb or an antigen-binding portion thereof described
25 herein, *e.g.*, that mediates depletion of CCR8-expressing cells, such that the subject is treated. In other embodiments, the disclosure provides a method for inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-CCR8 mAb or an antigen-binding portion described herein, *e.g.*, that mediates depletion of CCR8-expressing cells, such that growth of tumor cells in
30 the subject is inhibited. In certain embodiments of these methods, the anti-CCR8 mAb, when bound to CCR8 on the surface of a cell, mediates depletion of the cell with an EC_{50} of about 10 pM or lower. In certain other embodiments, the anti-CCR8 mAb, when bound to CCR8 on the surface of a cell, inhibits binding of CCL1 to CCR8 and inhibits

CCR8/CCL1 signaling with an IC_{50} of about 5 nM or lower. In additional embodiments, this disclosure provides an anti-CCR8 mAb or an antigen-binding portion thereof described herein, *e.g.* that mediates depletion of CCR8-expressing cells, for use in a method for treating a subject afflicted with a cancer or for use in a method for inhibiting growth of tumor cells in a subject, each method comprising administering to the subject a therapeutically effective amount of the anti-CCR8 mAb or antigen-binding portion thereof.

This disclosure further provides a method for treating a subject afflicted with a cancer comprising administering to the subject a combination of therapeutically effective amounts of (a) an anti-CCR8 Ab, *e.g.*, a mAb or an antigen-binding portion thereof described herein, *e.g.*, that mediates depletion of CCR8-expressing cells, and (b) an additional therapeutic agent for treating cancer, optionally wherein the additional therapeutic agent is a compound that reduces inhibition or increases stimulation of the immune system. In certain preferred embodiments, the additional therapeutic agent is an antagonistic Ab or antigen-binding portion thereof that binds specifically to PD-1, PD-L1, or CTLA-4. The method may also be a method for inhibiting growth of tumor cells in a subject.

Where such methods involve the use of a combination of therapeutic agents, they can be referred to in different manners. For example, where a method of treatment uses Agents (A) and (B), for instance to treat cancer, this can be referred to as: (i) Agent (A) and Agent (B) for use in a method of treating cancer; (ii) Agent (A) for use with Agent (B) in a method of treating cancer; or (iii) Agent (B) for use with Agent (A) in a method of treating cancer. Therefore, the above combination can be referred to as:

(i) (A) an anti-CCR8 mAb or an antigen-binding portion thereof, *e.g.*, that mediates depletion of CCR8-expressing cells, and (B) an additional therapeutic agent for treating cancer, for use in a method for treating a subject afflicted with a cancer or for use in a method for inhibiting growth of tumor cells in a subject, the method comprising administering to the subject a combination of therapeutically effective amounts of (A) the anti-CCR8 mAb or antigen-binding portion thereof, *e.g.*, that mediates depletion of CCR8-expressing cells, and (B) the additional therapeutic agent for treating cancer; or

(ii) (A) an anti-CCR8 mAb or an antigen-binding portion thereof, *e.g.*, that mediates depletion of CCR8-expressing cells, for use with (B) an additional therapeutic agent for treating cancer, in a method for treating a subject afflicted with a cancer or in a

method for inhibiting growth of tumor cells in a subject, the method comprising administering to the subject a combination of therapeutically effective amounts of (A) the anti-CCR8 mAb or antigen-binding portion thereof, *e.g.*, that mediates depletion of CCR8-expressing cells, and (B) the additional therapeutic agent for treating cancer; or

- 5 (iii) (B) an additional therapeutic agent for treating cancer for use with (A) an anti-CCR8 mAb or an antigen-binding portion thereof, *e.g.*, that mediates depletion of CCR8-expressing cells, in a method for treating a subject afflicted with a cancer or in a method for inhibiting growth of tumor cells in a subject, the method comprising administering to the subject a combination of therapeutically effective amounts of (B) the additional therapeutic agent for treating cancer, and (A) the anti-CCR8 mAb or antigen-binding portion thereof, *e.g.*, that mediates depletion of CCR8-expressing cells.

The disclosure also provides a kit comprising: (a) one or more dosages ranging from about 0.01 to about 20 mg/kg body weight, or about 0.1 to about 2,000 mg fixed dose, of an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, that binds specifically to CCR8 expressed on the surface of a cell and mediates depletion of the CCR8-expressing cell by ADCC; (b) optionally one or more dosages ranging from about 200 to about 1600 mg of a mAb or an antigen-binding portion thereof that binds specifically to PD-1, PD-L1 or CTLA-4; and (c) instructions for using the isolated Ab or portion thereof that binds specifically to CCR8, and optionally the mAb or portion thereof that binds specifically to PD-1, PD-L1 or CTLA-4, in the therapeutic methods disclosed herein.

Other features and advantages of the instant invention will be apparent from the following detailed description and examples which should not be construed as limiting. The contents of all cited references, including scientific articles, GenBank entries, patents and patent applications cited throughout this application are expressly incorporated herein by reference.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C show analyses of human *CCR8* and *FOXP3* gene-gene correlations in The Cancer Genome Atlas (TCGA). A: Mutual rank-based network of gene-gene correlation across all non-heme TCGA tumor RNA-seq identifies CCR8 as a Treg-selective marker. B: Analysis of hepatocellular carcinoma (HCC) single cell RNA-seq demonstrates that CCR8 is selectively expressed on FOXP3^{high} lymphocytes in HCC tumor samples. C: Spearman correlation analysis performed in FOXP3⁺ T lymphocytes

shows that CCR8 expression is associated with higher levels of FOXP3 expression.

Figures 2A-2F show that CCR8 expression, compared to other Treg-targeted molecules, is enriched on tumor Tregs. A: Flow cytometry analysis of CCR8, CCR4, CTLA-4 and CD25 positive frequencies in Tregs from tumor and blood of cancer patients (colorectal, kidney, lung, and melanoma) (n = 8-18). B: Relative expression levels (MFI) of Treg targets on CD4⁺FOXP3⁺ Tregs from the blood (n = 6-10) and tumors (n = 7-20) of cancer patients. C-E: Treg target frequencies on CD4⁺ Tconv from tumors (C) and blood (D), and CD8⁺ T cells from tumors (E) (n = 7-17) and peripheral blood samples (F) (n = 5-13) of cancer patients. Not all markers were analyzed for all patients, and not all patients have matching blood samples. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. A-B: Two-way ANOVA followed by Bonferroni's multiple comparisons test; C-F: One-way ANOVA Kruskal-Wallis test followed by Dunn's multiple comparisons.

Figures 3A-3C show flow cytometry analyses which demonstrate that CCR8 is highly expressed on tumor-infiltrating Tregs. The level of expression of CCR8 was measured in different T cell populations. A: Percentage of tumor-infiltration T lymphocyte subsets expressing CCR8. A significantly higher frequency of tumor-infiltrating Tregs expresses CCR8 compared to conventional CD4⁺FOXP3⁻ (CD4 Tconv) T cells and CD8⁺ T cells (CD8). B: Mean fluorescence intensity (MFI) of a PE-conjugated anti-CCR8 Ab bound to tumor-infiltrating T lymphocyte subsets. Tumor Tregs also express higher levels of CCR8 on a per cell basis as compared to CD4 Tconv and CD8 cells. C: Comparison of Treg CCR8 expression levels on CCR8⁺ Tregs in the blood and tumor tissue of cancer patients. There is a low level of expression of CCR8 on Tregs in the peripheral blood compared to a significantly higher level on tumor-infiltrating Tregs.

Figures 4A-4D show that CCR8 is differentially expressed on different Treg subpopulations. A: Percentage of cells within different T cell populations in PBMCs from healthy subjects expressing CCR8. CCR8 is predominantly expressed on peripheral Tregs. B: Percentage of cells within different Treg subpopulations in healthy subjects expressing CCR8. Within the Treg population, CCR8 is expressed more highly in the effector memory population (EM) and to a lesser degree in central memory cells (CM), with very little CCR8 expressed in naïve Tregs. C: Percentage of cells within conventional CD4 Tconv cells expressing CCR8. There is little CCR8 expression in any subpopulation of these CD4 Tconv cells. D: Mean fluorescence intensity (MFI) of anti-

CCR8 Ab bound to CCR8⁺ Tregs from peripheral blood or tumor-infiltrating Tregs from cancer patients. In cancer patients, there is higher CCR8 expression on a per cell basis on tumor-infiltrating Tregs than on Tregs from peripheral blood.

Figure 5 shows that CCR8 is expressed on the most immunosuppressive
 5 CD4⁺FOXP3^{high} Treg population. Tumors from 2 melanoma patients and 1 renal cell carcinoma (RCC) patient were dissociated and stained for FOXP3 and CCR8 expression. CCR8 is mainly expressed by the CD4⁺FOXP3^{high} population, which represents the most activated Tregs.

Figures 6A-6D show the percentages of CCR8⁺ and CCR8⁻ T cells that express
 10 CD25, CD39, and IL1R2. A: Percentages of CCR8⁺ and CCR8⁻ cells that express CD25; B: Percentages of CCR8⁺ and CCR8⁻ cells that express CD39; C: Percentages of CCR8⁺ and CCR8⁻ cells that express IL1R2; D: Percentages of stimulated CCR8⁺ and CCR8⁻ cells that express IL1R2.

Figures 7A-7D show the percentages of CCR4⁺ and CCR4⁻ T cells that express
 15 CD25, CD39, and IL1R2. A: Percentages of CCR4⁺ and CCR4⁻ cells that express CD25; B: Percentages of CCR4⁺ and CCR4⁻ cells that express CD39; C: Percentages of CCR4⁺ and CCR4⁻ cells that express IL1R2; D: Percentages of stimulated CCR4⁺ and CCR4⁻ cells that express IL1R2.

Figures 8A and 8B show the percentages of CCR8⁺ and CCR8⁻ T cells that
 20 express the activation marker HLA-DR (8A), and the percentages of CCR4⁺ and CCR4⁻ T cells that express this marker (8B).

Figures 9A - 9C show the percentages of stimulated CCR8⁺ and CCR8⁻ T cells that express IFN γ , IL-2, and granzyme B. A: Percentages of stimulated CCR8⁺ and CCR8⁻ cells that express IFN γ ; B: Percentages of stimulated CCR8⁺ and CCR8⁻ cells that
 25 express IL-2; C: Percentages of stimulated CCR8⁺ and CCR8⁻ cells that express granzyme B.

Figures 10A-10C show the percentages of stimulated CCR4⁺ and CCR4⁻ T cells that express IFN γ , IL-2, and granzyme B. A: Percentages of stimulated CCR4⁺ and CCR4⁻ cells that express IFN γ ; B: Percentages of stimulated CCR4⁺ and CCR4⁻ cells that
 30 express IL-2; C: Percentages of stimulated CCR4⁺ and CCR4⁻ cells that express granzyme B.

Figures 11A and 11B show the binding of different anti-hCCR8 mAbs to hCCR8-expressing cell lines or to activated human Tregs. A: Anti-hCCR8 Abs were generated

exhibiting varying ranges of binding affinities (EC_{50} bins in nM) to hCCR8-transfected cell lines (293F, CHO, Raji) and activated human Tregs. B: EC_{50} values (nM) of a selected subset of anti-CCR8 mAbs binding to activated Tregs are shown.

Figures 12A and 12B show the crystal structure of the 4A19 Fab fragment bound to the hCCR8 N-terminal peptide. A: 4A19 Fab fragment bound to the singly sulfated (at tyr-17) hCCR8 N-terminal peptide at 2.03 Å resolution. The epitope bound by the 4A19 mAb comprises residues 15-21 with the sulfated tyrosine-17 residue at the center of the epitope; B: 4A19 Fab fragment bound to the doubly sulfated (at tyr-15 and tyr-17) hCCR8 N-terminal peptide at 1.80 Å resolution. The epitope bound by the 4A19 mAb comprises residues 12-22.

Figures 13A and 13B show tissue cross-reactivity of different anti-hCCR8 mAbs applied at 1 µg/ml (top panel) and 3 µg/ml (top panel) to normal human PBMCs and normal human thymus, respectively. A: Binding of mAbs 18Y12 (left panels), 16B13 (middle panels) and 4A19 (right panels) to PBMCs. MAb 18Y12 and 4A19 do not bind to the PBMCs whereas 16B13 shows high-level binding to a target which is unlikely to be CCR8. B: Binding of mAbs 18Y12 (left panels), 16B13 (middle panels) and 4A19 (right panels) to thymus tissue. MAb 18Y12 does not show any staining whereas positive staining was observed with mAb 4A19 in rare and scattered immune cells in the medulla of the thymus. MAb 16B13 shows intense and diffuse staining in the vast majority of immune cells in the thymus, with predominate cytoplasmic and/or peri-nuclear patterns.

Figures 14A-14C show that anti-hCCR8 mAbs block the binding of human CCL1 (hCCL1) to hCCR8. A: Anti-hCCR8 mAbs exhibit varying capacities to block the binding of hCCL1 to hCCR8 on hCCR8-transfected CHO cells as assessed by inhibition of Ca flux. B: The percentage blockade of CCL1 signaling (CCL1-induced Ca flux) is shown for 7 selected anti-hCCR8 Abs. C: Ca flux-blocking IC_{50} curves are shown for 4 selected anti-CCR8 mAbs.

Figures 15A and 15B show that anti-hCCR8 mAbs mediate CD16 crosslinking of CD16 expression reporter cells, which reflects the ADCC potential of anti-CCR8 mAbs. Co-cultures of CD16-expressing luciferase reporter cells with CCR8-expressing Raji cells or activated Tregs were treated with anti-CCR8 Abs (with either nonfucosylated (nf) or wild-type hIgG1 backbones). A: A range of CD16 cross-linking capacities was observed. B: CD16 cross-linking using activated Treg as targets for a select set of anti-hCCR8 mAbs with the hIgG1-nf backbone is depicted.

Figure 16 shows that anti-CCR8 Abs mediate killing of activated Tregs by allogeneic NK cells. Activated Tregs were co-cultured with primed allogeneic NK cells and titrated anti-hCCR8 Abs. Cell death was measured by Annexin-V positivity on Tregs.

Figures 17A and 17B show that anti-CCR8 mAbs mediate killing of patient tumor Tregs by allogeneic NK cells *in vitro*. Enzymatically dissociated patient endometrial tumors were co-cultured with primed allogeneic NK cells for 24 hours at 37°C. The anti-CCR8 Ab, 14S15, specifically depleted patient tumor Tregs (Figure 17A), but not conventional CD4⁺ T cells (Figure 17B). In contrast, an anti-CCR4 Ab (CCR4) mediated depletion of both Tregs and conventional CD4⁺ T cells (Figures 17A and 17B).

Figures 18A-18I show that anti-hCCR8-hIgG1-nf Abs mediate CCR8⁺ Treg depletion in *ex vivo* patient tumor slice culture system without the addition of allogeneic NK cells. A-C: Representative results for the depletion of peripheral blood Tregs (A), CD4⁺ Tconv (B) and CD8⁺ T cells (C) upon treatment with 14S15-IgG1-nf or anti-hCCR4-IgG1-nf *in vitro*. D-F: Representative plots from non-small cell lung carcinoma (NSCLC) tumor and allogeneic NK cell killing assays comparing 14S15-IgG1-nf (D), anti-hCCR4-IgG1-nf (E), and isotype (F) Abs. A-F: 14S15-IgG1-nf, closed circles; anti-hCCR4-IgG1-nf, squares; isotype IgG1-nf, open circles. G: Allogeneic NK and NSCLC tumor co-culture comparing 16B13-IgG1-nf to anti-hCCR8-inert and an isotype control Abs. G: isotype IgG1-nf, open circles; 14S15-IgG1-nf, closed circles; 14S15-IgG1-inert, triangles. H and I: Results from *ex vivo* primary intact tumor sections from renal cell carcinoma (H) and gastric cancer (I) cultured for 24 h in the presence of 16B13-IgG1-nf or IgG1-nf isotype control (3-5 technical replicates for each condition). H and I: isotype IgG1-nf, open circles; 14S15-IgG1-nf, closed circles. *P<0.05, ****P<0.0001. Ordinary one-way ANOVA with Tukey's multiple comparisons test (G). Mann-Whitney test (two-tailed) (G-I).

Figure 19 shows that anti-CCR8 Abs, with or without cross-linking, do not induce CCR8 internalization on activated Tregs. Activated Tregs were incubated with the anti-CCR8 mAb 4A19, a positive control Ab (anti-ICOS), and an isotype control, with or without an anti-human Fc cross-linking Ab. CCR8 expression on the Treg surface was assessed at various times.

Figures 20A-20D show depletion of tumor Tregs by anti-CCR8 when digested patient tumors are co-cultured with allogeneic natural killer (NK) cells *in vitro*. A: MAb 4A19 (CCR8-nf) induced measurable depletion of patient tumor Tregs, more so than the

depletion caused by a nonfucosylated anti-hCCR4 mAb (CCR4-nf). B: Conversely, the anti-CCR4-nf Ab, but not mAb 4A19, induced depletion of CD4⁺ Teff cells. C: Neither anti-CCR8 nor anti-CCR4 depleted CD8⁺ Teffs. D: In contrast to 4A19 (CCR8-nf), neither a control anti-keyhole limpet hemocyanin (KLH)-nf mAb with an irrelevant targeting arm (isotype) nor the 4A19 mAb with an inert backbone (CCR8-inert) depleted Tregs.

Figures 21A-21D show that anti-CCR8 inhibits growth of CT26 colon cancer in a mouse model. A: Treatment of CT26 colon cancer with anti-CCR8-mIgG2a, an anti-mCCR8 Ab having a mIgG2a isotype derived from BioLegend's rat anti-mCCR8 mAb, Clone SA214G2, significantly reduced tumor growth and increased the number of tumor-free mice. Individual tumor volumes are depicted. B: Changes in mean tumor volumes. C: Tumor Treg depletion was observed with the anti-CCR8 treatment, while the number of splenic Tregs was unaffected by anti-CCR8 treatment (D).

Figures 22A-22F show the effects of anti-CCR8 on T lymphocyte populations in mice having the CT26 colon adenocarcinoma as analyzed by flow cytometry. A: Percentage of CCR8-expressing CD4⁺FOXP3⁺ Tregs (Treg), FOXP3⁺CD4⁺ effector cells (CD4eff), and CD8⁺ T (CD8T) cells in spleen, blood, tumor Tregs and skin. B: Percentage of CCR8-expressing Double Negative CD4⁺CD8⁺ (DN), Single Positive CD4⁺CD8⁺ (CD4 SP), Single Positive CD4⁺CD8⁺ (CD8 SP), and Double Positive CD4⁺CD8⁺ (DP) thymocytes. C: Percentage of Foxp3⁺ Tregs in spleen, blood, tumor and skin following treatment with the anti-CCR8-mIgG2a mAb and an isotype control. D: Percentage of DP, CD8 SP, CD4 SP and DN thymocytes in the thymus following treatment with anti-CCR8-mIgG2a and an isotype control. E: Percentage of CD4⁺ T cells in the skin following treatment with anti-CCR8-mIgG2a and an isotype control. F: Percentage of CD8⁺ T cells in the skin following treatment with anti-CCR8-mIgG2a and an isotype control.

Figures 23A and 23B show that anti-CCR8 inhibits growth of MC38 colon cancer in a mouse model. A: Treatment of MC38 colon cancer with anti-CCR8-mIgG2a significantly reduced tumor growth and increased the number of tumor-free mice. B: Changes in mean tumor volumes.

Figures 24A-24D show that anti-mCCR8 Ab-induced Treg depletion leads to potent single agent efficacy and increased pharmacodynamic and pharmacokinetic responses in the MC38 colon cancer mouse model. A: Single dose of anti-mCCR8 mAb

leads to a dose-dependent decrease in tumor volume, Treg (% Foxp3⁺CD4⁺) depletion (B), and an increase in the percentage of tumor-infiltrating CD8⁺ T cells (C). Error bars indicate standard deviation of the mean. D: The anti-mCCR8 mAb demonstrates non-linear PK in the dosing range of 0.03 to 3 mg/kg.

5 Figures 25A-25C show the effects on tumor growth of the combination of a mouse anti-mPD-1 Ab and a mouse anti-mCCR8 Ab compared to anti-PD-1 or anti-CCR8 Ab therapy alone, as measured by changes in the tumor volumes in a MB49 murine bladder cancer model. A: Anti-CCR8-mIgG2a and anti-PD-1 induce moderate and low levels, respectively, of tumor growth inhibition but in combination demonstrate synergistic efficacy in completely inhibiting tumor growth. B: Treatment with anti-CCR8-mIgG2a in the presence or absence of anti-PD-1 significantly decreased tumor Treg frequencies, but increased anti-tumor CD8⁺ T cell frequencies (C).

10 Figure 26 shows the effects on tumor growth of the combination of a mouse anti-mPD-1 Ab and a mouse anti-mCCR8 Ab compared to anti-PD-1 or anti-CCR8 Ab therapy alone, as measured by changes in the tumor volumes in a 4T1 murine breast cancer model. Anti-PD-1 shows no activity in inhibiting tumor growth, with tumor growth closely mirroring that in the mice treated with a combination of negative control Abs, while anti-CCR8-mIgG2a induces a moderate level of tumor growth inhibition. Anti-CCR8 interacts synergistically with anti-PD-1 to almost completely inhibit tumor growth.

15 Figures 27A and 27B show that an anti-mCCR8 Ab comprising an inert Fc constant region exhibits anti-tumor activity in an SA1N fibrosarcoma mouse model. A: Anti-CCR8-mIgG2a treatment potently reduced tumor growth with all of the 9 mice being tumor-free by Day 25 post-implantation. Blockade of CCR8 with an Fc-inert Ab (Anti-CCR8-mIgG1-D265A) partially reduced tumor growth. B: Depletion of tumor Tregs by treatment with anti-CCR8 was achieved with anti-CCR8-mIgG2a treatment but not with the Fc-inert anti-CCR8-mIgG1-D265A.

20 Figures 28A-28F show that Fc receptor engagement is required for anti-mCCR8 Ab activity in the MC38 tumor model. Mean (A) and individual growth curves of MC38 tumors implanted in C57BL/6 mice treated with anti-CCR8-mIgG2a (n=10) (B), anti-CCR8-mIgG1-D265A (n=10) (C) or IgG2a isotype control (n=10) (D) on Days 7, 10 and 14 post-tumor implantation at 200 µg/mouse/treatment. E: MC38 tumors were harvested on Day 15 post-implantation and Treg depletion was assessed by flow cytometry (n=5 per

group) $**P < 0.01$. One-way ANOVA Kruskal-Wallis test with Dunn's multiple comparisons. F: Proportions of Ccr8^{+/+} and Ccr8^{-/-} donor-derived Tregs in MC38 tumors and peripheral tissues at 13 days post-MC38 tumor implantation. $**P < 0.01$. Two-way ANOVA followed by Bonferroni's multiple comparisons test.

5 Figures 29A-29I show that anti-CCR8-mIgG2a induces productive memory responses in a heterologous re-challenge model. Mice implanted with CT26 tumors were randomized once tumors reached 100-120 mm³. A-C: Growth curves and Treg depletion in CT26 tumors treated with (A) anti-CCR8-mIgG2a (n=8), (B) anti-CTLA4-mIgG2a (n=8) or (C) mIgG2a isotype control (n=8) on 1, 4, and 8 days after randomization at 0.2
10 mg/mouse/treatment. Six tumors from each group were analyzed by flow cytometry to assess Treg depletion (D) and frequency of AH1-Tetramer⁺ CD8⁺ T cells (E) on day 9 post randomization. F: Frequency of AH1-Tetramer⁺ CD8⁺ T cells in the blood 92 days after treatment. G: Frequency of AH1-Tetramer⁺ CD8⁺ effector memory T cells (TEM) in the blood 5 days after challenge with LM-AH1A5. H and I: Intracellular cytokine staining
15 for (H) IFN γ ⁺ and (I) polyfunctional IFN γ ⁺TNF α ⁺ CD8⁺ T cells in the spleen after stimulation with AH1A5 peptide for 5 h. ns, not significant; * $P < 0.05$, $**P < 0.01$. One-way ANOVA Kruskal-Wallis test with Dunn's multiple comparison's (D-I).

 Figures 30A and 30B show the variable expression of CCR8 in multiple human cancers. Immunohistochemistry (IHC) was conducted in 17 cancer types/subtypes from
20 two sets of samples (full-size tissue sections and MTB sets). A: Full-size tissue sections showing whole-slide image analysis of CCR8⁺ cells in formalin-fixed paraffin-embedded (FFPE) slides of 6 tumor types/subtypes with 14-24 samples per tumor type. B: Multi-tumor blocks (MTB) set showing 16 tumor types/subtypes with 20 cases/tumor types. Each MTB contained 5 cases of a single indication per FFPE block and 1 hyperplastic
25 tonsil sample as positive control.

DETAILED DESCRIPTION OF THE INVENTION

 The present invention relates to mAbs that bind specifically and with high affinity to CCR8 expressed on a cell surface, and to methods for treating cancers in a subject comprising administering to the subject an anti-CCR8 Ab as monotherapy or in
30 combination with an anticancer agent such as an immune checkpoint inhibitor, a chemotherapeutic agent and/or radiation therapy. The effects of CCR8-mediated Treg depletion alone and in combination with PD-1 blockade in potently inhibiting tumor

growth are demonstrated herein in multiple, diverse preclinical mouse tumor models.

Terms

In order that the present disclosure may be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided
5 herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

“Administering”, “administer” or “administration” refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. A preferred
10 route for administration of therapeutic Abs such as anti-CCR8 and anti-PD-1 Abs is intravenous (IV) administration. Other routes of administration include subcutaneous (SC), intraperitoneal (IP), intramuscular (IM), spinal or other parenteral routes of administration, for example by injection or infusion. The phrase “parenteral administration” as used herein means modes of administration other than enteral and
15 topical administration, usually by injection, and includes, without limitation, intravenous, intraperitoneal, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. Alternatively, an Ab
20 of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

An “antibody” (Ab) shall include, without limitation, a glycoprotein
25 immunoglobulin (Ig) which binds specifically to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding portion thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region of an IgG Ab comprises three constant domains, C_{H1} , C_{H2} and C_{H3} . Each light
30 chain comprises a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region of an IgG Ab comprises one constant domain, C_L . The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with

regions that are more conserved, termed framework regions (FR). Each V_H and V_L comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. A variety of methods have been used to delineate the CDR domains within an Ab, including the Kabat, Chothia, AbM, contact, and IMGT definitions. The constant regions of the Abs may mediate the binding of the Ig to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

10 As used herein, and in accordance with conventional usage, an Ab that is described as comprising “a” heavy chain and/or “a” light chain refers to an Ab that comprise “at least one” of the recited heavy and/or light chains, and thus will encompass Abs having two or more heavy and/or light chains. Specifically, Abs so described will encompass conventional Abs having two substantially identical heavy chains and two substantially identical light chains. Ab chains may be substantially identical but not entirely identical if they differ due to post-translational modifications, including, for example, C-terminal cleavage of lysine residues, and alternative glycosylation patterns.

An Ig may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. “Isotype” refers to the Ab class or subclass (*e.g.*, IgM, IgG1, or IgG4) that is encoded by the heavy chain constant region genes. The term “antibody” includes, by way of example, both naturally occurring and non-naturally occurring Abs, monoclonal and polyclonal Abs, chimeric and humanized Abs, human or nonhuman Abs, wholly synthetic Abs, and single chain Abs. A nonhuman Ab may be humanized partially or fully by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term “antibody” also includes an antigen-binding fragment or an antigen-binding portion of any of the aforementioned Ig’s, and includes a monovalent and a divalent fragment or portion, and a single chain Ab.

30 An “isolated” Ab refers to an Ab that is substantially free of other Abs having different antigenic specificities (*e.g.*, an isolated Ab that binds specifically to CCR8 is substantially free of Abs that bind specifically to antigens other than CCR8, such as Abs that bind to CCR4). An isolated Ab that binds specifically to human CCR8 (hCCR8) may,

however, have cross-reactivity to other antigens, such as CCR8 polypeptides from different species such as mouse and cynomolgus monkey. Moreover, in certain contexts, an isolated Ab may also mean an Ab that is purified so as to be substantially free of other cellular material and/or chemicals. By comparison, an “isolated” nucleic acid refers to a
5 nucleic acid composition of matter that is markedly different, *i.e.*, has a distinctive chemical identity, nature and utility, from nucleic acids as they exist in nature. For example, an isolated DNA, unlike native DNA, is a free-standing portion of a native DNA and not an integral part of a larger structural complex, the chromosome, found in nature. Further, an isolated DNA, unlike native DNA, can be used as a PCR primer or a
10 hybridization probe for, among other things, measuring gene expression and detecting biomarker genes or mutations for diagnosing disease or predicting the efficacy of a therapeutic. In addition, in certain contexts, an isolated nucleic acid may mean a nucleic acid that is purified so as to be substantially free of other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, using standard techniques well
15 known in the art.

The term “monoclonal” Ab (mAb) refers to a non-naturally occurring preparation of Ab molecules of single molecular composition, *i.e.*, Ab molecules whose primary sequences are essentially identical and which exhibit a single binding specificity and affinity for a particular epitope. A mAb is an example of an isolated Ab. MABs may be
20 produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

A “chimeric” Ab refers to an Ab in which the variable regions are derived from one species and the constant regions are derived from another species, such as an Ab in which the variable regions are derived from a mouse Ab and the constant regions are
25 derived from a human Ab.

A “human” mAb (HuMAb) refers to a mAb having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the Ab contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human Abs of the
30 invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term “human” Ab, as used herein, is not intended to include Abs in which CDR sequences derived from the

germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms “human” Abs and “fully human” Abs are used synonymously.

5 A “humanized” mAb refers to a mAb in which some, most or all of the amino acids outside the CDR domains of a non-human mAb are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an Ab, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions,
10 insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the Ab to bind to a particular antigen. A “humanized” Ab retains an antigenic specificity similar to that of the original Ab.

An “anti-antigen” Ab refers to an Ab that binds specifically to an antigen. For example, an anti-CCR8 Ab is an Ab that binds specifically to CCR8, whereas an anti-PD-
15 1 Ab is an Ab that binds specifically to PD-1. As used herein, an “anti-PD-1/anti-PD-L1” Ab is an Ab that is used to disrupt the PD-1/PD-L1 signaling pathway, which may be an anti-PD-1 Ab or an anti-PD-L1 Ab.

An “antigen-binding portion” or “antigen-binding fragment” of an Ab refers to one or more fragments of an Ab, *e.g.*, a mAb, that retain the ability to bind specifically to
20 the antigen bound by the whole Ab.

“Antibody-dependent cell-mediated cytotoxicity” (“ADCC”) refers to an *in vitro* or *in vivo* cell-mediated cytotoxic activity in which nonspecific effector cells that express Fc receptors (FcRs) on the effector cell surface (*e.g.*, natural killer (NK) cells, macrophages, neutrophils and eosinophils) recognize the Fc region of the Abs bound to
25 surface antigens on a target cell and actively lyses the target cell. In principle, any effector cell with an activating FcR can be triggered to mediate ADCC. Therapeutic Abs of the present invention for use in human subjects are preferably anti-hCCR8 Abs that have been specifically modified to mediate enhanced ADCC activity against cells expressing CCR8. ADCC activity of an Ab can be measured as described, for example, in
30 any of Examples 17-20.

“Enhanced ADCC” or “enhanced ADCC activity” of a modified Ab of the present invention refer to ADCC activity levels greater than ADCC induced by an unmodified Ab. A modified anti-CCR8 IgG1 Ab of the present invention exhibiting enhanced ADCC,

for example, is a modified form of the Ab that induces greater ADCC than the Ab with its native IgG1 constant domain. A nonfucosylated (nf) mAb is an example of a modified Ab that induces enhances ADCC via improved binding of IgG to activating FcγRIIIA. In certain embodiments, the level of enhancement in ADCC activity is at least a 2-fold, preferably at least a 10-fold, more preferably at least a 100-fold reduction in the EC₅₀ *e.g.*, as measured by a reduction in the EC₅₀ for cell lysis in a NK cell lysis assay, for example, the NK cell lysis assay described in Example 17.

A “cancer” refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth divide and grow results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream.

“C-C Motif Chemokine Receptor 8” (“CCR8”; also known in the art as, for example, CY6, TER1, CCR-8, CKRL1, CDw198, CMKBR8, GPRCY6, CMKBRL2, or CC-CCR-8) is a seven-transmembrane GPCR; this GPCR has been shown to be expressed primarily on intratumoral FOXP3^{hi} Tregs. The term “CCR8” as used herein includes human CCR8 (hCCR8), variants, isoforms, species homologs of hCCR8 such as mouse CCR8 (mCCR8), and analogs having at least one common epitope with hCCR8. The complete hCCR8 and mCCR8 amino acid sequences can be found under GENBANK® Accession Nos. AAI07160.1 and NP_031746.1, respectively.

A “cell surface receptor” refers to molecules or complexes of molecules expressed on the surface of a cell that are capable of receiving a signal and transmitting the signal across the plasma membrane of the cell.

“Effector function” refers to the interaction of an Ab Fc region with an FcR or ligand, or a biochemical event that results therefrom. Exemplary “effector functions” include Clq binding, complement dependent cytotoxicity (CDC), FcR binding, FcγR-mediated effector functions such as ADCC and Ab dependent cell-mediated phagocytosis (ADCP), and down-regulation of a cell surface receptor (*e.g.*, the B cell receptor; BCR). Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.*, an Ab variable domain).

An “Fc receptor” or “FcR” is a receptor that binds to the Fc region of an immunoglobulin. FcRs that bind to an IgG Ab comprise receptors of the FcγR family, including allelic variants and alternatively spliced forms of these receptors. The FcγR

family consists of three activating receptors (FcγRI, FcγRIII, and FcγRIV in mice; FcγRIA, FcγRIIA, and FcγRIIIA in humans) and one inhibitory receptor (FcγRIIB). Various properties of human FcγRs are summarized in Table 1. The majority of innate effector cell types co-express one or more activating FcγR and the inhibitory FcγRIIB, whereas NK cells selectively express one activating Fc receptor (FcγRIII in mice and FcγRIIIA in humans) but not the inhibitory FcγRIIB in mice and humans.

Table 1. Properties of Human FcγRs

Fcγ	Allelic variants	Affinity for human IgG	Isotype preference	Cellular distribution
FcγRI	None described	High (K _D ~10 nM)	IgG1=3>4>>2	Monocytes, macrophages, activated neutrophils, dendritic cells?
FcγRIIA	H131	Low to medium	IgG1>3>2>4	Neutrophils, monocytes, macrophages, eosinophils, dendritic cells, platelets
	R131	Low	IgG1>3>4>2	
FcγRIIIA	V158	Medium	IgG1=3>>4>2	NK cells, monocytes, macrophages, mast cells, eosinophils, dendritic cells?
	F158	Low	IgG1=3>>4>2	
FcγRIIB	I232	Low	IgG1=3=4>2	B cells, monocytes, macrophages, dendritic cells, mast cells
	T232	Low	IgG1=3=4>2	

An “Fc region” (fragment crystallizable region), “Fc domain” or “Fc” refers to the C-terminal region of the heavy chain of an Ab that mediates the binding of the Ig to host tissues or factors, including binding to FcRs located on various cells of the immune system (*e.g.*, effector cells) or to the first component (C1q) of the classical complement system. Thus, the Fc region is a polypeptide comprising the constant region of an Ab excluding the first constant region Ig domain. In IgG, IgA and IgD Ab isotypes, the Fc region is composed of two identical protein fragments, derived from the second (C_{H2}) and third (C_{H3}) constant domains of the Ab’s two heavy chains; IgM and IgE Fc regions contain three heavy chain constant domains (C_H domains 2-4) in each polypeptide chain. For IgG, the Fc region comprises Ig domains Cγ2 and Cγ3 and the hinge between Cγ1 and Cγ2. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position C226 or P230 to the C-terminus of the heavy chain, wherein the numbering is according to the EU index as in Kabat. The C_{H2} domain of a

human IgG Fc region extends from about amino acid 231 to about amino acid 340, whereas the C_{H3} domain is positioned on C-terminal side of a C_{H2} domain in an Fc region, *i.e.*, it extends from about amino acid 341 to about amino acid 447 of an IgG. As used herein, the Fc region may be a native sequence Fc or a variant Fc.

5 “Fucosylation” and “nonfucosylation,” as used herein, refer to the presence and absence, respectively, of a core fucose residue on the N-linked glycan at position N297 of an Ab. Unless otherwise indicated, or is clear from the context, amino acid residue numbering in the Fc region of an Ab is according to the EU numbering convention (the EU index as in Kabat *et al.*, 1991).

10 An “immune response” refers to a biological response within a vertebrate against foreign agents, which response protects the organism against these agents and diseases caused by them. The immune response is mediated by the action of a cell of the immune system (for example, a T lymphocyte, B lymphocyte, NK cell, macrophage, eosinophil, mast cell, dendritic cell or neutrophil) and soluble macromolecules produced by any of
15 these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from the vertebrate’s body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

20 The term “immunotherapy” refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response.

 The term “monotherapy” refers to a single type of treatment such as, for example, the administration to a patient of a single drug, or the use of radiation therapy or surgery
25 alone, to treat a disease or condition. The administration of a drug by itself does not constitute monotherapy if in the same course of treatment it is preceded or followed by another type of treatment for the disease or condition, such as the administration of an additional drug.

 In contrast, “combination therapy” refers to a treatment modality that combines at
30 least two types of therapy such as, for example, the administration to a patient of two or more drugs, or the administration of a drug plus radiation therapy or surgery, to treat a disease or condition. These two or more treatments need not be delivered concurrently to the patient but are part of the same course of treatment. In certain embodiments, the

different therapies are administered simultaneously. In other embodiments, the administration of one therapy overlaps with the administration of at least one other therapy. In further embodiments, the different therapies are administered sequentially.

“Potentiating an immune response” means increasing the effectiveness or
5 potency of an immune response, which could be an existing or an induced immune response, in a subject. This increase in effectiveness and potency may be achieved, for example, by reducing or overcoming mechanisms that suppress an endogenous host immune response, by stimulating mechanisms that enhance the endogenous host immune response, or by enhancing the immune response elicited by an
10 immunotherapeutic agent.

“Programmed Death-1” (PD-1) refers to an immunoinhibitory receptor belonging to the CD28 family that is expressed predominantly on previously activated T cells *in vivo*, and binds to two ligands, PD-L1 and PD-L2. The term “PD-1” as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-
15 1, and analogs having at least one common epitope with hPD-1. The complete hPD-1 amino acid sequence can be found under GENBANK® Accession No. U64863.

“Programmed Death Ligand-1” (PD-L1) is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that downregulate T cell activation and cytokine secretion upon binding to PD-1. The term “PD-L1” as used herein includes
20 human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GENBANK® Accession No. Q9NZQ7.

A “subject” includes any human or nonhuman animal. The term “nonhuman animal” includes, but is not limited to, vertebrates such as nonhuman primates, sheep,
25 dogs, and rodents such as mice, rats and guinea pigs. In preferred embodiments, the subject is a human. The terms “subject” and “patient” are used interchangeably herein.

A “therapeutically effective amount” or “therapeutically effective dosage” of a drug or therapeutic agent is any amount of the drug or agent that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a
30 disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, a prevention or reduction of impairment or disability due to the disease affliction, or otherwise an amelioration of disease symptoms in the subject. In addition, the terms

“effective” and “effectiveness” with regard to a treatment includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the drug to promote disease regression, *e.g.*, cancer regression, in the patient. Physiological safety refers to an acceptable level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (adverse effects) resulting from administration of the drug. The efficacy of a therapeutic agent can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

By way of example for the treatment of tumors, a therapeutically effective amount of an anti-cancer agent preferably inhibits cell growth or tumor growth by at least about 20%, preferably by at least about 40%, more preferably by at least about 60%, even more preferably by at least about 80%, and still more preferably by about 100% relative to untreated subjects. The ability of an agent or treatment to inhibit tumor growth can be evaluated in an animal model system such as any of the CT26 colon adenocarcinoma, MC38 colon adenocarcinoma, SAIN fibrosarcoma, 4T1 mammary carcinoma, and MB49 bladder carcinoma mouse tumor models, which is predictive of efficacy in human tumors. Alternatively, tumor growth inhibition can be measured by evaluating the ability of the agent or treatment to inhibit cell growth *in vitro* using assays known to the skilled practitioner. In preferred embodiments of the invention, tumor regression may be observed and continue for a period of at least about 30 days in a human subject, more preferably at least about 60 days, or even more preferably at least about 6 months.

A therapeutically effective amount of a drug includes a “prophylactically effective amount,” which is any amount of the drug that, when administered alone or in combination with an another therapeutic agent to a subject at risk of developing a disease (*e.g.*, a subject having a pre-malignant condition who is at risk of developing a cancer) or of suffering a recurrence of the disease, inhibits the development or recurrence of the disease (*e.g.*, a cancer). In preferred embodiments, the prophylactically effective amount prevents the development or recurrence of the disease entirely. “Inhibiting” the development or recurrence of a disease means either lessening the likelihood of the disease’s development or recurrence, or preventing the development or recurrence of the disease entirely.

“Treatment” or “therapy” of a subject refers to any type of intervention or process performed on, including the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease.

As used herein, the indefinite articles “a” or “an” should be understood to refer to “one or more” of any recited or enumerated component.

The term “about”, when applied to numeric value, refers to a value that is reasonably close to the stated value and within an acceptable error range as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean a range of plus or minus 50% of a stated reference value, preferably a range of plus or minus 25%, or more preferably a range of plus or minus 10%. When particular values are provided in the application, the meaning of “about”, unless otherwise stated, should be understood to be within an acceptable error range for that particular value according to the practice in the art.

The term “substantially the same” or “essentially the same” refers to a sufficiently high degree of similarity between two or more numeric values, compositions or characteristics that one of skill in the art would consider the difference between these values, compositions or characteristics to be of little or no biological and/or statistical significance within the context of the property being measured. The difference between numeric values being measured may, for example, be less than about 50%, preferably less than about 25%, and more preferably less than about 10%.

As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

Various aspects of the invention are described in further detail in the following subsections.

Expression of CCR8 Specifically in Highly Immunosuppressive Tumor-Infiltrating Tregs

A variety of experiments reported herein demonstrate that CCR8 is expressed specifically by Tregs in human tumors, and unlike CCR4, a Treg depletion target currently undergoing clinical trials, CCR8 is selectively expressed on suppressive tumor

Tregs and minimally on proinflammatory Teffs. Analysis of human *CCR8* and *FOXP3* gene-gene correlations in The Cancer Genome Atlas (TCGA; National Cancer Institute, 2021) (Example 1) showed that *CCR8* expression has the highest correlation with *FOXP3* (master transcriptional regulator of Tregs) in most cancer types (Figure 1A).

5 *CCR8* is expressed on tumor *FOXP3*^{hi} Tregs, but rarely observed on Tregs and Teffs in the peripheral blood. *CCR8* is also selectively expressed on *FOXP3*^{hi} lymphocytes in HCC tumor samples but not in *FOXP3*^{mid} and *FOXP3*^{neg} CD8 and CD4 Teffs in patient tumors (Figure 1B). *CCR8* expression is associated with higher levels of *FOXP3* expression and canonical markers of Tregs (*IL2RA*, *IKZF2*, *BATF*) whereas lower
10 expression of *CCR8* is associated with cytotoxic T cell markers (*GZMA*, *CD8A*; Figure 1C).

Comparing the expression profiles of different Treg-associated molecules (Example 3), *CCR8* was shown to be more highly expressed on tumor-associated *FOXP3*⁺ Tregs than on Treg from patient-matched blood, whereas there was little
15 difference in expression of *CCR4*, *CTLA-4*, and *CD25* on Tregs from tumor and blood (Figure 2A). The high per-cell abundance of *CCR8* on *FOXP3*⁺ tumor Tregs compared to peripheral blood Tregs (Figure 2B), coupled with the low frequency of expression of *CCR8* on CD4⁺ Tconv (Figure 2C and D) and CD8⁺ T cells (Figure 2E and F) in both tumor and peripheral blood suggest that *CCR8* represents a highly selective therapeutic
20 marker for targeting tumor Tregs with a low risk of compromising anti-tumor Teff cell populations.

Flow cytometric analysis of the level of expression of *CCR8* in different subsets of T lymphocytes associated with human tumors (Example 4) showed that *CCR8* is expressed by a high proportion of tumor-resident Tregs (median 82%) as defined by
25 *FOXP3* (Figure 3A). In contrast, a much lower proportion of tumor-infiltrating CD4⁺ T cells and CD8⁺ T cells express *CCR8* (medians 12.65% and 4.55%, respectively; Figure 3B). Whereas a small proportion of CD4 Tconv cells express *CCR8*, the expression level of *CCR8* on a per cell basis is significantly higher on Tregs than on CD4 Tconv cells (median MFI 2106 vs. 132, *P* < 0.0001), while CD8⁺ T cells express negligible levels of
30 *CCR8* (Figure 3B). Tregs in the peripheral blood was also shown to express *CCR8* but at a significantly lower level than on tumor-infiltrating Tregs (Figure 3C).

The differential expression of *CCR8* on different human Treg subpopulations was also analyzed by flow cytometry (Example 5). In PBMCs from healthy subjects, *CCR8*

was found to be predominantly expressed on peripheral blood Tregs, but only on a small fraction (median 21%) of these peripheral Tregs (Figure 4A) compared to the expression on about a median 82% of tumor-infiltrating Tregs (*cf.* Figure 3A). Thus, anti-CCR8 Ab-mediated cell depletion would likely not significantly affect the peripheral Treg compartment due to the low proportion of CCR8⁺ Tregs in the peripheral Treg population. This implies a lower risk of autoimmune toxicity associated with depleting tumor-associated Tregs by targeting CCR8 compared to indiscriminate depletion of non-tumor associated Tregs. Within the Treg population, CCR8 is expressed more highly in the effector memory population and to a lesser degree in central memory cells, with very little CCR8 expressed on naïve Tregs (Figure 4B) or conventional CD4⁺ T cells (Figure 4C). In cancer patients, there is higher CCR8 expression on a per cell basis on tumor-infiltrating Tregs than on Tregs from peripheral blood as measured by the MFI of a bound anti-CCR8 Ab (Figure 4D).

In comparison to other Treg-targeting agents in the clinic (CCR4-mogamulizumab and CTLA-4-Ipi-nf), CCR8 is specifically expressed on the most activated and immunosuppressive subset of FOXP3^{hi} tumor Tregs associated with poor survival (Plitas *et al.*, 2016; Wang *et al.*, 2019), effectively excluding Tregs expressing granzyme and other effector cytokines. Two melanoma tumors and a RCC tumor were dissociated and stained for FOXP3 and CCR8 expression (Example 6). Figure 5 shows that CCR8 is expressed on the most immunosuppressive CD4⁺ FOXP3^{high} Treg population, which represents the most activated Tregs. In the melanoma samples where there is a clear FOXP3^{mid} population, most of the CCR8 expression is found in the FOXP3^{high} T cells with the level of CCR8 expression significantly lower in the FOXP3^{mid} and FOXP3^{neg} tumor T cells that are mainly effector cells expressing granzyme and other effector cytokines. In the RCC sample where most of the patient FOXP3⁺ T cells exhibit high levels of FOXP3, CCR8 expression overlaps with FOXP3 expression. FOXP3^{high} CD4⁺ T cells have been shown to be true Tregs, in contrast to FOXP3^{mid} CD4⁺ T cells, which can be activated conventional T cells or resting Tregs.

The expression of functionally relevant molecules in CCR8⁺ versus CCR8⁻ tumor-infiltrating T cell populations was examined (Example 7). CCR8⁺ cells from patient tumor samples were shown to co-express several proteins with potential immunosuppressive functions such as CD25, IL1R2, and CD39 (Figures 6A-D) whereas a comparable enrichment was not seen in CCR4⁺ T cells (Figures 7A-D). Using HLA-DR

as an activation marker, CCR8 expression also correlates with a higher level of HLA-DR expression (Figure 8A) and thus identifies activated Tregs whereas CCR4 expression does not (Figure 8B). Therefore, depletion of CCR8⁺ cells would remove activated immunosuppressive Tregs expressing CD25, CD39 and IL1R2 whereas Tregs expressing these molecules would not be removed by CCR4-targeted depletion. Further, stimulation of dissociated patient tumor cells *ex vivo* with phorbol 12-myristate 13-acetate (PMA) and ionomycin induced minimal IL-2, IFN γ , and granzyme B production in the CCR8⁺ fraction of CD4⁺ T cells (Figures 9A-C). In contrast, CCR4⁺CD4⁺ T cells were major producers of IFN γ and IL-2 (Figures 10A and B), suggesting that targeted depletion of CCR4⁺, but not CCR8⁺ cells, may be detrimental to anti-tumor immunity.

The data revealed in Examples 1-7 and summarized above show that not all CD4⁺FOXP3⁺ T cells in the tumor express CCR8, and the CCR8⁺ fraction appears to represent the more immunosuppressive subset of FOXP3⁺ cells. Thus, CCR8 expression marks a subset of highly suppressive Treg that are enriched in the tumor and may hinder anti-tumor immunity. The high specificity of CCR8 expression to tumor-specific Tregs and, in particular, the most activated and immunosuppressive CD4⁺ FOXP3^{high} intratumoral Tregs, makes CCR8 an optimal target for mediating depletion of these highly immunosuppressive Tregs through ADCC using an anti-CCR8 Ab.

Accordingly, anti-hCCR8 mAbs were generated in experiments described herein (Example 8) and screened to identify Abs that exhibit multiple properties desirable in a therapeutic Ab for treating cancer (Examples 9-31). It is demonstrated herein that a surrogate mouse anti-mCCR8-mIgG2a Ab potently inhibited tumor growth in multiple mouse tumor models, and nonfucosylated (nf) anti-hCCR8 Abs depleted human tumor Tregs in *ex vivo* patient tumor samples and *in vitro* while sparing proinflammatory CD4⁺ and CD8⁺ Teff cells that drive anti-tumor immune responses (Examples 20 and 22). Depletion of tumor-associated Tregs reduces their immunosuppressive effect and thereby enhances the overall immune response in fighting cancer. Moreover, because CCR8 is rarely expressed on Tregs and Teffs in peripheral blood or in other tissues, targeting Tregs poses minimal toxicity risks. In comparison, anti-hCCR4-IgG1-nf treatment *in vitro* led to depletion of both Treg and CD4⁺ Tconv cells in the periphery and in the tumor microenvironment (Examples 20 and 22). This is consistent with evidence that mogamulizumab (anti-CCR4) depletes Teff populations in peripheral blood in the clinic (Kurose *et al.*, 2015).

Generation of Anti-hCCR8 MAbs

Chemokine receptors, including CCR8, have traditionally been “very difficult antigens to develop Ab against” because of their low profiles on the cell surface and relative inaccessibility to Ab binding (WO 2007/044756). Also, Abs generated against peptides corresponding to extracellular domains of chemokine receptors often fail to recognize the intact receptor on the cell, probably because of differences in secondary structure. Due to these difficulties, efforts to generate Abs against chemokine receptors have had a low success rate (WO 2007/044756). CCR8 is a particularly difficult GPCR, which has been described as an “extremely challenging” target against which to generate Abs (Harbour BioMed, 2020).

Initial efforts to generate anti-hCCR8 Abs by immunizing mice with hCCR8-expressing cells were unsuccessful. However, anti-hCCR8 mAbs were generated in the present study (Example 8) after trying immunization of different rodents with a variety of hCCR8 antigens and various combinations of these antigens.

Immunization of rodents

Humanized or human anti-CCR8 mAbs were generated by immunizing different rodents, including regular C57Bl/6 mice, different strains of transgenic mice that express a human Ig repertoire, a specifically generated CCR8^{-/-} knockout mouse strain, rats and hamsters, with immunogens comprising a variety of hCCR8 antigens including Chinese hamster ovary (CHO), mouse BAF3 and human HEK 293F cells overexpressing hCCR8, or purified plasma membranes from these cells. These immunogens were boosted with chemically synthesized peptides from the hCCR8 N-terminus (residues 1-35 of hCCR8) that importantly contained singly or doubly sulfated tyrosine residues at positions 15 and/or 17, conjugated to either bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Certain of the mAbs generated in non-transgenic mice were subsequently modified to humanized or chimeric derivatives.

Proteins often display post-translational modifications, some of which, *e.g.*, glycosylation, have been very well studied and documented. In comparison, the sulfation of certain extracellular tyrosine residues has been little studied and, to date, there are only 32 confirmed molecules that have been documented to contain sulfated tyrosines (Mehta *et al.*, 2020). Even though additional proteins with sulfated tyrosines are yet to be discovered, sulfated tyrosines are estimated to be fairly rare (Moore, 2003). As a chemical moiety, they are very different from all other amino acids and therefore represent an

excellent focal point to generate Abs with very high specificity and low off-target binding. Chemokine receptors that include CCR8 constitute one of the few protein classes that have been well documented to have sulfated tyrosines on their N-terminus (Ludeman and Stone, 2014). For several of these receptors, tyrosine sulfation has been found to be
5 crucial for ligand, *i.e.*, chemokine, engagement (Zhu *et al.*, 2011). Thus, it is likely that an Ab that engages the tyrosine sulfates will disrupt binding between chemokine and chemokine receptor and thereby prevent chemokine receptor activation. As discussed elsewhere herein, Abs of the invention that bind to an epitope comprising at least one amino acid within a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂
10 (SEQ ID NO: 109), *e.g.* comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or all the amino acids within a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109) and, in particular, wherein amino acids Y₁₅ and Y₁₇ are sulfated, are of particular interest and Example 11 shows that mAb 4A19 binds to the doubly sulfated CCR8 N-terminus with a K_D of about 1.6 nM (*see* Table 5).

15 *Reduced fucosylation, nonfucosylation and hypofucosylation of anti-CCR8 mAbs*

The interaction of Abs with FcγRs can be enhanced by modifying the glycan moiety attached to each Fc fragment at the N297 residue (EU numbering). In particular, the absence of core fucose residues strongly enhances ADCC via improved binding of IgG to activating FcγRIIIA without altering antigen binding or complement-dependent
20 cytotoxicity (CDC; Natsume *et al.*, 2009). Binding of an nf Ab to CCR8 on human Tregs facilitates engagement of the FcγR on NK cells and myelomonocytic Teffs. This FcγR engagement drives NK cell activation leading to enhanced Treg killing by ADCC. There is convincing evidence that afucosylated tumor-specific Abs translate into enhanced therapeutic activity in mouse models *in vivo* (Nimmerjahn and Ravetch, 2005; Mössner *et al.*, 2010).
25

Modification of Ab glycosylation can be accomplished by, for example, expressing the Ab in a host cell with altered glycosylation machinery. Certain of the anti-hCCR8 mAbs disclosed herein possess reduced or eliminated fucosylation and exhibit enhanced ADCC, which is particularly useful in the methods of the present invention.
30 The anti-hCCR8 mAbs disclosed herein may therefore be generated in a form in which they possess reduced or eliminated fucosylation, *e.g.*, by expressing the anti-hCCR8 mAbs in cells with altered glycosylation machinery, and as a result exhibit enhanced ADCC, which is particularly useful in the methods of the present invention. Cells with

altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant Abs of this disclosure to thereby produce an Ab, *e.g.*, a mAb, with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase enzyme, FUT8 (α -(1,6) fucosyltransferase; *see* U.S. Publication No. 2004/0110704; Yamane-Ohnuki *et al.*, 2004), such that Abs expressed in these cell lines lack fucose in their carbohydrates. EP 1176195 also describes a cell line with a functionally disrupted FUT8 gene as well as cell lines that have little or no activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the Ab, for example, the rat myeloma cell line YB2/0 (ATCC CRL 1662). Many other methods for producing Abs with Ab with reduced fucosylation have been described in the art.

Hypofucosylated or nf chimeric, humanized or human anti-hCCR8 Abs disclosed herein were produced by expression in Expi293 Fut8^{-/-} knock-out cells (Thermo Fisher Scientific, San Diego, CA) lacking the FUT8 enzyme essential to fucosylation.

Because nf Abs exhibit greatly enhanced ADCC compared with fucosylated Abs, Ab preparations need not be completely free of fucosylated heavy chains to be useful in the methods of the present invention. Residual levels of fucosylated heavy chains will not significantly interfere with the ADCC activity of a preparation of substantially nf heavy chains. Abs produced in conventional CHO cells, which are fully competent to add core fucose to N-glycans, may nevertheless comprise from a few percent up to 15% nf Abs. Nf Abs may exhibit about 10-fold higher affinity for CD16, and up to 30- to 100-fold enhancement of ADCC activity, so even a small increase in the proportion of nf Abs may drastically increase the ADCC activity of a preparation. Any preparation comprising more nf Abs than would be produced in normal CHO cells, *e.g.*, wild-type CHO cells having unaltered glycosylation machinery, in culture may exhibit some level of enhanced ADCC.

Such Ab preparations are referred to herein as preparations having reduced fucosylation. Depending on the original level of nonfucosylation obtained from normal CHO cells, *e.g.*, wild-type CHO cells, reduced fucosylation preparations may comprise as little as 50%, 30%, 20%, 10% and even 5% nf Abs. Reduced fucosylation may be functionally defined as preparations exhibiting about a 2-fold or greater enhancement of ADCC compared with Abs prepared in normal CHO cells, and not with reference to any fixed percentage of nf species.

However, as used herein unless otherwise indicated, the level of nonfucosylation is structurally defined. Specifically, “nonfucosylated” (nf) or “afucosylated” (terms used

synonymously) Ab preparations are Ab preparations comprising greater than 95% nf Ab heavy chains, including 100%; “hypofucosylated” refers to Ab preparations in which 80 to 95% of heavy chains lack fucose; and “hypofucosylated or nonfucosylated” refers to Ab preparations in which 80% or more of heavy chains lack fucose.

5 The level of fucosylation in an Ab preparation may be determined by a method known in the art, including but not limited to gel electrophoresis, liquid chromatography (LC), and mass spectrometry (MS). Unless otherwise indicated, for the purposes of the present invention, the level of fucosylation in an Ab preparation is determined by hydrophilic interaction chromatography (or hydrophilic interaction liquid
10 chromatography, HILIC). To determine the level of fucosylation of an Ab preparation, samples are denatured treated with PNGase F to cleave N-linked glycans, which are then analyzed for fucose content. LC/MS of full-length Ab chains is an alternative method to detect the level of fucosylation of an Ab preparation, but mass spectroscopy is inherently less quantitative.

15 *Therapeutic anti-hCCR8 mAbs must have a high potential for ADCC*

Because of the great difficulty we encountered in generating anti-CCR8 Abs in preliminary immunization experiments, immunizations were ultimately done with a wide variety of antigens as described above and in Example 8. Hybridomas generated were screened as described in Example 9 to confirm Ab binding to CCR8 and to a CCR8 N-
20 terminal peptide. Selected mouse Abs were humanized as described in Example 10.

Also provided is a method of generating an Ab against CCR8, said method comprising immunizing a rodent at least once with one or more hCCR8 antigens in an immunization schedule which comprises one or more immunizations, wherein the immunization schedule includes in at least one of the immunizations administering a
25 hCCR8 antigen which is a KLH-conjugated, hCCR8 peptide including residues Y15 and Y17 of hCCR8 and wherein residues Y15 and Y17 are sulphotyrosine residues. Preferably, residues both Y15 and Y17 are sulphotyrosine residues.

Optionally, the hCCR8 peptide comprises or consists of at least the N-terminal-most 25, 30, or 35 amino acids of hCCR8. Optionally, the hCCR8 peptide consists of the
30 N-terminal-most 35 amino acids of hCCR8.

Optionally, the rodent is a mouse, rat or hamster. Preferably, the rodent is a mouse.

Optionally, the rodent is immunized with a cell expressing hCCR8 or preferably a

plasma membrane-enriched fraction isolated from a cell expressing CCR8 in at least one of the immunizations, alone or in combination with the KLH-conjugated, hCCR8 peptide. Optionally the immunization schedule includes immunizing the rodent with HEK 293F cells expressing hCCR8 and with a KLH-conjugated, N-terminal hCCR8 peptide.

- 5 Optionally the immunization schedule includes immunizing the rodent with plasma membrane enriched fractions of BAF3 cells expressing hCCR8 and with a KLH-conjugated, hCCR8 peptide.

10 In certain aspects, the present disclosure relates to an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, that specifically binds to CCR8 expressed on the surface of a cell and mediates depletion of the CCR8-expressing cell by ADCC. In certain embodiments, the CCR8 to which the mAb or antigen-binding portion thereof binds is hCCR8, the sequence of which is set forth as SEQ ID NO: 1. In other embodiments, the CCR8 is mCCR8, the sequence of which is set forth as SEQ ID NO: 120.

15 Human IgG1 and IgG3 Ab isotypes are able to mediate ADCC through binding to activating Fcγ receptors, particularly the CD16 (FcγRIIIa) receptor expressed by human NK cells and monocytes (*see* Table 1). Many therapeutic Abs that have been commercialized have the human IgG1 isotype, which can induce strong ADCC and CDC when compared with other human Ab isotypes. Additionally, therapeutic IgG1 Abs have long-term stability in blood mediated via binding to the neonatal Fc receptor (FcRn). The activity of several therapeutic Abs, including anti-CD20 rituximab (RITUXAN®) (Dall'Ozzo *et al.*, 2004), anti-Her2 trastuzumab (HERCEPTIN®) (Gennari *et al.*, 2004), anti-tumor necrosis factor-α (anti-TNF-α) infliximab (REMICADE®) (Louis *et al.*, 2004), and anti-RhD (Miescher *et al.*, 2004) is mediated, at least in part, by ADCC.

25 In certain embodiments of the disclosed invention, the anti-CCR8 mAb or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG3 isotype. In preferred embodiments, the anti-CCR8 mAb or antigen-binding portion thereof is of a human IgG1.

30 This invention also provides a modified anti-hCCR8 mAb, or an antigen-binding portion thereof, which comprises a modified heavy chain constant region that binds with higher affinity to a FcγR and mediates enhanced ADCC compared to the corresponding unmodified mAb or antigen-binding portion thereof. In certain embodiments, the modified anti-hCCR8 mAb or antigen-binding portion thereof mediates at least: (a) about

2 times enhanced ADCC activity; (a) about 5 times enhanced ADCC activity; (c) about 10 times enhanced ADCC activity; (d) about 30 times enhanced ADCC activity, or (e) about 100 times enhanced ADCC activity, *e.g.*, as measured by a reduction in the EC₅₀ for cell lysis in a NK cell lysis assay, for example, the NK cell lysis assay that is described in Example 17. In certain embodiments, the modified anti-hCCR8 mAb or antigen-binding portion thereof comprises a modified IgG1 heavy chain constant region which exhibits reduced fucosylation.

In further embodiments, the modified anti-hCCR8 mAb or antigen-binding portion thereof comprises a modified IgG1 heavy chain constant region which is hypofucosylated or nonfucosylated. In certain other embodiments, the modified mAb or antigen-binding portion thereof comprises a modified IgG1 heavy chain constant region which contains a mutation, or a multiplicity of mutations, that mediate enhanced ADCC. In further embodiments, the mutation or multiplicity of mutations is chosen from G236A; S239D; F243L; E333A; G236A/I332E; S239D/I332E; S267E/H268F; S267E/S324T; H268F/S324T; G236A/S239D/I332E; S239D/A330L/I332E; S267E/H268F/S324T; and G236A/S239D/A330L/I332E. In certain embodiment, the modified anti-hCCR8 mAb or antigen-binding portion thereof comprises a modified IgG1 heavy chain constant region which is hypofucosylated or nonfucosylated, and further contains a mutation, or a multiplicity of mutations, such as are listed above, that mediate enhanced ADCC.

Functional Screening for Anti-hCCR8 MAbs that Mediate ADCC

Human, humanized and chimeric anti-hCCR8 mAb clones were functionally screened to identify Abs that exhibit properties desirable in a therapeutic Ab, including Abs that bind to hCCR8 with high affinity (Example 11), bind specifically to CCR8-expressing human cells (Example 14), block binding of the CCL1 ligand to CCR8 (Example 15), mediate ADCC of CCR8-expressing cells, including Tregs, when bound to CCR8 on the surface of such cells (Examples 17, 19 and 20), not cause internalization of cell CCR8 (Example 21), promote depletion of human tumor-associated Tregs *in vitro* (Example 22) and in *ex vivo* human tumor slice samples (Example 20), mediate depletion of tumor Tregs specifically while sparing CCR8⁺ T cells in normal tissues (Example 24), and reduce tumor growth in preclinical mouse tumor models when administered to the mice as monotherapy or in combination with a checkpoint inhibitor (Examples 23-29).

Clones were selected for further characterization after being initially shown to bind to CCR8 on human cells with sub-nanomolar EC₅₀ values, and to bind specifically to

CCR8-expressing cells with no cross-reactivity to diverse human tissues that do not express CCR8. The DNA encoding the variable regions in these Abs was sequenced by next generation sequencing and clones were selected for diversity based on sequence homology and limited potential sequence liabilities, *e.g.*, asparagine deamidation, methionine oxidation and glycosylation sites. Based on their potency in mediating ADCC of CCR8-expressing cells, binding kinetics, and sequence family diversity, certain selected clones mAbs were further tested for functions deemed to be desirable in a therapeutic Ab, including the ability to inhibit tumor growth in mouse tumor models and were subjected to sequence optimization to mitigate sequence liabilities, optimize binding affinities and revert to germline amino acids. Select mAbs were also analyzed for their biophysical properties through a variety of means such as analytical size exclusion chromatography, capillary isoelectric focusing, hydrophobicity assessments, thermal stability, and aggregation potential, to identify clones suitable for further development.

Characterization of binding affinity of anti-CCR8 mAbs to CCR8

Certain of the anti-CCR8 mAbs of this invention bind to hCCR8 with high affinity. Abs typically bind specifically to their cognate antigen with high affinity, reflected by a dissociation constant (K_D) of 1 μ M to 10 pM or lower. Any K_D greater than about 100 μ M is generally considered to indicate nonspecific binding. As used herein, an IgG Ab that “binds specifically” to an antigen refers to an Ab that binds to the antigen and substantially identical antigens with high affinity, which means having a K_D of about 100 nM or lower, preferably about 10 nM or lower, more preferably about 5 nM or lower, and even more preferably between about 5 nM and 0.1 nM or lower, but does not bind with high affinity to unrelated antigens. An antigen is “substantially identical” to a given antigen if it exhibits a high degree of sequence identity to the given antigen, for example, if it exhibits at least 80%, at least 90%, preferably at least 95%, more preferably at least 97%, or even more preferably at least 99% sequence identity to the sequence of the given antigen.

The term “ K_D ,” as used herein, is intended to refer to the dissociation constant for a particular Ab-antigen interaction, which is obtained from the ratio of k_{off} to k_{on} (*i.e.*, k_{off}/k_{on}) and is expressed as a molar concentration (*e.g.*, nM). The term “ k_{on} ” refers to the association rate or “on rate” for the association of an Ab and its antigen interaction, whereas the term “ k_{off} ” refers to the dissociation rate for the Ab-antigen complex. K_D values for Abs can be determined using methods well established in the art, such as

surface plasmon resonance (SPR), kinetic exclusion assay (KinExA®; Sapidyne Instruments, Boise, ID), or bio-layer interferometry (BLI; ForteBio, Fremont, CA). K_D values determined by different methods for a single Ab can vary considerably, for example, up to a 1,000-fold. Thus, in comparing the K_D values for different Abs, it is
5 important that these K_D values be determined using the same method. Where not explicitly stated, and unless the context indicates otherwise, K_D values for Ab binding disclosed herein were determined by SPR using a BIACORE® biosensor system (GE Healthcare, Chicago, IL).

Binding affinities for an Ab binding to a target such as hCCR8 can also be
10 determined by measuring the EC_{50} for binding to CCR8-expressing cell lines, which is the concentration of the Ab that achieves half of the maximal binding. Studies of mAbs binding to CHO, 293F, Raji cell lines expressing CCR8 exhibited EC_{50} of under 1 nM (Figure 11A). When bound against activated Tregs, these Abs exhibited a wider range of binding affinities, with less than half of the Abs exhibiting binding with EC_{50} 's less than 1
15 nM (Figure 11A). The select set of mAbs shown in Figure 11B exhibit EC_{50} 's ranging from picomolar to nanomolar.

Accordingly, in certain embodiments of the disclosed invention, the anti-CCR8 mAb or antigen-binding portion thereof specifically binds to human CCR8-expressing CHO cells with an EC_{50} of: about 10 nM or lower; preferably about 5 nM or lower;
20 preferably about 2 nM or lower; more preferably about 1.7 nM or lower; more preferably about 1 nM or lower; more preferably about 0.5 nM or lower; and even more preferably about 0.1 nM or lower. In certain preferred embodiments, the anti-hCCR8 mAb or antigen-binding portion thereof binds to hCCR8-expressing CHO cells with an EC_{50} of about 0.1 nM. In certain other preferred embodiments, the anti-hCCR8 mAb or antigen-
25 binding portion thereof binds to hCCR8-expressing CHO cells with an EC_{50} of about 1.7 nM. In certain embodiments, the mAb binds with an EC_{50} of between about 0.1 nM and about 10 nM. In certain other embodiments, the EC_{50} is between about 0.1 nM and about 2 nM. In certain other embodiments, the EC_{50} is between about 0.5 nM and about 5 nM. In certain preferred embodiments, the EC_{50} is between about 1 nM and about 2 nM. In
30 other embodiments, the mAb or antigen-binding portion thereof binds to human hCCR8 with an EC_{50} of between about 0.5 nM and about 1 nM. In certain preferred embodiments, the EC_{50} value is measured by the binding assay described in Example 11.

In certain other embodiments of the present invention, the anti-hCCR8 mAb or

antigen-binding portion thereof specifically binds to activated human Tregs with an EC_{50} of: about 50 nM or lower, about 14 nM or lower, about 10 nM or lower; preferably about 5 nM or lower; more preferably about 2 nM or lower; more preferably about 0.5 nM or lower; more preferably about 0.3 nM or lower; even more preferably about 0.1 nM or lower; and yet more preferably about 0.03 nM or lower. In certain preferred
5 embodiments, the anti-hCCR8 mAb or antigen-binding portion thereof binds to hCCR8-expressing CHO cells with an EC_{50} of about 1.7 nM. In certain embodiments, the mAb binds with an EC_{50} of between about 0.03 nM and about 10 nM. In certain preferred
10 embodiments, the EC_{50} is between about 0.1 nM and about 5 nM. In more preferred
10 embodiments, the mAb or antigen-binding portion thereof binds to human hCCR8 with an EC_{50} of between about 0.2 nM and about 2 nM. In certain preferred embodiments, the EC_{50} value is measured by the binding assay described in Example 11.

The Fab fragment of a selected anti-hCCR8 mAb, 4A19, was shown by X-ray crystallography to bind to an epitope comprising residues 15-21 in the N-terminal peptide
15 of hCCR8 (Example 11), with the sulfated tyrosine-17 residue at the center of the epitope (Figure 12A). Accordingly, in certain embodiments, the anti-hCCR8 mAb or antigen-binding portion thereof described herein binds to a N-terminal epitope of human CCR8 as determined by X-ray crystallography, wherein the epitope comprises at least one amino acid within a peptide having the sequence Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁ (SEQ ID NO: 2). In
20 certain preferred embodiments, the epitope peptide comprises a sulfated tyrosine-17 residue. In certain embodiments, the epitope comprises 2, 3, 4, 5, 6, or all the amino acids within a peptide having the sequence of SEQ ID NO: 2.

Binding of the doubly sulfated peptide (Y15 and Y17 sulfated) to the 4A19 Fab fragment confirmed the identity and orientation of the center of the epitope. It
25 additionally allowed the delineation of a more extended linear epitope comprising the N-terminal residues 12-22 (VTDYYYPDIFS; SEQ ID NO: 109) of hCCR8 (Figure 12B). The extension of the epitope from the one revealed by the monosulfated peptide is likely a consequence of the sulfo-Y15 ordering a larger segment of the hCCR8 N-terminal peptide, allowing visualization of its interactions with the 4A19 Ab. The epitope sequence
30 observed in the structure with the doubly sulfated peptide may constitute the entire epitope bound by mAb 4A19 since this Ab was generated using an immunization strategy that involved multiple immunizations with a hCCR8 N-terminal peptide to enhance the immune response to the hCCR8 N-terminus. If other amino acid residues in the N-

terminal peptide formed part of the epitope, this would likely be seen in the crystal structure but it is not (Figure 12B).

Accordingly, in certain preferred embodiments, the epitope peptide bound by 4A19 comprises sulfated tyrosine-15 and tyrosine-17 residues. In certain embodiments, amino acids Y₁₅ and/or Y₁₇ of this peptide are sulfated. In certain preferred embodiments, both amino acids Y₁₅ and Y₁₇ of this peptide are sulfated.

This disclosure provides an Ab, *e.g.*, a mAb, or an antigen-binding portion thereof, which is capable of mediating ADCC and which specifically binds to an epitope on hCCR8, the sequence of which is set forth as SEQ ID NO: 1, wherein the epitope is located in the N-terminal domain of hCCR8 within a peptide spanning approximately amino acid residues 15 to 21 (Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁; SEQ ID NO: 2) as determined by X-ray crystallography. In certain embodiment, the epitope comprises at least one amino acid within a peptide having the sequence Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁ (SEQ ID NO: 2). In other embodiments, the epitope comprises 2, 3, 4, 5, 6, or all the amino acids within a peptide having the sequence Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁ (SEQ ID NO: 2). In certain preferable embodiments, the epitope comprises all 7 of the amino acids having the sequence of SEQ ID NO: 2.

This disclosure also provides an Ab, *e.g.*, a mAb, or an antigen-binding portion thereof, which is capable of mediating ADCC and which specifically binds to an epitope on hCCR8, the sequence of which is set forth as SEQ ID NO: 1, wherein the epitope is located in the N-terminal domain of hCCR8 within a peptide spanning approximately amino acid residues 12 to 22 (V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂; SEQ ID NO: 109) as determined by X-ray crystallography. In certain embodiment, the epitope comprises at least one amino acid within a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109). In other embodiments, the epitope comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or all the amino acids within a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109). In certain preferable embodiments, the epitope comprises all 11 of the amino acids having the sequence of SEQ ID NO: 109. In certain other embodiments, the epitope consists of a peptide having the sequence of SEQ ID NO: 109.

In certain aspects of this invention, the anti-CCR8 mAb or antigen-binding portion thereof binds to the N-terminal peptide of hCCR8 comprising sulfated tyr-15 and tyr-17 residues (*e.g.*, a peptide of N-terminal CCR8 residues 1-35 sulfated at positions tyr-15

and tyr-17 (CCR8-2sulfo)) with a K_D of: about 100 nM or lower; preferably about 50 nM or lower; preferably about 10 nM or lower; more preferably about 5 nM or lower; more preferably about 1.6 nM or lower; more preferably about 1.0 nM or lower; and even more preferably about 0.5 nM or lower; and yet more preferably about 0.1 nM or lower. In certain preferred embodiments, the anti-hCCR8 mAb or antigen-binding portion thereof binds to the N-terminal epitope peptide, *e.g.*, a peptide of N-terminal CCR8 residues 1-35 sulfated at positions Tyr15 and Tyr17 (CCR8-2sulfo)) with a K_D of about 1.6 nM. In certain embodiments, the mAb binds with a K_D of between about 100 nM and about 0.1 nM. In certain preferred embodiments, the K_D is between about 50 nM and about 0.5 nM. In more preferred embodiments, the mAb or antigen-binding portion thereof binds with a K_D of between about 10 nM and about 1 nM. In yet more preferred embodiments, the mAb or antigen-binding portion thereof binds with a K_D of between about 2 nM and about 1 nM. In certain preferred embodiments, the K_D value is measured by the SPR method described in Example 11.

Sulfation of tyrosine (tyr-15 and tyr-17) in the N-terminus of hCCR8 is required for binding of the 4A19 Ab (*see* Example 11). If only one tyrosine residue, tyr-15, is sulfoylated, the 4A19 Fab fragment binds less tightly to the N-terminal peptide, evidenced by a K_D of more than 10-fold higher and about 1,000-fold higher than the doubly sulfated peptide, while there is approximately a 10-fold decrease in affinity if only tyr-17 is sulfated (Table 5). Accordingly, this invention provides an anti-hCCR8 mAb or an antigen-binding portion thereof which binds to a N-terminal peptide of hCCR8 comprising a single sulfated residue, tyr-17 (CCR8-sulfoY17), of: about 100 nM or lower; preferably about 50 nM or lower; preferably about 25 nM or lower; more preferably about 10 nM or lower; and even more preferably about 1.0 nM or lower. In certain preferred embodiments, the anti-hCCR8 mAb or antigen-binding portion thereof binds to the singly sulfoylated epitope peptide with a K_D of about 20 nM. In certain embodiments, the mAb binds with a K_D of between about 100 nM and about 1 nM. In certain preferred embodiments, the K_D is between about 50 nM and about 10 nM. In more preferred embodiments, the mAb or antigen-binding portion thereof binds with a K_D of between about 30 nM and about 20 nM. In certain preferred embodiments, the K_D value is measured by the SPR method described in Example 11.

Tissue Cross-Reactivity of Anti-hCCR8 MAbs

Since a therapeutic anti-CCR8 mAb will be used to deplete target cells expressing

CCR8, it is important that the mAb bind specifically to the intended target cells, *i.e.*, tumor-infiltrating Tregs, and not to other essential cell types in the body whose depletion would induce toxic or undesirable side effects. Candidate mAbs were, therefore, tested for binding to a wide variety of normal human tissue types (Example 14). Anti-hCCR8 mAbs 18Y12 and 4A19 were shown to bind to rare and scattered immune cells primarily in the medulla of the thymus and dermis of the skin whereas no binding was observed in numerous other tissues examined. Another mAb, 16B13, was observed to bind nonspecifically to PBMCs and a variety of human tissues including immune cells in lymphoid organs and lymphoid-rich tissues, and to many tissues where immune cells were present. The staining exhibited a cytoplasmic pattern. Abs such as 16B13 that bind nonspecifically to targets other than CCR8 on the cell surface are not suitable for therapeutic use in targeting CCR8-expressing cells for depletion notwithstanding other desirable functional properties they may exhibit.

Accordingly, this disclosure provides an anti-CCR8 mAb or antigen-binding portion thereof which binds specifically to CCR8-expressing cells such as tumor Tregs and rare and scattered immune cells in the medulla of the thymus and dermis of the skin but does not bind to a wide variety of human tissues including cerebrum, cerebellum, heart, liver, lung, kidney, tonsil, spleen, thymus, colon, stomach, pancreas, adrenal, pituitary, skin, peripheral nerve, testis or uterus tissue, or PBMCs. For example, the anti-CCR8 mAb or antigen-binding portion thereof may bind specifically to tumor-infiltrating Tregs but not bind to PBMCs, *e.g.*, not show cytoplasmic staining in fixed PBMCs. Non-binding of the Ab to the above recited list of cells and tissues may be established, for instance, by carrying out standard staining with the relevant Abs, *e.g.*, by the methods described in Example 14, *e.g.*, on fixed tissue samples.

Inhibition of CCR8/Ligand Signaling by Anti-CCR8 MAbs

In Examples 15 and 16, blockade of hCCL1 and mCCL1 binding to hCCR8 and mCCR8, respectively, and blockade of CCR8/CCL1 signaling by anti-CCR8 mAbs was tested by conducting calcium (Ca) flux assays on CCR8-expressing CHO cell lines since CCL1 engagement of CCR8 on CHO cells induces Ca flux. CCL1 is the only ligand known to bind to CCR8. Studies have shown that CCL1 binding to CCR8 can enhance Treg suppression in *in vitro* assays as well as suppress autoimmune inflammatory responses in mouse models (Barsheshet *et al.*, 2017). A blocking anti-CCR8 Ab in an SA1N fibrosarcoma model also demonstrated partial anti-tumor efficacy (Figure 28A).

Therefore, in certain embodiments of the present invention, an anti-CCR8 Ab or antigen-binding portion thereof of this invention inhibits binding of CCL1 to CCR8, for example hCCR8 or mCCR8, and inhibits CCR8/CCL1 signaling. In preferred embodiments, inhibition of Ca flux is measured as described in Examples 15 and 16 for hCCR8 and
5 mCCR8, respectively.

In certain embodiments, the anti-hCCR8 mAb inhibits CCR8/CCL1 signaling with an IC_{50} of about 10 nM or lower; about 5 nM or lower; preferably about 1 nM or lower; more preferably about 0.5 nM or lower; more preferably about 0.1 nM or lower; even more preferably about 0.01 nM or lower. In certain preferred embodiment, the anti-
10 hCCR8 mAb inhibits CCR8/CCL1 signaling with an IC_{50} of about 0.5 nM. In certain embodiments, the anti-hCCR8 Ab inhibits CCR8/CCL1 signaling with an IC_{50} of between about 0.01 nM and about 10 nM. In certain other embodiments, the anti-hCCR8 Ab inhibits CCR8/CCL1 signaling with an IC_{50} of between about 0.05 nM and about 5 nM. In certain preferred embodiments, the anti-hCCR8 Ab inhibits CCR8/CCL1 signaling
15 with an IC_{50} of between about 0.1 nM and about 1 nM. In more preferred embodiments, the anti-hCCR8 Ab inhibits CCR8/CCL1 signaling with an IC_{50} of between about 0.2 nM and about 0.5 nM. These IC_{50} values are based on the assay described in Example 15.

ADCC-Mediated Killing of CCR8-Expressing Cells by Anti-CCR8 MAbs

The capacity of the anti-hCCR8 and anti-mCCR8 mAbs to induce ADCC-
20 mediated killing of CCR8-expressing cells was indirectly evaluated by measuring their ability to induce crosslinking of human or mouse reporter cells that express Fc receptors. In certain embodiments, a mAb or antigen-binding portion thereof of the invention mediates depletion of the CCR8-expressing cell with an EC_{50} , as measured by a CD16 cross-linking assay. In preferred embodiments, ADCC potential is measured by the cross-
25 linking assays described in Examples 17 and 18 for hCCR8 and mCCR8, respectively.

In certain embodiments, the anti-hCCR8 mAb or antigen-binding portion thereof mediates depletion of a CCR8-expressing cell with an EC_{50} , as measured by a CD16 cross-linking assay, of about 100 pM or lower; preferably about 30 pM or lower; preferably about 10 pM or lower; preferably about 3 pM or lower; more preferably about
30 1 pM or lower; more preferably about 0.5 pM or lower; more preferably about 0.1 pM or lower; or even more preferably about 0.05 pM or lower. In certain preferred embodiment, the anti-hCCR8 mAb mediates depletion of the CCR8-expressing cell with an EC_{50} of about 0.7 pM. In certain embodiments, the anti-hCCR8 Ab mediates depletion of the

CCR8-expressing cell with an EC₅₀ of between about 0.05 pM and about 50 pM, preferably between about 0.1 pM and about 10 nM; more preferably between about 0.3 nM and about 7 nM; and even more preferably between about 0.6 nM and about 3 nM. These EC₅₀ values are based on the CD16 cross-linking assay described in Example 17.

5 The direct killing of activated Tregs and patient tumor Tregs was demonstrated in Examples 19 and 20. In certain embodiments, the anti-hCCR8 mAb or antigen-binding portion thereof mediates depletion of activated Tregs with an EC₅₀, as measured by an apoptosis assay, of about 500 pM or lower; preferably about 100 pM or lower; preferably about 30 pM or lower; more preferably about 15 pM or lower; even more preferably
10 about 5 pM or lower; or yet more preferably about 1 pM or lower. In certain preferred embodiments, the anti-hCCR8 mAb mediates depletion of the CCR8-expressing cell with an EC₅₀ of about 13 pM. In certain embodiments, the anti-hCCR8 mAb mediates depletion of the CCR8-expressing cell with an EC₅₀ of between about 1 pM and about 500 pM, preferably between about 5 pM and about 100 pM; and more preferably between
15 about 10 pM and about 50 pM. These EC₅₀ values are based on the apoptosis assay described in Example 19.

No Internalization of CCR8 by Anti-CCR8 mAbs

Abs specific for certain cell surface receptors induce internalization of the receptor through receptor-mediated endocytosis which may be essential for targeted
20 delivery of certain drugs, toxins, or enzymes for therapeutic applications. As shown in Figure 19, an anti-Inducible T cell Co-Stimulator (ICOS) mAb does not cause internalization of the ICOS receptor in the absence of a cross-linking Ab, but in the presence of the cross-linking Ab causes significant ICOS internalization. In contrast, an anti-CCR8 mAb causes no internalization of CCR8 either in the presence or absence of a
25 cross-linking Ab. Internalization of CCR8 by an anti-CCR8 Ab would reduce its ability to mediate Treg depletion due to removal of the receptor from the Treg cell surface. Therefore, the lack of CCR8 internalization by anti-CCR8 Abs further validates the therapeutic potential of these Abs. CCR8 internalization may be measured by standard techniques used in the art, *e.g.*, the experimental protocol in Example 21.

30 Accordingly, this invention provides an anti-CCR8 mAb or antigen-binding portion thereof that, when bound to CCR8 on the surface of a cell, does not cause internalization of CCR8 either in the presence or absence of a cross-linking Ab. In certain preferred embodiments, the cell expressing CCR8 on the cell surface is a Treg.

Depletion of Human Tumor Tregs In Vitro and in Ex-Vivo Patient Tumor Samples

The capacity of the anti-hCCR8 mAbs to mediate the depletion of tumor Tregs was evaluated in an *in vitro* system in which digested patient tumors were co-cultured with allogeneic NK cells (Example 22). The anti-hCCR8 mAb, 4A19, was shown to induce depletion of tumor Tregs without reducing the population of T effs. MAb 4A19 was more effective at Treg depletion than the anti-CCR4-nf mAb in this *in vitro* assay system (Figure 20A) and did not induce depletion of CD4⁺ effector T cells whereas the anti-CCR4-nf Ab measurably caused depletion of these cells (Figure 20B). Neither anti-CCR8 nor anti-CCR4 depleted CD8⁺ effector T cells (Figure 20C).

The ability of the anti-hCCR8 mAbs to induce depletion of tumor Tregs was also evaluated in an *ex vivo* assay system using sliced patient tumors (Example 20). MAb 16B13-IgG1-nf was shown to mediate Treg depletion without the addition of allogeneic NK cells (Figures 18H and 18I).

In certain embodiments, an anti-CCR8 mAb or antigen-binding portion thereof of the invention induces depletion of tumor Tregs *in vitro* without reducing the population of CD4⁺ or CD8⁺ effector T cells. In preferred embodiments, depletion of tumor Tregs *in vitro* is measured using the assay described in Example 22.

In certain other embodiments, an anti-CCR8 mAb or antigen-binding portion thereof of the invention induces depletion of tumor Tregs in *ex vivo* patient tumor tissue samples. In certain preferred embodiments, depletion of tumor Tregs in the *ex vivo* patient tumor tissue samples is measured using the assay described in Example 20.

Anti-mCCR8-Mediated Depletion of Tumor Tregs Specifically, and Not CCR8 T Cells in Normal Tissues Samples

In addition to tumor Tregs, CCR8 is also expressed on a small subset of thymic T cells, as well as skin resident T cells, which are a rare population found in the skin. In the CT26 mouse syngeneic tumor model, which exhibits a very similar CCR8 expression profile to humans, the anti-mCCR8 depleting mAb, anti-CCR8-mIgG2a, selectively depleted CCR8⁺ Tregs in the tumor, but did not deplete CCR8⁺ T cells in the skin, thymus, spleen, or blood (Example 24). The highly specific depleting activity of anti-CCR8-mIgG2a in the tumor but not in other CCR8-expressing organs may be due to the relatively low frequency of FcγRIV-expressing cells in proximity to Ab-bound target cells or to the lower CCR8 surface density on the CCR8⁺ T cells in the skin, thymus, spleen and blood. This strongly supports the view that an anti-CCR8 Ab having optimized

affinity for activating FcγRs, such as a human or humanized IgG1-nf anti-CCR8 mAb like 4A19 or 14S15, will enable potent tumor Treg depletion in human patients without inadvertent depletion of CCR8⁺ T cells in the skin where CD16 expression is low.

In certain embodiments of the disclosed invention, an anti-hCCR8 mAb or
5 antigen-binding portion thereof specifically induces depletion of tumor Tregs without depleting CCR8⁺ T cells in the skin, thymus, spleen and blood. In certain preferred embodiments, depletion of tumor Tregs *in vivo* is measured using the assay described in Example 24.

Anti-CCR8 MAbs that Bind to the Same CCR8 Epitope as Does a Reference Ab

10 The present invention also provides an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which binds to the same epitope of hCCR8 as does a reference Ab, wherein the reference Ab comprises:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 3 and a V_L comprising consecutively linked amino acids having the
15 sequence set forth as SEQ ID NO: 15;

(b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;

(c) a V_H comprising consecutively linked amino acids having the sequence set
20 forth as SEQ ID NO: 5 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 17;

(d) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;

(e) a V_H comprising consecutively linked amino acids having the sequence set
25 forth as SEQ ID NO: 7 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 19;

(f) a V_H comprising consecutively linked amino acids having the sequence set
30 forth as SEQ ID NO: 8 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 20;

(g) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 9 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 21;

(h) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 10 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 22;

5 (i) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 11 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 23;

(j) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 12 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 24;

10 (k) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 13 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 25;

(l) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 14 and a V_L comprising consecutively linked amino acids having
15 the sequence set forth as SEQ ID NO: 26; or

(m) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116.

In certain preferred embodiments an isolated Ab, preferably a mAb, or an antigen-
20 binding portion thereof is provided, which binds to the same epitope of hCCR8 as does a reference Ab, wherein the reference Ab comprises:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;

25 (b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 1144 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116; or

(c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the
30 sequence set forth as SEQ ID NO: 18.

In certain preferred embodiments, an isolated Ab, preferably a mAb, or an antigen-binding portion thereof is provided, which binds to the same epitope of hCCR8 as does a reference Ab, wherein the reference Ab comprises a V_H comprising consecutively

linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18.

Anti-CCR8 MAbs that Cross-Compete with a Reference Ab for Binding to CCR8

Also encompassed within the scope of the disclosed invention is an isolated Ab,
5 preferably a mAb, or an antigen-binding portion thereof, which specifically binds to hCCR8 expressed on the surface of a cell, and cross-competes with a reference Ab or a reference antigen-binding portion thereof for binding to hCCR8. The ability of a pair of Abs to “cross-compete” for binding to an antigen, *e.g.*, CCR8, indicates that a first Ab binds to substantially the same epitope region of the antigen as, and sterically hinders the
10 binding of, a second Ab to that particular epitope region and, conversely, the second Ab binds to substantially the same epitope region of the antigen as, and sterically hinders the binding of, the first Ab to that epitope region. Thus, the ability of a test Ab to competitively inhibit the binding of, for example, mAb 14S15 or 4A19 to hCCR8, demonstrates that the test Ab binds to substantially the same epitope region of hCCR8 as
15 does mAb 14S15 or 4A19.

A first Ab is considered to bind to “substantially the same epitope” as does a second Ab if the first Ab reduces the binding of the second Ab to an antigen by at least about 40%. Preferably, the first Ab reduces the binding of the second Ab to the antigen by more than about 50% (*e.g.*, at least about 60% or at least about 70%). In more preferred
20 embodiments, the first Ab reduces the binding of the second Ab to the antigen by more than about 70% (*e.g.*, at least about 80%, at least about 90%, or about 100%). The order of the first and second Abs can be reversed, *i.e.* the “second” Ab can be first bound to the surface and the “first” is thereafter brought into contact with the surface in the presence of the “second” Ab. The Abs are considered to “cross-compete” if a competitive reduction in
25 binding to the antigen is observed irrespective of the order in which the Abs are added to the immobilized antigen.

Cross-competing Abs are expected to have functional properties very similar to the properties of the reference Abs by virtue of their binding to substantially the same epitope region of an antigen such as a CCR8 receptor. The higher the degree of cross-competition, the more similar will the functional properties be. For example, two cross-
30 competing Abs are expected to have essentially the same functional properties if they each inhibit binding of the other to an epitope by at least about 80%. This similarity in function is expected to be even closer if the cross-competing Abs exhibit similar affinities

for binding to the epitope as measured by the dissociation constant (K_D).

Cross-competing anti-antigen Abs can be readily identified based on their ability to detectably compete in standard antigen binding assays, including BIACORE® analysis, ELISA assays or flow cytometry, using either recombinant antigen molecules or cell-surface expressed antigen molecules. By way of example, a simple competition assay to identify whether a test Ab competes with mAb 4A19 for binding to hCCR8 may involve: (1) measuring the binding of 4A19, applied at saturating concentration, to a BIACORE® chip (or other suitable medium for SPR analysis) onto which hCCR8 is immobilized, and (2) measuring the binding of 4A19 to a hCCR8-coated BIACORE® chip (or other medium suitable) to which the test Ab has been previously bound. The binding of 4A19 to the hCCR8-1-coated surface in the presence and absence of the test Ab is compared. A significant (*e.g.*, more than about 40%) reduction in binding of 4A19 in the presence of the test Ab indicates that both Abs recognize substantially the same epitope such that they compete for binding to the hCCR8 target. The percentage by which the binding of a first Ab to an antigen is inhibited by a second Ab can be calculated as: $[1 - (\text{detected binding of first Ab in presence of second Ab}) / (\text{detected binding of first Ab in absence of second Ab})] \times 100$. To determine whether the Abs cross-compete, the competitive binding assay is repeated except that the binding of the test Ab to the hCCR8-coated chip in the presence of 4A19 is measured.

Any of the anti-CCR8 Abs disclosed herein may serve as a reference Ab in cross-competition assays. In certain embodiments, the reference Ab comprises:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 3 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 15;

(b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;

(c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 5 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 17;

(d) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;

(e) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 7 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 19;

5 (f) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 8 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 20;

(g) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 9 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 21;

10 (h) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 10 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 22;

(i) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 11 and a V_L comprising consecutively linked amino acids having
15 the sequence set forth as SEQ ID NO: 23;

(j) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 12 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 24;

(k) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 13 and a V_L comprising consecutively linked amino acids having
20 the sequence set forth as SEQ ID NO: 25;

(l) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 14 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 26; or

25 (m) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116.

In certain preferred embodiments, the reference Ab comprises:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the
30 sequence set forth as SEQ ID NO: 16;

(b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 1144 and a V_L comprising consecutively linked amino acids having

the sequence set forth as SEQ ID NO: 116; or

(c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18.

5 In certain preferred embodiments the reference Ab comprises a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18.

Structurally Defined Anti-CCR8 MAbs

10 The present disclosure also provides an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which specifically binds to hCCR8 expressed on the surface of a cell, and comprises the CDR1, CDR2 and CDR3 domains in each of:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 3 and a V_L comprising consecutively linked amino acids having the
15 sequence set forth as SEQ ID NO: 15;

(b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;

(c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 5 and a V_L comprising consecutively linked amino acids having the
20 sequence set forth as SEQ ID NO: 17;

(d) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;

(e) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 7 and a V_L comprising consecutively linked amino acids having the
25 sequence set forth as SEQ ID NO: 19;

(f) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 8 and a V_L comprising consecutively linked amino acids having the
30 sequence set forth as SEQ ID NO: 20;

(g) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 9 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 21;

(h) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 10 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 22;

5 (i) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 11 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 23;

(j) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 12 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 24;

10 (k) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 13 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 25;

(l) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 14 and a V_L comprising consecutively linked amino acids having
15 the sequence set forth as SEQ ID NO: 26; or

(m) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116.

Preferred is an isolated Ab, preferably a mAb, or an antigen-binding portion
20 thereof, which specifically binds to hCCR8 expressed on the surface of a cell, and comprises the CDR1, CDR2 and CDR3 domains in each of:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;

25 (b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 1144 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116; or

(c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the
30 sequence set forth as SEQ ID NO: 18.

Preferred is an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which specifically binds to hCCR8 expressed on the surface of a cell, and comprises the CDR1, CDR2 and CDR3 domains in each of a V_H comprising

consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18.

Different methods have been developed to delineate the CDR domains within an Ab. The approach of Kabat and co-workers (Wu and Kabat, 1970; Kabat *et al.*, 1983),
 5 was based on the assumption that CDRs include the most variable positions in Abs and therefore could be identified by aligning the fairly limited number of Ab sequences then available. Based on this alignment, Kabat *et al.* introduced a numbering scheme for the residues in the hypervariable regions and determined which positions mark the beginning
 10 and the end of each CDR (<http://bioinf.org.uk/abs/simkab.html>).

In addition to the widely used Kabat definition, others including the Chothia (Chothia *et al.*, 1987; 1989; Al-Lazikani *et al.*, 1997;
<http://bioinf.org.uk/abs/chothia.html>), AbNum (Abhinandan and Martin, 2008; *see AbNum*; available at <http://www.bioinf.org.uk/abs/abnum/>), AbM
 15 (<http://www.bioinf.org.uk/abs/>; Martin *et al.*, 1989), contact (<http://bioinf.org.uk/abs/>; MacCallum *et al.*, 1996) and IMGT (Lefranc *et al.*, 2003; <http://www.imgt.org>) definitions that seek to address deficiencies of the Kabat definitions, have been employed. The Kabat definition is the most commonly used method to predict CDR domains, notwithstanding it was developed when no structural information on Abs was available.

20 Where not explicitly stated, and unless the context indicates otherwise, CDRs disclosed herein have been identified using the Kabat definition. The amino acid sequences for the 6 CDR domains as defined using the Kabat method, as well as the amino acid sequences for the V_H , V_L , heavy chain and light chain for mAbs 16B13, 14S15, 14S15h, 18Y12, 4A19, 2M18, 15C17, 13T20, 10R3, 8D55, 1V11, 11K16 and
 25 12F27 are shown in Table 10.

The present invention provides isolated Abs, preferably mAbs, comprising the following CDR domains as defined by the Kabat method:

(a) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 27; a heavy chain variable region
 30 CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 28; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 29; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ

ID NO: 30; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 31; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 32;

5 (b) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 33; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 34; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 35; a light chain variable region
10 CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 36; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 37; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 38;

15 (c) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 39; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 40; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 41; a light chain variable region
20 CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 42; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 43; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 44;

25 (d) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 45; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 46; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 47; a light chain variable region
30 CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 48; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 49; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ

ID NO: 50;

(e) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 51; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ
5 ID NO: 52; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 53; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 54; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 55; and a light chain variable region
10 CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 56;

(f) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 57; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ
15 ID NO: 58; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 59; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 60; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 61; and a light chain variable region
20 CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 62;

(g) heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 63; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ
25 ID NO: 64; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 65; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 66; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 67; and a light chain variable region
30 CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 68;

(h) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 69; a heavy chain variable region

CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 70; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 71; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 72; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 73; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 74;

(i) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 75; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 76; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 77; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 78; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 79; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 80;

(j) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 81; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 82; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 83; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 84; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 85; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 86;

(k) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 87; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 88; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 89; a light chain variable region

CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 90; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 91; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 92;

(l) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 93; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 94; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 95; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 96; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 97; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 98; or

(m) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 103; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 104; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 105; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 106; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 107; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 108.

Preferred isolated Abs, preferably mAbs, comprise the following CDR domains as defined by the Kabat method:

(a) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 33; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 34; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 35; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ

ID NO: 36; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 37; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 38;

5 (b) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 103; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 104; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 105; a light chain variable region
10 CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 106; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 107; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 108; or

15 (c) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 45; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 46; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 47; a light chain variable region
20 CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 48; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 49; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 50.

25 In certain preferred embodiments, the isolated Abs, preferably mAbs, comprise the following CDR domains as defined by the Kabat method: a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 45; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 46; a heavy chain variable
30 region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 47; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 48; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth

as SEQ ID NO: 49; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 50.

The disclosed invention also encompasses an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which specifically binds to hCCR8 expressed on the surface of a cell, wherein the isolated Ab or antigen-binding portion thereof comprises:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 3 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 15;

(b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;

(c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 5 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 17;

(d) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;

(e) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 7 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 19;

(f) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 8 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 20;

(g) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 9 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 21;

(h) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 10 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 22;

(i) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 11 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 23;

(j) a V_H comprising consecutively linked amino acids having the sequence set

forth as SEQ ID NO: 12 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 24;

(k) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 13 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 25;

(l) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 14 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 26; or

(m) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116.

A preferred isolated Ab, preferably a mAb, or an antigen-binding portion thereof, specifically binds to hCCR8 expressed on the surface of a cell and comprises:

(a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;

(b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116; or

(c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;

A preferred isolated Ab, preferably a mAb, or an antigen-binding portion thereof, specifically binds to hCCR8 expressed on the surface of a cell and comprises a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18.

Anti-CCR8 Abs comprising V_H and V_L regions having amino acid sequences that are highly similar or homologous to the amino acid sequences of any of the above anti-CCR8 Abs and which retain the functional properties of these Abs are also suitable for use in the present methods. For example, suitable Abs include mAbs comprising a V_H and/or V_L region each comprising consecutively linked amino acids having a sequence that is at least 80% identical to the amino acid sequence set forth in SEQ ID Nos. 6 and/or

18, respectively. In further embodiments, for example, the V_H and/or V_L amino acid sequences exhibits at least 85%, 90%, 95%, or 99% identity to the sequences set forth in SEQ ID Nos. 6 and/or 18, respectively. As used herein, the percent sequence identity between two amino acid sequences is a function of the number of identical positions shared by the sequences relative to the length of the sequences compared (*i.e.*, % identity = number of identical positions/total number of positions being compared x 100), taking into account the number of any gaps, and the length of each such gap, introduced to maximize the degree of sequence identity between the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using mathematical algorithms that are well known to those of ordinary skill in the art.

Where anti-CCR8 Abs comprising V_H and V_L regions having amino acid sequences that are highly similar or homologous to the amino acid sequences of any of the above anti-CCR8 Abs and which retain the functional properties of these Abs are disclosed, they may have 100% identity within at least 1, 2, 3, 4, 5, or all 6 CDRs and at least 85%, 90%, 95%, or 99% identity to the relevant full V_H and/or V_L sequence.

For example, an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which specifically binds to hCCR8 expressed on the surface of a cell, may comprise:

(a) a V_H comprising consecutively linked amino acids having a sequence that is at least 85%, 90%, 95%, or 99% identical to the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having a sequence that is at least 85%, 90%, 95%, or 99% identical to the sequence set forth as SEQ ID NO: 16, optionally wherein the V_H comprises at least 1, 2 or all 3 of the CDRs as defined for SEQ ID NO:4 (SEQ ID Nos 33-35) and the V_H comprises at least 1, 2, or all 3 of the CDRs as defined for SEQ ID NO:16 (SEQ ID Nos 36-38);

(b) a V_H comprising consecutively linked amino acids having a sequence that is at least 85%, 90%, 95%, or 99% identical to the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having a sequence that is at least 85%, 90%, 95%, or 99% identical to the sequence set forth as SEQ ID NO: 116, optionally wherein the V_H comprises at least 1, 2 or all 3 of the CDRs as defined for SEQ ID NO: 115 (SEQ ID NOs. 103-105) and the V_L comprises at least 1, 2, or all 3 of the CDRs as defined for SEQ ID NO: 116 (SEQ ID NOs. 106-108); or

(c) a V_H comprising consecutively linked amino acids having a sequence that is at least 85%, 90%, 95%, or 99% identical to the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having a sequence that is at least 85%, 90%, 95%, or 99% identical to the sequence set forth as SEQ ID NO: 18, optionally
5 wherein the V_H comprises at least 1, 2 or all 3 of the CDRs as defined for SEQ ID NO: 6 (SEQ ID NOs. 45-47) and the V_L comprises at least 1, 2 or all 3 of the CDRs as defined for SEQ ID NO: 18 (SEQ ID NOs. 48-50).

Likewise, for any of the other isolated Abs referred to herein, the Ab may comprise a V_H comprising consecutively linked amino acids having a sequence that is at
10 least 85%, 90%, 95%, or 99% identical to the reference V_H sequence, and a V_L comprising consecutively linked amino acids having a sequence that is at least 85%, 90%, 95%, or 99% identical to the reference V_H sequence, optionally wherein the V_H comprises at least 1, 2 or all 3 of the CDRs as defined for the reference V_H sequence, and the V_L comprises at least 1, 2, or all 3 of the CDRs as defined for the reference V_L sequence.

15 The present invention further encompasses an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which optionally specifically binds to hCCR8 expressed on the surface of a cell, wherein the isolated Ab or antigen-binding portion thereof comprises:

(a) a heavy chain comprising consecutively linked amino acids having the
20 sequence set forth as SEQ ID NO: 99 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 111;

(b) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 100 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 112;

25 (c) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 101 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 113;

(d) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked
30 amino acids having the sequence set forth as SEQ ID NO: 114;

(e) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 117 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 118; or

(f) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 110 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 119.

Preferred is an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which optionally specifically binds to hCCR8 expressed on the surface of a cell, wherein the isolated Ab or antigen-binding portion thereof comprises:

(a) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 100 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 112;

(b) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 117 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 118; or

(c) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114.

Preferred is an isolated Ab, preferably a mAb, or an antigen-binding portion thereof, which optionally specifically binds to hCCR8 expressed on the surface of a cell, wherein the isolated Ab or antigen-binding portion thereof comprises:

(a) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114.

In certain embodiments, the isolated anti-CCR8 Ab or antigen-binding portion thereof of this invention is a human Ab or fragment thereof. In other embodiments, it is a humanized Ab or fragment thereof. In further embodiments, it is a chimeric Ab or fragment thereof. In other embodiments, the isolated anti-CCR8 Ab or antigen-binding portion thereof is a mouse Ab or fragment thereof. For administration to human subjects, the Abs are preferably chimeric Abs or, more preferably, humanized or human Abs. Such chimeric, humanized, human or mouse mAbs can be prepared and isolated by methods well known in the art.

Anti-CCR8 Abs disclosed herein also include antigen-binding fragments that are capable of mediating ADCC, in addition to full-length Abs.

Anti-CCR8 Immunoconjugates

In another aspect, the present invention relates to any one of the isolated anti-

hCCR8 Abs disclosed herein, or an antigen-binding portion thereof, linked to a cytolytic agent, such as a cytotoxin or a radioactive isotope. Such conjugates are referred to herein as “immunoconjugates”. Cytotoxins can be conjugated to Abs of the invention using linker technology available in the art. Methods for preparing radioimmunoconjugates are also established in the art.

Bispecific Molecules

In another aspect, the present invention relates to bispecific molecules comprising any one of the isolated anti-hCCR8 mAbs disclosed herein, or an antigen-binding portion thereof, linked to a binding domain that has a different binding specificity than the anti-hCCR8 mAb or antigen-binding portion thereof. The binding domain may be a functional molecule, *e.g.*, another Ab, antigen-binding portion of an Ab, or a ligand for a receptor), such that the bispecific molecule generated binds to at least two different binding sites or target molecules.

Nucleic Acids Encoding Anti-hCCR8 MAbs and Use for Expressing Abs

Another aspect of the disclosure pertains to nucleic acids that encode any of the isolated anti-hCCR8 Abs of the invention. The disclosure provides an isolated nucleic acid encoding any of the anti-CCR8 mAbs or antigen-binding portions thereof described herein.

An “isolated” nucleic acid refers to a nucleic acid composition of matter that is markedly different, *i.e.*, has a distinctive chemical identity, nature and utility, from nucleic acids as they exist in nature. For example, an isolated DNA, unlike native DNA, is a free-standing portion of a native DNA and not an integral part of a larger structural complex, the chromosome, found in nature. Further, an isolated DNA, unlike native DNA, can be used as a PCR primer or a hybridization probe for, among other things, measuring gene expression and detecting biomarker genes or mutations for diagnosing disease or predicting the efficacy of a therapeutic. An isolated nucleic acid may also be purified so as to be substantially free of other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, using standard techniques well known in the art.

Nucleic acids of the invention can be obtained using standard molecular biology techniques. For Abs expressed by hybridomas (*e.g.*, hybridomas prepared from transgenic mice carrying human Ig genes as described in Example 8), cDNAs encoding the light and heavy chains or variable regions of the Ab made by the hybridoma can be obtained by

standard PCR amplification techniques. Once DNA fragments encoding V_H and V_L segments are obtained, these DNA fragments can be further manipulated using standard recombinant DNA techniques, for example, to convert the variable region DNAs to full-length Ab chain genes, to Fab fragment genes, or to a scFv gene. For Abs obtained from
5 an Ig gene library (*e.g.*, using phage display techniques), nucleic acids encoding the Ab can be recovered from the library.

A nucleic acid of the invention can be, for example, RNA or DNA such as cDNA or genomic DNA. In preferred embodiments, the nucleic acid is a cDNA.

The disclosure also provides an expression vector comprising an isolated nucleic
10 which encodes an anti-CCR8 mAb or antigen-binding portion thereof. The disclosure further provides a host cell comprising said expression vector. Eukaryotic cells, and most preferably mammalian host cells, are preferred as host cells for expressing Abs because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active Ab. Preferred
15 mammalian host cells for expressing the recombinant Abs of the invention include Chinese Hamster Ovary (CHO) cells (Kaufman and Sharp, 1982), NSO myeloma cells, COS cells and SP2 cells.

The host cell may be used in a method for preparing an anti-CCR8 mAb or an antigen-binding portion thereof, which method comprises expressing the mAb or antigen-
20 binding portion thereof in the host cell and isolating the mAb or antigen-binding portion thereof from the host cell. The host cell may be used *ex vivo* or *in vivo*. The DNAs encoding the Ab heavy and light chains can be inserted into separate expression vectors or, more typically, are both inserted into the same vector. The V_H and V_L segments of an Ab can be used to create full-length Abs of any isotype by inserting DNAs encoding these
25 variable regions into expression vectors already encoding heavy chain and light chain constant regions of the desired isotype such that the V_H segment is operatively linked to the C_H segment(s) within the vector and the V_κ segment is operatively linked to the C_L segment within the vector. Cell lines that lack the α -(1,6) fucosyltransferase (FUT8) enzyme can be used to produce mAbs that lack fucose in their carbohydrates (*see, e.g.*,
30 U.S. Publication No. 2004/0110704; Yamane-Ohnuki *et al.*, 2004; EP 1176195).

Another aspect of this invention relates to a transgenic mouse comprising human Ig heavy and light chain transgenes, wherein the mouse expresses any of the anti-CCR8 HuMAbs disclosed herein. The invention also encompasses a hybridoma prepared from

said mouse, wherein the hybridoma produces the HuMAb.

Therapeutic Anti-CCR8 Methods

Since the anti-hCCR8 Abs generated in the present study do not bind to mCCR8, a mouse anti-mCCR8 mAb was used as a surrogate for human or humanized nf IgG1 anti-hCCR8 mAbs to test the efficacy of anti-CCR8 as an anti-cancer drug in mouse tumor models. Selection of the appropriate Ab isotype can be used to enhance CCR8-mediated tumor Treg depletion by ADCC/ADCP in mice, which has been demonstrated to be primarily driven by interaction of the Ab with FcγRIV⁺ cells (Nimmerjahn *et al.*, 2010) that are abundant in murine tumors (Simpson *et al.*, 2013). An anti-mCCR8 mAb having a mouse IgG2a isotype, designated anti-CCR8-mIgG2a, was used as a surrogate for the humanized or human anti-hCCR8 mAbs that would likely be used as a human therapeutic. Anti-CCR8-mIgG2a is derived from the commercial rat anti-mCCR8 mAb sold by BioLegend as Clone SA214G2, which was modified to change the rat IgG2b isotype to a mouse IgG2a isotype. Like the human anti-hCCR8 mAbs, A419 and 14S15, anti-CCR8-mIgG2a is an N-terminal binder and its mIgG2a backbone provides maximal FcγR binding for driving an ADCC response similar to the human nf format.

Anti-CCR8-mIgG2a was demonstrated to have high ADCC potential (Example 18), similar to the high ADCC potential of several of the anti-hCCR8 mAbs tested (Example 17), and to block binding of mCCL1 to mCCR8-expressing cells (Example 16) though with lower potency than several of the anti-hCCR8 mAbs tested block binding of hCCL1 to hCCR8-expressing cells (Example 15). The anti-hCCR8 mAbs also exhibit potent ADCC activity (Examples 19 and 20). Anti-CCR8-mIgG2a also binds to mCCR8-expressing cells with lower affinity than certain of the disclosed anti-hCCR8 mAbs (Example 13). This suggests that anti-CCR8-mIgG2a is a suitable surrogate for the anti-hCCR8 mAbs in direct studies on the inhibition of tumor growth in mouse models, though the anti-hCCR8 Abs may be even more potent in inhibiting tumor growth in human subjects given their higher-affinity binding to their cognate hCCR8 target, higher activity in blocking binding of the CCL1 ligand to CCR8, and higher ADCC potential.

A variety of syngeneic mouse tumor models were used to determine anti-tumor activity of the anti-CCR8-mIgG2a mouse surrogate mAb alone and in combination with anti-PD-1. Anti-CCR8-mIgG2a mAb-mediated Treg depletion and subsequent pro-inflammatory responses (increases in CD8, CD4, interferon-γ, GranzymeB, and Ki67) induced robust tumor growth inhibition, with a high percentage of complete tumor

clearance, as a single agent in immunogenic mouse tumor models including the CT26 and MC38 colon adenocarcinoma models and SA1N fibrosarcoma model (see Examples 23-26 and 29), and in combination with anti-PD-1 in the immunotherapy-resistant models, MB49 and 4T1. In contrast, treatment with CCR8-mIgG1-D265A, a variant Ab comprising the Fc-inert mIgG1-D265A heavy chain (Baudino *et al.*, 2008), had minimal effects on tumor regression (Examples 29 and 30). To determine whether there is a dose-dependent relationship between Treg depletion and tumor efficacy upon anti-CCR8 Ab treatment, a single dose monotherapy study was conducted in the MC38 model and a dose-dependent pharmacokinetic/pharmacodynamic/efficacy relationship was observed (Example 26). In addition, increased and titratable CCL1 ligand changes were observed in tumor supernatants upon depletion (data not shown), which might serve as a surrogate biomarker.

In the MB49 mouse bladder carcinoma, anti-CCR8-mIgG2a showed only partial anti-tumor activity though this was stronger than the activity of an anti-PD-1 mAb (Example 27). Similarly, in the 4T1 mouse breast cancer model, anti-CCR8-mIgG2a also showed only partial anti-tumor activity but this tumor was completely resistant to treatment with the anti-PD-1 mAb (Example 28). In both tumor models, anti-CCR8-mIgG2a potentiates the immune response induced by anti-PD-1, resulting in a striking increase in the potency of the anti-tumor response. The combination of the two Abs is synergistic in strongly inhibiting tumor growth in these recalcitrant tumor models. Abs are considered herein to interact synergistically if the anti-tumor efficacy of the combination of these Abs is greater than the sum of the anti-tumor efficacy exhibited by each Ab individually.

Tumor Treg-specific depletion in human tumor explant models was also demonstrated upon treatment with the 14S15, 16B13 and 4A19 mAbs (Example 20).

Treatment of cancer with an anti-CCR8 mAb as monotherapy

The data provided herein demonstrate the generation of anti-hCCR8 mAbs that bind to hCCR8 on the surface of activated Tregs with high affinity (EC_{50} = about 0.1-2 nM; Example 11), induce potent killing of Tregs via ADCC in an *in vitro* system where primary activated human Tregs are co-cultured with allogeneic activated NK cells (EC_{50} = about 10-60 pM; Example 19), and mediate the depletion specifically of CCR8⁺ tumor-infiltrating Tregs in human tumor explant and mouse models (Examples 20, 22 and 24). These mAbs exhibit robust inhibition of tumor growth in diverse immunogenic and

immunotherapy-resistant mouse tumor models (Examples 23, 25, 27-29), and the data strongly support clinical evaluation of CCR8 depletion, as monotherapy or in combination with immune checkpoint blockade, as a novel immunotherapy for cancer.

Accordingly, as supported by the data provided in the Examples, this disclosure provides a method for treating a subject afflicted with a cancer, comprising administering to the subject a therapeutically effective amount of any one of the Treg-depleting anti-CCR8 Abs, *e.g.*, mAbs, immunoconjugates or bispecific molecules disclosed herein, or a pharmaceutical composition comprising any one of said Abs, *e.g.*, anti-CCR8 mAbs, immunoconjugates or bispecific molecules, such that the subject is treated.

The disclosure also provides a method for inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount any one of the Treg-depleting anti-CCR8 Abs, *e.g.*, mAbs, immunoconjugates or bispecific molecules disclosed herein, or a pharmaceutical composition comprising any one of said anti-CCR8 Abs, *e.g.*, mAbs, immunoconjugates or bispecific molecules, such that growth of tumor cells in the subject is inhibited.

Treatment of cancer with an anti-CCR8 Ab in combination with another anti-cancer agent

This disclosure provides a method for treating a subject afflicted with a cancer, comprising administering to the subject a therapeutically effective amount of: (a) any one of the Treg-depleting anti-CCR8 Abs, immunoconjugates or bispecific molecules disclosed herein, or a pharmaceutical composition comprising any one of said anti-CCR8 Abs, immunoconjugates or bispecific molecules; and (b) an additional therapeutic agent for treating cancer, optionally wherein the additional therapeutic agent is a compound that reduces inhibition, or increases stimulation, of the immune system, such that the subject is treated.

The disclosure also a method for inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of: (a) any one of the Treg-depleting anti-CCR8 Abs, immunoconjugates or bispecific molecules disclosed herein, or a pharmaceutical composition comprising any one of said anti-CCR8 Abs, immunoconjugates or bispecific molecules; and (b) an additional therapeutic agent for treating cancer. In certain preferred embodiments, the additional therapeutic agent is a compound that reduces inhibition, or increases stimulation, of the immune system, such that growth of tumor cells in the subject is inhibited.

In other preferred embodiments of any of the present methods, the subject is a human patient.

Treg-mediated immunosuppression is potentially a major obstacle to optimal anti-tumor immune responses in immuno-oncology. Many of the molecules targeted by cancer immunotherapies, such as PD-1, CTLA-4, LAG3, TIM3, and TIGIT, are upregulated on Tregs (Kumar *et al.*, 2018). Consequently, these T cell-based immunotherapies have the potential to augment Treg responses as well. For example, PD-1 blockade may enhance Treg suppression and increase Treg proliferation (Kamada *et al.*, 2019), and there is evidence of Treg expansion following anti-CTLA-4 therapies in the clinic (Kavanagh *et al.*, 2008). Therefore, a Treg-depleting agent not only improves anti-tumor responses as monotherapy, but also enhances the activities of other immunotherapies as disclosed herein (*see* Examples 27 and 28). Thus, the disclosure provides a method for potentiating an anti-tumor immune response elicited by a therapeutic agent in a subject afflicted with a cancer, wherein the therapeutic agent is an immunotherapeutic agent such as cancer immunotherapies, such as an antibody binding specifically to PD-1, CTLA-4, LAG3, TIM3, or TIGIT.

In mouse tumor models, potent combinatorial anti-tumor activity was observed in mice treated with both anti-CCR8-mIgG2a and anti-PD-1 (Examples 27 and 28). Despite similar increases in CD8⁺ T cell frequency in the anti-CCR8-monotherapy and the combination treatments, anti-PD-1 may be required for enhancing effector functionality. In addition, it has been demonstrated that anti-CCR8 treatment results in long-lasting, antigen specific CD8⁺ T cell memory (Example 31). These findings have important clinical implications for the treatment of advanced cancers, which often develop resistance to anti-PD1/PD-L1 therapies (Jenkins *et al.*, 2018) and present with high rates of recurrence (Mahvi *et al.*, 2018). Overall, these studies highlight the specificity and targetability of CCR8 on tumor Treg, and support the evaluation of anti-CCR8 depleting Abs in the treatment of advanced solid tumors as monotherapy or in combination with another immunotherapy such as anti-PD-1, anti-PD-L1 or anti-CTLA-4.

In certain embodiments of the disclosed methods, the additional therapeutic agent is a compound that reduces inhibition of the immune system. For example, the additional therapeutic agent may be a small-molecule compound, a macrocyclic peptide, a fusion protein, or an Ab, *e.g.*, a mAb. In further embodiments, the additional therapeutic agent is an antagonistic agent, such as an antagonistic mAb, that binds specifically to Programmed

Death-1 (PD-1), Programmed Death Ligand-1 (PD-L1), Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4), Lymphocyte Activation Gene-3 (LAG-3), B and T lymphocyte Attenuator (BTLA), T cell Immunoglobulin and Mucin domain-3 (TIM-3), Killer Immunoglobulin-like Receptor (KIR), Killer cell Lectin-like Receptor G1 (KLRG-1),
5 Adenosine A2a Receptor (A2aR), T Cell Immunoreceptor with Ig and ITIM Domains (TIGIT), V-domain Ig Suppressor of T cell activation (VISTA), proto-oncogene tyrosine-protein kinase MER (MerTK), Natural Killer Cell Receptor 2B4 (CD244), or CD160.

In certain preferred embodiments, the additional therapeutic agent is an antagonistic Ab or antigen-binding portion thereof that binds specifically to PD-1. In
10 further embodiments, the Ab that binds specifically to PD-1 is chosen from nivolumab, pembrolizumab, cemiplimab, spartalizumab, camrelizumab, sintilimab, tislelizumab, toripalimab, dostarlimab, retifanlimab, and pimivalimab, *e.g.* chosen from nivolumab, pembrolizumab, cemiplimab, spartalizumab, camrelizumab, sintilimab, tislelizumab, and, *e.g.* toripalimab, *e.g.* chosen from nivolumab and pembrolizumab.

15 In other preferred embodiments, the additional therapeutic agent is an antagonistic Ab or antigen-binding portion thereof that binds specifically to PD-L1. In further embodiments, the Ab that binds specifically to PD-L1 is chosen from atezolizumab, durvalumab, avelumab, envafoleimab, BMS-936559, CK-301, CS-1001, SHR-1316, CBT-502, BGB-A333, and KN035, *e.g.* chosen from atezolizumab, durvalumab, avelumab,
20 envafoleimab, BMS-936559, CK-301, CS-1001, SHR-1316, CBT-502, BGB-A333, *e.g.* chosen from atezolizumab, durvalumab and avelumab.

In other preferred embodiments, the additional therapeutic agent is an antagonistic Ab or antigen-binding portion thereof that binds specifically to CTLA-4. In further
25 *e.g.*, chosen from ipilimumab.

The disclosure further provides a method for potentiating an anti-tumor immune response elicited by a therapeutic agent in a subject afflicted with a cancer, comprising administering to the subject a therapeutically effective amount of any one of the Treg-depleting anti-CCR8 Abs, immunoconjugates or bispecific molecules disclosed herein, or
30 a pharmaceutical composition comprising any one of said anti-CCR8 Abs, immunoconjugates or bispecific molecules, such that the subject experiences a stronger immune response against the cancer compared to the immune response elicited by the therapeutic agent alone. In certain preferred embodiments of this method, the therapeutic

agent is a checkpoint inhibitor, for example an anti-PD-1, anti-PD-L1 or anti-CTLA-4 mAb. In certain preferred embodiments of this method, the therapeutic agent is the anti-PD-1 Ab nivolumab. In other preferred embodiments, the therapeutic agent is the anti-PD-1 Ab pembrolizumab. In certain preferred embodiments, the therapeutic agent is the anti-PD-L1 Ab atezolizumab. In other preferred embodiments, the therapeutic agent is the anti-PD-L1 Ab durvalumab. In further preferred embodiments, the therapeutic agent is the anti-PD-L1 Ab avelumab. In certain preferred embodiments of this method, the therapeutic agent is the anti-CTLA-4 Ab ipilimumab. In certain other embodiments, the therapeutic agent is radiotherapy.

10 *Cancers Treatable by Disclosed Methods*

Immuno-oncology, which relies on using the practically infinite flexibility of the immune system to attack and destroy cancer cells, is applicable to treating a very broad range of cancers (*see, e.g.*, Yao *et al.*, 2013; Callahan *et al.*, 2016; Pianko *et al.*, 2017; Farkona *et al.*, 2016; Kamta *et al.*, 2017). For example, the anti-PD-1 Ab, nivolumab, has been shown to be effective in treating many different types of cancers (*see, e.g.*, Brahmer *et al.*, 2015; Guo *et al.*, 2017; Pianko *et al.*, 2017; WO 2013/173223), and is currently undergoing clinical trials in multiple solid and hematological cancers. Accordingly, the disclosed methods, employing CCR8-mediated depletion of tumor-infiltrating Tregs as monotherapy or in combination with another immunotherapy such as immune checkpoint inhibition, are applicable to treating a wide variety of both solid and liquid tumors.

Broad spectrum of cancers amenable to treatment

Because the Abs used in the cancer treatment methods disclosed herein do not directly target cancer cells but, instead, target and enhance the immune system by depleting immunosuppressant Tregs, optionally in combination with immune checkpoint inhibition, which facilitates the immune system in attacking and destroying cancer cells, these Abs are applicable to the treatment of a broad range of cancers. The efficacy of nivolumab in treating diverse cancers has already been demonstrated, evidenced by the approval of this drug to treat advanced melanoma, advanced non-small cell lung cancer, metastatic renal cell carcinoma, classical Hodgkin lymphoma, advanced squamous cell carcinoma of the head and neck, metastatic urothelial carcinoma, MSI-H or dMMR metastatic colorectal cancer, hepatocellular carcinoma, small cell lung cancer, and esophageal squamous cell carcinoma (Drugs.com - Opdivo Approval History: <https://www.drugs.com/history/opdivo.html>), with clinical trials in many other cancers

ongoing. Similarly, anti-PD-L1 drugs such as atezolizumab (TECENTRIQ®), durvalumab (IMFINZI®) and avelumab (BAVENCIO®) have been gaining approvals in a variety of indications. Accordingly, a wide variety of different cancers are treatable using an anti-CCR8 Ab, and optionally the combination of anti-CCR8 and anti-PD-1/PD-L1 Abs. The high efficacy demonstrated for this combination of therapeutics allows a focus on cancers plagued by large unmet medical need.

In certain embodiments, the disclosed cancer therapy methods may be broadly used to treat a cancer which is a solid tumor. For example, in certain embodiments, the solid tumor is a cancer selected from squamous cell carcinoma, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), squamous NSCLC, non-squamous NSCLC, head and neck cancer, breast cancer, cancer of the esophagus, gastric cancer, gastrointestinal cancer, cancer of the small intestine, liver cancer, hepatocellular carcinoma (HCC), pancreatic cancer (PAC), kidney cancer, renal cell carcinoma (RCC), bladder cancer, cancer of the urethra, cancer of the ureter, colorectal cancer (CRC), colon cancer, colon carcinoma, cancer of the anal region, endometrial cancer, prostate cancer, a fibrosarcoma, neuroblastoma, glioma, glioblastoma, germ cell tumor, pediatric sarcoma, sinonasal natural killer, melanoma, skin cancer, bone cancer, cervical cancer, uterine cancer, carcinoma of the endometrium, carcinoma of the fallopian tubes, ovarian cancer, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, testicular cancer, cancer of the endocrine system, thyroid cancer, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the penis, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain cancer, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, solid tumors of childhood, environmentally-induced cancers, virus-related cancers, cancers of viral origin, advanced cancer, unresectable cancer, metastatic cancer, refractory cancer, recurrent cancer, and any combination thereof. In certain embodiments, the cancer is an advanced, unresectable, metastatic, refractory cancer, and/or recurrent cancer.

Based on the demonstration of effective treatment of different cancers with anti-CCR8 in mouse models (Examples 23-29), certain tumor types are expected to be particularly amenable to treatment with an anti-CCR8 Ab. Accordingly, in certain embodiments, the solid tumor is a cancer chosen from colon adenocarcinoma, bladder carcinoma, mammary carcinoma, and fibrosarcoma. The finding that anti-CCR8 is

effective in shrinking tumors in mouse models in which anti-PD-1 shows little efficacy, such as the MB49 bladder (Example 27) and the 4T1 breast cancer model (Example 28) suggests that anti-CCR8 may be very broadly applicable, and more broadly effective than anti-PD-1, in treating cancers and the combination of anti-CCR8 with checkpoint blockade, *e.g.*, anti-PD-1, anti-PD-L1 or anti-CTLA-4 may have even broader application to treating diverse cancers.

Single-cell RNA-seq analysis was performed on human tumors for differential gene expression analysis of CCR8⁺ Tregs (Example 32). The relatively high expression of CCR8 and CD8A, and their high CCR8/CD8A ratio, identify head and neck squamous cell carcinoma (HNSC), lung adenocarcinoma (LUAD), stomach adenocarcinoma (STAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), esophageal carcinoma (ESCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD) and cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) as tumor types that are expected to be particularly amenable to treatment with an anti-CCR8 Ab. Accordingly, in certain

embodiments, the solid tumor is a cancer chosen from HNSC, LUAD, STAD, LUSC, PAAD, READ, ESCA, BRCA, COAD, CESC, follicular lymphoma, acute lymphocytic leukemia and lymphoma as tumor types that are expected to be particularly amenable to treatment with an anti-CCR8 Ab.

CCR8 expression, evaluated in 17 tumor types or subtypes by IHC on formalin-fixed, paraffin-embedded (FFPE) tissue samples, was found to be abundant in head and neck squamous cell carcinoma (HNSCC; also referred to herein as squamous cell carcinoma of the head and neck [SCCHN]) and least abundant in glioblastoma multiforme (GBM; Example 33). On the basis that tumors that express high levels of CCR8 are more likely to respond to treatment with an anti-CCR8 Ab, these tumor profiling data support the prioritization of HNSCC, cervical, CRC, non-small cell lung cancer-squamous cell carcinoma (NSCLC-SCC), NSCLC-adenocarcinoma (NSCLC-ADC), pancreatic, gastric, bladder, and breast cancers for anti-CCR8 therapy. Accordingly, in certain embodiments, the solid tumor is a cancer chosen from HNSCC, cervical, CRC, NSCLC-SCC, NSCLC-ADC, pancreatic, gastric, bladder, and breast cancers.

In certain embodiments, the present therapy methods may be used to treat a cancer which is a hematological malignancy. Hematological malignancies include liquid tumors

derived from either of the two major blood cell lineages, *i.e.*, the myeloid cell line (which produces granulocytes, erythrocytes, thrombocytes, macrophages and mast cells) or the lymphoid cell line (which produces B, T, NK and plasma cells), including all types of leukemias, lymphomas, and myelomas. Hematological malignancies that may be treated
5 using the present therapy methods include, for example, cancers selected from acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), Hodgkin's lymphoma (HL), non-Hodgkin's lymphomas (NHLs), multiple myeloma, smoldering myeloma, monoclonal gammopathy of undetermined significance (MGUS), advanced,
10 metastatic, refractory and/or recurrent hematological malignancies, and any combinations of said hematological malignancies.

TARGET (Therapeutically Applicable Research to Generate Effective Treatments, <https://ocg.cancer.gov/programs/target>) analysis also indicated that, among hematological malignancies examined, follicular lymphoma, acute lymphocytic leukemia
15 and lymphoma were found to have the highest relative expression of *CCR8* and should be prioritized for treatment with an anti-*CCR8* mAb (Example 32). Thus, in certain embodiments of the present therapeutic methods, the hematological malignancy is follicular lymphoma or acute lymphocytic leukemia and lymphoma.

In certain other embodiments, the hematological malignancy is a cancer selected
20 from acute, chronic, lymphocytic (lymphoblastic) and/or myelogenous leukemias, such as ALL, AML, CLL, and CML; lymphomas, such as HL, NHLs, of which about 85% are B cell lymphomas, including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), mantle cell lymphoma, marginal zone B-cell lymphomas (mucosa-associated lymphoid tissue
25 (MALT) lymphoma, nodal marginal zone B-cell lymphoma, and splenic marginal zone B-cell lymphoma), Burkitt lymphoma, lymphoplasmacytoid lymphoma (LPL; also known as Waldenström's macroglobulinemia (WM)), hairy cell lymphoma, and primary central nervous system (CNS) lymphoma, NHLs that are T cell lymphomas, including precursor T-lymphoblastic lymphoma/leukemia, T-lymphoblastic lymphoma/leukemia (T-Lbly/T-
30 ALL), peripheral T-cell lymphomas such as cutaneous T-cell lymphoma (CTLC, *i.e.*, mycosis fungoides, Sezary syndrome and others), adult T-cell lymphoma/leukemia, angioimmunoblastic T-cell lymphoma, extranodal natural killer/T-cell lymphoma nasal type, enteropathy-associated intestinal T-cell lymphoma (EATL), anaplastic large-cell

lymphoma (ALCL), and peripheral T-cell lymphoma unspecified, acute myeloid lymphoma, lymphoplasmacytoid lymphoma, monocytoid B cell lymphoma, angiocentric lymphoma, intestinal T-cell lymphoma, primary mediastinal B-cell lymphoma, post-transplantation lymphoproliferative disorder, true histiocytic lymphoma, primary effusion
 5 lymphoma, diffuse histiocytic lymphoma (DHL), immunoblastic large cell lymphoma, and precursor B-lymphoblastic lymphoma; myelomas, such as multiple myeloma, smoldering myeloma (also called indolent myeloma), monoclonal gammopathy of undetermined significance (MGUS), solitary plasmacytoma, IgG myeloma, light chain myeloma, nonsecretory myeloma, and amyloidosis; and any combinations of said
 10 hematological malignancies.

In further embodiments, the hematological malignancy is selected from acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), a T cell lymphoma, Hodgkin's lymphoma (HL), non-Hodgkin's lymphomas (NHLs), multiple myeloma,
 15 smoldering myeloma, monoclonal gammopathy of undetermined significance (MGUS), advanced, metastatic, refractory and/or recurrent hematological malignancies, and any combinations of said hematological malignancies.

The present methods are also applicable to treatment of advanced, metastatic, refractory and/or recurrent hematological malignancies.

20 For a clinical trial of anti-CCR8 in treating cancer (Example 34), certain solid tumors were selected based on the results of the different pre-clinical studies noted above: demonstrated efficacy of anti-CCR8 in mouse tumor models (Examples 23-29); RNA expression of CCR8 and CD8A in the 33 tumor types represented in the Cancer Genome Atlas (National Cancer Institute, 2021), identifying tumor types having relatively high
 25 expression of CCR8 and enrichment for CD8A expression (Example 32); and levels of CCR8 expression in 17 tumor types or subtypes as measured by IHC (Example 33). The selected tumor types are NSCLC, SCCHN, MSS-CRC, gastric/gastroesophageal (GE) junction adenocarcinoma, and cervical cancer (squamous cell carcinoma [SCC] or adenocarcinoma). Although limited data are available for advanced tumors, this selection
 30 is supported by high CCR8 expression levels or presence of Tregs correlating with worse prognosis in NSCLC, CRC, gastric, and cervical cancers (Shah *et al.*, 2011; Yi *et al.*, 2018; Zhao *et al.*, 2016; Tao *et al.*, 2012; Xu *et al.*, 2020). Reports on the prognosis for SCCHN is mixed, perhaps in part because of the varying methods and molecular markers

used to detect the presence of Tregs (Saleh *et al.*, 2020). Accordingly, in certain embodiments of the disclosed methods of treating cancer, the solid tumor is a cancer chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer. In certain preferred embodiments, the solid tumor is NSCLC. In other preferred
5 embodiments, the solid tumor is SCCHN. In other preferred embodiments, the solid tumor is MSS-CRC. In further preferred embodiments, the solid tumor is gastric/GE junction cancer. In yet other preferred embodiments, the solid tumor is cervical cancer.

Medical Uses of Anti-CCR8 and Anti-PD-1/Anti-PD-L1 Abs

This disclosure also provides an isolated anti-CCR8 Ab, preferably a mAb or an
10 antigen-binding portion thereof, for use in a method for treating a subject afflicted with a cancer. The disclosure further provides an isolated anti-CCR8 Ab, preferably a mAb or an antigen-binding portion thereof, and a checkpoint inhibitor such as an isolated anti-PD-1/anti-PD-L1 Ab, preferably a mAb or an antigen-binding portion thereof, for use in combination in a method for treating a subject afflicted with cancer comprising dual Treg
15 depletion and blockade of the checkpoint pathway, *e.g.*, the PD-1/PD-L1 signaling pathway. The anti-CCR8 Ab may be used as monotherapy or in combination with a checkpoint inhibitor, such as anti-PD-1/anti-PD-L1 Ab, for treatment of the full range of cancers disclosed herein.

One aspect of the disclosed invention entails the use of an isolated anti-CCR8 Ab
20 or an antigen-binding portion thereof of the invention for the preparation of a medicament for treating a subject afflicted with a cancer. The anti-CCR8 Ab may be used alone or in combination with a checkpoint inhibitor such as an isolated anti-PD-1/anti-PD-L1 Ab or an antigen-binding portion thereof for the preparation of the medicament for treating the cancer patient. Uses of any such anti-CCR8 Ab and anti-PD-1/anti-PD-L1 Ab for the
25 preparation of medicaments are broadly applicable to the full range of cancers disclosed herein.

This disclosure also provides an anti-CCR8 Ab or an antigen-binding portion thereof in combination with a checkpoint inhibitor such as an isolated anti-PD-1/anti-PD-L1 Ab or an antigen-binding portion thereof for use in methods of treating cancer
30 corresponding to all the embodiments of the methods of treatment employing this combination of therapeutics described herein.

Anti-CCR8 Abs Suitable for Use in the Disclosed Therapeutic Methods

An anti-CCR8 Ab suitable for use in the disclosed methods is an isolated Ab,

preferably a mAb or antigen-binding portion thereof, that binds specifically to CCR8 expressed on the surface of a cell with high specificity and affinity and mediates depletion of the CCR8-expressing cell by ADCC. Such an Ab exhibits one or more properties that are important for therapeutic efficacy. In particular, the isolated Ab or antigen-binding portion thereof exhibits at least one of the following properties:

(a) specifically binds to CCR8 expressed on the surface of a cell with an EC_{50} of about 20 nM or lower, preferably about 2 nM or lower, preferably about 1 nM or lower;

(b) binds specifically to rare and scattered immune cells in the medulla of the thymus and dermis of the skin but, for example, does not bind to human cerebrum, cerebellum, heart, liver, lung, kidney, tonsil, spleen, thymus, colon, stomach, pancreas, adrenal, pituitary, skin, peripheral nerve, testis or uterus tissue, or PBMCs. For example, the anti-CCR8 mAb or antigen-binding portion thereof may bind specifically to tumor-infiltrating Tregs but not bind to PBMCs, *e.g.*, not show cytoplasmic staining in fixed PBMCs. Non-binding of the Ab to the above recited list of cells and tissues may be established, for instance, by carrying out standard staining with the relevant Abs, *e.g.* by the methods described in Example 14, *e.g.*, on fixed tissue samples;

(c) inhibits binding of CCL1 to CCR8 and inhibits CCR8/CCL1 signaling with an IC_{50} of about 5 nM or lower;

(d) when bound to CCR8 on the surface of a cell mediates depletion of the cell with an EC_{50} of about 100 pM or lower, preferably about 60 pM or lower, preferably about 40 pM or lower, more preferably about 13 pM or lower, more preferably 10 pM or lower;

(e) when bound to CCR8 on the surface of a cell does not cause internalization of CCR8 either in the presence or absence of a cross-linking Ab;

(f) inhibits growth of tumor cells in a subject when administered as monotherapy to the subject; and

(g) inhibits growth of tumor cells in a subject when administered to the subject in combination with an additional therapeutic agent for treating a cancer, optionally wherein the additional therapeutic agent is an immune checkpoint inhibitor, optionally wherein the checkpoint inhibitor is an anti-PD-1, anti-PD-L1 or anti-CTLA-4 Ab.

In certain embodiments, the isolated Ab or antigen-binding portion thereof exhibits at least 2 or 3, preferably 4, 5 or 6 of the aforementioned properties. In more preferred embodiments, the isolated Ab or antigen-binding portion thereof exhibits all of

the aforementioned properties. For example, in certain preferred embodiments, the isolated Ab or antigen-binding portion thereof:

(a) specifically binds to CCR8 expressed on the surface of a cell with an EC_{50} of about 20 nM or lower, preferably about 2 nM or lower, preferably about 1 nM or lower;

5 (b) when bound to CCR8 on the surface of a cell mediates depletion of the cell with an EC_{50} of about 100 pM or lower, about 60 pM or lower, preferably about 40 pM or lower, more preferably about 13 pM or lower, more preferably 10 pM or lower;

(c) inhibits growth of tumor cells in a subject when administered as monotherapy to the subject; and

10 (d) inhibits growth of tumor cells in a subject when administered to the subject in combination with an additional therapeutic agent for treating a cancer, optionally wherein the additional therapeutic agent is a checkpoint inhibitor.

In other preferred embodiments, the isolated Ab or antigen-binding portion thereof:

15 (a) specifically binds to CCR8 expressed on the surface of a cell with an EC_{50} of about 2 nM or lower;

(b) when bound to CCR8 on the surface of a cell mediates depletion of the cell with an EC_{50} of about 40 pM or lower; and

20 (c) inhibits growth of tumor cells in a subject when administered to the subject in combination with an additional therapeutic agent for treating a cancer, optionally wherein the additional therapeutic agent is an anti-PD-1, anti-PD-L1, or anti-CTLA-4 Ab.

In certain embodiments, the isolated Ab, *e.g.*, a mAb, or antigen-binding portion thereof exhibiting one or more, up to all, of the aforementioned functional properties further binds to an epitope located in the N-terminal domain of hCCR8 with a K_D of about 10 nM or lower, wherein the epitope comprises a peptide having the sequence Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁ (SEQ ID NO: 2) and sulfated tyr-15 and/or tyr-17 residues. In certain other embodiments, the isolated Ab, *e.g.*, a mAb, or antigen-binding portion thereof exhibiting one or more, up to all, of the aforementioned functional properties further binds to an epitope located in the N-terminal domain of human CCR8 with a K_D of about 10 nM or lower, wherein the epitope comprises a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109) and sulfated tyr-15 and tyr-17 residues.

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In certain embodiments the isolated Ab, preferably a mAb, or antigen-binding portion thereof may have the above properties and/or comprise the CDR1, CDR2 and CDR3 domains in each of a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16. For example, such an Ab or antigen-binding portion thereof may comprise the following CDRs as defined by the Kabat method: a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 33; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 34; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 35; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 36; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 37; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 38. As another example, such an Ab or antigen-binding portion thereof may comprise a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16. As a further example, such an Ab may comprise a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 100 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 112. Optionally, the Ab has reduced fucosylation of its heavy chain, or a hypofucosylated or nonfucosylated heavy chain constant region as described elsewhere herein.

In certain embodiments the isolated Ab, preferably a mAb, or antigen-binding portion thereof may have the above properties and/or comprise the CDR1, CDR2 and CDR3 domains in each of a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116. For example, such an Ab or antigen-binding portion thereof may comprise the following CDRs as defined by the Kabat method: a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 103; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth

as SEQ ID NO: 104; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 105; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 106; a light chain variable region CDR2 comprising
5 consecutively linked amino acids having the sequence set forth as SEQ ID NO: 107; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 108. As another example, such an Ab or antigen-binding portion thereof may comprise a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively
10 linked amino acids having the sequence set forth as SEQ ID NO: 116. As a further example, such an Ab may comprise a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 117 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 118. Optionally, the Ab has reduced fucosylation of its heavy chain, or a hypofucosylated or
15 nonfucosylated heavy chain constant region as described elsewhere herein.

In certain embodiments the isolated Ab, preferably a mAb, or antigen-binding portion thereof may have the above properties and/or comprise the CDR1, CDR2 and CDR3 domains in each of a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino
20 acids having the sequence set forth as SEQ ID NO: 18. For example, such an Ab or antigen-binding portion thereof may comprise a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 45; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 46; a heavy chain variable region
25 CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 47; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 48; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 49; and a light chain variable region CDR3 comprising consecutively linked
30 amino acids having the sequence set forth as SEQ ID NO: 50. In another example, such an Ab or antigen-binding portion thereof may comprise a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18. In a

further example, such an Ab may comprise a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114. Optionally, the Ab has reduced fucosylation of its heavy chain, or a
5 hypofucosylated or nonfucosylated heavy chain constant region as described elsewhere herein.

Combination Therapy

Human CCR8⁺ Tregs also co-express PD-1 at high levels (data not shown). Treatment with an anti-PD-1 mAb may activate PD-1⁺ Tregs, potentially explaining
10 hyperprogression or primary resistance in nivolumab-treated patients with gastric cancer (Kamada *et al.*, 2019). Therefore, Treg depletion likely improves overall response rates to anti-PD-1 therapy. This combination is also directed supported by preclinical mouse studies where the synergistic effects of anti-CCR8 with anti-PD-1 were observed in MB49 and 4T-1 tumor models (Examples 27 and 28).

15 Although the efficacy of combination therapy with an anti-CCR8 Ab and a checkpoint inhibitor have been demonstrated herein using an anti-PD-1 Ab, several other costimulatory and inhibitory receptors and ligands that regulate T cell responses have been identified. Examples of stimulatory receptors include Inducible T cell Co-Stimulator (ICOS), CD137 (4-1BB), CD134 (OX40), CD27, Glucocorticoid-Induced TNFR-Related
20 protein (GITR), and HerpesVirus Entry Mediator (HVEM), whereas examples of inhibitory receptors in addition to PD-1/PD-L1 include Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4), B and T Lymphocyte Attenuator (BTLA), T cell Immunoglobulin and Mucin domain-3 (TIM-3), Lymphocyte Activation Gene-3 (LAG-3), Killer Immunoglobulin-like Receptor (KIR), adenosine A2a receptor (A2aR), Killer
25 cell Lectin-like Receptor G1 (KLRG-1), Natural Killer Cell Receptor 2B4 (CD244), CD160, T cell Immunoreceptor with Ig and ITIM domains (TIGIT), and the receptor for V-domain Ig Suppressor of T cell Activation (VISTA), (Mellman *et al.*, 2011; Pardoll, 2012; Baitsch *et al.*, 2012). These receptors and their ligands provide targets for
30 therapeutics designed to stimulate, or prevent the suppression, of an immune response so as to thereby attack tumor cells (Weber, 2010; Mellman *et al.*, 2011; Pardoll, 2012). Stimulatory receptors or receptor ligands are targeted by agonist agents, whereas inhibitory receptors or receptor ligands are targeted by blocking agents. Because many of the immune checkpoints are initiated by ligand-receptor interactions, they can be readily

blocked by Abs or modulated by recombinant forms of ligands or receptors. One or more of the costimulatory and inhibitory receptors and ligands that regulate T cell responses, other than PD-1/PD-L1, may provide targets for synergizing with the anti-CCR8 Abs disclosed herein for inhibiting tumor growth. For example, in certain embodiments, the anti-CCR8 Ab is combined with an anti-CTLA-4 Ab. In other embodiments, the anti-CCR8 Ab is combined with an anti-LAG-3 Ab.

Radiotherapy leads to an increase in Treg infiltration post treatment in murine models (Muroyama *et al.*, 2017) and in humans (unpublished data). Increased Treg infiltration may lead to undesirable suppression of anti-tumor immunity, and CCR8-mediated depletion may potentiate the efficacy of radiation therapy.

The present disclosure provides anti-CCR8 mAbs that are effective in potentiating an immune response by enhancing the anti-tumor efficacy of treatments such as checkpoint inhibition or radiotherapy and which exhibit at least one, several or all of the following desirable characteristics: (a) specifically binding to hCCR8 expressed on the surface of a cell with an EC₅₀ of about 1 nM or lower; (b) binding specifically to rare and scattered immune cells in the medulla of the thymus and dermis of the skin but, for example, not binding to human cerebrum, cerebellum, heart, liver, lung, kidney, tonsil, spleen, thymus, colon, stomach, pancreas, adrenal, pituitary, skin, peripheral nerve, testis or uterus tissue, or PBMCs. For example, the anti-CCR8 mAb or antigen-binding portion thereof may bind specifically to tumor-infiltrating Tregs but not bind to PBMCs, *e.g.*, not show cytoplasmic staining in fixed PBMCs. Non-binding of the Ab to the above-recited list of cells and tissues may be established, for instance, by carrying out standard staining with the relevant Abs, *e.g.* by the methods described in Example 14, *e.g.* on fixed tissue samples; (c) inhibiting binding of CCL1 to CCR8 and inhibiting CCR8/CCL1 signaling with an IC₅₀ of about 5 nM or lower; (d) when bound to CCR8 on the surface of a cell mediates depletion of the cell with an EC₅₀ of about 50 pM or lower; (e) when bound to CCR8 on the surface of a cell not causing internalization of CCR8 either in the presence or absence of a cross-linking Ab; (f) inhibiting growth of tumor cells in a subject when administered as monotherapy to the subject; and (g) inhibiting growth of tumor cells in a subject when administered to the subject in combination with an additional therapeutic agent for treating a cancer, optionally wherein the additional therapeutic agent is an anti-PD-1, anti-PD-L1, or anti-CTLA-4 Ab.

Certain anti-CCR8 mAbs that may be used in the therapeutic methods, compositions or kits described herein include mAbs that bind specifically to hCCR8 on a cell surface with high affinity, mediate depletion of the cell with an EC₅₀ of about 10 pM or lower, and exhibit at least two other, and preferably all, of the preceding properties. In certain embodiments the isolated Ab, preferably a mAb, or antigen-binding portion thereof may have at least one of the above properties and/or comprise the CDR1, CDR2 and CDR3 domains in each of a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16. For example, such an Ab or antigen-binding portion thereof may comprise the following CDRs as defined by the Kabat method: a V_H CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 33; a V_H CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 34; a V_H CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 35; a V_L CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 36; a V_L CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 37; and a V_L CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 38. In another example, such an Ab or antigen-binding portion thereof may comprise a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16. In a further example, such an Ab may comprise a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 100 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 112. Optionally, the Ab has reduced fucosylation of its heavy chain, or a hypofucosylated or nonfucosylated heavy chain constant region as described elsewhere herein.

In certain embodiments the isolated Ab, preferably a mAb, or antigen-binding portion thereof may have at least one of the above properties and/or comprise the CDR1, CDR2 and CDR3 domains in each of a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116. For example, such an Ab or antigen-binding portion thereof may comprise the following CDRs as defined by

the Kabat method: a V_H CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 103; a V_H CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 104; a V_H CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 105; a V_L CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 106; a V_L CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 107; and a V_L CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 108. As another example, such an Ab or antigen-binding portion thereof may comprise a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116. As a further example, such an Ab may comprise a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 117 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 118. Optionally, the Ab has reduced fucosylation of its heavy chain, or a hypofucosylated or nonfucosylated heavy chain constant region as described elsewhere herein.

In certain embodiments the isolated Ab, preferably a mAb, or antigen-binding portion thereof may have at least one of the above properties and/or comprise the CDR1, CDR2 and CDR3 domains in each of a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18. For example, such an Ab or antigen-binding portion thereof may comprise the following CDRs as defined by the Kabat method: a V_H CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 45; a V_H CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 46; a V_H CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 47; a V_L CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 48; a V_L CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 49; and a V_L CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 50. In another example, such an Ab or antigen-binding portion thereof may comprise a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising

consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18. In a further example, such an Ab or antigen-binding portion thereof may comprise a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114. Optionally, the Ab has reduced fucosylation of its heavy chain, or a hypofucosylated or nonfucosylated heavy chain constant region as described elsewhere herein.

Anti-PD-1/Anti-PD-L1 Abs Suitable for Use in the Disclosed Therapeutic Methods

Anti-PD-1 Abs suitable for use in the methods for cancer treatment, compositions or kits disclosed herein include isolated Abs, preferably mAbs or antigen-binding portions thereof, that bind to PD-1 with high specificity and affinity, block the binding of PD-L1 and/or PD-L2 to PD-1, and inhibit the immunosuppressive effect of the PD-1 signaling pathway. Similarly, anti-PD-L1 Abs suitable for use in these methods are isolated Abs, preferably mAbs or antigen-binding portions thereof, that bind to PD-L1 with high specificity and affinity, block the binding of PD-L1 to PD-1 and CD80 (B7-1), and inhibit the immunosuppressive effect of the PD-1 signaling pathway. In any of the therapeutic methods disclosed herein, an anti-PD-1 or anti-PD-L1 Ab includes an antigen-binding portion or fragment that binds to the PD-1 receptor or PD-L1 ligand, respectively, and exhibits functional properties similar to those of whole Abs in inhibiting receptor-ligand binding and reversing the inhibition of T cell activity, thereby upregulating an immune response.

Anti-PD-1 mAbs

MAbs that bind specifically to PD-1 with high affinity have been disclosed in U.S. Patent No. 8,008,449. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 7,488,802, 8,168,757, 8,354,509, and 9,205,148. The anti-PD-1 mAbs disclosed in U.S. Patent No. 8,008,449 have been demonstrated to exhibit several or all of the following characteristics: (a) binding to human PD-1 with a K_D of about 50 nM or lower, as determined by the SPR (BIAcore®) biosensor system; (b) not substantially binding to human CD28, CTLA-4 or ICOS; (c) increasing T-cell proliferation, interferon- γ production and IL-2 secretion in a Mixed Lymphocyte Reaction (MLR) assay; (d) binding to human PD-1 and cynomolgus monkey PD-1; (e) inhibiting the binding of PD-L1 and PD-L2 to PD-1; (f) releasing inhibition imposed by Treg cells on proliferation and interferon- γ production of CD4⁺CD25⁻ T cells; (g) stimulating antigen-specific memory

responses; (h) stimulating Ab responses; and (i) inhibiting tumor cell growth *in vivo*. Anti-PD-1 Abs usable in the disclosed methods of treatment, compositions or kits include mAbs that bind specifically to human PD-1 with high affinity and exhibit at least five, and preferably all, of the preceding characteristics. For example, an anti-PD-1 Ab suitable for use in the therapeutic methods disclosed herein (a) binds to human PD-1 with a K_D of about 10 nM to 0.1 nM, as determined by SPR (BIAcore®); (b) increases T-cell proliferation, interferon- γ production and IL-2 secretion in a MLR assay; (c) inhibits the binding of PD-L1 and PD-L2 to PD-1; (d) reverses inhibition imposed by Tregs on proliferation and interferon- γ production of CD4⁺CD25⁻ T cells; (e) stimulates antigen-specific memory responses; and (f) inhibits tumor cell growth *in vivo*.

Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802, 8,168,757 and 8,354,509, U.S. Publication No. 2016/0272708, and PCT Publication Nos. WO 2008/156712, WO 2012/145493, WO 2014/179664, WO 2014/194302, WO 2014/206107, WO 2015/035606, WO 2015/085847, WO 2015/112800, WO 2015/112900, WO 2016/106159, WO 2016/197367, WO 2017/020291, WO 2017/020858, WO 2017/024465, WO 2017/024515, WO 2017/025016, WO 2017/025051, WO 2017/040790, WO 2017/106061, WO 2017/123557, WO 2017/132827, WO 2017/133540, the disclosure of each of which is incorporated herein by reference in its entirety.

In certain embodiments, the anti-PD-1 mAb is selected from the group consisting of nivolumab (OPDIVO®; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538), pembrolizumab (KEYTRUDA®; formerly designated lambrolizumab and MK-3475; *see* WO 2008/156712A1), PDR001 (*see* WO 2015/112900), MEDI-0680 (formerly designated AMP-514; *see* WO 2012/145493), REGN-2810 (*see* WO 2015/112800), JS001 (*see* Liu and Wu, 2017), BGB-A317 (*see* WO 2015/035606 and US 2015/0079109), INCSHR1210 (SHR-1210; *see* WO 2015/085847; Liu and Wu, 2017), TSR-042 (ANB011; *see* WO 2014/179664), GLS-010 (WBP3055; *see* Liu and Wu, 2017), AM-0001 (*see* WO 2017/123557), STI-1110 (*see* WO 2014/194302), AGEN2034 (*see* WO 2017/040790), and MGD013 (*see* WO 2017/106061).

In certain preferred embodiments of any of the therapeutic methods described herein comprising administration of an anti-PD-1 Ab, the anti-PD-1 Ab is nivolumab, OPDIVO®, which has already been approved by the U.S. Food and Drug Administration (FDA) for treating multiple different cancers. Nivolumab is a fully human

IgG4 (S228P) PD-1 immune checkpoint inhibitor Ab that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of anti-tumor T-cell functions (described as mAb C5 in U.S. Patent No. 8,008,449; Wang *et al.*, 2014). In other preferred embodiments, the anti-PD-1 Ab is pembrolizumab (KEYTRUDA®; a humanized monoclonal IgG4 Ab directed against PD-1 and described as h409A11 in U.S. Patent No. 8,354,509), which has also been approved for multiple cancer indications.

Anti-PD-1 Abs usable in the disclosed methods, compositions or kits also include isolated Abs, preferably mAbs, that bind specifically to human PD-1 (hPD-1) and cross-compete for binding to human PD-1 with any one of the anti-PD-1 Abs described herein, *e.g.*: nivolumab (5C4; *see, e.g.*, U.S. Patent No. 8,008,449; WO 2013/173223) and pembrolizumab. Abs that cross-compete with a reference Ab, *e.g.*, nivolumab or pembrolizumab, for binding to an antigen, in this case human PD-1, can be readily identified in standard PD-1 binding assays such as BIACORE® analysis, ELISA assays or flow cytometry (*see, e.g.*, WO 2013/173223). In certain embodiments, the anti-PD-1 Ab binds to the same epitope as any of the anti-PD-1 Abs described herein, *e.g.*, nivolumab or pembrolizumab.

An anti-PD-1 Ab usable in the methods of the disclosed invention also includes an antigen-binding portion, including a Fab, F(ab')₂, Fd or Fv fragment, a sdAb, a scFv, di-scFv or bi-scFv, a diabody, a minibody or an isolated CDR (*see* Hollinger and Hudson, 2005; Olafsen and Wu, 2010, for further details).

In certain embodiments, the isolated anti-PD-1 Ab or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1, IgG2, IgG3 or IgG4 isotype. In certain preferred embodiments, the anti-PD-1 Ab or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG4 isotype. In other embodiments, the anti-PD-1 Ab or antigen-binding portion thereof is of a human IgG1 isotype. In certain other embodiments, the IgG4 heavy chain constant region of the anti-PD-1 Ab or antigen-binding portion thereof contains an S228P mutation (numbered using the Kabat system; Kabat *et al.*, 1983) which replaces a serine residue in the hinge region with the proline residue normally found at the corresponding position in IgG1 isotype Abs. This mutation, which is present in nivolumab, prevents Fab arm exchange with endogenous IgG4 Abs, while retaining the low affinity for activating Fc receptors associated with wild-type IgG4 Abs (Wang *et al.*, 2014). In yet other

embodiments, the Ab comprises a light chain constant region which is a human kappa or lambda constant region.

In other embodiments of the present methods, the anti-PD-1 Ab or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. For administration to human subjects, the anti-PD-1 Ab is preferably a chimeric Ab or, more preferably, a humanized or human Ab. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art, *e.g.*, as described in U.S. Patent No. 8,008,449.

Anti-PD-L1 mAbs

Because anti-PD-1 and anti-PD-L1 target the same signaling pathway and have been shown in clinical trials to exhibit comparable levels of efficacy in a variety of cancers (*see, e.g.*, Brahmer *et al.*, 2012; WO 2013/173223), an anti-PD-L1 Ab may be substituted for the anti-PD-1 Ab in the combination therapy methods disclosed herein.

Anti-PD-L1 Abs suitable for use in the disclosed methods, compositions or kits are isolated Abs that bind to PD-L1 with high specificity and affinity, block binding of PD-L1 to PD-1 and to CD80, and inhibit the immunosuppressive effect of the PD-1 signaling pathway. MAbs that bind specifically to PD-L1 with high affinity have been disclosed in U.S. Patent No. 7,943,743. Other anti-PD-L1 mAbs have been described in, for example, U.S. Patent Nos. 8,217,149, 8,779,108, 9,175,082 and 9,624,298, and PCT Publication No. WO 2012/145493. The anti-PD-1 HuMAbs disclosed in U.S. Patent No. 7,943,743 have been demonstrated to exhibit one or more of the following characteristics: (a) binding to human PD-1 with a K_D of about 50 mM or lower, as determined by SPR (BIAcore®); (b) increasing T-cell proliferation, interferon- γ production and IL-2 secretion in a MLR assay; (c) stimulating Ab responses; (d) inhibiting the binding of PD-L1 to PD-1; and (e) reversing the suppressive effect of Tregs on T cell effector cells and/or dendritic cells. Anti-PD-L1 Abs for use in the therapeutic methods disclosed herein include isolated Abs, preferably mAbs, that bind specifically to human PD-L1 with high affinity and exhibit at least one, in some embodiments at least three, and preferably all, of the preceding characteristics. For example, an anti-PD-L1 Ab suitable for use in these methods (a) binds to human PD-1 with a K_D of about 50 mM to 0.1 mM, as determined by surface plasmon resonance (BIAcore®); (b) increases T-cell proliferation, interferon- γ production and IL-2 secretion in a MLR assay; (c) inhibits the binding of PD-L1 to PD-1 and to CD80; and (d) reverses the suppressive effect of Tregs

on T cell effector cells and/or dendritic cells.

A suitable anti-PD-L1 Ab for use in the present methods is BMS-936559 (formerly MDX-1105; designated 12A4 in U.S. Patent No. 7,943,743). Other suitable anti-PD-L1 Abs include atezolizumab (TECENTRIQ®; previously known as RG7446 and MPDL3280A; designated YW243.55S70 in U.S. Patent No. 8,217,149; *see, also*,
5 Herbst *et al.*, 2014), durvalumab (IMFINZI®; previously known as MEDI-4736; designated 2.14H9OPT in U.S. Patent No. 8,779,108), avelumab (BAVENCIO®; previously known as MSB-0010718C; designated A09-246-2 in U.S. Patent No. 9,624,298), STI-A1014 (designated H6 in U.S. Patent No. 9,175,082), CX-072 (*see* WO
10 2016/149201), KN035 (*see* Zhang *et al.*, 2017), LY3300054 (*see, e.g.*, WO 2017/034916), and CK-301 (*see* Gorelik *et al.*, 2017).

In certain preferred embodiments of any of the therapeutic methods described herein comprising administration of an anti-PD-L1 Ab, the anti-PD-L1 Ab is atezolizumab (TECENTRIQ®). In other preferred embodiments, the anti-PD-L1 Ab is
15 durvalumab (IMFINZI®). In further preferred embodiments, the anti-PD-L1 Ab is avelumab (BAVENCIO®).

Anti-PD-L1 Abs suitable for use in the disclosed methods, compositions or kits also include isolated Abs that bind specifically to human PD-L1 and cross-compete for binding to human PD-L1 with a reference Ab which may be any one of the anti-PD-L1
20 Abs disclosed herein, *e.g.*, BMS-936559 (12A4; *see, e.g.*, U.S. Patent No. 7,943,743; WO 2013/173223), atezolizumab, durvalumab, avelumab or STI-A1014. The ability of an Ab to cross-compete with a reference Ab for binding to human PD-L1 demonstrates that such Ab binds to the same epitope region of PD-L1 as the reference Ab and is expected to have very similar functional properties to that of the reference Ab by virtue of its binding to
25 substantially the same epitope region of PD-L1. In some embodiments, the anti-PD-L1 Ab binds the same epitope as any of the anti-PD-L1 Abs described herein, *e.g.*, atezolizumab, durvalumab, avelumab or STI-A1014. Cross-competing Abs can be readily identified based on their ability to cross-compete with a reference Ab such as atezolizumab or avelumab in standard PD-L1 binding assays such as BIAcore®
30 analysis, ELISA assays or flow cytometry that are well known to persons skilled in the art (*see, e.g.*, WO 2013/173223).

In certain preferred embodiments, the isolated anti-PD-L1 Abs for use in the present methods are mAbs. In other embodiments, especially for administration to human

subjects, these Abs are preferably chimeric Abs, or more preferably humanized or human Abs. Chimeric, humanized and human Abs can be prepared and isolated by methods well known in the art, *e.g.*, as described in U.S. Patent No. 7,943,743.

In certain embodiments, the anti-PD-L1 Ab or antigen-binding portion thereof
5 comprises a heavy chain constant region which is of a human IgG1, IgG2, IgG3 or IgG4 isotype. In certain other embodiments, the anti-PD-L1 Ab or antigen-binding portion thereof is of a human IgG1 or IgG4 isotype. In further embodiments, the sequence of the IgG4 heavy chain constant region of the anti-PD-L1 Ab or antigen-binding portion thereof contains an S228P mutation. In other embodiments, the Ab comprises a light
10 chain constant region which is a human kappa or lambda constant region.

Anti-PD-L1 Abs of the invention also include antigen-binding portions of the above Abs, including Fab, F(ab')₂, Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, nanobodies, diabodies, triabodies, tetrabodies, and isolated CDRs, that bind to PD-L1 and exhibits functional properties similar to those of whole Abs in inhibiting
15 receptor binding and up-regulating the immune system.

Pharmaceutical Compositions and Dosage Regimens

MAbs disclosed herein and used in any of the therapeutic methods described may be constituted in a composition, *e.g.*, a pharmaceutical composition containing an Ab and a pharmaceutically acceptable carrier. This invention also provides compositions
20 comprising any of the disclosed immunoconjugates or bispecific molecule and a pharmaceutically acceptable carrier. As used herein, a "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier for a composition containing an Ab is suitable for intravenous (IV), intramuscular, subcutaneous (SC), parenteral, spinal or
25 epidermal administration (*e.g.*, by injection or infusion).

An option for SC injection is based on Halozyme Therapeutics' ENHANZE® drug-delivery technology, involving a co-formulation of an Ab, *e.g.*, a mAb, with recombinant human hyaluronidase enzyme (rHuPH20) that removes traditional
30 limitations on the volume of biologics and drugs that can be delivered subcutaneously due to the extracellular matrix (U.S. Patent No. 7,767,429). It may be possible to co-formulate two Abs used in combination therapy into a single composition for SC administration.

A pharmaceutical composition of the invention may include one or more

pharmaceutically acceptable salts, anti-oxidants, aqueous and non-aqueous carriers, and/or adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents.

Dosage regimens are adjusted to provide the optimum desired response, *e.g.*, a
5 maximal therapeutic response and/or minimal adverse effects. For administration of an anti-CCR8, anti-PD-1 or anti-PD-L1 Ab or an antigen-binding portion thereof, including for combination use, the dosage may range from about 0.01 to about 20 mg/kg, preferably from about 0.1 to about 10 mg/kg, of the subject's body weight. For example, dosages can be about 0.1, 0.3, 1, 2, 3, 5 or 10 mg/kg body weight, and more preferably, about 0.3,
10 1, 3, or 10 mg/kg body weight. Alternatively, a fixed or flat dose, *e.g.*, about 0.1 to about 2,000 mg, preferably about 1 to about 1,000 mg such as about 0.3, 1, 3, 5, 10, 30, 60, 100, 150, 200, 240, 300, 400, 500, 600, 800 or 1,000 mg, of the Ab or antigen-binding portion thereof, instead of a dose based on body weight, may be administered. Flat (vs. weight-based) dosing is attractive due to the ease of preparation, a reduced risk of medication
15 errors, and the observation that for most biologics the two dosing approaches perform similarly (Wang *et al.*, 2009).

The dosing schedule is typically designed to achieve exposures that result in sustained receptor occupancy (RO) based on typical pharmacokinetic properties of an Ab. An exemplary treatment regime entails administration once per week, once every 2
20 weeks, once every 3 weeks, once every 4 weeks, once a month, once every 3-6 months or longer. In certain preferred embodiments, the anti-CCR8, anti-PD-1 or anti-PD-L1 Ab or antigen-binding portion thereof is administered to the subject once every 2 weeks. In other preferred embodiments, the Ab or antigen-binding portion thereof is administered once every 3 weeks or once every 4 weeks. The dosage and scheduling may change
25 during a course of treatment. The first-in-human (FIH) starting flat dose of 0.3 mg (4 µg/kg) IV Q2W for 4A19 was derived using the totality of data generated from a mix of pharmacology- and toxicology-based approaches with the goal of ensuring adequate safety while minimizing the participants' exposure to potentially sub-efficacious doses and risk of cytokine release.

30 In the clinical trial described in Example 34, the totality of data generated from a mix of pharmacology- and toxicology-based approaches were employed to determine dosages used in the first-in-human (FIH) clinical study. In the dose escalation phase of the anti-CCR8 monotherapy arm, a flat dose of 0.3, 1, 3, 10, 30, 100, 300 or 800 mg of

4A19 is administered intravenously (IV) to the subject once every 2 weeks (Q2W). In the combination therapy arm, the same doses of 4A19 are administered in the dose escalation phase in combination with nivolumab administered IV at the FDA-approved flat dose of 480 mg once every 4 weeks (Q4W). For dose expansion, single-arm and randomized cohorts are opened to include different tumor types and dose levels from the escalation phase, with treatment as monotherapy or combination therapy continuing until progression, unacceptable toxicity, withdrawal of consent, completion of 26 cycles of study therapy (104 weeks), or the study ends, whichever occurs first.

Accordingly, in certain embodiments of the disclosed therapy methods, an anti-CCR8 Ab is administered to the subject as monotherapy, or in combination with an immune checkpoint inhibitor, *e.g.*, an anti-PD-1 or anti-PD-L1 Ab, at a flat dose of about 0.3 to about 800 mg Q2W. More specifically, in certain embodiments, the anti-CCR8 Ab is administered at 0.3, 1, 3, 10, 30, 100, 300 or 800 mg Q2W. In certain preferred embodiments, the anti-CCR8 Ab is mAb 4A19. In other preferred embodiments, the anti-CCR8 Ab is mAb 14S5 or 14S15h. In certain embodiments, the anti-CCR8 Ab is administered to the subject at a flat dose of 3 mg Q2W. In certain other embodiments, the anti-CCR8 Ab is administered at 10 mg Q2W. In other embodiments, the anti-CCR8 Ab is administered at a flat dose of 30 mg Q2W. In yet other embodiments, the anti-CCR8 Ab is administered to the subject at a flat dose of 100 mg Q2W.

For combination therapy, in certain embodiments, the immune checkpoint inhibitor is an anti-PD-1, anti-PD-L1, or anti-CTLA-4 Ab. In certain preferred embodiments, the anti-PD-1 Ab is nivolumab. In preferred embodiments, nivolumab is administered to the subject at the flat dose of 480 mg Q4W.

When used in combinations, a subtherapeutic dosage of one or both Abs, *e.g.*, a dosage of an anti-CCR8, anti-PD-1 and/or anti-PD-L1 Ab or antigen-binding portion thereof may be used. As used herein, a “subtherapeutic” dose or dosage of a therapeutic agent, such as a therapeutic Ab, refers to a dose that is lower than the typical or approved monotherapy dose. For example, a dosage of nivolumab that is lower than the initially FDA-approved 3 mg/kg every 2 weeks, for instance, 1.0 mg/kg or less every 2, 3 or 4 weeks, is regarded as a subtherapeutic dosage. Nivolumab has subsequently been approved by the FDA at 240 mg every two weeks or 480 mg every 4 weeks. Thus, for example, a dosage of nivolumab that is lower than the approved 480 mg every 4 weeks, for instance, 120 mg or less every 2, 3 or 4 weeks, is regarded as a subtherapeutic dosage.

RO data from 15 subjects who received 0.3 mg/kg to 10 mg/kg dosing with nivolumab indicate that PD-1 occupancy appears to be dose-independent in this dose range. Across all doses, the mean occupancy rate was 85% (range, 70% to 97%), with a mean plateau occupancy of 72% (range, 59% to 81%) (Brahmer *et al.*, 2010). Thus, 0.3 mg/kg dosing
5 may allow for sufficient exposure to lead to significant biologic activity.

In contrast, as used herein, a “sub-efficacious” dose or dosage of a therapeutic agent, such as a therapeutic Ab, refers to a dose that is lower than the dose required for significant biologic activity and, therefore, does not cause any meaningful therapeutic effect when administered as monotherapy or in combination therapy.

10 The synergistic interaction observed in mouse tumor models between the anti-CCR8 and anti-PD-1/anti-PD-L1 Abs or antigen-binding portions thereof may permit the administration of one or both of these therapeutics to a cancer patient at subtherapeutic dosages. In certain embodiments of the disclosed combination therapy methods, the anti-CCR8 Ab or antigen-binding portion thereof is administered at a subtherapeutic dose to a
15 cancer patient. In other embodiments, the anti-PD-1/anti-PD-L1 Ab or antigen-binding portion thereof is administered to the patient at a subtherapeutic dose. In further embodiments, the anti-PD-1/anti-PD-L1 and anti-CCR8 Abs or antigen-binding portions thereof are each administered to the patient at a subtherapeutic dose.

The administration of such a subtherapeutic dose of one or both Abs may reduce
20 adverse events compared to the use of higher doses of the individual Abs in monotherapy. Thus, the success of the disclosed methods of combination therapy may be measured not only in improved efficacy of the combination of Abs relative to monotherapy with these Abs, but also in increased safety, *i.e.*, a reduced incidence of adverse events, from the use of lower dosages of the drugs in combination relative to the monotherapy doses.

25 In certain embodiments of any of the methods disclosed herein, the anti-CCR8, anti-PD-1 and/or anti-PD-L1 Abs are formulated for intravenous (IV) administration or for subcutaneous (SC) injection. In certain embodiments, the anti-CCR8 Ab or antigen-binding portion thereof and the anti-PD-1/anti-PD-L1 Ab or antigen-binding portion thereof are administered sequentially to the subject. “Sequential” administration means
30 that one of the anti-CCR8 and anti-PD-1/anti-PD-L1 Abs is administered before the other. Either Ab may be administered first; *i.e.*, in certain embodiments, the anti-PD-1/anti-PD-L1 Ab is administered before the anti-CCR8 Ab, whereas in other embodiments, the anti-CCR8 Ab is administered before the anti-PD-1/anti-PD-L1 Ab. In certain embodiments,

each Ab is administered by IV infusion, for example, by infusion over a period of about 60 minutes. In other embodiments, at least one Ab is administered by SC injection.

In certain embodiments of sequential IV administration, for the convenience of the patient, the anti-CCR8 and anti-PD-1/anti-PD-L1 Abs or portions thereof are
5 administered within 30 minutes of each other. Typically, when both the anti-CCR8 and anti-PD-1/anti-PD-L1 Abs are to be delivered by IV administration on the same day, separate infusion bags and filters are used for each infusion. The infusion of the first Ab is promptly followed by a saline flush to clear the line of the Ab before starting the infusion of the second Ab. In other embodiments, the two Abs are administered within 1,
10 2, 4, 8, 24 or 48 h of each other.

The delivery of at least one Ab by SC administration reduces health care practitioner time required for administration and shortens the time for drug administration. For example, the use of SC injection could cut the time needed for IV administration, typically about 30-60 min, to about 5 min. In certain embodiments of
15 sequential SC administration, the anti-CCR8 and anti-PD-1/anti-PD-L1 Abs or portions thereof are administered within 10 min of each other.

Because checkpoint inhibitor Abs have been shown to produce very durable responses, in part due to the memory component of the immune system (*see, e.g.,* WO 2013/173223; Lipson *et al.*, 2013; Wolchok *et al.*, 2013), the activity of an administered
20 anti-PD-1/anti-PD-L1 Ab may be ongoing for several weeks, several months, or even several years. In certain embodiments, the present combination therapy methods involving sequential administration entail administration of an anti-CCR8 Ab to a patient who has been previously treated with an anti-PD-1/anti-PD-L1 Ab. In further embodiments, the anti-CCR8 Ab is administered to a patient who has been previously
25 treated with, and progressed on, an anti-PD-1/anti-PD-L1 Ab. In other embodiments, the present combination therapy methods involving sequential administration entail administration of an anti-PD-1/anti-PD-L1 Ab to a patient who has been previously treated with an anti-CCR8 Ab, optionally a patient whose cancer has progressed after treatment with the anti-CCR8 Ab.

30 In certain other embodiments, the anti-PD-1/anti-PD-L1 and anti-CCR8 mAbs are administered concurrently, either admixed as a single composition in a pharmaceutically acceptable formulation for concurrent administration, or concurrently as separate compositions with each Ab in formulated in a pharmaceutically acceptable composition.

In certain preferred embodiments, the anti-PD-1/anti-PD-L1 and anti-CCR8 mAbs are administered concurrently as separate compositions with each Ab in formulated in a pharmaceutically acceptable composition. In other embodiments, the anti-PD-1/anti-PD-L1 and anti-CCR8 mAbs are administered concurrently as a single composition in a pharmaceutically acceptable formulation.

This disclosure provides a hypofucosylated or nonfucosylated isolated Ab, preferably a mAb, which specifically binds to hCCR8 expressed on the surface of a cell, wherein the isolated Ab comprises a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114, for use in a method of treating a subject afflicted with a cancer, wherein the cancer is chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer.

In certain embodiments, the disclosure also provides a hypofucosylated or nonfucosylated isolated Ab, preferably a mAb, which specifically binds to hCCR8 expressed on the surface of a cell, wherein the isolated Ab comprises a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114, for use in a method of treating in combination with another therapeutic agent a subject afflicted with a cancer, wherein the cancer is chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer, and wherein the other therapeutic agent is an anti-PD-1, an anti-PD-L1, or an anti-CTLA-4 Ab.

In certain other embodiments, the disclosure further provides a hypofucosylated or nonfucosylated isolated Ab, preferably a mAb, which specifically binds to hCCR8 expressed on the surface of a cell, wherein the isolated Ab comprises a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114, for use in a method of treating in combination with another therapeutic agent a subject afflicted with a cancer, wherein the cancer is chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer, wherein the other therapeutic agent is nivolumab, and wherein the anti-CCR8 Ab is administered to the subject at a dose of 1-30 mg once every 2 weeks and nivolumab is administered to the subject at a dose of 240 mg once every 2 weeks or 480 mg once every

4 weeks.

This disclosure also provides a method for treating a subject afflicted with a cancer comprising administering to the subject a therapeutically effective amount of a hypofucosylated or nonfucosylated anti-CCR8 mAb which comprises a heavy chain
5 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114 such that the subject is treated, wherein the cancer is chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer.

10 In certain embodiments, the disclosure also provides a method for treating a subject afflicted with a cancer comprising administering to the subject therapeutically effective amounts of: (a) a hypofucosylated or nonfucosylated anti-CCR8 mAb which comprises a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked
15 amino acids having the sequence set forth as SEQ ID NO: 114; and (b) another therapeutic agent, such that the subject is treated, wherein the cancer is chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer, and wherein the other therapeutic agent is an anti-PD-1, an anti-PD-L1, or an anti-CTLA-4 Ab.

20 In certain other embodiments, the disclosure further provides a method for treating a subject afflicted with a cancer comprising administering to the subject therapeutically effective amounts of: (a) a hypofucosylated or nonfucosylated anti-CCR8 mAb which comprises a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked
25 amino acids having the sequence set forth as SEQ ID NO: 114; and (b) another therapeutic agent, such that the subject is treated, wherein the cancer is chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer, wherein the other therapeutic agent is nivolumab, and wherein the anti-CCR8 Ab is administered to the subject at a dose of 1-30 mg once every 2 weeks and nivolumab is administered to
30 the subject at a dose of 240 mg once every 2 weeks or 480 mg once every 4 weeks.

The disclosure further provides a method for inhibiting growth of tumor cells in a subject comprising administering to the subject a therapeutically effective amount of a hypofucosylated or nonfucosylated anti-CCR8 mAb which comprises a heavy chain

comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114 such that growth of tumor cells in the subject is inhibited, wherein the cancer is chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer.

In certain embodiments, the disclosure also provides a method for inhibiting growth of tumor cells in a subject comprising administering to the subject therapeutically effective amounts of: (a) a hypofucosylated or nonfucosylated anti-CCR8 mAb which comprises a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114; and (b) another therapeutic agent, such that growth of tumor cells in the subject is inhibited, wherein the cancer is chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer, and wherein the other therapeutic agent is an anti-PD-1, an anti-PD-L1, or an anti-CTLA-4 Ab.

In certain other embodiments, the disclosure further provides a method for inhibiting growth of tumor cells in a subject comprising administering to the subject therapeutically effective amounts of: (a) a hypofucosylated or nonfucosylated anti-CCR8 mAb which comprises a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114; and (b) another therapeutic agent, such that growth of tumor cells in the subject is inhibited, wherein the cancer is chosen from NSCLC, SCCHN, MSS-CRC, gastric/GE junction cancer, and cervical cancer, wherein the other therapeutic agent is nivolumab, and wherein the anti-CCR8 Ab is administered to the subject at a dose of 1-30 mg once every 2 weeks and nivolumab is administered to the subject at a dose of 240 mg once every 2 weeks or 480 mg once every 4 weeks.

Kits

Also within the scope of the present invention are kits comprising an anti-CCR8 Ab. Kits typically include a label indicating the intended use of the contents of the kit and instructions for use. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit. Accordingly, this disclosure provides a kit for treating a subject afflicted with a cancer, the kit comprising: (a) one or

more dosages ranging from about 0.01 to about 20 mg/kg body weight, *e.g.*, about 0.1 or 1 mg/kg, or about 0.1 to about 2,000 mg fixed dose, *e.g.*, about 3, 10, 30 or 100 mg, of an Ab, *e.g.*, a mAb or an antigen-binding portion thereof that binds specifically to CCR8 and mediates depletion of a CCR8-expressing cell by ADCC; (b) optionally one or more
5 dosages ranging from about 0.1 to about 20 mg/kg body weight, *e.g.*, about 2 to about 10 mg/kg, or about 200 to about 1600 mg fixed dose, *e.g.*, about 240 or 480 mg, of an Ab, *e.g.*, a mAb or an antigen-binding portion thereof that binds specifically to PD-1, PD-L1 or CTLA-4; and (c) instructions for using the Ab or portion thereof that binds specifically to CCR8, and optionally the Ab or portion thereof that binds specifically to PD-1, PD-L1
10 or CTLA-4, in any of the therapeutic methods disclosed herein.

The disclosure further provides a kit for treating a subject afflicted with a cancer, the kit comprising: (a) one or more dosages ranging from about 0.01 to about 20 mg/kg body weight, *e.g.*, about 0.1 or 1 mg/kg, or about 0.1 to about 2,000 mg fixed dose, *e.g.*, about 3, 10, 30 or 100 mg, of an Ab, *e.g.*, a mAb or an antigen-binding portion thereof
15 that binds specifically to CCR8 and mediates depletion of a CCR8-expressing cell by ADCC; (b) one or more dosages of about 0.1 to about 20 mg/kg body weight, *e.g.*, about 2 or 3 mg/kg, or 200 to about 1600 mg fixed dose, *e.g.*, about 240 or 480 mg, of an anti-PD-1/anti-PD-L1 mAb or an antigen-binding portion thereof; and (c) instructions for using the anti-CCR8 mAb and the anti-PD-1/anti-PD-L1 mAb, in any of the combination
20 therapy methods disclosed herein.

In certain embodiments, the kit comprises one or more fixed dosages of the anti-CCR8 Ab ranging from about 0.1 to about 2,000 mg, preferably about 0.3 to about 1,000 mg such as about 0.3, 1, 3, 5, 10, 30, 60, 100, 150, 200, 240, 300, 400, 500, 600, 800 or 1,000 mg, of the Ab or antigen-binding portion thereof, instead of a dose based on body
25 weight. In certain embodiments, the kit comprises 0.3, 1, 3, 10, 30, 100, 300 and 800 mg of the anti-CCR8 Ab for administration once every 2 weeks. In certain other embodiments, the kit comprises about 100 to about 600 mg of the anti-PD-1/PD-L1 Ab, *e.g.*, 240 mg of nivolumab for administration once every 2 weeks or 480 mg of nivolumab for administration once every 4 weeks. In certain preferred embodiments, the anti-CCR8
30 Ab is mAb 4A19. In other preferred embodiments, the anti-CCR8 Ab is mAb 14S15 or 14S15h. In certain preferred embodiments, the anti-PD-1 Ab is nivolumab. In other preferred embodiments, the anti-PD-1 Ab is pembrolizumab.

In certain embodiments, the Abs may be co-packaged in unit dosage form. In

certain preferred embodiments for treating human patients, the kit comprises an anti-human PD-1 Ab disclosed herein, *e.g.*, nivolumab or pembrolizumab.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references cited
5 throughout this application are expressly incorporated herein by reference.

EXAMPLE 1

CORRELATION OF CCR8 EXPRESSION WITH FOXP3 EXPRESSION IN TUMOR Tregs

CCR8 is a Treg-Selective Marker

10 TCGA samples (Goldman *et al.*, 2019; Wang and Liu, 2019; batch-corrected FPKM values from UCSC Xena PanCan Repository) from tumors of non-hematopoietic origin were divided into tumor categories (TCGA designations, tumor only) and RNA-seq data for each transcript was transformed into a standard distribution (mean = 0, standard deviation = 1) within tumor type, after which all tumor RNA data were combined.
15 Mutual-Rank distance measurements (Siemers *et al.*, 2017; Huttenhower *et al.*, 2007) were performed from the Pearson correlation matrix of log-transformed transcripts.

Mutual rank-based network of gene-gene correlation across all non-heme tcga tumor RNA-seq, shown in Figure 1A, identified CCR8 as a Treg-selective marker as transcript abundances for CCR8 exhibited both a strong and selective correlation to
20 *FOXP3* when compared with other gene neighbors in most cancer types from TCGA. It is notable that costimulatory/coinhibitory molecules (CTLA4, ICOS, TIGIT) occupy a part of the network intermediary to FOXP3 and canonical T cell markers (*e.g.*, CD3E, IL2RB, SLAMF1). Line weights and distances indicate strength of mutual-rank associations.

CCR8 Is Selectively Expressed on FOXP3^{high} Lymphocytes in Hepatocellular Carcinoma

25 Data were plotted logarithmically scaled as log2 (counts + 1) from Zheng *et al.* (2017), NCBI Gene Expression Omnibus: GSE98638, and plots created with the Fauxflow R/Shiny toolkit (<https://github.com/NathanSiemers/FauxFlow>). A small amount of Gaussian noise was added to each point to aid in visualization.

As shown in Figure 1B, CCR8 is selectively expressed on FOXP3^{high} lymphocytes
30 in hepatocellular carcinoma tumor samples, and there is an absence of CCR8 expression in FOXP3^{mid} CD8 and CD4 populations.

Association of CCR8 Expression with Expression of Other Genes

Spearman correlation analysis was performed in FOXP3⁺ (expression ≥ 1) T lymphocytes (expression ≥ 1) between CCR8 expression ≥ 1 vs. CCR8 expression < 1 , and unadjusted Spearman P values were plotted vs. the ratio of mean gene expression of associated markers.

Figure 1C shows that transcripts associated with CCR8 positivity and negativity within FOXP3⁺ cells in Zheng *et al.* (2017). CCR8 expression is associated with higher levels of FOXP3 expression and canonical markers of Tregs (IL2RA, IKZF2, BATF), whereas lower expression of CCR8 is associated with cytotoxic T cell markers (GZMA, CD8A).

EXAMPLE 2

TISSUE PROCESSING

Human Tumor Dissociation

Tumor samples were minced with dissecting lab scissors and further mechanically dissociated with a Dounce homogenizer or enzymatically digested using a human tumor dissociation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) in conjunction with a gentleMACS dissociator (Miltenyi Biotec) according to the manufacturer's instructions. Cell suspensions were filtered through a 70 μ m cell strainer.

Human Treg Isolation and Stimulation

PBMCs were isolated from a leukopak (AllCells) using a Ficoll density gradient (GE Healthcare). CD25⁺ cells were magnetically enriched using anti-CD25 MicroBeads II (Miltenyi Biotec). Enriched cells were stained for CD25 (4E3, Miltenyi Biotec), CD127 (A019D5, BioLegend), CD45RA (HI100, BioLegend), and CD4 (SK3, BD Biosciences). CD4⁺ CD127^{low} CD25^{high} CD45RA⁺ Tregs were sorted on a BD FACSAria II. Isolated naïve Tregs were expanded using Dynabeads Human T-Activator CD3/CD28 (Thermo Fisher Scientific) (3 beads to 1 Treg) in Roswell Park Memorial Institute (RPMI) cell culture medium (Corning, Corning, NY) with 10% fetal bovine serum (FBS; GE Healthcare, Chicago, IL). After 3 days, IL-2 (PeproTech) was added at 300 U/ml. Tregs were expanded and cryopreserved. Thawed Tregs were restimulated using CD3/CD28 Dynabeads (1 bead to 1 Treg) in RPMI medium with 10% FBS and 100 U/ml of IL-2.

Human Skin Processing

Skin biopsies from normal female abdominal human skin, age 43, was digested overnight in a MACS C tube (Miltenyi Biotec) using a whole skin dissociation kit (Miltenyi Biotec) without enzyme P. Cell suspensions were washed, filtered, and stained.

5 *Mouse Tissue Processing*

Tumors were minced and then enzymatically digested for 30 min at 37°C using 250 U/ml Collagenase IV (Worthington Biochemical, Lakewood, NJ) and DNase I (MilliporeSigma, Burlington, MA). Shaved skin was minced and then enzymatically digested for 60 min at 37°C with 500 µg/ml Collagenase XI (MilliporeSigma), 500 µg/ml
10 Hyaluronidase (MilliporeSigma), and 100 µg/ml DNase I. Spleen and thymus were dissociated mechanically using a gentleMACS dissociator (Miltenyi Biotec). Red blood cells (RBCs) in dissociated spleen and blood samples were lysed with ammonium-chloride-potassium solution (Thermo Fisher Scientific) for 5 min.

Study Approval

15 Human tumor, skin, spleen, patient blood, and healthy blood leukopack samples were collected through commercial providers (BioIVT, Westbury, NY; MT Group, Avaden, BioOptions, Discovery Life Sciences, Los Osos, CA; AllCells, Alameda, CA) or the Cooperative Human Tissue Network (CHTN). All samples were collected from donors giving written informed consent at IRB-approved study locations in the United
20 States.

Blood from healthy donors was obtained from a blood donation program administered by the Bristol Myers Squibb (BMS) Occupational Health department in compliance with all relevant ethical regulations. All animal experiments complied with institutional guidelines and were approved by the IACUC at BMS under protocol 1612-
25 01.

EXAMPLE 3

CCR8 MARKS SUPPRESSIVE FOXP3⁺ CELLS

WITH LOW PROINFLAMMATORY POTENTIAL IN HUMAN TUMORS

Expression profiles of several Treg-associated molecules were compared by flow
30 cytometry.

Flow cytometry

For flow cytometry, in general, cell suspensions were stained with amine reactive

viability dyes. Human cells were then blocked with rat, mouse, and human A/B serum (GEMCELL™; Gemini Bio, West Sacramento, CA), HUMAN TRUSTAIN FcX™ (BioLegend), and TRUE-STAIN MONOCYTE BLOCKER™ (BioLegend). Mouse cells were blocked with FcR blocking reagent (Miltenyi Biotec). Cells were surface stained, and intracellular staining was performed using the FOXP3 Transcription Factor Staining Buffer Set (Thermo Fisher Scientific) using manufacturer's protocols. For binding of unconjugated mAbs, an appropriate secondary Ab (Jackson ImmunoResearch Laboratories) was used. Samples were acquired using a BD LSRFortessa X-20 and analyzed using FlowJo (BD Biosciences). The complete list of flow cytometry Abs is provided in Table 2.

Table 2: Abs used for flow cytometric analyses

Target	Species	Clone	Fluorophore	Manufacturer
CD45	Human	HI10	AF488	Biolegend
CCR4	Human	L291H4	PE	Biolegend
CCR4	Human	L291H4	BV510	Biolegend
CD16	Human	3G8	AF647	Biolegend
CD8a	Human	SK1	PerCP-eFluor 710	Thermo Fisher
CD56	Human	CMSSB	PE-eFluor 610	Thermo Fisher
FOXP3	Human	236A/E7	PE-Cy7	Thermo Fisher
CCR8	Human	433H	BV421	BD Biosciences
CD15	Human	W6D3	BV510	BD Biosciences
CD4	Human	SK3	BV650	BD Biosciences
CD4	Human	SK3	BV605	BD Biosciences
CTLA4	Human	BNI3	BV421	BD Biosciences
HLA-DR	Human	G46-6	BUV395	BD Biosciences
PD-1	Human	EH12.1	BUV737	BD Biosciences
CD3	Human	UCHT1	BUV805	BD Biosciences
CD3	Human	UCHT1	APC	BD Biosciences
CD39	Human	A1	BV786	Biolegend
Granzyme B	Human	QA16A02	PE	Biolegend

Target	Species	Clone	Fluorophore	Manufacturer
IFNg	Human	4S.B3	PE-eFluor 610	Thermo Fisher
IL-1R2	Human	MA5-23626	APC	Thermo Fisher
IL-2	Human	MQ1-17H12	BUV737	BD Biosciences
CD19	Human	SJ25C1	BV510	BD Biosciences
CD25	Human	2A3	APC-R700	BD Biosciences
CD3e	Mouse	145-2C11	BV421	Biolegend
CCR8	Mouse	SA214G2	AF647	Biolegend
CCR8	Mouse	SA214G2	PE	Biolegend
IFNg	Mouse	XMG1.2	PE-Cy7	Biolegend
TNFa	Mouse	MP6-XT22	APC	Biolegend
FOXP3	Mouse	FJK-16a	PE-eFluor 610	Thermo Fisher
CD8a	Mouse	53-6.7	BUV805	BD Biosciences
CD8b	Mouse	H35-17.2	BV650	BD Biosciences
CD4	Mouse	RM4-4	BV786	BD Biosciences
CD4	Mouse	RM4-5	BV786	BD Biosciences
CD45	Mouse	30-F11	BUV395	BD Biosciences
CD90.1	Mouse	OX-7	BB700	BD Biosciences
CD90.2	Mouse	30-H12	BV421	Biolegend
CD45.2	Mouse	104	APC	Biolegend
CD45.1	Mouse	A20	BUV395	BD Biosciences
Fixable Viability Stain 575V	NA	NA	NA	BD Biosciences

CCR8 Is a Marker for Tumor-Associated Tregs

CCR8 was highly expressed on FOXP3⁺ Tregs isolated from solid tumor surgical resections but expression was significantly lower on Tregs from patient-matched blood.

- 5 In contrast, CCR4, CTLA-4, and CD25 were expressed at similar frequencies by Tregs from tumor and blood (Figure 2A). The per-cell abundance of CCR8 on FOXP3⁺ Tregs

was higher on tumor Tregs compared to peripheral blood Tregs (Figure 2B), whereas CCR4 expression was lower in tumor Tregs compared to peripheral blood Tregs. On CD4⁺FOXP3⁻ conventional T cells (CD4⁺ Tconv), CCR8 was less frequently expressed than CCR4 and CD25 in both tumor and peripheral blood (Figures 2C and D). Blood and tumor-infiltrating CD8⁺ T cells showed marginal expression of all four molecules, with the exception of CD25 in the blood (Figures 2E and 2F). These data suggest that CCR8 is a highly selective marker for targeting tumor Tregs with a relatively low risk of compromising anti-tumor Teff cell populations.

EXAMPLE 4

10 EXPRESSION OF CCR8 ON DIFFERENT SUBSETS OF T LYMPHOCYTES

The level of expression of CCR8 of different subsets of T lymphocytes associated with human tumors was analyzed by flow cytometry. Excised human tumors (ampullary, colorectal (CRC), duodenal, head and neck, melanoma, NSCLC, ovarian, parotid, renal cell carcinoma (RCC), stomach, thyroid, tongue) were mechanically or enzymatically dissociated. To compare CCR8 expression levels in Tregs in the peripheral blood and tumor-infiltrating Tregs, resected tumors from CRC, RCC, melanoma, and NSCLC patients were used. After dissociation or digestion the cells were filtered and stained for viability before the labeling of surface markers, including with phycoerythrin (PE)-conjugated Abs that bind to CCR8 (anti-hCCR8 Ab Clone L263G8; BioLegend), CD3, CD4, and CD8). Upon the completion of surface staining, the cells were washed, then fixed and permeabilized for intracellular staining for FOXP3. After washing, the cell samples were processed on a flow cytometer and data analyzed using Flowjo software (FlowJo, Ashland, OR) to calculate the mean fluorescence intensity (MFI). Tregs are defined as CD3⁺CD4⁺FOXP3⁺ T cells, while CD4 Tconv cells, or conventional CD4⁺ T cells, are CD3⁺CD4⁺FOXP3⁻ T cells.

As shown in Figure 3A, CCR8 is expressed by a high proportion of tumor-resident Tregs (median 82%) as defined by FOXP3, a known Treg-associated transcription factor. In contrast, a significantly smaller fraction of conventional tumor-infiltrating CD4⁺ T cells and CD8⁺ T cells express CCR8 (medians 12.65% and 4.55%, respectively). Although a small proportion of CD4 Tconv cells express CCR8, the expression level of CCR8 on a per cell basis is significantly higher on Tregs than on CD4 Tconv cells (median MFI 2106 vs. 132, $P < 0.0001$), while CD8⁺ T cells express negligible levels of CCR8 (Figure 3B). Tregs in the peripheral blood also express CCR8 but at much lower

levels than seen on tumor-infiltrating Tregs (Figure 3C). The high expression level of CCR8 specifically on Tregs makes CCR8 a suitable target for depleting the immunosuppressive cell population through ADCC using an anti-CCR8 Ab.

EXAMPLE 5

5 EXPRESSION OF CCR8 ON SUBPOPULATIONS OF PERIPHERAL Tregs

To investigate the level of expression of CCR8 by different subpopulations of peripheral Tregs, PBMCs were isolated using a density gradient from 5 healthy donors and stained for CCR8, CD3, CD4, CD8a, FOXP3 (intracellular), CCR8 and CD45RO. T cells were subdivided into CD4⁺FOXP3⁺ T cells (Tregs), CD4⁺FOXP3⁻ T cells (CD4
10 Tconv), and CD8 T cell (CD3⁺CD8a⁺) subsets. These subsets were further divided into naïve (CD45RO⁻CCR7⁺), effector memory (EM; CD45RO⁺CCR7⁻), and central memory (CM, CD45RO⁺CCR7⁺) populations for examination of CCR8 expression. Cells were processed through a flow cytometer and analyzed using Flowjo software. PBMCs were also isolated from a subpopulation of cancer patients and stained for CCR8 expression as
15 described in Example 4 for human tumor-infiltrating lymphocytes.

The results are shown in Figure 4. Figure 4A shows that CCR8 is expressed on a small fraction of peripheral blood Tregs from healthy subjects (median 21%) compared to the expression on a high percentage of tumor-infiltrating Tregs (median 82%; *cf.* Figure 3A). Within the Treg population from healthy subjects, CCR8 is expressed more highly in
20 the effector memory population (EM) and to a lesser degree in central memory cells (CM) (Figure 4B), while very little CCR8 expression is observed on naïve Tregs (Figure 4B) or CD4⁺ conventional T cells (Figure 4C). Within the CCR8⁺ Treg population, tumor-associated CCR8⁺ Tregs express more CCR8 on a per cell basis than peripheral CCR8⁺ Tregs as measured by the MFI of the bound anti-CCR8 Ab (Figure 4D).

25 EXAMPLE 6

EXPRESSION OF CCR8 ON THE MOST SUPPRESSIVE FOXP3^{HIGH} Tregs

The expression of CCR8 on FOXP3⁻ Tregs and Tregs expressing medium and high levels of expressing FOXP3 was investigated by flow cytometry. Excised human tumors from 2 melanoma and 1 RCC patients were mechanically dissociated using
30 dissection scissors and a dounce. After dissociation, the cells were filtered and stained for viability before the labeling of surface markers with Abs that bind to CCR8, CD3, CD4, and CD8 as described in Example 4. Upon the completion of surface staining, the cells

were washed, then fixed and permeabilized for intracellular staining for FOXP3. After washing, the samples were processed on a flow cytometer and data analyzed using Flowjo software. Cells were gated on the CD3⁺CD4⁺ population for analysis.

As shown in Figure 5, CCR8 is mainly expressed by the CD4⁺FOXP3^{high} population, which represent the most activated Tregs. In samples where there is a clear FOXP3^{mid} population as in the 2 melanoma patient tumor samples, most of the CCR8 expression is found in the FOXP3^{high} T cells. The level of CCR8 expression is significantly lower in the FOXP3^{mid} populations. In some instances, most of the patient FOXP3⁺ T cells exhibit high levels FOXP3 (Figure 5, RCC). In these cases, CCR8 expression overlaps with FOXP3 expression. FOXP3^{high}CD4⁺ T cells have been shown to be true Tregs, in contrast to FOXP3^{mid} CD4⁺ T cells, which can be activated conventional T cells or resting Tregs.

EXAMPLE 7

CCR8⁺ Tregs OVEREXPRESS IMMUNOSUPPRESSIVE MOLECULES AND UNDEREXPRESS PROINFLAMMATORY MARKERS

To investigate the expression of immunosuppressive and proinflammatory proteins by tumor-associated, CCR8- and CCR4-expressing T cells, resected lung tumors obtained from the clinician by overnight delivery were enzymatically digested using a kit (Miltenyi Biotec) into single-cell suspensions. Dissociated tumor cell suspensions were rested overnight in RPMI medium (Corning) containing 10% FBS. On the following day, cells were cultured for 4 h in the presence (for cell stimulation) or absence of phorbol myristate acetate (PMA) and ionomycin, with the addition of brefeldin A (BFA) and monensin (ThermoFisher, Waltham, MA) to block the transport of proteins at 37°C. After the 4 h culture, the cells were stained for surface and intracellular markers associated with Treg suppression and inflammation, and analyzed by flow cytometry.

Figures 6A-D show that CCR8⁺ Tregs in patient tumors are enriched for immune suppressive molecules, whereas a comparable enrichment is not seen in CCR4⁺ T cells (Figures 7A-D). The majority (about 80%) of the CD25⁺ cells are found in the CCR8⁺ fraction whereas less than 20% of CD25⁺ cells are found in the CCR8⁻ fraction (Figure 6A). Similarly, virtually all the CD39⁺ cells are found in the CCR8⁺ fraction whereas less than 40% of cells expressing this marker are CCR8⁻ cells (Figure 6B). About one third of cells expressing IL1R2 are CCR8⁺ cells but no IL1R2-expressing cells are found in the CCR8⁻ fraction (Figure 6C), and the disparity between IL1R2 expression in CCR8⁺ and

CCR8⁺ T cells is even greater when measured in stimulated T cells (Figure 6D). Thus, in terms of immunosuppression, CCR8⁺ T cells from patient tumor samples are enriched for several proteins with suppressive functions such as CD25, CD39, and IL1R2. CD39 is an ectonucleotidase that converts ATP to AMP and then to adenosine, which is suppressive to T cells (Cekic *et al.*, 2011), whereas CD25 (the IL-2 receptor used by Tregs for the sequestration of IL-2) and IL1R2 (a decoy receptor for IL-1) potentially act as sinks for proinflammatory cytokines IL-2 (Pandiyani *et al.*, 2012) and IL-1D/E (Garlanda *et al.*, 2013), respectively.

In contrast to T cells that are CCR8⁺ or CCR8⁻, there are smaller differentials in the levels of expression of CD25 (Figure 7A), CD39 (Figure 7B) and IL1R2 (Figures 7C and 7D) in CCR4⁺ and CCR4⁻ T cells. Therefore, targeted depletion of CCR8⁺ cells would remove the immunosuppressive Tregs expressing CD25, CD39 and IL1R2 whereas CCR4-targeted depletion would leave behind Tregs expressing these molecules.

Using HLA-DR as an activation marker, CCR8 expression also correlates with a higher level of HLA-DR expression (Figure 8A) and thus identifies activated Tregs whereas CCR4 expression does not (Figure 8B).

Stimulation of enzymatically dissociated patient tumor cultures *ex vivo* with PMA and ionomycin revealed that the CCR8⁺ fraction of CD4⁺ T cells contained the vast majority of IFN γ - (Figure 9A), IL-2- (Figure 9B), and granzyme B- (Figure 9C) producing cells. In contrast, CCR4⁺CD4⁺ T cells were major producers of IFN γ (Figure 10A) and IL-2 (Figure 10B) in this assay, suggesting that targeted depletion of CCR4-expressing, but not CCR8-expressing, cells may be detrimental to anti-tumor immunity. CCR4⁺ and CCR4⁻ T cells produced comparable levels of granzyme B (Figure 10C).

EXAMPLE 8

25 GENERATION OF MAbs AGAINST CCR8

Anti-hCCR8 mAbs

Humanized, chimeric or human anti-CCR8 mAbs were generated by immunizing different rodents, including regular C57Bl/6 mice, different strains of transgenic mice that express a human Ig repertoire, and also a specifically generated mouse strain harboring a CCR8 knockout, with a variety of human CCR8 (hCCR8) antigens. A selection of Abs generated in non-transgenic mice were subsequently converted to humanized or chimeric Abs.

Immunizations with CCR8 antigens

To generate Abs against hCCR8, cohorts of 2-5 rodents were immunized with hCCR8 antigens, with each cohort being subjected to an immunization strategy comprising a unique combination of CCR8 antigen, dose, injection route, adjuvant, animal strain, animal age, and immunization timing. A total of 222 animals in 58 cohorts were immunized. Homozygous CCR8 knockout mice (derived from C57BL/6), 5 different strains of human Ig transgenic mice, wild-type mice (BALB/C, C57BL/6), and Armenian hamsters were immunized.

Immunizations were done with a wide variety of antigens comprising one or more of 17 antigen compositions containing full or partial hCCR8 protein sequences. Typically, mice were immunized via either footpad or base of tail up to 12 times with 2.5×10^6 CCR8 overexpressing cells (transfected or transduced HEK 293F, BA/F3, or CHO stable lines), plasma membrane-enriched fractions isolated from those cells via differential centrifugation, detergent-solubilized, lipid-stabilized CCR8 proteins (proteoliposome, bicelle, micelle) derived from 293 cells transiently overexpressing hCCR8, or cell lines with CCR8 transmembrane mutations for enhanced stability (Abilita Bio, San Diego, CA). Similar immunizations with cells and plasma membrane fractions were delivered via intraperitoneal and subcutaneous injections.

In many cases, 3-10 μg of a keyhole limpet hemocyanin (KLH)-conjugated, 35-residue peptide with two sulfotyrosine residues corresponding to the N-terminal sequence of hCCR8 was used as the antigen. In some instances, this N-terminal peptide was given on the 3rd, 5th, and 7th doses along with hCCR8 293F cells in a 10-immunization schedule, with the goal of enhancing and maturing the Ab response against the CCR8 N-terminus, as Ab binding to this peptide sequence was associated with efficacy in downstream assays. In other instances, this N-terminal peptide was given in combination with every cell or plasma membrane fraction immunization, or alone with no other antigens given.

In some cases, mice were immunized with CCR8-encoding DNA plasmids via tibialis anterior and quadriceps intramuscular injections, followed by the previously described cell or N-terminal peptide immunizations. Constrained peptides mimicking the second extracellular loop of CCR8, virus-like particles with CCR8, and other recombinantly engineered CCR8 materials (apolipoprotein, and cell lines with CCR8 transmembrane mutations for enhanced stability) were also used. Some cell- and plasma

membrane-based antigens were hapten-labeled using picrylsulfonic acid (Sigma-Aldrich) to increase immunogenicity. In some cases, alternating hCCR8 and cynomolgus CCR8 (cCCR8) antigens were given during immunization. RIBI adjuvant (Sigma-Aldrich) containing monophosphoryl lipid A was typically delivered with immunizations, either mixed 1:1 with antigens, or delivered as an adjacent injection so as not to disrupt lipid bilayers and CCR8 protein conformation. Animals received immunizations over varying periods between 18 and 177 days.

To monitor immune responses, titrated serum from retroorbital or tail bleeds was screened by flow cytometry and ELISA as described below, typically after 4-6 weeks of immunizations. Serum was screened for Ab binding to multiple CCR8 overexpressing cell lines, corresponding negative control cell lines not overexpressing CCR8, and the sulfated N-terminal peptide of CCR8 conjugated to bovine serum albumin (BSA). CCR8-specific and CCR8 non-specific Ab responses were measured in each animal, and animals with sufficient titers of anti-CCR8 Ig were selected for final immunizations 6 and 3 days before sacrifice and tissue harvest to create hybridoma fusions. Superior CCR8-specific Ab titer was observed in CCR8 knockout mice compared to other mouse strains, and limited efforts at immunizing rats and hamsters also did not lead to any Abs. The sulfated N-terminal peptide of CCR8 conjugated to KLH carrier protein was one of the best immunogens, and worked alone or best in combination with plasma membrane fractions. Conversely, not all of the antigens worked; for example, use of apolipoprotein and membrane mutant cell lines as immunogens did not lead to any Abs.

Generation of Hybridomas Producing MAbs to CCR8

Lymphoid organs, including spleens and lymph nodes, were isolated from mice immunized as described above. Most typically, popliteal, inguinal, and iliac lymph nodes from mice immunized via footpad and base of tail with CCR8 immunogens were collected. Hybridomas were generated by fusions with immortalized mouse myeloma cells derived from the P3X63AgU.1 cell line (ATCC CRL-1597) by electric field-based electrofusion using a cell fusion electroporator (BTX, Holliston, MA). The resulting cells were plated in flat-bottom microtiter plates in Medium E (StemCell Technologies, Cambridge, MA) supplemented with aminopterin (Sigma-Aldrich, St. Louis, MO) for selection of hybridomas.

Anti-mCCR8 mAbs

The isotype of the commercially available rat IgG2b anti-mCCR8 mAb (Clone

SA214G2; BioLegend) was changed to mIgG2a or mIgG1-D265A by cloning DNAs encoding the variable regions into two different pTT5 vectors (National Research Council of Canada) and expressed using the Expi293 expression system.

EXAMPLE 9

5 SCREENING AND SELECTION OF ANTI-HUMAN CCR8 MAbs

Screening for MAbs that Selectively Bind to Human CCR8

In order to generate mAbs that bind to hCCR8, rodents, including human Ig transgenic mice, were immunized with a variety of hCCR8 antigens, and hybridomas were generated as described in Example 8. After 10-13 days of culture and growth media replacement, hybridoma culture supernatants were collected from individual wells and screened to identify wells with secreted CCR8-specific Abs. All supernatants were initially screened against at least two cell lines, one overexpressing hCCR8 and a corresponding control cell line not overexpressing CCR8. Ab binding on cells was measured through image-based fluorometric microvolume assay technology (FMAT) screening, high throughput 384-well flow cytometry using the Intellicyt iQue Screener (Sartorius, Albuquerque, NM), or an adherent cell-based enzyme-linked immunoassay (ELISA). Initial binding assays were sometimes performed in parallel to maximize identification of CCR8 Abs. Supernatants from approximately 157,250 culture wells in 66 hybridoma fusions were screened for hCCR8 Abs.

20 Hybridomas from positive wells were transferred to 24-well plates with new culture media, allowed to grow for 2-3 days, then screened again by flow cytometry to confirm Ab binding to CCR8. Briefly, 75-100 μ l of hybridoma culture supernatant and CCR8-overexpressing cells (such as CHO or 293F) or control cells (such as GFP-CHO or parental 293F) were co-incubated for 30-60 min, washed, and incubated with anti-mouse IgG Fc or anti-human IgG Fc secondary Ab conjugated to AF647, APC, or PE (Jackson ImmunoResearch, West Grove, PA). After incubation and washing, fluorescence was measured by flow cytometry. Approximately 328 hybridoma clones were identified producing anti-hCCR8 Ab, *i.e.*, these were FACS⁺ hybridoma supernatants obtained approximately 2 weeks after fusion and included low-affinity binders that showed binding in the initial screen for binding to CCR8-expressing cell lines but not to control cell lines. There was a high attrition rate in advancing Abs for a variety of reasons, *e.g.*, clones were lost when an anti-CCR8-producing cell could not be isolated, certain clones did not perform well in CD16 crosslinking or Treg depletion assays, Ab yields in some clones

were unacceptably low, and certain Abs contained sequence liabilities that could not be circumvented. Of the 328 FAC⁺ clones, about 90 were not screened extensively, and of the remainder, about 55 advanced to the stage of being cloned into a human backbone for detailed testing. About 15% of these Abs, including mAbs 4A19, 14S15, 18Y12, 16B13, 10R3 and 8D55 were deemed to be good therapeutic candidates based on their performance in various functional assays (*see, e.g.*, Examples 11, 14, 15, 17, 19-23, and 25-29). Accordingly, Abs described herein as Abs that mediate depletion of CCR8-expressing cells include mAbs 4A19, 14S15, 14S15h, 18Y12, 16B13, 10R3 and 8D55.

To broadly categorize the epitopes of confirmed anti-CCR8 specific Abs, culture supernatants were also screened by ELISA to measure binding to CCR8 N-terminal peptides. Briefly, BSA-conjugated peptide (2 µg/ml) representing the sulfated hCCR8 N-terminus was coated onto high-binding 96-well plates (Corning) overnight at 4°C. Plates were blocked with BSA and washed, then 100 µl of culture supernatant were added for 30-60 min on a plate shaker. After washing, an appropriate anti-Fc secondary Ab conjugated to horseradish peroxidase (HRP) was added, and the plates were developed with ABTS or HRP substrate (SurModics, Eden Prairie, MN) and absorbance measured at 405 or 650 nm on a Sunrise microplate reader (TECAN; Männedorf, Switzerland). Abs generated against hCCR8 predominantly, but not exclusively, bound by ELISA to the BSA-conjugated N-terminal peptide sequence with two sulfated tyrosine residues. Among these Abs, ELISA binding to alternate BSA-conjugated N-terminal peptides containing only one sulfated tyrosine was generally greatly reduced, and binding was generally lost when neither of the tyrosine residues were sulfated.

Anti-CCR8 Ab-secreting hybridomas were subcloned once or twice to ensure monoclonality. Briefly, approximately 700 viable hybridoma cells were plated in 5 ml of semi-solid methylcellulose medium (StemCell Technologies) with AF488-conjugated anti-human or anti-mouse IgG Ab (Jackson ImmunoResearch) used to detect hybridoma-secreted IgG. After 7 days, hybridoma colonies arising from single cells with desirable properties (distance from other colonies, IgG secretion levels, colony size, and colony circularity as measured by the ClonePix2 system (Molecular Devices, San Jose, CA) were picked to 96-well plate cultures and allowed to grow 2-4 days. Culture supernatant was screened by flow cytometry as previously described to confirm CCR8 Ab binding. Stable hybridoma subclones were cultured *in vitro* to generate Ab for affinity purification and further characterization. DNA sequences encoding the Ab heavy and light chains were

obtained by standard sequencing techniques (Sanger sequencing and next generation sequencing). Predicted masses from Ab amino acid sequences were compared to known purified Ab masses obtained via mass spectroscopy to ensure sequencing accuracy.

Overall, in screening the Abs generated, binding to CCR8 as described above and, more importantly, CD16 crosslinking (Example 17), were the two main assays used in early screening to determine which Abs showed promise as therapeutic Abs to be characterized further. Binding titrations on Abs against hCCR8-overexpressing 293F cells (Example 11) generally distinguished the strong vs. weak binders, and weak binders typically performed poorly in functional assays, and therefore were generally not advanced to the next stage of screening. so they were not included in that next step in the funnel. Abs with good or moderate binding or activity in those assays were advanced into similar assays on primary Tregs, *e.g.*, binding (Example 11), calcium flux (Example 15) and NK-mediated ADCC (Example 19), with the latter considered one of the most important for comparing Ab effectiveness and for selecting lead candidates. Because it was limited by primary cells, the cell line-based CD16 crosslinking assay was used as an ADCC surrogate assay, which generally correlated well with the results of Treg ADCC/depletion assays (Examples 19, 20 and 22).

In the primary Treg ADCC assays, the Y max (% Annexin V+ cells) considered at as important if not more important than the EC₅₀ value as certain Abs. Abs with the highest ADCC Ymax (while binding specifically) were considered superior to Abs with a comparable EC₅₀ but a lower Ymax.

EXAMPLE 10

HUMANIZATION OF MOUSE ANTI-CCR8 MABs

Humanization of Mouse Anti-CCR8 MAb into 4A19 MAb

A mouse anti-hCCR8 mAb was engineered to humanize the framework to the closest human germline sequences which were hIgHV1-8*01 and hIGJ4 for the heavy chain, as well as IGKV2D-30 and hIGKJ2 for the light chain. Several potential sequence liabilities were removed. A single unpaired cysteine in the heavy chain CDR1 was mutated to serine (C35S). A potential isomerization site at an Asp-Gly sequence motif was removed by mutating the aspartate to glutamine (D52Q). Four other mutations at this site were attempted (D52E, D52S, D52V, D52A) and the best variant, D52Q, was chosen on the basis of the ability to bind with high affinity to a Raji cell line expressing CCR8. A potential glycosylation site in the framework of the heavy chain variable domain was

removed by replacing an asparagine by aspartate (N72D). This mutation was chosen on the basis of aspartate occurring in the germline with a frequency of 27%. A valine in the CDR1 of the light chain was replaced by a phenylalanine (V27F) and resulted in a molecule with increased binding. The resulting mAb, 4A19, showed binding to Raji cells expressing CCR8 that was comparable to the parent 9D7 Ab (10 nM vs. 3 nM for 9D7 and 4A19, respectively).

Table 3. Functional Characterization Data for Representative Anti-hCCR8 MAbs

mAb	Type of mAb	Immunogen
4A19	Nonfucosylated humanized IgG1 anti-hCCR8	hCCR8 293F cells (+ KLH hCCR8-N-Sulf peptide in 3 immunizations)
14S15 ^a	Nonfucosylated chimeric mouse-human ^b anti-hCCR8	hCCR8-encoding DNA from In-Cell-Art (Nantes, France), which produced no detectable anti-CCR8 serum titer, then KLH hCCR8-N-Sulf Peptide
18Y12	Nonfucosylated chimeric mouse-human ^b anti-hCCR8	KLH hCCR8-N-Sulf peptide
16B13	Nonfucosylated IgG1 human	hCCR8 BAF3 plasma membranes + KLH hCCR8-N-Sulf peptide
2M18	human IgG1 anti-hCCR8	hCCR8 BAF3 plasma membranes + KLH hCCR8-N-Sulf peptide
15C17	human IgG1 anti-hCCR8	CCR8 293 plasma membranes + KLH/BSA N-sulf-hCCR8 Peptide
13T20	human IgG1 anti-hCCR8	CCR8 293 plasma membranes + KLH/BSA N-sulf-hCCR8 Peptide
10R3	human IgG1 anti-hCCR8	hCCR8 BAF3 plasma membranes + KLH hCCR8-N-Sulf Peptide
8D55	human IgG1 anti-hCCR8	hCCR8 BAF3 plasma membranes + KLH hCCR8-N-Sulf Peptide
1V11	human IgG1 anti-hCCR8	hCCR8 BAF3 plasma membranes + KLH hCCR8-N-Sulf Peptide
11K16	human IgG1 anti-hCCR8	hCCR8 BAF3 plasma membranes + KLH hCCR8-N-Sulf Peptide
12F27	Nonfucosylated chimeric ^c (mFc/hFab)	hCCR8 293 plasma membranes + KLH hCCR8-N-Sulf Peptide

^a The mAb designated 14S15 herein refers to a chimeric derivative of a mouse anti-hCCR8 mAb in which the mouse Fc region was replaced by a nf IgG1 human Fc. A humanized, affinity matured, and sequence liability-fixed version of this mAb was generated as described below in this Example and is designated 14S15h.

^b These mAbs are chimeric Abs comprising a mouse Fab grafted onto a human Fc.

^c This mAb is a chimeric Ab in the opposite sense, in that it was generated in a transgenic mouse to comprise a mouse Fc and a fully human Fab. These mice were so designed to produce Abs comprising a mouse Fc to help with affinity maturation.

A representative number of anti-CCR8 mAbs generated are listed in Table 3, which indicates the type of mAb and the immunogen used to generate them. Human anti-hCCR8 Abs were produced recombinantly as hIgG1 isotypes using the Expi293 expression system (Thermo Fisher Scientific). Nf (hIgG1-nf) Abs were expressed in
 5 Expi293 Fut8^{-/-} cells.

Several of the Abs in Table 3, namely mAbs 2M18, 15C17, 13T20, 1V11, 11K16 and 12F27 are Abs that performed poorly in screening assays, *e.g.*, CD16 crosslinking and Treg ADCC assays (comparatively high EC₅₀ values and comparatively low Ymax values) and were therefore deemed to be unpromising therapeutic candidates. However,
 10 they are included in Table 3 to emphasize the fact that the vast majority of CCR8-binding Abs generated, were not considered to be strong therapeutic candidates.

Humanization of Mouse Anti-CCR8 MAb into 14S15h MAb

Another mouse anti-CCR8 mAb was engineered to humanize the framework to the closest human germline sequences, which were hIgHV3-15 for the heavy chain and
 15 IGKV2-18 for the light chain. Humanization of the framework led to a decrease in binding of the human Fab as measured by SPR (K_D of 4.2 nM compared to 1.4 nM for the starting mouse Ab, 9G10). Affinity maturation of the heavy chain variable domain was performed using 3 different NNK libraries and 4 cycles of panning with 200 nM, 50 nM, 10 nM and 1 nM biotinylated CCR8 N-terminal peptide from Anaspec (Freemont, CA)
 20 and increasing wash stringencies. A single substitution of N30G in the heavy chain CDR1 region, resulted in affinity maturation. Furthermore, a potential deamidation site at an Asn-Gly sequence motif was removed by mutating the asn to gln (N28Q, Kabat numbering). The resulting humanized, affinity-matured antibody and sequence liability-removed 14S15h had a fully restored and even increased affinity for the CCR8 N-
 25 terminal peptide (K_D of 0.64 nM for 14S15h Fab fragment). Assays described herein involving the 14S15 mAb is the chimeric version of this mAb as indicated in Table 3.

EXAMPLE 11

ANTI-CCR8 mAbs THAT BIND WITH HIGH AFFINITY TO hCCR8-EXPRESSING CELLS

30 *EC₅₀ Values for Binding of Anti-hCCR8 MAbs to Tregs*

Anti-hCCR8 mAbs were incubated with activated human Tregs or human CCR8-expressing cell lines (293F, CHO, Raji). Activated Tregs prepared from previously

isolated and expanded Tregs were stimulated for 2 days with anti-CD3/CD28 activation beads (ThermoFisher) in the presence of 100 units/ml of recombinant human IL-2. The Abs were serially diluted from a starting concentration of 30 µg/ml. An appropriate PE-conjugated secondary Ab (Jackson ImmunoResearch) against the primary anti-CCR8 Ab was applied, incubated for 15 min at 4°C, and then washed off. Samples were processed on a flow cytometer and data was analyzed using Flowjo and shown in Figure 11. EC₅₀ values for the binding of selected anti-CCR8 mAbs to activated Tregs, was calculated using GraphPad Prism (GraphPad Software, La Jolla, CA).

As shown in Figure 11, a representative sample of the anti-CCR8 mAbs generated exhibit binding to various hCCR8-transfected cell lines and activated Tregs with a range of affinities. Abs were generally initially screened based on their binding affinities to CCR8-expressing cells lines (CHO, 293F, Raji), and it was found that most of the Abs screened exhibited half-maximal effective concentrations (EC₅₀) of under 1 nM (Figure 11A). However, when these pre-screened Abs were tested for binding to activated Tregs, a wider range of binding affinities was observed, with fewer than half of the Abs exhibiting binding with EC₅₀'s below 1 nM (Figure 11A). A select subset of Abs having EC₅₀ values ranging from picomolar to nanomolar is presented in Figure 11B, and the respective EC₅₀ values are provided in Table 4.

Table 4. Affinity (EC₅₀) of anti-CCR8 mAbs binding to activated Tregs

Anti-CCR8 MAb	Median EC ₅₀ (nM)
8D55	0.284
10R3	14.1
14S15	0.252
16B13	0.149
4A19	1.65
18Y12	0.120

20

Since anti-hCCR8 mAbs including 18D55, 10R3, 14S15, 16B13, 4A19 and 18Y12 have been shown to have a high potential for eliciting ADCC (Example 17), or directly eliciting the efficient depletion (Examples 19, 20 and 22), of tumor-infiltrating Tregs it is clear that Abs which bind to CCR8 on activated Tregs with an affinity (EC₅₀) in the nM range (*e.g.*, about 20 nM or lower, but the limit may be higher, *e.g.*, in excess of 100 nM) can efficiently mediate Treg depletion. However, to be useful as a therapeutic

25

that specifically targets tumor-infiltrating Tregs, the binding to CCR8 must be highly specific (*cf.* mAb 16B13 which binds with high affinity to CCR8 but also strongly binds to a target that is not CCR8 (Example 14)).

K_D Values for Binding of Anti-CCR8 MAb, 4A19, to the CCR8 N-Terminus Peptide

5 The dissociation constant (K_D) for the binding of mAb 4A19 to an N-terminal peptide of human CCR8 was measured by surface plasmon resonance (SPR). SPR measurements were conducted on a Biacore T200 at 37°C in HBST buffer (10 mM HEPES (pH 7.4), 150 mM NaCl, 0.05% with 1g/l BSA). A CM4 chip with immobilized anti-human kappa capture pAb (Southern Biotech, Birmingham, AL), EDA (ethylene diamine)-blocked. CCR8 Abs (both Fabs and mAbs) were captured, and CCR8 N10
10 terminal peptides (200 nM, 40 nM, 8 nM or 1.6 nM) were injected as analytes for 2 min with a flow rate of 100 μ l/min. All data were double-referenced and fitted to a 1:1 Langmuir binding model with mass transfer using Biacore T200 Evaluation Software 3.1. Peptides of CCR8 residues 1-35 were either non-sulfated (CCR8-nosulfo), sulfated at
15 Tyr15 (CCR8-sulfoY15), at Tyr17 only (CCR8-sulfoY17), or at both positions Tyr15 and Tyr17 (CCR8-2sulfo), were produced by Anaspec (Fremont, CA).

Table 5. Affinity (K_D) of anti-CCR8 mAb, 4A19, Fab fragment binding to CCR8 N-Terminus

MAb	Ligand	k_a (1/Ms)	K_d (1/s)	K_D (M)
4A19	CCR8-2sulfo	2.5×10^7	4.1×10^{-2}	1.6×10^{-9}
4A19	CCR8-sulfoY17	4.3×10^6	8.4×10^{-2}	2.0×10^{-8}
4A19	CCR8-sulfoY15	Rough estimate:		$\sim 1.3 \times 10^{-6}$
4A19	CCR8-nosulfo	No binding		

20 The results summarized in Table 5 show that the 4A19 Fab fragment binds to the CCR8 N-terminus with a K_D of 1.6 nM. Sulfoylated tyrosine is required for binding as the non-sulfoylated peptide does not show any binding to the 4A19 Fab fragment. The absence of the sulfoylation on Y15 leads (CCR8-sulfoY17) to an approximately 10-fold decrease in affinity compared to the doubly sulfoylated peptide, while the absence of
25 sulfoylation on Y17 (CCR8-sulfoY15) leads to a decrease in 4A19 binding of approximately 1,000-fold (Table 5).

EXAMPLE 12

CRYSTAL STRUCTURE OF 4A19 BOUND TO CCR8 N-TERMINAL PEPTIDE

Binding of 4A19 Fab to singly sulfated peptide

The Fab region (domains VH and CH1) of the 4A19 heavy chain was fused to a
5 8His tag and cloned into a pTT5 vector. A 4A19 Fab fragment comprising this modified
4A19 heavy chain and the 4A19 light chain was expressed in HEK cells. The supernatant
was purified over a nickel affinity column, and the eluate was buffer-exchanged into
phosphate-buffered saline (PBS) and sized over a size exclusion column (GE Superdex-
200) with running buffer containing 10 mM Tris pH 7.4, 150 mM NaCl. The appropriate
10 main peak was pooled and concentrated to 7-10 mg/ml. Residues 1-35 of human CCR8
with only Y17 sulfated was chemically synthesized by Bio-Synthesis (Lewisville, TX).
Lyophilized peptide was dissolved in 10 mM Tris pH 7.4, 150 mM NaCl, and mixed in a
5:1 molar ratio with the 4A19 Fab fragment. Crystallization was carried out by mixing the
protein solution with equal parts of 25% PEG3350 and cryoprotected using Al's oil.
15 Crystals were diffracted to a resolution of 2.03 Å in space group C2. Collected data were
indexed using XDS, scaled and truncated using ccp4i, and refined using refmac5.

Figure 12A shows the crystal structure of the 4A19 Fab fragment (displayed in
surface representation) bound to the CCR8 N-terminal peptide (shown in stick
representation) at 2.03 Å resolution, revealing that the epitope bound by 4A19 comprises
20 residues 15-21 of CCR8 with the sulfated tyrosine-17 at its center.

Binding of 4A19 Fab to doubly sulfated peptide

The 4A19 Fab fragment was produced as described in the previous section. A
peptide consisting of residues 1-35 of hCCR8 with both Y15 and Y17 sulfated was
chemically synthesized by Anaspec. The Fab fragment was buffer exchanged into 50 mM
25 NaCl, 10 mM Tris pH 8.0, and the doubly sulfated peptide was added to the Fab fragment
at a 2-fold molar excess. Crystals were grown by sitting-drop vapor diffusion at 20°C by
mixing 200 nl of concentrated protein sample at 12.8 mg/ml with 200 nl of mother liquor
(0.2 M ammonium acetate, 20% PEG 3350, pH 7.2). Crystals appeared within 7 days and
x-ray diffraction data were collected to 1.80 Å resolution at beamline 17-ID (IMCA-
30 CAT) at the Advanced Photon Source. Data were processed in space group P21 using
XDS and the structure was determined by molecular replacement with Phaser and refined
with Phenix.

Figure 12B shows the crystal structure of the 4A19 Fab fragment (displayed in

surface representation) bound to the CCR8 N-terminal peptide (shown in stick representation). The 4A19 Ab recognizes both tyr sulfate residues at positions Y15 and Y17. Molecular interactions for the CCR8 epitope were calculated using PISA software. The CCR8 residues V12, D14, Y(SO3)15, Y16, Y(SO3)17, P18, I20, F21, and S22 show
 5 change in accessible surface area $> 3\text{\AA}^2$ when in complex with the 4A19 Fab fragment.

EXAMPLE 13

BINDING OF ANTI-mCCR8 MAb TO mCCR8-EXPRESSING CELLS

Binding of Anti-CCR8-mIgG2a MAb to mCCR8-Expressing CHO Cells

Anti-CCR8-mIgG2a (the mouse IgG2a isotype of a commercial rat anti-mCCR8
 10 mAb [BioLegend Clone SA214G2], which is used herein as a surrogate for ADCC-eliciting anti-hCCR8 therapeutic Abs disclosed herein) was serially diluted from a starting concentration of 30 $\mu\text{g/ml}$ and incubated with 5×10^5 mCCR8-expressing CHO cells. After incubation, the cells were washed and then stained with an anti-mouse IgG, PE-conjugated secondary Ab (Jackson ImmunoResearch) on ice for 30 min. Cells were
 15 washed and analyzed on a flow cytometer. The mean fluorescence intensity was calculated using Flowjo. The EC_{50} , calculated using GraphPad Prism, was determined to be 6.44 nM. This is significantly higher than the EC_{50} 's of many of the anti-hCCR8 Abs analyzed above which are about 0.05 nM, for binding to hCCR8-expressing CHO cells.

Binding of Anti-CCR8 MAb to Mouse Thymocytes and Primary Activated Mouse Splenic Tregs

The binding of anti-CCR8-mIgG2a to mouse thymocytes and primary activated Tregs from mouse splenocytes was assayed by flow cytometry. Thymic T cells express CCR8 constitutively whereas CCR8 expression is induced on splenic Tregs. To induce CCR8 expression on mouse splenocytes, CD4^+ T cells were isolated and activated with
 25 anti-CD3/CD28 beads at a ratio of 3:1 and 2,000 U/ml of recombinant mouse recombinant IL-2 (rIL-2). Thymic CD4^+ T cells, isolated the same day, or CD4^+ splenic T cells, activated for 48 h, were mixed with titrations of anti-CCR8-mIgG2a or anti-KLH-mIgG2a control Ab. Binding of Abs to cell surface CCR8 was detected by fluorescently labeled anti-mouse IgG Ab. Relative cell binding was measured as frequency of total
 30 CD4^+ cells positive for fluorescently conjugated secondary Ab.

The anti-mCCR8 Ab was shown to bind to CCR8 on the surface of thymic CD4^+ cells ($\text{EC}_{50} = 4 \text{ nM}$) and activated mouse splenic CD4^+ cells ($\text{EC}_{50} = 6.16 \text{ nM}$).

EXAMPLE 14

TISSUE CROSS-REACTIVITY OF DIFFERENT ANTI-hCCR8 MAbs

Anti-hCCR8 mAbs, 18Y12, 16B13 and 4A19 were tested at 1 and 3 µg/ml for binding to CCR8-expressing cell lines, PBMCs, and diverse tissue types (n=1-2/each).

5 *CCR8 Transfectants*

All 3 Abs bound strongly to hCCR8-transfected CHO and 293F cell lines (data not shown). These transfected cell lines were used as positive controls in subsequent tissue staining experiments.

Normal Human PBMCs

- 10 As shown in Figure 13A, mAbs 18Y12 and 4A19 did not bind to PBMCs. However, 16B13 showed strong binding to a target which was not CCR8. Although 16B13 shows attractive properties, e.g., in binding to activated Tregs with high affinity (Example 11), efficiently inhibiting calcium flux by CCR8-expressing cells (Example 15), and exhibiting high ADCC potential or activity (Examples 17 and 19), the non-
15 specific binding to PBMCs observed with this mAb indicates its use in therapy would present problems with off-target depletion of cells, other than tumor-infiltrating Tregs, that do not express CCR8.

Diverse Human Tissue Types

- MAbs 18Y12 and 4A19 was tested against a panel of 18 normal human tissue
20 types including cerebrum, cerebellum, heart, liver, lung, kidney, tonsil, spleen, thymus, colon, stomach, pancreas, adrenal, pituitary, skin, peripheral nerve, testis, uterus, and PBMC smear (n=1-2/each) with hCCR8-expressing CHO and 293F cells were used as positive controls.

- Positive staining was observed in rare and scattered immune cells primarily in the
25 medulla of the thymus (Figure 13B; 18Y12-left panels and 4A19-right panels) and dermis of the skin (data not shown), whereas no positive staining was observed in other tissues examined (data not shown). A similar expression profile was observed in mouse tumor models (data not shown).

- MAb 16B13 was tested against a panel of 21 normal human tissue types including
30 cerebrum, cerebellum, heart, liver, lung, kidney, tonsil, spleen, thymus, colon, stomach, pancreas, adrenal, thyroid, pituitary, skin, peripheral nerve, prostate, testis, uterus, and PBMC smear (n=1-2/each) with hCCR8-transfected CHO cells used as positive control.

The most profound staining was observed in immune cells primarily in lymphoid organs (thymus [Figure 13B; middle panels], tonsil, spleen) and lymphoid-rich tissues (colon and small intestine; data not shown). The staining was strong and diffuse in the vast majority of immune cells, particularly in lymphocyte-rich regions (*e.g.*, much stronger staining in the white pulp than red pulp in the spleen), with predominate cytoplasmic and/or peri-nuclear patterns. This staining was observed in many tissues where immune cells were present, including Kuepfer cells in the liver, alveolar macrophages in the lung, and mesangial-like cells in the glomeruli of the kidney.

In PBMC smears, no or minimum staining was observed in non-fixed (but air-dried) smears, while peri-nuclear and cytoplasmic staining was seen in acetone-fixed (*i.e.* permeabilized) slides, which further support the observation that the positive labeling is intracellular.

Strong and diffuse staining (primarily at the higher 3 $\mu\text{g/ml}$ concentration) was also observed in smooth muscle cells in the muscularis in the guts (small intestine, colon, stomach), arrector pilli muscle in the skin, and the stroma of the prostate, as well as vasculatures in most tissues. The staining exhibited a cytoplasmic pattern.

Strong and diffuse peri-nuclear and/or cytoplasmic staining was revealed in neurons and glial cells in the cerebrum and cerebellum, as well as subset of cells in the neurohypophysis and spindle interstitial cells in the peripheral nerves.

Strong and diffuse cytoplasmic staining was also observed in subset of epithelial cells, including mesothelium in the heart, subset of distal or collecting tubule epithelium in the kidney, endocrine cells in the pituitary, airway epithelia in the lung, ductal epithelium in the pancreas, basal layer of seminiferous tubule epithelium in the testis, and gland epithelium in the endometrium of the uterus. The staining tended to be more profound at high concentration (3 $\mu\text{g/ml}$) in most cases.

EXAMPLE 15

ANTI-hCCR8 MABs BLOCK BINDING OF hCCL1 TO hCCR8-EXPRESSING CELLS

Blockade of hCCL1 binding to hCCR8 by anti-hCCR8 mAbs was tested by conducting calcium (Ca) flux assays on hCCR8-expressing CHO cells (hCCR8-CHOs) since CCL1 engagement of CCR8 on CHO cells induces calcium flux. hCCR8-CHOs were seeded and incubated for 2 h at 37°C in the presence of FLIPR Calcium 6 dye (Molecular Devices) and probenecid (Thermo Fisher Scientific). After incubation, different anti-CCR8 mAbs were added to the cells and left at room temperature for 15

min before the addition of 10 nM of recombinant human CCL1 (R&D Systems, Minneapolis, MN). The Ca flux fluorescent signal was measured by Max-Min using a FLIPR Tetra system (Molecular Devices). The half-maximal inhibitory concentration (IC₅₀) was calculated using GraphPad Prism.

5 Table 6. Blockade of calcium flux on CCR8-expressing cells by anti-CCR8 mAbs

Anti-CCR8 MAbs	IC ₅₀ (nM)
4A19	0.4645
16B13	0.2600
18Y12	0.2314
14S15	1.331
KLH-g1fnf	N/A
Rituximab (anti-CD20)	N/A

The capacity to block CCL1 binding to CCR8 can have an anti-suppressive effect on Tregs, and this function was used as a selection criterion in selecting anti-CCR8 therapeutic Ab candidates. About 52% (50 out of 96) anti-hCCR8 mAbs tested blocked CCL1 binding on hCCR8-CHO cells (Figure 14A). A selected subset of these Abs blocked CCL1-induced Ca flux of CCR8-CHO cells from 51%-94% (Figure 14B). IC₅₀ curves for the blockade of Ca flux on hCCR8-CHOs are shown for 4 select anti-CCR8 mAbs in Figure 14C (IC₅₀ range 0.23 nM to 1.331 nM), and the IC₅₀ values for these mAbs are shown in Table 6.

15 EXAMPLE 16

ANTI-mCCR8 MAbs BLOCK BINDING OF mCCL1 TO mCCR8-EXPRESSING CELLS

Blockade of mCCL1 binding to mCCR8 by anti-CCR8-mIgG2a was tested by conducting Ca flux assays on mCCR8-expressing CHO cells (mCCR8-CHOs) as described in Example 15 except that mCCR8-CHOs were used instead of hCCR8-CHOs. The IC₅₀, calculated using GraphPad Prism, was determined to be 19.34 nM.

In order for anti-CCR8-mIgG2a serve as an appropriate mouse surrogate Ab for testing the therapeutic efficacy of anti-CCR8 Abs designed for human use, it is important that this mouse Ab exhibit similar functional characteristics to its hCCR8 Ab counterparts. Similar to anti-hCCR8 mAbs inhibiting hCCL1-induced Ca flux (Figure 14, Table 6), anti-CCR8-mIgG2a was found to inhibit mCCL1-induced Ca flux in mCCR8-

CHO cells, though less efficiently with an IC₅₀ value of 19.34 nM compared to IC₅₀ values of about 0.2 nM to 1.3 nM observed for the anti-hCCR8 Abs tested in Example 15.

EXAMPLE 17

ADCC POTENTIAL OF ANTI-hCCR8 MAbs MEASURED BY CAPACITY FOR CD16 CROSS-LINKING

The ADCC potential of anti-hCCR8 mAbs was measured by the capacity of these Abs to mediate crosslinking of CD16 expression reporter cells. Activated human Tregs or hCCR8-expressing Raji cells were co-cultured in a 1:5 ratio with CD16-expressing Jurkat NFAT luciferase reporter cells at 37°C for 4 h in the presence of an anti-CCR8 Ab or a control Ab. BIO-GLO™ (Promega, Madison, WI) was added to the cells and incubated for 5 min at room temperature, and then the luminescence was read for measurement of luciferase activity. The half-maximal effective concentration (EC₅₀) was calculated using GraphPad Prism.

NK cells mediate ADCC through the engagement, or cross-linking, of Fc-γ receptor 3A (CD16). The capacity of anti-hCCR8 mAbs to mediate ADCC of target cells (hCCR8-expressing Raji cells or activated human Tregs) was measured by a CD16-expressing cell line as described above. Sixteen percent of anti-hCCR8 mAbs tested with the human IgG1 (hIgG1) backbone exhibited 80-100% of maximal CD16 engagement with CCR8-expressing Raji cells as targets (Figure 15A). Selected mAbs were modified to the nf hIgG1 format (hIgG1-nf) and screened in the same manner.

Table 7. Cross-linking of CD16 expression reporter cells by anti-CCR8 mAbs

Anti-CCR8 MAb	EC ₅₀ (pM)
8D55	6.58
10R3	3.02
14S15	8.02
16B13	0.67
4A19	0.69
18Y12	0.698

A majority of these anti-CCR8 hIgG1-nf mAbs achieved 80-100% CD16 cross-linking (Figure 15A). Twenty-seven percent of anti-CCR8 hIgG1-nf mAbs tested achieved maximal CD16 engagement with activated human Tregs as target cells (Figure

15A). EC₅₀ values for the CD16 cross-linking capacities of a select set of anti-hCCR8 hIgG1-nf mAbs tested are shown in Figure 15B and Table 7.

EXAMPLE 18

ADCC POTENTIAL OF ANTI-mCCR8 MABs MEASURED BY CAPACITY FOR CROSS-LINKING OF MOUSE FcγRIV⁺ REPORTER CELLS

5 The capacity of anti-CCR8-mIgG2a to induce ADCC-mediated killing of CCR8-expressing cells was indirectly evaluated by measuring its ability to induce crosslinking of mouse FcγRIV⁺ reporter cells (Promega). In this assay, FcγRIV⁺ effector cells expressing firefly luciferase were co-cultured with primary activated Tregs from mouse
10 splenocytes or mCCR8-expressing CHO cells at a 1:5 ratio. Cells were co-cultured at 37°C for 4 h in the presence of a titration of anti-CCR8-mIgG2a, anti-CCR8-mIgG1-D265A having an inert backbone, or anti-KLH-mIgG2a control Abs. Target cell killing activity was measured as crosslinking and activation of FcγRIV⁺ effector cells that shows an increase in bioluminescent signal (RLU) produced upon incubation of cells with the
15 BIO-GLO™ (Promega) luciferin substrate.

Specific crosslinking of effector cells was observed (EC₅₀ = 6.34 nM), manifested by a high luciferase signal, at higher concentrations of anti-CCR8-mIgG2a. This showed that the Ab bound to target cells and engaged the FcγRIV in effector cells. The specificity of this interaction was assessed by using a non-target specific Ab (anti-KLH-mIgG2a)
20 and a non-FcγRIV-engaging Ab (anti-CCR8-mIgG1-D265A), neither of which showed any luciferase signaling.

EXAMPLE 19

ANTI-CCR8 MABs PROMOTE NK CELL-MEDIATED CYTOTOXICITY OF Tregs

Nonfucosylated anti-hCCR8 mAbs, which exhibit enhanced effector function,
25 were tested for their ability to promote NK cell-mediated apoptosis of activated Treg cells, prepared as described in Example 2. Allogenic NK cells for use as effectors were isolated using Ficoll gradient separation of PBMCs from whole blood followed by magnetic bead negative selection (Miltenyi Biotec, Sunnyvale, CA) to remove non-NK cells. NK cells were isolated from fresh healthy donor PBMCs (Human NK Cell Isolation
30 Kit, Miltenyi Biotec) and cultured for 24 h in Myelocult H5100 medium (StemCell Technologies) with 1 μM hydrocortisone (StemCell Technologies) and 500 U/ml of recombinant human IL-2 (PeproTech, Cranbury, NJ) to increase NK cell activation.

NK cells were fluorescently labeled with CellTrace Violet (Thermo Fisher Scientific) and combined with Treg cells at a ratio of 5:1 in RPMI medium with 10% ultra-low IgG FBS and 1 mM sodium pyruvate. Titrations of anti-hCCR8 Abs and isotype control Ab were added and incubated with the cells for 3 h at 37°C. Cells were stained with Annexin-V FITC-conjugated Ab and 7-AAD in buffer containing Ca²⁺, and cell fluorescence was measured by flow cytometry using a BD Fortessa X-20 cytometer (BD Biosciences, San Jose, CA). The percentage of apoptotic Tregs among total Tregs (Annexin-V positive, CellTrace Violet negative) was determined through standard flow cytometry analysis.

ADCC of activated human Tregs by allogeneic NK cells in the presence of anti-hCCR8-hIgG1-nf mAbs was used to assess the capacity of anti-CCR8 Abs to mediate depletion of tumor-infiltrating Tregs. After screening of a larger pool of anti-hCCR8 Abs through CCR8 binding (Example 9), CCL1 blocking (Example 15) and CD16-crosslinking assays (Example 17), a subset of Abs were evaluated for their ability to mediate ADCC of activated Tregs through NK cells. The median percentages of cells subject to apoptosis induced by selected anti-hCCR8-hIgG1-nf Abs are depicted in Figure 16, showing that these percentages range from around 34% to 99%. The EC₅₀ values for the killing of activated Tregs by allogeneic NK cells mediated by different anti-CCR8 Abs are shown in Table 8, with values ranging from about 13 pM to 304 nM.

Table 8. ADCC killing of activated Tregs mediated by anti-CCR8 mAbs

Anti-CCR8 MAb	Median EC ₅₀ (pM)
8D55	38.6
10R3	56.6
14S15	39.7
16B13	13.4
4A19	13.1
18Y12	18.6
12F27	3.04 x 10 ⁵

EXAMPLE 20

ANTI-CCR8 MAbs PROMOTE DEPLETION OF Tregs IN PATIENT TUMORS

Nonfucosylated anti-hCCR8 mAbs were tested for their ability to promote NK

cell-mediated apoptosis of tumor Tregs from human patients. Briefly, dissociated patient endometrial tumors were purchased from Discovery Life Sciences. NK cells from a healthy donor were isolated and primed overnight for ADCC as described in Example 19 and fluorescently labeled (CellTrace Violet, ThermoFisher). These NK cells (1.875×10^5 cells/well) were then incubated with endometrial tumor cells (2.5×10^5 cells/well) for 24 h with titrations of anti-hCCR8 (14S15) and control Abs (the anti-CCR4 mAb, mogamulizumab, and anti-KLH-nf isotype) in RPMI medium with 10% ultra-low IgG FBS and 1mM sodium pyruvate at 37°C. The resulting cells were stained for Tregs and other lymphocyte populations and processed through a flow cytometer. Treg and conventional T cell frequencies were analyzed using Flowjo software.

To determine whether anti-hCCR8 Abs can specifically mediate ADCC of patient tumor-infiltrating Tregs, allogeneic NK cells were co-cultured with digested endometrial tumor cells containing tumor-infiltrating Tregs. It has previously been shown that mogamulizumab, an anti-CCR4-hIgG1nf Ab, can mediate Treg killing in cancer patients. As shown in Figure 17, both mogamulizumab and the anti-CCR8 mAb, 14S15, induce tumor Treg killing, with 14S15 being more potent (Figure 17A). MAb 14S15 specifically depletes the endometrial tumor Tregs (Figure 17A) but not conventional $CD4^+FOXP3^-$ T cells (Figure 17B). In contrast, mogamulizumab also depletes $CD4^+FOXP3^-$ conventional T cells. 14S15 does not deplete any other lymphocyte subsets (data not shown).

In a further experiment, several Abs against hCCR8 with the following human heavy chain variants were generated: 1) hIgG1; 2) nf IgG1 (IgG1-nf), which exhibit significantly higher FcγR binding affinity than IgG1 and can outcompete endogenous IgG for binding to CD16 for ADCC/ADCP (Iida, 2006); and 3) IgG1-L234A-L235E-G237A (hIgG1-inert), which minimally engages FcγRs (Hezareh *et al.*, 2001). Anti-human CCR8 clones 16B13 and 14S15 as IgG1-nf Abs bound to *in vitro* activated human $FOXP3^+$ Tregs, blocked CCL1 binding, and cross-linked CD16 upon binding to CCR8 on activated Tregs (Examples 11, 15 and 17; Campbell *et al.*, 2021, in which 16B13 is designated CCR8.1, and 14S15 is designated CCR8.2). Clone 16B13-IgG1 was also capable of cross-linking CD16 albeit at a lesser potency than its IgG1-nf counterpart (Campbell *et al.*, 2021), whereas 16B13-IgG1-inert was entirely unable to mediate CD16 cross-linking (Campbell *et al.*, 2021).

The capacity of anti-hCCR8-IgG1-nf Abs to deplete $FOXP3^+$ Tregs from peripheral blood and primary human tumor specimens was subsequently determined.

PBMC depletion assays were performed by incubating 2.0×10^5 PBMCs in culture media and 100 U/ml IL-2 (Peprotech) in a U-bottom 96-well plate (Corning) for 48 h at 37°C. After two days, the relevant Abs were added and cells were further incubated for 96 h, followed by flow cytometric analysis.

5 Although 14S15-IgG1-nf depleted a subset of peripheral blood Tregs (less than 50%) (Figure 18A), a nf anti-human CCR4 Ab (anti-hCCR4-IgG1-nf, mogamulizumab biosimilar) removed more than 90% of these cells (Figure 18A) as well as approximately half of peripheral CD4⁺ Tconv (Figure 18B). Neither Ab affected the CD8⁺ T cell population (Figure 18C). Thus, treatment of activated PBMCs with an anti-hCCR8-IgG1-
10 nf Ab depletes a subset of peripheral blood Tregs, but importantly does not deplete Teff cells. In breast cancer patients, TCR sequencing revealed a significant clonal overlap between peripheral blood CCR8⁺ Tregs and tumor Tregs (Wang *et al.*, 2019), suggesting either that (1) Treg clones from the periphery migrate into the tumor and expand and enact Treg suppression, or (2) activated Treg from the tumor microenvironment expand
15 into the periphery. Both scenarios could concurrently occur and lead to infiltration by circulating CCR8⁺ Tregs into tumor metastatic sites to mediate immune suppression. Regardless of the mechanism, these results suggest that the wholesale depletion of CCR8⁺ Tregs may serve as a method of systemically removing tumor specific Tregs in the periphery or in tumors.

20 To test for anti-CCR8-mediated depletion in *ex vivo* tumor samples, cell suspensions of NSCLC surgical resections were cultured with activated allogeneic NK cells from healthy donor blood. Addition of 14S15-IgG1-nf resulted in a decrease in the frequency of FOXP3⁺ Tregs (Figure 18D) without any effect on overall CD4⁺ Tconv (Figure 18E) or CD8⁺ T cell frequencies (Figure 18F). In contrast, anti-hCCR4-IgG1-nf
25 depleted about 30% of CD4⁺ Tconv (Figure 18E) in addition to depleting Tregs (Figure 18D). No Treg depletion was observed after treatment with 16B13-IgG1-inert (Figure 18G).

 The ability of 16B13-IgG1-nf and 4A19 to deplete Tregs in *ex vivo* patient tumor samples in the absence of allogeneic NK cells was tested. For the 16B13 study, fresh clear
30 cell renal cell carcinoma (RCC) and gastric tumor tissues were encased in agar and sequential 300-μM thick slices were created using a COMPRESSTOME® vibrating microtome (Precisionary Instruments; Greenville, NC) until all the tumor tissue was sliced. Tissue slices were cultured between two pieces of collagen hemostat (Becton

Dickinson, Warwick, RI) in a 6-well tissue culture plates containing 5 ml of RPMI 1640 cell culture medium (Corning) with 10% FBS supplemented with 1 mM sodium pyruvate and 55 nM 2-mercaptoethanol (Thermo Fisher Scientific). Slice cultures were incubated at 37°C on a plate shaker rotating at 80 rpm to facilitate media perfusion. After 24 h, slice culture wells were treated with 16B13-IgG1-nf or anti-KLH-nf hIgG1 isotype control. After 3 days of incubation with the mAbs, tumor tissue slices were enzymatically dissociated using a kit (Miltenyi Biotec) into single-cell suspensions and stained for flow cytometry analyses. The percentage of FOXP3⁺ Tregs out of CD4⁺ T cells was determined.

As shown in Figures 18H and 18I, treatment of renal cell and gastric tumors with 16B13-IgG1-nf exhibited an approximately 50% reduction in the frequency of Treg cells in the *ex vivo* patient tumor slice culture system without the addition of allogeneic NK cells, confirming that endogenous tumor-resident FcγR⁺ cells are sufficient to mediate ADCC/ADCP against CCR8⁺ target cells *in situ*.

For the 4A19 study, resected melanoma tumor was sliced and cultured for 3 days with 10 μg/ml of 4A19, mogamulizumab biosimilar, or anti-KLH-nf hIgG1 isotype control. After 3 days, the treated tumor slices were dissociated into single cell suspensions for flow cytometry analysis. Overall, the tumor slices treated with 4A19 showed reduced percentage of FOXP3⁺ CD4 Tregs when compared to the mogamulizumab biosimilar- and isotype-treated slices.

EXAMPLE 21

ANTI-CCR8 MAbs DO NOT INDUCE CELL INTERNALIZATION OF CCR8

To determine whether anti-CCR8 Abs induce internalization of the CCR8 receptor upon binding to CCR8 on the surface of a cell, Tregs were activated as described in Example 2. An anti-CCR8 mAb (4A19), a positive control Ab (anti-ICOS Ab), and an isotype control were each added at a concentration of 10 μg/ml to 4 x 10⁴ activated Tregs and incubated at 37°C for 5 time periods: 0, 30, 60, 90, and 120 min. In addition, a goat anti-human Fc-γ Ab was added to a subset of samples at 5 μg/ml to crosslink the primary Abs. After each time period, a sodium azide solution was added to the Tregs and the mixture placed on ice to prevent further receptor internalization. Finally, all of the samples were washed twice with a 0.05% sodium azide solution, then stained with an appropriate phycoerythrin (PE)-conjugated secondary Ab. Cells were fixed with 2% paraformaldehyde (PFA), then analyzed on a cytometer. The level of CCR8 expression on

the cell surface was measured by the median fluorescence intensity as determined using Flowjo software.

As shown in Figure 19, the anti-ICOS mAb in the absence of a cross-linking Ab caused minimal internalization of ICOS over the 120-min time period, but in the presence of the cross-linking Ab caused significant ICOS internalization. In contrast, the anti-CCR8 mAb, 4A19, caused no internalization of CCR8 either in the presence or absence of a cross-linking Ab. Treatment of activated Tregs by the isotype Ab with or without a cross-linking secondary Ab did not cause internalization of CCR8.

EXAMPLE 22

10 DEPLETION OF HUMAN TUMOR Tregs *IN VITRO*

Donor natural killer (NK) cells were isolated and activated using Myelocult H5100 (StemCell), 1 μ M hydrocortisone, and 500 U/ml of IL-2 (PeproTech) for 24 h. Frozen dissociated human lung adenocarcinoma tissue (Discovery Life Sciences) was thawed and co-cultured *ex vivo* in a 1:1 ratio with 2.5×10^5 allogeneic NK cells for 24 h in the presence of an anti-hCCR8 (mAb 4A19), anti-hCCR4 hIgG1-nf (a mogamulizumab biosimilar), or a control nf keyhole limpet hemocyanin (KLH-nf) mAb. The cells were stained and Treg depletion was measured by flow cytometry using Flowjo software to calculate cell populations.

As shown in Figure 20, mAb 4A19 induced measurable depletion of tumor Tregs without impacting the frequency or number of T effs. This was demonstrated in allogeneic NK killing assays with digested patient tumors and patient tumor slice explant systems. MAb 4A19 was more effective at Treg depletion than the anti-CCR4-nf Ab *in vitro* (Figure 20A). Moreover, in contrast to the anti-CCR4-nf Ab, mAb 4A19 did not induce depletion of CD4⁺ effector T cells (Figure 20B). Neither anti-CCR8 nor anti-CCR4 depleted CD8⁺ effector T cells (Figure 20C).

An Ab with an irrelevant targeting arm (anti-KLH-nf) or inert backbone (anti-CCR8-inert) did not deplete Tregs, suggesting that target-mediated Fc γ R coengagement through the nf backbone is required for Treg depletion (Figure 20D).

EXAMPLE 23

30 ANTI-CCR8 MABs REDUCE TUMOR GROWTH IN CT26 MOUSE TUMOR MODEL

Diverse mouse tumor models, namely syngeneic CT26, MC38 and SA1N models, as well as tumor models MB49 and 4T1 that were resistant to anti-PD-1 treatment, were

used to test the effects of anti-CCR8 mAbs. All Ab treatments were performed at 200 µg/mouse, and tumors were measured twice weekly for all studies.

The anti-tumor activity of an anti-mCCR8 mAb (anti-CCR8-mIgG2a) was assessed in the CT26 mouse colon adenocarcinoma model. Fifteen-week-old female
5 BALB/cAnNHsd mice were each implanted subcutaneously (SC) with 10^6 CT26 tumor cells. Mice were randomized into treatment groups of 10 mice/group 7 days post-tumor implantation when tumors reached a median size of approximately 100 mm³. Abs (anti-CCR8-mIgG2a or a control mIgG2a isotype Ab), formulated in PBS, were administered at 200 µg/mouse in a volume of 200 µl via IP injections at Days 7, 10, and 14 post-
10 implantation. Tumor volumes, body weights and clinical observations were noted to establish efficacy and tolerability of test agents. Tumor caliper measurements were converted into tumor volumes using the formula: volume = $\frac{1}{2}$ (length x width x height). Tumor measurements were recorded twice per week for up to 35 days post-implantation. On study, mice received sterile rodent chow and water *ad libitum* and were housed in
15 sterile filter-top cages with 12-h light/dark cycles. All mouse experiments were conducted in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International.

The data show that anti-CCR8-mIgG2a potently reduced the rate of tumor growth, with 8 out of the 10 mice being tumor-free by Day 28 post-implantation, whereas
20 treatment with a control mIgG2a Ab did not inhibit tumor growth (Figure 21A). For the experiments performed in mouse tumor models, tumor-free mice were monitored for tumor recurrences for ≥ 25 days following the last administration of Ab. Measurements for the control-treated mice stopped after about 35 days post-tumor implantation, after which the remaining mice were euthanized. The mean tumor volumes of the treated mice
25 are shown in Figure 21B, which confirms the strong inhibition of tumor growth by the anti-CCR8 mAb and uninhibited growth of tumors treated with the isotype control. These data show that the anti-CCR8 Ab is highly effective in inhibiting tumor growth in this CT26 mouse model and is associated with depletion of tumor-infiltrating Tregs.

For analysis of tumor-infiltrating lymphocytes, mice tumors were harvested 16
30 days post-implantation. Tumors were digested with collagenase, dissociated mechanically using an OctoMacs dissociator (Miltenyi Biotech), and filtered through sterile 100-µM strainers. Samples were blocked for Fc receptors by incubation with FcR Blocking Reagent (Miltenyi Biotech), followed by surface staining for lymphocyte populations,

fixation, permeablization and intracellular staining for Foxp3. Cells were then washed and processed through a flow cytometer. Analysis was performed using FlowJo software and graphed using GraphPad Prism.

As shown in Figure 21C, treatment of mice harboring CT26 tumors with anti-CCR8-mIgG2a drastically reduced the percentage of Foxp3⁺ Tregs in the CD4⁺ T cell population, compared to mice treated with the isotype control, whereas the Treg population in the spleen was unaffected by treatment with anti-CCR8-mIgG2a (Figure 21D). These data indicate that CCR8 is expressed specifically on the surface of tumor-associated Tregs but not expressed on peripheral Tregs, and further, that binding of an Ab to CCR8 mediates depletion of tumor-associated Tregs by ADCC.

EXAMPLE 24

ANTI-mCCR8 MEDIATES DEPLETION OF ONLY TUMOR Tregs, SPARING CCR8⁺ T CELLS IN NORMAL TISSUES

Murine tissue lymphocyte populations were evaluated in the CT26 mouse colon adenocarcinoma model. Female BALB/C mice were injected subcutaneously with 10⁶ CT26 tumor cells. Mice were randomized by tumor size into treatment groups after 12 days, when tumor volumes measured by calipers had reached about 100-150 mm³. Mice were administered a 200-μg flat dose (approximately 10 mg/kg for mice weighing an average of 21.2g) of either anti-CCR8-mIgG2a or a mIgG2a isotype control mAb 3 times on Days 12, 14, and 19 after implantation. On Day 20, 24 h after the third Ab dose, mice were sacrificed, blood samples collected, and tumor, skin, spleen, and thymus tissues were excised.

Tissues were processed into single-cell suspensions for flow cytometry. Tumors were minced and enzymatically digested, spleen and thymus tissue were mechanically dissociated, and RBCs in dissociated spleen and blood samples were depleted as described in Example 2. Cell suspensions from processed tissues were blocked for Fc receptors by incubation with FcR Blocking Reagent (Miltenyi Biotec), followed by surface staining for lymphocyte populations, fixation, permeablization and intracellular staining for Foxp3. Cells were then washed and processed through a flow cytometer. Analysis was performed using FlowJo software and results graphed using GraphPad Prism.

Among T cells infiltrating the mouse CT26 tumor, CD4⁺FOXP3⁺ Tregs (Treg) were the most enriched for CCR8 expression compared to FOXP3⁻CD4⁺ effector

(CD4^{eff}) or CD8⁺ T (CD8T) cells (Figure 22A). CCR8 expression was also observed on a population of skin-resident T cells, with very little expression on T cells in the spleen or blood (Figure 22A). In the thymus, CCR8 was expressed on a subset of CD4⁺CD8⁻ (single positive) thymocytes (Figure 22B). Following treatment with anti-CCR8-mIgG2a, tumor infiltrating Tregs were depleted, whereas Tregs in the spleen, blood, and skin were not depleted (Figure 22C). Thymocytes (Figure 22D) as well as CD4⁺ (Figure 22E) and CD8⁺ (Figure 22F) T cells in the skin were also unaffected. Thus, in the mouse CT26 tumor model, treatment with an anti-mCCR8 depleting Ab selectively depleted CCR8⁺ Tregs in the tumor, but not the relatively low number of CCR8⁺ T cells in the skin, thymus, spleen, or blood.

EXAMPLE 25

ANTI-CCR8 MAbs REDUCE TUMOR GROWTH IN MC38 MOUSE TUMOR MODEL

The anti-tumor activity of anti-CCR8-mIgG2a was measured in the MC38 mouse colon adenocarcinoma model. Fifteen-week-old female FOXP3_IRES-EGFP (C57BL/6) mice (Jackson Laboratory, Bar Harbor, ME) were each implanted SC with 10⁶ MC38 tumor cells and randomized into treatment groups of 10 mice/group 7 days post-tumor implantation. Abs (anti-CCR8-mIgG2a or a control mIgG2a Ab) were administered at 200 µg/mouse in a volume of 200 µl via IP injections at Days 7, 10, and 14 post-implantation. Tumor measurements were recorded twice per week for up to 51 days post-implantation, after which the mice were euthanized. Data analysis and graphing was performed using GraphPad Prism.

The data show that anti-CCR8-mIgG2a potently reduced the rate of tumor growth, with 7 out of the 10 mice having tumor volumes below 10 mm³ by Day 51 post-implantation, compared to mean volumes of over 1,100 mm³ for tumors showing uninhibited growth, whereas treatment with a control mIgG2a Ab did not inhibit tumor growth (Figure 23A). The mean tumor volumes of the treated mice are shown in Figure 23B, which confirms the strong inhibition of tumor growth by anti-CCR8-mIgG2a and uninhibited growth of tumors treated with the isotype control. These data show that the anti-CCR8 Ab is highly effective in inhibiting tumor growth in this MC38 mouse model.

EXAMPLE 26

PHARMACODYNAMIC AND PHARMACOKINETIC RESPONSES
TO ANTI-CCR8 MAb TREATMENT IN MC38 MOUSE TUMOR MODEL

C57BL/6 mice were injected SC with 1.0×10^6 MC38 cells. Tumors were
5 measured with calipers two-dimensionally, and tumor volume was calculated as $L \times (W^2/2)$, where L = length (the longer of the 2 measurements), and W = width. Mice were randomized into 6 groups of 25 mice each (21 mice for the isotype control) when they reached a mean tumor volume of about 77 mm^3 , and dosed by IP injection with anti-CCR8-mIgG2a or a mIgG2a isotype control, diluted in PBS, on Day 10 post-implantation
10 at the doses shown in Table 9. Animals were checked daily for postural, grooming, and respiratory changes, as well as lethargy. Tumors and group body weights were recorded at least twice a week until death, euthanasia or end of study period. Animals were euthanized if the tumor reached a volume greater than approximately $2,000 \text{ mm}^3$ or appeared ulcerated.

15 To investigate the effect of the anti-mCCR8 mAb on tumor-infiltrating lymphocytes, tumors ($n = 7$ mice/treatment group) were harvested on Days 3, 5, and 10 post-treatment. Single cell suspensions from tumors were obtained by using gentleMACS C tubes. Cell suspensions were passed through a $70\text{-}\mu\text{m}$ filter then pelleted. After resuspension, cells were counted using Vi-cell counter (Beckman Coulter, Miami, FL).
20 Cells were then plated in 96-well plates with 3.0×10^6 cells per well and stained for immune cell subsets and functional markers using the flow cytometry Abs. Ab fluorescence was detected by flow cytometry on the Fortessa X-20 cytometer (BD Biosciences), and the results were analyzed using FlowJo software.

To evaluate the pharmacokinetics (PK) of the anti-mCCR8 mAb, whole blood
25 samples ($n = 4$ mice/time point) were obtained by tail bleeds or terminal bleeds at 4, 24, 72, 120, 168 and 240 hours post-dose. Blood samples were coagulated and centrifuged at 4°C ($1,500$ to $2,000 \times g$) to obtain serum. Serum samples were stored at -80°C until analysis. Mouse serum samples were analyzed using immune-capture LC-MS/MS for detection of the anti-mCCR8 mAb. Reference material was used to prepare calibrators
30 and quality control samples. The range of the anti-mCCR8 mAb calibration curve was from 200 to $200,000 \text{ ng/ml}$ in mouse serum. The upper and lower limits of quantification were $200,000$ and 200 ng/ml , respectively (i.e., ULOQ $200,000 \text{ ng/ml}$, LLOQ 200 ng/ml). The PK parameters were obtained by non-compartmental analysis of serum

concentration versus time data using Phoenix WinNonlin software (Certara, Menlo Park, CA). The area under the curve from time zero to the last sampling time [AUC (0-T)] and the area under the curve from time zero to infinity [AUC (INF)] were calculated using a combination of linear and log trapezoidal summations. The CLT Vss, and $T_{1/2}$ were estimated after IP administration. Specifically, CLT is the total clearance of drug and Vss is the volume of (drug) distribution at steady-state. Estimations of half-life ($T_{1/2}$) were made using a minimum of 3 terminal time points with quantifiable concentrations.

Consistent with the results in Example 25, treatment with a single dose of anti-mCCR8 mAb led to reduced tumor growth in the MC38 model. At Day 10 post-treatment, the mean tumor volumes were as shown in Figure 24A and Table 9.

Table 9. ADCC killing of activated Tregs mediated by anti-CCR8 mAbs

Group	Ab Treatment (Dose)	Tumor Vol. at Day 10 (mm ³)	% of Tregs	% of CD8 ⁺ T cells
1	Control (10 mg/kg)	629	59.5 ± 12.8	10.1 ± 2.0
2	CCR8 (0.03 mg/kg)	453	57.3 ± 13.8	17.8 ± 11.3
3	CCR8 (0.1 mg/kg)	438	51.4 ± 13.8	21.2 ± 8.4
4	CCR8 (0.3 mg/kg)	375	28.9 ± 5.2	29.0 ± 15.0
5	CCR8 (1 mg/kg)	161	38 ± 14.7	31.7 ± 15.5
6	CCR8 (3 mg/kg)	207	21.6 ± 6.5	32.0 ± 13.1

Immune-phenotyping studies in the MC38 model further showed that anti-mCCR8 induces Treg depletion in a dose-dependent manner. At Day 5 post-treatment, the mean (± SD) percentage of Tregs (% Foxp3⁺/CD4⁺ T cells) in the mice treated with anti-mCCR8 at the lower doses of 0.03 and 0.1 mg/kg remained comparable to that of the isotype control-treated group (see Figure 24B; Table 9).

On Day 5 post treatment, the frequency (%) of tumor-infiltrating CD8⁺ T cells increased in anti-mCCR8 mAb-treated groups compared to the isotype control-treated group as shown in Figure 24C; Table 9.

A single IP dose of anti-CCR8-mIgG2a demonstrates non-linear PK in the dosing range of 0.03 to 3 mg/kg indicating target-mediated drug disposition (TMDD). Compared with 0.3 mg/kg dosing, the $T_{1/2}$ increases by approximately three-fold compared to the $T_{1/2}$ for the 1 and 3 mg/kg doses (Figure 24D).

EXAMPLE 27

ANTI-CCR8 AND ANTI-PD-1 MAbs SYNERGISTICALLY REDUCE TUMOR GROWTH IN MB49 MOUSE BLADDER CARCINOMA TUMOR MODEL

The anti-tumor activity of anti-CCR8-mIgG2a was measured as a single agent or
5 in combination with an anti-mPD-1 Ab (clone 4H2) in the MB49 murine bladder
carcinoma model, which is known to be less responsive to anti-mPD-1 monotherapy.
Mice were also treated with a control mIgG2a Ab or with 4H2 alone. Anti-mPD-1 clone
4H2 is a chimeric rat-mouse anti-mPD-1 mAb constructed from a rat IgG2a anti-mouse
PD-1 Ab in which the Fc-portion was replaced with an Fc-portion from a mouse IgG1
10 isotype to generate a mAb with reduced binding to mouse FcR's (Li *et al.*, 2009). It
blocks binding of mPD-L1 and mPD-L2 to mPD-1, stimulates a T cell response, and
exhibits anti-tumor activity in mice, and was generated as previously described (Li *et al.*,
2009) and expressed in CHO cells.

Fifteen-week-old male C57BL/6 Foxp3-IRES-EGFP mice were injected SC with
15 2×10^5 MB49 tumor cells and randomized into treatment groups of 10 mice/group 9 days
post-tumor implantation. MAbs were administered at 200 μ g/mouse via IP injections on
Days 10, 13, and 18 post-implantation. Tumor measurements were recorded twice per
week up to Day 39, after which the mice were euthanized.

For analysis of tumor-infiltrating lymphocytes, mouse tumors were harvested 17
20 days post-implantation. Tumors were digested with collagenase, dissociated mechanically
using an OctoMacs dissociator (Miltenyi Biotech), and filtered through sterile 100- μ m
strainers. Samples were blocked for Fc receptors with FcR Blocking Reagent followed by
surface staining for lymphocyte populations. Cells were then washed and processed
through a flow cytometer. Analysis was performed using FlowJo software and graphed
25 using GraphPad Prism.

As shown in Figure 25A, single-agent anti-CCR8 and anti-PD-1 treatments
induced partial anti-tumor activity in the mouse MB49 tumor model, with the anti-CCR8
mAb having a stronger effect than the anti-PD-1 mAb. However, administration of anti-
CCR8 and anti-PD-1 mAbs in combination had a synergistic effect in almost completely
30 inhibiting tumor growth, resulting in 8 out of 10 mice being tumor-free. This
demonstrates that anti-PD-1 treatment can synergistically enhance the anti-tumor activity
of anti-CCR8 in a less immunogenic tumor model. A combination of Abs is considered
synergistic if the anti-tumor effect of the combination is greater than the sum of the level

of inhibition exhibited by each Ab individually.

Figure 25B shows the percentage of tumor-associated CD4⁺ T cells that are Tregs following the various Ab treatments. Despite similar depletion levels of tumor Tregs in both the anti-CCR8 single agent and anti-CCR8/anti-PD-1 combination treatments, the single-agent anti-CCR8 treatment arm was insufficient at inhibiting tumor growth.

Figure 25C shows the percentage of tumor-associated CD45⁺ T cells that are CD8⁺ T cells following the various Ab treatments. CD8⁺ T cell expansion levels were found to be similar for the single-agent and combination arms. However, the quality of the CD8⁺ T cell responses may be improved with the combination treatment.

10

EXAMPLE 28

ANTI-CCR8 AND ANTI-PD-1 MABs SYNERGISTICALLY REDUCE TUMOR GROWTH IN 4T1 MURINE BREAST CANCER MODEL

The anti-tumor activity of anti-CCR8-mIgG2a was measured as a single agent or in combination with an anti-PD-1 Ab (clone 4H2) in the 4T1 mammary carcinoma model, another mouse tumor model that is resistant to anti-mPD-1 monotherapy. Mice were also treated with 4H2 alone and with a combination of control mIgG2a and mIgG1-D265A Abs. Fifteen-week-old female Balb/C mice (Envigo, Indianapolis, IN) were injected SC with 10⁶ 4T1 tumor cells and randomized into treatment groups of 10 mice per group 7 days post-tumor implantation when tumors reached a median size of approximately 100 mm³. Abs were administered at 200 µg/mouse via IP injections on Days 7, 10, and 14 post-implantation. Tumor measurements were recorded twice per week up to Day 27, after which the mice were euthanized. Data analysis and graphing was performed using GraphPad Prism.

As shown in Figure 26, single agent anti-CCR8 treatment induced partial anti-tumor activity in the mouse 4T1 tumor model whereas anti-PD-1 treatment alone did not exhibit any anti-tumor efficacy in this model. Notwithstanding the absence of tumor growth inhibition by anti-PD-1, the combination of this Ab and the anti-CCR8 Ab produced a strong synergistic interaction, evidenced by a massive enhancement of the anti-tumor activity observed with antiCCR8, with 7 out of 10 mice having tumor volumes below 20 mm³, compared to mean volumes of about 1,500 mm³ for tumors showing uninhibited growth. Together with the MB49 data (Example 27), this demonstrates that in anti-PD-1-resistant tumor models, anti-CCR8 treatment can induce synergistic anti-tumor effects when combined with anti-PD-1 treatment.

EXAMPLE 29

ANTI-CCR8 MAbs REDUCE TUMOR GROWTH
IN SA1N FIBROSARCOMA MOUSE MODEL

The anti-tumor activity of anti-CCR8-mIgG2a was measured and compared to that of CCR8-mIgG1-D265A, a variant with the Fc-inert mIgG1-D265A heavy chain, in the SA1N fibrosarcoma mouse model. Female A/J mice were each injected SC with 10^6 SA1N tumor cells and randomized into treatment groups of 9 mice per group 5 days post-tumor implantation. Abs (anti-CCR8-mIgG2a, anti-CCR8-mIgG1-D265A or a control mIgG2a Ab) were administered at 200 μ g/mouse in a volume of 200 μ l via IP injections at Days 5, 8, and 12 post-implantation. Tumor measurements were recorded twice per week for up to 57 days post-implantation, after which the mice were euthanized. Data analysis and graphing was performed using GraphPad Prism.

For analysis of tumor-infiltrating lymphocytes, mice tumors were harvested 16 days post-implantation. Tumors were dissociated mechanically and filtered through sterile 100- μ M strainers. Samples were blocked for Fc receptors with FcR Blocking Reagent followed by surface staining for lymphocyte populations, fixation, permeabilization and intracellular staining for Foxp3. Cells were then washed and processed through a flow cytometer. Analysis was performed using FlowJo software and graphed using GraphPad Prism.

The data show that anti-CCR8-mIgG2a potently reduced the rate of tumor growth, with 9 out of the 9 mice tumor-free by Day 25 post-implantation, whereas treatment with a control mIgG2a mAb did not inhibit tumor growth (Figure 27A). Measurements for the control-treated mice stopped after about 57 days post-tumor implantation, after which the remaining mice were euthanized. These data show that the anti-CCR8 Ab is highly effective in inhibiting tumor growth in this SA1N mouse model. Interestingly, treatment of mice with the non-depleting anti-CCR8 mAb (CCR8-mIgG1-D265A) resulted in partial inhibition of tumor growth (Figure 27A), indicating that at least in the SA1N tumor model, CCR8 engagement by CCL1 may be enhancing immune suppression by Tregs in the tumor environment.

Figure 27B shows the percentage of tumor-associated CD4⁺ T cells that are Tregs following the various Ab treatments. Despite the anti-tumor activity observed with the non-depleting anti-CCR8-mIgG1-D265A treatment, only the depleting anti-CCR8-mIgG2a treatment resulted in tumor Treg depletion. Blockade of CCL1-mediated

enhancement of Treg suppression may have resulted in increased pro-inflammatory and anti-tumor responses.

EXAMPLE 30

ANTI-TUMOR EFFICACY REQUIRES AN Fc-ENGAGING ISOTYPE OF ANTI-CCR8

- 5 To further interrogate the relative contribution of CCR8 blockade versus Ab-mediated Treg cell depletion in anti-tumor immunity, an anti-mCCR8 Ab (Clone SA214G2 from BioLegend) was engineered into Fc-engaging (anti-CCR8-mIgG2a) and non-Fc-engaging or inert (anti-CCR8-mIgG1-D265A) isoforms, and MC38 tumor-bearing mice were treated with either anti-CCR8-mIgG2a or anti-CCR8-mIgG1-D265A.
- 10 The mIgG2a isotype strongly engages Fc receptors to promote ADCC and ADCP, while the mIgG1-D265A version does not (Baudino *et al.*, 2008; Nimmerjahn *et al.*, 2010). Treatment with anti-CCR8-mIgG2a induced significant tumor regression (Figures 28A and D), whereas anti-CCR8-mIgG1-D265A treatment had no discernable effect on tumor growth in this MC38 model (Figures 28A and C), compared to treatment with the IgG2a
- 15 isotype control (Figures 28A and B). Importantly, the frequency of Tregs in tumors was reduced by 75% following treatment with anti-CCR8-mIgG2a whereas anti-CCR8-mIgG1-D265A treatment did not cause a drop in the frequency of tumor Tregs (Figure 28E). This mirrors the result of the *in vitro* experiments showing that whereas mAb 4A19 mediated depletion of tumor Tregs, an anti-CCR8 Ab with an inert backbone (anti-CCR8-
- 20 inert) did not deplete Tregs, suggesting that target-mediated FcγR coengagement through the nf backbone is required for Treg depletion (Example 22). It is also similar to the result observed with the SA1N tumor model where, although, treatment with anti-CCR8-mIgG1-D265A resulted in partial inhibition of tumor growth (Example 29), the immunosuppressive tumor Tregs were not depleted (Figure 27B).
- 25 Indeed, despite similar binding characteristics, anti-CCR8-mIgG2a effectively cross-linked FcγRIV in the presence of CCR8-expressing target cells, while the CCR8-mIgG1-D265A isoform did not (data not shown; *see* Campbell *et al.*, 2021). It was also verified that both the anti-CCR8-mIgG2a and anti-CCR8-mIgG1-D265A Abs blocked CCL1 binding to similar extents (data not shown; *see* Campbell *et al.*, 2021).
- 30 It can be concluded that CCR8 blockade is not generally required for anti-tumor activity, but in certain highly immunogenic tumors, such as SA1N, blockade of CCL1 binding may result in partial inhibition of tumor growth due to an increase in pro-inflammatory cytokines. Greater tumor regression is produced by the depletion of

immunosuppressive tumor Tregs, which requires an Fc-engaging anti-CCR8 Ab.

FOXP3⁺ Treg Migration into Tumors Is CCR8-Independent

As an orthogonal approach, mixed bone marrow chimeras were conducted with congenic Ccr8^{+/+} and Ccr8^{-/-} donor bone marrow to determine whether CCR8 promotes chemotactic migration of Tregs into developing tumors.

Bone marrow chimera

Female recipient mice (WT-CD45.2-Thy1.1; Jackson Laboratory) were given 2 doses of 4.5 Gy 3 h apart, followed by intravenous delivery of congenic CCR8^{+/+} (WT-CD45.1-Thy1.2; Jackson Laboratory) and CCR8^{-/-} (CCR8KO/B6N-CD45.2; Jackson Laboratory) bone marrow. Mice were maintained on antibiotic water, monitored for 8 weeks, and bled at 8 weeks post-transplant to assess donor cell reconstitution. 10⁶ MC38 cells were then implanted SC. Tumors, whole blood, lymph nodes, and splenocytes were harvested after 12 days for immune-monitoring.

Following the 8-week reconstitution phase, equivalent donor immune cell reconstitution was observed in the blood (data not shown). Recipient mice were then subcutaneously implanted with MC38 tumors. CCR8-deficient FOXP3⁺ cells accumulated in tumors similarly to their wild-type counterparts (Figure 28F), confirming that FOXP3⁺ Treg migration into tumors is CCR8-independent.

EXAMPLE 31

ANTI-CCR8-MEDIATED Treg DEPLETION ENHANCES MEMORY POTENTIAL OF TUMOR-SPECIFIC CD8⁺ T CELLS

The effect of Treg depletion on the generation of long-lived memory CD8⁺ T cells recognizing the tumor antigen, AH1 (Huang *et al.*, 1996), was measured.

BALB/c mice were subcutaneously injected with 10⁶ CT26 tumor cells and randomized when tumors reached a volume of 100-120 mm³. Mice were treated retro-orbitally with 200 µg per dose of anti-CCR8-mIgG2a and anti-mCTLA-4-IgG2a treatment Abs or an IgG2a isotype control on Days 1, 4, and 8 after staging. Tumors were harvested on Day 9, and surgically resected on Day 11. After 3 months, mice were bled to quantify AH-1 specific CD8⁺ T cells. Mice were challenged with 10⁷ CFU recombinant *Listeria monocytogenes* expressing the AH-1 peptide (LM-AH1A5) intravenously. *Listeria* was cultured in sterile Brain Heart Infusion Broth, Modified (Teknova Inc., Hollister, CA) overnight to achieve stationary phase culture of 10⁹ CFU/ml and then

diluted with Hank's Balanced Salt solution (HBSS) to 10^8 CFU/ml for immunization. Five days post-challenge, spleen and blood were harvested and processed for flow cytometry.

As expected, anti-mCTLA4-IgG2a and anti-CCR8-mIgG2a induced potent anti-tumor responses (Figures 29A and B) compared to the control (Figure 29C), and Treg depletion (Figure 29D). One day after the last treatment, the frequency of AH-1 tetramer⁺ cells from resected tumors was trending higher in mice treated with either anti-mCTLA4-IgG2a or anti-CCR8-mIgG2a relative to control treated mice (Figure 29E), and AH-1 tetramer⁺ cells remained elevated in the blood and spleen 3 months after resection (Figure 29F). Upon challenge with a *Listeria monocytogenes* strain expressing AH-1 to assess AH-1 specific memory recall responses, mice initially treated with either anti-mCTLA4-IgG2a or anti-CCR8-mIgG2a mounted larger recall expansions of AH-1 tetramer⁺ effector memory cells compared to control mice (Figure 29G).

Anti-CCR8 treated mice also had a significantly larger magnitude of IFN γ ⁺ and polyfunctional IFN γ ⁺TNF α ⁺ CD8⁺ T cells in the spleen after AH1A5 peptide stimulation (Figures 29H and 29I), demonstrating that treatment of tumor-bearing mice with anti-CCR8-mIgG2a results in a boost in the frequency of tumor antigen-specific CD8⁺ T cells with heightened effector functions.

EXAMPLE 32

DIFFERENTIAL EXPRESSION OF CCR8 ON TUMOR CELLS

Gene expression data for CCR8 and CD8A was used to prioritize tumor types that would benefit from treatment with an anti-CCR8 Ab. The RNA-seq gene expression data from bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), Head and Neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), mesothelioma (MESO), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), sarcoma (SARC),

skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), THCA, UCEC and UVM were analyzed from data generated by the TCGA Research Network accessed at <https://www.cancer.gov/tcga>. The Therapeutically Applicable Research to Generate Effective Treatments (TARGET, <https://ocg.cancer.gov/programs/target>) initiative, data
 5 available at <https://portal.gdc.cancer.gov/projects> and M2GEN Oncology Research Information Exchange Network (ORIEN) <https://www.oriencancer.org/> datasets were used to explore hematological tumors.

TMM normalized log2 transformed CPM values from *CCR8* and *CD8A* and the ratio of *CCR8/CD8A* were compared across tumor types. HNSC, LUAD, STAD, LUSC,
 10 PAAD, READ, ESCA, BRCA, COAD, CESC, follicular lymphoma acute lymphocytic leukemia and lymphoma were found to have the highest relative expression of *CCR8* across the tumor types explored. KIRC, LUAD, LUSC, SKCM, STAD, CESC, MESO, HNSC, PAAD and BRCA express the highest relative expression of *CD8A* across tumor types. The ratio of *CD8A/CCR8* was used to enrich for *CD8A* positive tumors that also
 15 have *CCR8* expression. HNSC, STAD, LUAD BRCA, LUSC, PAAD, LAML, CESC, SKCM and MESO express the highest relative expression of *CD8A/CCR8* across tumor types analyzed.

The relatively high expression of *CCR8* and *CD8A*, and their high *CCR8/CD8A* ratio, identify HNSC, LUAD, STAD, LUSC, PAAD, READ, ESCA, BRCA, COAD,
 20 CESC, follicular lymphoma, acute lymphocytic leukemia and lymphoma as tumor types that are expected to be particularly amenable to treatment with an anti-*CCR8* Ab.

EXAMPLE 33

CCR8 EXPRESSION IN MULTIPLE HUMAN CANCERS

To profile *CCR8* expression across multiple cancer types to support indication
 25 selection, with the underlying hypothesis that tumor types with higher levels of *CCR8* expression are most likely to respond to *CCR8* mAb therapy, immunohistochemistry (IHC) was conducted in 17 cancer types or subtypes from two sets of samples (TR-TP and MTB sets).

The TR-TP sample set comprised full-size formalin-fixed, paraffin-embedded
 30 (FFPE) slides of 6 tumor types/subtypes including colorectal adenocarcinoma (CRC), head and neck squamous cell carcinoma (HNSCC), non-small cell lung adenocarcinoma (NSCLC-AD), non-small cell lung squamous cell carcinoma (NSCLC-SQC), pancreatic carcinoma, and small cell lung carcinoma (SCLC), with 14-24 samples per tumor type,

were studied. FFPE tissue samples were obtained from various commercial tissue vendors.

In addition, multi-tumor blocks (MTBs) containing 16 tumor types/subtypes including bladder, breast, cervical, CRC, endometrial, gastric, GBM, HNSCC, melanoma, 5 NHL, NSCLC-AD, NSCLC-SQC, ovarian, RCC, prostate, and pancreatic carcinoma, with 20 cases/tumor types, were studied. Each MTB contained 5 cases of a single indication per FFPE block and 1 hyperplastic tonsil as positive control. The MTBs were custom made by Discovery Life Sciences.

To detect CCR8 tissue binding, an automated IHC assay was developed and 10 validated with a commercial mouse anti-hCCR8 mAb, Clone 433H (BD Biosciences), and the Leica Bond RX System (Leica Biosystems, Buffalo Grove, IL). Slides were deparaffinized and rehydrated following routine histology procedures. Wet slides were loaded onto the Leica Bond RX automated IHC stainer for Heat Induced Epitope Retrieval (HIER) and staining. HIER was performed with the Leica Bond retrieval 15 solution ER2 (pH 9) at 100°C for 30 min, and CCR8 IHC staining was done with the Bond polymer refine detection system (Leica). Briefly, peroxidase blocking (Leica) was performed for 10 min followed by non-specific binding blocking using Dako serum-free protein block (Agilent, Santa Clara, CA) supplemented with 0.5% human gamma 20 globulins (Sigma) for 20 min. The 433H primary Ab was incubated for 60 min at 0.25 µg/ml followed by rabbit anti-mouse linker and then anti-rabbit Poly-HRP-IgG (Leica) for 8 min each. Finally, slides were reacted with the DAB substrate-chromogen solution (Leica) for 10 min. Slides were then counterstained with hematoxylin (Leica) for 5 min and dehydrated, cleared, and coverslipped following routine histological procedures. Stained slides were scanned using a Leica AT Turbo scanner and whole-slide image 25 analysis was performed using HALO® software (Indica Labs, Albuquerque, NM). CCR8 expression was reported as the percentage of positive cells out of total cells within the tumor region. In a small subset of samples, when image analysis score did not produce an accurate result as deemed by a Board-certified pathologist, manual visual scoring was performed by the pathologist.

30 Strong CCR8 positive staining was primarily distributed in a subset of immune cells showing both membranous and cytoplasmic patterns. Weak to moderate cytoplasmic staining in tumor cells was also observed in a small number of cases. Whole-slide image analysis of CCR8⁺ cells in the TR-TP full-size tissue sections is shown in Figure 30A,

while whole-slide image analysis of CCR8⁺ cells in the MTB set is shown in Figure 30B. Both the TR-TP and the MTB data sets indicate that among the 17 tumor types evaluated, CCR8 expression was most abundant in HNSCC and least abundant in GBM. In order of highest to lowest CCR8 expression, the tumor cohorts ranked as follows: HNSCC, cervical, CRC, NSCLC-SCC, NSCLC-ADC, pancreatic, gastric, bladder, breast carcinomas, ovarian cancer and GBM. The RCC, prostate, melanoma, endometrial and NHL tumor cohorts showed similar levels of CCR8 expression and were lower than the expression observed in the breast cancer tumor cohort.

Based on the hypothesis that tumors with high CCR8 expression are more likely to respond to CCR8 mAb therapy, these tumor profiling data support the prioritization of indication selection to HNSCC, cervical, CRC, NSCLC-SCC, NSCLC-ADC, pancreatic, gastric, bladder, and breast cancers.

EXAMPLE 34

PHASE 1/2 CLINICAL STUDY OF ANTI-CCR8 IN TREATING CANCER

A phase 1/2 study of the 4A19 anti-CCR8 mAb administered as monotherapy and in combination with the anti-PD-1 mAb, nivolumab, is conducted in subjects having select advanced solid tumors patients, namely NSCLC, SCCHN, CRC, gastric/gastroesophageal (GE) junction, and cervical cancer, to assess, among other things, the safety, tolerability and preliminarily the efficacy of administering 4A19 as a single agent and as a combination therapy with nivolumab.

Study Design

This study is composed of 4 parts: 4A19 administered as monotherapy in dose escalation (Part 1A) and dose expansion (Part 2A), and 4A19 administered in combination with nivolumab in dose escalation (Part 1B) and expansion (Part 2B).

In the dose escalation stage, to identify suitable doses of anti-CCR8 a range of doses of 4A19 is administered to at least 3 subjects for each dose, except for the lowest 2 dose levels of 4A19 monotherapy consisting of at least 1 subject per dose level to minimize the number of participants receiving 4A19 at potentially sub-efficacious doses. In Part 1A, 4A19 is administered intravenously (IV) to subjects at a flat dose of 0.3, 1, 3, 10, 30, 100, 300 and 800 mg, once every 2 weeks (Q2W). The first-in-human (FIH) starting flat dose of 0.3 mg (4 µg/kg) IV Q2W for 4A19 was derived using the totality of data generated from a mix of pharmacology- and toxicology-based approaches with the

goal of ensuring adequate safety while minimizing the participants' exposure to potentially sub-efficacious doses and risk of cytokine release.

In Part 1B, 4A19 is administered intravenously (IV) to subjects at the same flat doses in combination with nivolumab administered IV at the FDA-approved flat dose of 5 480 mg once every 4 weeks (Q4W).

For dose expansion, single-arm and randomized cohorts including different tumor types and dose levels from escalation are opened to administer monotherapy and combination therapy to at least 20 subjects per cohort.

All subjects complete up to 3 study periods:

10 (a) screening (up to 28 days), during which subjects are evaluated to verify they meet eligibility criteria and a fresh tumor biopsy is provided;

(b) treatment (up to 26 cycles of study therapy [104 weeks] from first treatment regardless of treatment delays, 28 days per cycle); and

(c) follow-up, including safety and survival follow-up (up to 2 years following the 15 end of treatment [EOT]) periods). The maximum duration of study participation is about 4 years (up to 28 days of screening, treatment of up to 2 years, and follow-up of up to 2 years).

The Ab drugs are administered to the subjects until progression, unacceptable toxicity, withdrawal of consent, completion of 26 cycles of study therapy (104 weeks), or 20 the study ends, whichever occurs first.

Table 10. SEQ ID NOs. and Amino acid Sequence Summary

SEQ ID NO.	Description of Sequence	Amino acid Sequence
1	Amino acid sequence of human CCR8	MDYTLDLSTV TVTDYYPDI FSSPCDAELI QTNGKLLAV FYCLLFVFS LGNSLVILVL VVCCKLRST DVYLLNLALS DLLFVFSFPF QTYLLDQWV FGTVMCKVVS GFYYIGFYSS MFFITLMSVD RYLAVHAVY ALKVRTIRMG TTLCLAVWLT AIMATIPLLV FYQVASEDGV LQCYSFYNNQ TLKWKIFTNF KMNILGLLIP FTIFMFCYIK ILHQLKRCQN HNKTKAIRLV LIVVIASLLF WVPFNVVLF TSLHSMHILD GCSISQQLTY ATHVTEIISF THCCVNPVIY AFVGEKFKKH LSEIFQKSCS QIFNYLGRQM PRESCEKSSS CQQHSSRSSS VDYIL
2	Amino acid residues 15-21 of human CCR8 sequence	YYYPDIF
3	Amino Acid Sequence for V _H in mAb 16B13	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SFNMNWRQA PGKGLEWISY ISSGSTTIAH ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCVRR LKGSFDYWGQG TLVTVSS

SEQ ID NO.	Description of Sequence	Amino acid Sequence
4	Amino Acid Sequence for V _H in mAb 14S15	EVQLVEFGGG LVQPKGSLKL SCVVS GFRFN PYAMN WVRQA PGKGLEWLGR IRSKSNNYET YYADSVKDRF IISRDDSQKM VYLQMN NVKT EDTAMYYCVG YSDLYVLDYW GQGTSTVSS
5	Amino Acid Sequence for V _H in mAb 18Y12	QVQLQQSGAE LVRPGASVTL SCKASGYTFT DYEMHWVKQT PVHGLEWIGA IDPKTGSTAY NQKFKGKAIM TADKSSSTAY MELRSLTSED SVVYYCTGLR RFVYWGQGT VTVSA
6	Amino Acid Sequence for V _H in mAb 4A19	QVQLVQSGAE VKKPGASVKV SCKASGYTFT DSEMH WVRQA TGQGLEWMGA IQPETGGTAY NQKFKARVTM TRDTSISTAY MELSSLRSED TAVYYCARRR RNFDYWGQGT LVTVSS
7	Amino Acid Sequence for V _H in mAb 2M18	EVQLVESGGG LVQPGGSLRL SCVVS GFTFS NFMNI WVRQV PGKGLEWISH ISRGTTINH ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARRR LGVFDYWGQG TLVTVSS
8	Amino Acid Sequence for V _H in mAb 15C17	QVQLVESGGG LVQPGGSLRL SCAASGFTFS SYDMN WVRQA PGKGLEWISY ISSSNGNKKH ADSVKGRFTI SRDNAENSLY LQMNSLRDED TAVYYCARRR QGVFDI WGQG TLVTVSS
9	Amino Acid Sequence for V _H in mAb 13T20	QVQLVESGGG LVKPRGSLRL SCAASGFTFS VAWMH WVRQA PGKGLEWVGR IKSKTDGGTT DYATPVKGRF TISRDDSKNT LYLQMHSLKT EDTAVYYCTV VTMVRGVLYD FWGQGT LVTVSS
10	Amino Acid Sequence for V _H in mAb 10R3	QVQLVESGGG VVQPGSLRL SCAASGFTFS SYAMH WVRQA PGKGLEWVGI ISYDGSNKYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TALYSCARVP NWRGA FDIWG QGTMTVSS
11	Amino Acid Sequence for V _H in mAb 8D55	QVQLVESGGG LVQPGGSLRL SCAASGFTFS NYNMI WVRQT PGKGLEWISY ISSRSIISY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARRW KGVFDYWGQG TLVTVSS
12	Amino Acid Sequence for V _H in mAb 1V11	QVQLVESGGG VVQPGSLRL SCAASGFTFS NYAMH WVRQA PGKGLDWLAV ISYDGSNKYH SDSVKGRFTI SRDNSKNTLY LQINSLRAED AAVYYCARRG ELGIGGF D YW GQGT LVTVSS
13	Amino Acid Sequence for V _H in mAb 11K16	QVQLVESGGG LVLPGGSLRL SCAASGFTFS SNNMI WVRQA PGKGLEWVSY ISSSSSTISY ADSVRGRFTI SRDNAKNSLF LQMNSLRDED TAVYYCARRW RGVFDYWGQG TLVTVSS
14	Amino Acid Sequence for V _H in mAb 12F27	QVQLVESGGG VVQPGSLRL SCAASGFSFR SYGIH WVRQA PGKGLEWVAV ISYEGSNKRY ADSVKGRFTI SRDNSKSTLS LQMNSLRAED TAVYYCAKGR RPSGEGAFDI WGQGTMTVSS
15	Amino Acid Sequence for V _L in mAb 16B13	DIQMTQSPSS LSASVGDRTV ITCRASQGIS SWLAWYQQKP EKAPKSLIYG ASRLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGQ GTKVEIK
16	Amino Acid Sequence for V _L in mAb 14S15	DIVMTQAAPS VPVTPGESVS ISCRSSKSL HSNGNTFLYW FLQRPQGSPQ LLIYRMSNLA SGVPHRFSGS GSGTAFTLRI SRVEAEDVGV YYCMQHLEYP FTFGSGTKLE IK
17	Amino Acid Sequence for V _L in mAb 18Y12	DVMTQTPLS LPVSLGDQAS ISCRSRQSLV HSSGYTYLHW YLQKPGQSPK LLIYRVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP FTFGSGTKLE IK

SEQ ID NO.	Description of Sequence	Amino acid Sequence
18	Amino Acid Sequence for V _L in mAb 4A19	DIVMTQTPLS LSVTPGQPAS ISCRSSQSLF HSSGNTYLHW YLQKPGQPPQ LLIIYKVSNR SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCSQSTHVP FTFGQGTKLE IK
19	Amino Acid Sequence for V _L in mAb 2M18	DIQMTQSPSS LSASVGDRVT ITCRASQGIS SWLAWYQQKP EKAPKSLIYA ASSLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGQ GTKVEIK
20	Amino Acid Sequence for V _L in mAb 15C17	DIVMTQSPSS LSASVGDRVT ITCRASQGIS RWLAWYQQKP EKAPKSLIYA ASSLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGG GTKVEIK
21	Amino Acid Sequence for V _L in mAb 13T20	DIVMTQSPGT LSLSPGERAT LSCRASQSVS SNYLAWYQQK PGQAPRLLIY GASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSRTFGG GTKVEIK
22	Amino Acid Sequence for V _L in mAb 10R3	EIVLTQSPDF QSVTPKEKVT ITCRASQSIG SHLHWYQQKP DQSPKLLIKY ASQSFSGVPS RFGSGSGSTD FSLTINSLET EDAATYFCHQ SYSLPLTFGG GTKVEIK
23	Amino Acid Sequence for V _L in mAb 8D55	DIVMTQSPSS LSASVGDRVT ITCRASQGIS SWLAWYQQKP EKAPKSLIYA ASSLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGQ GTKVEIK
24	Amino Acid Sequence for V _L in mAb 1V11	DIVMTQTPLS SPVTLGRPAS ISCRSSRSLSV HTDGNTYLNW LQQRPGQPPR LLIIYKISNR SGVPDRFSGS GAGTDFTLKI TRVEAEDVGV YYCMQATQFP LTFGPGTKVD IK
25	Amino Acid Sequence for V _L in mAb 11K16	DIVMTQSPSS LSASVGDRVT ITCRASQGIS SWLAWYQQKP EKAPKSLIYA ASSLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGQ GTKVEIK
26	Amino Acid Sequence for V _L in mAb 12F27	DIQMTQSPSS LSASVGDRVT ITCRASQGIS SWLAWYQQKP EKAPKSLIYA ASSLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YNSFPPTFGQ GTKVEIK
27	Amino Acid Sequence for V _H CDR1 in mAb 16B13	GFTFSSFNMN
28	Amino Acid Sequence for V _H CDR2 in mAb 16B13	YISSGSTTIA HADSVKG
29	Amino Acid Sequence for V _H CDR3 in mAb 16B13	RLKGSFDY
30	Amino Acid Sequence for V _L CDR1 in mAb 16B13	RASQGISSWL A
31	Amino Acid Sequence for V _L CDR2 in mAb 16B13	GASRLQS
32	Amino Acid Sequence for V _L CDR3 in mAb 16B13	QQYNSYPPT
33	Amino Acid Sequence for V _H CDR1 in mAb 14S15	GFRFNPYAMN
34	Amino Acid Sequence for V _H CDR2 in mAb 14S15	RIRSKSNYE TYYADSVKD
35	Amino Acid Sequence for V _H	YSDLYVLDY

SEQ ID NO.	Description of Sequence	Amino acid Sequence
	CDR3 in mAb 14S15	
36	Amino Acid Sequence for V _L CDR1 in mAb 14S15	RSSKSLLSN GNTFLY
37	Amino Acid Sequence for V _L CDR2 in mAb 14S15	RMSNLAS
38	Amino Acid Sequence for V _L CDR3 in mAb 14S15	MQHLEYPFT
39	Amino Acid Sequence for V _H CDR1 in mAb 18Y12	GYTFTDYEMH
40	Amino Acid Sequence for V _H CDR2 in mAb 18Y12	AIDPKTGSTA YNQKFKG
41	Amino Acid Sequence for V _H CDR3 in mAb 18Y12	LRRFVY
42	Amino Acid Sequence for V _L CDR1 in mAb 18Y12	RSSKSLLSN GNTFLY
43	Amino Acid Sequence for V _L CDR2 in mAb 18Y12	RVSNRFS
44	Amino Acid Sequence for V _L CDR3 in mAb 18Y12	SQSTHVPFT
45	Amino Acid Sequence for V _H CDR1 in mAb 4A19	GYTFTDSEMH
46	Amino Acid Sequence for V _H CDR2 in mAb 4A19	AIQPETGGTA YNQKFKA
47	Amino Acid Sequence for V _H CDR3 in mAb 4A19	RRRNFDY
48	Amino Acid Sequence for V _L CDR1 in mAb 4A19	RSSQSLFHSS GNTYLH
49	Amino Acid Sequence for V _L CDR2 in mAb 4A19	KVSNRFS
50	Amino Acid Sequence for V _L CDR3 in mAb 4A19	SQSTHVPFT
51	Amino Acid Sequence for V _H CDR1 in mAb 2M18	GFTFSNFMNI
52	Amino Acid Sequence for V _H CDR2 in mAb 2M18	HISRGGTTIN HADSVKG
53	Amino Acid Sequence for V _H CDR3 in mAb 2M18	RLGVFDY
54	Amino Acid Sequence for V _L CDR1 in mAb 2M18	RASQGISSWL A
55	Amino Acid Sequence for V _L CDR2 in mAb 2M18	AASSLQS
56	Amino Acid Sequence for V _L CDR3 in mAb 2M18	QQYNSYPPT
57	Amino Acid Sequence for V _H CDR1 in mAb 15C17	GFTFSSYDMN
58	Amino Acid Sequence for V _H CDR2 in mAb 15C17	YISSNNGNKH HADSVKG

SEQ ID NO.	Description of Sequence	Amino acid Sequence
59	Amino Acid Sequence for V _H CDR3 in mAb 15C17	RQGVFDI
60	Amino Acid Sequence for V _L CDR1 in mAb 15C17	RASQGISRWL A
61	Amino Acid Sequence for V _L CDR2 in mAb 15C17	AASSLQS
62	Amino Acid Sequence for V _L CDR3 in mAb 15C17	QQYNSYPPT
63	Amino Acid Sequence for V _H CDR1 in mAb 13T20	GFTFSVAWMH
64	Amino Acid Sequence for V _H CDR2 in mAb 13T20	RIKSKTDGGT TDYATPVKG
65	Amino Acid Sequence for V _H CDR3 in mAb 13T20	VTMVRGVLYD F
66	Amino Acid Sequence for V _L CDR1 in mAb 13T20	RASQSVSSNY LA
67	Amino Acid Sequence for V _L CDR2 in mAb 13T20	GASSRAT
68	Amino Acid Sequence for V _L CDR3 in mAb 13T20	QQYGSSRT
69	Amino Acid Sequence for V _H CDR1 in mAb 10R3	GFTFSYAMH
70	Amino Acid Sequence for V _H CDR2 in mAb 10R3	IISYDGSNKY YADSVKG
71	Amino Acid Sequence for V _H CDR3 in mAb 10R3	VPNWRGAFDI
72	Amino Acid Sequence for V _L CDR1 in mAb 10R3	RASQSIGSHL H
73	Amino Acid Sequence for V _L CDR2 in mAb 10R3	YASQSFS
74	Amino Acid Sequence for V _L CDR3 in mAb 10R3	HQSYSLPLT
75	Amino Acid Sequence for V _H CDR1 in mAb 8D55	GFTFSNYNMI
76	Amino Acid Sequence for V _H CDR2 in mAb 8D55	YISSRSIIS YADSVKG
77	Amino Acid Sequence for V _H CDR3 in mAb 8D55	RWKGVFDY
78	Amino Acid Sequence for V _L CDR1 in mAb 8D55	RASQGISSWL A
79	Amino Acid Sequence for V _L CDR2 in mAb 8D55	AASSLQS
80	Amino Acid Sequence for V _L CDR3 in mAb 8D55	QQYNSYPPT
81	Amino Acid Sequence for V _H CDR1 in mAb 1V11	GFTFSNYAMH
82	Amino Acid Sequence for V _H	YDGSNKYHSD SVKG

SEQ ID NO.	Description of Sequence	Amino acid Sequence
	CDR2 in mAb 1V11	
83	Amino Acid Sequence for V _H CDR3 in mAb 1V11	RGELGIGGFD Y
84	Amino Acid Sequence for V _L CDR1 in mAb 1V11	RSSRSLVHTD GNTYLN
85	Amino Acid Sequence for V _L CDR2 in mAb 1V11	KISNRFS
86	Amino Acid Sequence for V _L CDR3 in mAb 1V11	MQATQFPLT
87	Amino Acid Sequence for V _H CDR1 in mAb 11K16	GFTFSSNNMI
88	Amino Acid Sequence for V _H CDR2 in mAb 11K16	YISSSSSTIS YADSVRG
89	Amino Acid Sequence for V _H CDR3 in mAb 11K16	RWRGVFDY
90	Amino Acid Sequence for V _L CDR1 in mAb 11K16	RASQGISSWL A
91	Amino Acid Sequence for V _L CDR2 in mAb 11K16	AASSLQS
92	Amino Acid Sequence for V _L CDR3 in mAb 11K16	QQYNSYPPT
93	Amino Acid Sequence for V _H CDR1 in mAb 12F27	GFSFRSYGIH
94	Amino Acid Sequence for V _H CDR2 in mAb 12F27	VISYEGSNKR YADSVKG
95	Amino Acid Sequence for V _H CDR3 in mAb 12F27	GRRPSGEGAF DI
96	Amino Acid Sequence for V _L CDR1 in mAb 12F27	RASQGISSWL A
97	Amino Acid Sequence for V _L CDR2 in mAb 12F27	AASSLQS
98	Amino Acid Sequence for V _L CDR3 in mAb 12F27	QQYNSFPPT
99	Amino Acid Sequence for heavy chain mAb of 16B13	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SFNMNWVRQA PGKGLEWISY ISSGSTTIAH ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCVRR L KGSFDYWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTGPS SSLGTQTYIC NVNHPKPSNTK VDRKVEPKSC DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK
100	Amino Acid Sequence for	EVQLVEFGGG LVQPKGSLKL SCVVSQFRFN PYAMNWVRQA PGKGLEWLGR IRSKSNNYET

SEQ ID NO.	Description of Sequence	Amino acid Sequence
	heavy chain mAb of 14S15	YYADSVKDRF IISRDDSQKM VYLQMNNVKT EDTAMYYCVG YSDLYVLDYW GQGTSTVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTPEVTCVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
101	Amino Acid Sequence for heavy chain mAb of 18Y12	QVQLQSGAE LVRPGASVTL SCKASGYTFT DYEMHWVKQT PVHGLEWIGA IDPKTGSTAY NQKFKGKAIM TADKSSSTAY MELRSLTSED SVVYCTGLR RFVYWGQGT VTVSAASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KRVEPKSCDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTL PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMEAL HNHYTQKSL LSPGK
102	Amino Acid Sequence for heavy chain mAb of 4A19	QVQLVQSGAE VKKPGASVKV SCKASGYTFT DSEMHWRQA TGQGLEWMGA IQPETGGTAY NQKFARVTM TRDTSISTAY MELSSLRSED TAVVYCARRR RNFDYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKRVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VSVLTVLHQD DWLNGKEYKC KVSNAKPAP IEKTISKAKG QPREPQVYTL PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMEAL LHNHYTQKSL SLSPG
103	Amino Acid Sequence for V_H CDR1 in mAb 14S15h	GFRFGPYAMN
104	Amino Acid Sequence for V_H CDR2 in mAb 14S15h	RIRSKSNYE TTYADSVKD
105	Amino Acid Sequence for V_H CDR3 in mAb 14S15h	YSDLYVLDY
106	Amino Acid Sequence for V_L CDR1 in mAb 14S15h	RSSKSLHSQ GNTFLY
107	Amino Acid Sequence for V_L CDR2 in mAb 14S15h	RMSNLAS
108	Amino Acid Sequence for V_L CDR3 in mAb 14S15h	MQHLEYPT

SEQ ID NO.	Description of Sequence	Amino acid Sequence
109	Amino acid residues 12-22 of human CCR8 sequence	VTDYYPDIFS
110	Amino Acid Sequence for heavy chain of mAb 12F27	QVQLVESGGG VVQPGRSLRL SCAASGFSTR SYGIHWVRQA PGKGLEWVAV ISYEGSNKRY ADSVKGRFTI SRDNSKSTLS LQMNSLRAED TAVYYCAKGR RPSGEGAFDI WQGTMTVTS SASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSV TVPSSSLGTQ TYICNVNHKP SNTKVDKRV PKSCDKTHC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPPP VLDSDGSFFL YSKLTVDKSR WQQGNVFPSC VMHEALHNHY TQKSLSLSPG K
111	Amino Acid Sequence for light chain of mAb 16B13	DIQMTQSPSS LSASVGDRTV ITCRASQGIS SWLAWYQQKP EKAPKSLIYG ASRLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YNSYPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
112	Amino Acid Sequence for light chain of mAb 14S15	DIVMTQAAPS VPVTPGESVS ISCRSSKSL HSNQNTFLYW FLQRPQGSPQ LLIYRMSNLA SGVPHRFSGS GSGTAFTLRI SRVEAEDVGV YYCMQHLEYP FTFGSGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC
113	Amino Acid Sequence for light chain of mAb 18Y12	DVVMTQTPLS LPVSLGDQAS ISCRSRQSLV HSSGYTYLHW YLQKPGQSPK LLIYRVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP FTFGSGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC
114	Amino Acid Sequence for light chain of mAb 4A19	DIVMTQTPLS LSVTPGQPAS ISCRSSQSLF HSSGNTYLHW YLQKPGQPPQ LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCSQSTHVP FTFGQGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC
115	Amino Acid Sequence for V_H in mAb 14S15h	EVQLVESGGG LVKPGGSLRL SCAASGFRFG PYAMNWVRQA PGKGLEWVGR IRSKSNYET YYADSVKDRF TISRDDSKNT LYLQMNLSKT EDTAVYYCVG YSDLYVLDYW GQGTTLTVSS
116	Amino Acid Sequence for V_L in mAb 14S15h	DIVMTQTTPS LPVNPGEPA S ISCRSSKSL HSQGNFTLYW YLQKPGQSPQ LLIYRMSNLA SGVPDRFSGS GSGSDFTLKI SWVEAEDVGV YYCMQHLEYP FTFGPGTKVD IK

SEQ ID NO.	Description of Sequence	Amino acid Sequence
117	Amino Acid Sequence for heavy chain of mAb 14S15h	EVQLVESGGG LVKPGGSLRL SCAASGFRFG PYAMNWVRQA PGKGLEWVGR IRSKSNNYET YYADSVKDRF TISRDDSKNT LYLQMNSLKT EDTAVYYCVG YSDLYVLDYW GQGTLLTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTPEVTCVVDVVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
118	Amino Acid Sequence for light chain of mAb 14S15h	DIQMTQTPPS LPVNPGEFAS ISCRSSKSL HSQGNFTFLYW YLQKPGQSPQ LLIYRMSNLA SGVPDRFSGS GSGSDFTLKI SWVEAEDVGV YYCMQHLEYP FTFPGGTKVD IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNQESVTE QDSKSTYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC
119	Amino Acid Sequence for light chain mAb of 12F27	DIQMTQSPSS LSASVGDRTV ITCRASQGIS SWLAWYQQKP EKAPKSLIYA ASSLQSGVPS RFGSGSGSTD FTLTISSLQP EDFATYYCQQ YNSFPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSK STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
120	Amino acid sequence of mouse CCR8	MDYTMENPVT MTDYYPDFFT APCDAEFLLR GSMLYLAILY CVLFVLGLLG NSLVILVLVG CKKLRSITDI YLLNLAASDL LFVLSIPFQT HNLLDQWVFG TAMCKVVSGL YYIGFFSSMF FITLMSVDRY LAIVHAVYAI KVRTASVGTA LSLTVWLAAY TATIPLMVFY QVASEDGMLQ CFQFYEEQSL RWKLFTHFEI NALGLLLPFA ILLFCYVRIL QQLRGCLNHN RTRAIKLVLT VVIVSLLFWV PFNVALFLTS LHDHLILDGC ATRQRLALAI HVTEVISFTH CCVNPVIYAF IGEKFKKHLM DVFQKSCSHI FLYLGRQMPV GALERQLSSN QRSSHSTLD DIL

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CLAIMS

What is claimed is:

1. A monoclonal antibody, or an antigen-binding portion thereof, that specifically binds to C-C Motif Chemokine Receptor 8 (CCR8) expressed on the surface of a cell and mediates depletion of the CCR8-expressing cell by antibody-dependent cellular cytotoxicity (ADCC).
2. The monoclonal antibody or antigen-binding portion thereof of claim 1, which specifically binds to human CCR8 (hCCR8), the sequence of which is set forth as SEQ ID NO: 1.
3. The monoclonal antibody or antigen-binding portion thereof of claim 1 or 2, which comprises a heavy chain constant region which is of a human IgG1 or IgG3 isotype.
4. A modified anti-hCCR8 monoclonal antibody, or an antigen-binding portion thereof, which comprises a modified heavy chain constant region that binds with higher affinity to an Fc γ receptor (Fc γ R) and mediates enhanced ADCC compared to the monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, optionally which mediates at least:
 - (a) 2 times enhanced ADCC activity;
 - (a) 5 times enhanced ADCC activity; or
 - (c) 10 times enhanced ADCC activity;as measured by a reduction in the EC₅₀ for cell lysis in the NK cell lysis assay described in Example 17.
5. The modified anti-hCCR8 monoclonal antibody or antigen-binding portion thereof of claim 4, comprising a modified IgG1 heavy chain constant region which exhibits reduced fucosylation, optionally comprising a modified IgG1 heavy chain constant region which is hypofucosylated or nonfucosylated.
6. The modified anti-hCCR8 monoclonal antibody or antigen-binding portion thereof of claim 4 or 5, comprising a modified IgG1 heavy chain constant region which contains a mutation, or a multiplicity of mutations, that mediate enhanced ADCC,

optionally wherein the mutation or multiplicity of mutations is chosen from G236A; S239D; F243L; E333A; G236A/I332E; S239D/I332E; S267E/H268F; S267E/S324T; H268F/S324T; G236A/S239D/I332E; S239D/A330L/I332E; S267E/H268F/S324T; and G236A/S239D/A330L/I332E.

- 5 7. The monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, which
- (i) specifically binds to human CCR8-expressing Chinese Hamster Ovary (CHO) cells with an EC_{50} , as measured by the binding assay as described in Example 11, of:
- 10 (a) about 10 nM or lower;
- (b) about 5 nM or lower;
- (c) about 1.7 nM or lower;
- (d) about 1 nM or lower;
- (e) about 0.5 nM or lower;
- 15 (f) about 0.1 nM or lower;
- (g) about 0.1 nM;
- (h) about 1.7 nM;
- (i) between about 0.1 nM and about 10 nM;
- (j) between about 0.1 nM and about 2 nM;
- 20 (k) between about 0.5 nM and about 5 nM;
- (l) between about 1 nM and about 2 nM; or
- (m) between about 0.5 nM and about 1 nM;
- and/or
- (ii) specifically binds to activated regulatory T cells (Tregs) with an EC_{50} , as
- 25 measured by the binding assay as described in Example 11, of:
- (a) about 50 nM or lower;
- (b) about 14 nM or lower;
- (c) about 5 nM or lower;
- (d) about 2 nM or lower;
- 30 (e) about 0.5 nM or lower;
- (f) about 0.3 nM or lower;
- (g) about 0.1 nM or lower;
- (h) about 0.03 nM or lower;

- (i) about 1.7 nM;
 - (j) between about 0.03 nM and about 10 nM;
 - (k) between about 0.1 nM and about 5 nM; or
 - (l) between about 0.2 nM and about 2 nM;
- 5 and/or
- (iii) binds to a N-terminal peptide of human CCR8 comprising sulfated tyrosine-15 and tyrosine-17 residues with a K_D , as measured by surface plasmon resonance (SPR) as described in Example 11, of:
 - (a) about 100 nM or lower;
 - 10 (b) about 50 nM or lower;
 - (c) about 10 nM or lower;
 - (d) about 5 nM or lower;
 - (e) about 1.6 nM;
 - (f) about 1.0 nM or lower;
 - 15 (g) about 0.5 nM or lower;
 - (h) about 0.1 nM or lower;
 - (i) between about 100 nM and about 0.1 nM;
 - (j) between about 50 nM and about 0.5 nM;
 - (k) between about 10 nM and about 1 nM; or
 - 20 (l) between about 2 nM and about 1 nM;
- and/or
- (iv) binds to a N-terminal peptide of human CCR8 comprising a single sulfated residue, tyrosine-15, with a K_D , as measured by SPR as described in Example 11, of:
 - 25 (a) about 100 nM or lower;
 - (b) about 50 nM or lower;
 - (c) about 25 nM or lower;
 - (d) about 10 nM or lower;
 - (f) about 1.0 nM or lower;
 - 30 (e) about 20 nM;
 - (i) between about 100 nM and about 1 nM;
 - (j) between about 50 nM and about 10 nM; or
 - (k) between about 30 nM and about 20 nM.

8. The monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, which binds specifically to rare and scattered immune cells in the medulla of the thymus and dermis of the skin but does not bind to human cerebrum, cerebellum, heart, liver, lung, kidney, tonsil, spleen, thymus, colon,
5 stomach, pancreas, adrenal, pituitary, skin, peripheral nerve, testis or uterus tissue, or peripheral blood mononuclear cells (PBMCs).
9. The monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, which further inhibits binding of C-C Motif Chemokine Ligand 1 (CCL1) to CCR8 and inhibits CCR8/CCL1 signaling as measured by inhibition
10 of calcium flux as described in Example 15.
10. The monoclonal antibody or antigen-binding portion thereof of the preceding claims, which
- (i) inhibits CCR8/CCL1 signaling with an IC_{50} of:
- 15 (a) about 10 nM or lower;
(b) about 5 nM or lower;
(c) about 1 nM or lower;
(d) about 0.5 nM or lower;
(e) about 0.1 nM or lower;
(f) about 0.01 nM or lower;
20 (g) between about 0.01 nM and about 10 nM;
(h) between about 0.05 nM and about 5 nM;
(i) between about 0.1 nM and about 1 nM; or
(j) about 0.46 nM;
- and/or
- 25 (ii) mediates depletion of the CCR8-expressing cell with an EC_{50} , as measured by a CD16 cross-linking assay as described in Example 17, of:
- 30 (a) about 100 pM or lower;
(b) about 30 pM or lower;
(c) about 10 pM or lower;
(d) about 3 pM or lower;
(e) about 1 pM or lower;
(e) about 0.5 pM or lower;

- (f) about 0.1 pM or lower;
 (g) about 0.05 pM or lower;
 (h) about 0.7 pM;
 (i) between about 0.05 pM and about 50 pM;
 5 (j) between about 0.1 pM and about 10 nM;
 (k) between about 0.3 nM and about 7 nM; or
 (l) between about 0.6 nM and about 3 nM;
 and/or
- (iii) mediates depletion of activated Tregs with an EC₅₀, as measured
 10 by an apoptosis assay as described in Example 19, of:
 (a) about 500 pM or lower;
 (b) about 100 pM or lower;
 (c) about 30 pM or lower;
 (d) about 15 pM or lower;
 15 (e) about 5 pM or lower;
 (f) about 1 pM or lower;
 (g) about 13 pM;
 (h) between about 1 pM and about 500 pM;
 (i) between about 5 pM and about 100 pM; or
 20 (j) between about 10 pM and about 50 pM.
11. The monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, which binds to an epitope located in the N-terminal domain of human CCR8 as determined by X-ray crystallography, wherein the epitope comprises at least one amino acid within a peptide having the sequence
 25 V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109), optionally wherein
 (i) the epitope comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or all the amino acids within a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109); optionally wherein
 (ii) the epitope comprises all 11 of the amino acids in the peptide having the
 30 sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109); optionally wherein
 (iii) both amino acids Y₁₅ and Y₁₇ are sulfated.

12. A monoclonal antibody, or an antigen-binding portion thereof, which is capable of mediating ADCC and which specifically binds to an epitope on human C-C Motif Chemokine Receptor 8 (hCCR8), the sequence of which is set forth as SEQ ID NO: 1, wherein the epitope is located in the N-terminal domain of hCCR8 within a peptide spanning approximately amino acid residues 12 to 22 (V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂; SEQ ID NO: 109) as determined by X-ray crystallography, optionally wherein :
- (i) the epitope comprises at least one amino acid within a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109);
 - (ii) the epitope comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or all the amino acids within a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109);
 - (iii) the epitope comprises 11 of the amino acids in the peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109); or
 - (iv) the epitope consists of all 11 of the amino acids in the peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109).
13. The monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, wherein
- (i) the antibody or portion thereof, when bound to CCR8 on the surface of a cell, does not cause internalization of CCR8 either in the presence or absence of a cross-linking antibody; and/or
 - (ii) the antibody or portion thereof promotes depletion of human tumor-associated Tregs *in vitro*; and/or
 - (iii) the antibody or portion thereof specifically induces depletion of tumor Tregs without depleting CCR8⁺ T cells in non-tumor tissue, optionally wherein the non-tumor tissue is the skin, thymus, spleen or blood; and/or
 - (iv) the antibody or portion thereof inhibits growth of tumor cells in a subject when administered as monotherapy to the subject; and/or
 - (v) the antibody or portion thereof inhibits growth of tumor cells in a subject when administered to the subject in combination with an additional therapeutic agent for treating a cancer.
14. The monoclonal antibody or antigen-binding portion thereof of any one of the

preceding claims, which exhibits at least one, two, three, four, five or six of the following properties:

- (a) specifically binds to CCR8 expressed on the surface of a cell with an EC₅₀ of about 2 nM or lower;
 - 5 (b) binds specifically to rare and scattered immune cells in the medulla of the thymus and dermis of the skin but does not bind to human cerebrum, cerebellum, heart, liver, lung, kidney, tonsil, spleen, thymus, colon, stomach, pancreas, adrenal, pituitary, skin, peripheral nerve, testis or uterus tissue, or peripheral blood mononuclear cells (PBMCs);
 - 10 (c) inhibits binding of CCL1 to CCR8 and inhibits CCR8/CCL1 signaling with an IC₅₀ of about 5 nM or lower;
 - (d) when bound to CCR8 on the surface of a cell mediates depletion of the cell with an EC₅₀ of about 10 pM or lower;
 - (e) when bound to CCR8 on the surface of a cell does not cause
15 internalization of CCR8 either in the presence or absence of a cross-linking antibody;
 - (f) promotes depletion of human tumor-associated Tregs *in vitro* using the assay described in Example 22;
 - (g) promotes depletion of human tumor-associated Tregs in *ex-vivo* human
20 tumor slice samples using the assay described in Example 20;
 - (h) mediates depletion of tumor Tregs specifically while sparing CCR8⁺ T cells in non-tumor tissues;
 - (i) inhibits growth of tumor cells in a subject when administered as monotherapy to the subject; and
 - 25 (j) inhibits growth of tumor cells in a subject when administered to the subject in combination with an additional therapeutic agent for treating a cancer; optionally wherein the monoclonal antibody or antigen-binding portion thereof exhibits all of those properties:
15. The monoclonal antibody or antigen-binding portion thereof of claim 34 or 35,
30 which further binds to an epitope located in the N-terminal domain of human CCR8 with a K_D of about 10 nM or lower, wherein the epitope comprises a peptide having the sequence V₁₂T₁₃D₁₄Y₁₅Y₁₆Y₁₇P₁₈D₁₉I₂₀F₂₁S₂₂ (SEQ ID NO: 109) and sulfated tyrosine-15 and tyrosine-17 residues.

16. A modified anti-hCCR8 monoclonal antibody, or an antigen-binding portion thereof, which comprises a modified heavy chain constant region that binds with higher affinity to an Fc γ receptor (Fc γ R) and mediates at least 2, 5 or 10 times enhanced ADCC compared to the monoclonal antibody or antigen-binding portion thereof of claim 14 or 15.
17. The monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, which binds to the same epitope as does a reference antibody, wherein the reference antibody comprises:
- (a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 3 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 15;
 - (b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;
 - (c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 5 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 17;
 - (d) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;
 - (e) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 7 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 19;
 - (f) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 8 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 20;
 - (g) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 9 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 21;
 - (h) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 10 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 22;
 - (i) a V_H comprising consecutively linked amino acids having the sequence set

- forth as SEQ ID NO: 11 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 23;
- (j) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 12 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 24;
- (k) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 13 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 25;
- (l) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 14 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 26; or
- (m) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116.
18. The monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, which cross-competes for binding to hCCR8 with a reference antibody, wherein the reference antibody comprises:
- (a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 3 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 15;
- (b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;
- (c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 5 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 17;
- (d) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;
- (e) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 7 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 19;
- (f) a V_H comprising consecutively linked amino acids having the sequence set

- forth as SEQ ID NO: 8 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 20;
- (g) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 9 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 21;
- (h) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 10 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 22;
- (i) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 11 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 23;
- (j) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 12 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 24;
- (k) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 13 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 25;
- (l) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 14 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 26; or
- (m) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116.
19. The monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, which comprises the CDR1, CDR2 and CDR3 domains in each of:
- (a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 3 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 15;
- (b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;
- (c) a V_H comprising consecutively linked amino acids having the sequence set

- forth as SEQ ID NO: 5 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 17;
- (d) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;
- (e) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 7 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 19;
- (f) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 8 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 20;
- (g) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 9 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 21;
- (h) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 10 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 22;
- (i) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 11 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 23;
- (j) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 12 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 24;
- (k) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 13 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 25;
- (l) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 14 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 26; or
- (m) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116.

20. The monoclonal antibody or antigen-binding portion thereof of claim 19, which

comprises the following CDR domains as defined by the Kabat method:

- 5 (a) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 27; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 28; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 29; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 30; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 31; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 32;
- 10 (b) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 33; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 34; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 35; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 36; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 37; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 38;
- 15 (c) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 39; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 40; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 41; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 42; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 43; and a light chain variable region CDR3 comprising consecutively
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- linked amino acids having the sequence set forth as SEQ ID NO: 44;
- (d) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 45; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 46; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 47; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 48; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 49; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 50;
- (e) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 51; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 52; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 53; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 54; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 55; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 56;
- (f) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 57; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 58; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 59; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 60; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 61; and a light chain variable region CDR3 comprising consecutively

- linked amino acids having the sequence set forth as SEQ ID NO: 62;
- (g) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 63; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 64; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 65; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 66; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 67; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 68;
- (h) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 69; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 70; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 71; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 72; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 73; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 74;
- (i) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 75; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 76; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 77; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 78; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 79; and a light chain variable region CDR3 comprising consecutively

- linked amino acids having the sequence set forth as SEQ ID NO: 80;
- (j) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 81; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 82; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 83; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 84; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 85; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 86;
- (k) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 87; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 88; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 89; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 90; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 91; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 92;
- (l) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 93; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 94; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 95; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 96; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 97; and a light chain variable region CDR3 comprising consecutively

- linked amino acids having the sequence set forth as SEQ ID NO: 98; or
- (m) a heavy chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 103; a heavy chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 104; a heavy chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 105; a light chain variable region CDR1 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 106; a light chain variable region CDR2 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 107; and a light chain variable region CDR3 comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 108.
21. The monoclonal antibody or antigen-binding portion thereof of claim 19 or 20, which comprises:
- (a) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 3 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 15;
- (b) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 4 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 16;
- (c) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 5 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 17;
- (d) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 6 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 18;
- (e) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 7 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 19;
- (f) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 8 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 20;

- (g) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 9 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 21;
- 5 (h) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 10 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 22;
- (i) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 11 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 23;
- 10 (j) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 12 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 24;
- (k) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 13 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 25;
- 15 (l) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 14 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 26; or
- (m) a V_H comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 115 and a V_L comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 116.
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22. The monoclonal antibody or antigen-binding portion thereof of claim 20 or 21, which comprises:
- (a) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 99 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 111;
- 25 (b) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 100 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 112;
- 30 (c) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 101 and a light chain comprising

- consecutively linked amino acids having the sequence set forth as SEQ ID NO: 113;
- 5 (d) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 102 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 114;
- (e) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 117 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 118; or
- 10 (f) a heavy chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 110 and a light chain comprising consecutively linked amino acids having the sequence set forth as SEQ ID NO: 119.
- 15 23. The monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, which is a chimeric antibody, a humanized antibody, a human antibody, or a fragment thereof.
24. An immunoconjugate comprising the monoclonal antibody or antigen-binding portion thereof of any one of the preceding claims, linked to a cytolytic agent,
- 20 optionally wherein the cytolytic agent is a cytotoxin or a radioactive isotope.
25. A bispecific molecule comprising the monoclonal antibody or antigen-binding portion thereof of any one of claims 1-23, linked to a binding domain that has a different binding specificity than the monoclonal antibody or antigen binding portion thereof.
- 25 26. A composition comprising:
- (a) the monoclonal antibody or antigen-binding portion thereof of any one of claims 1-23;
- (b) the immunoconjugate of claim 24; or
- (c) the bispecific molecule of claim 25,
- 30 and a pharmaceutically acceptable carrier.

27. An isolated nucleic acid encoding the monoclonal antibody or antigen-binding portion thereof of any one of claims 1-23.
28. An expression vector comprising the nucleic acid of claim 27.
29. A host cell comprising the expression vector of claim 28.
- 5 30. A method for preparing an anti-CCR8 antibody or antigen-binding portion thereof which comprises expressing the antibody or antigen-binding portion thereof in the host cell of claim 29 and isolating the antibody or antigen-binding portion thereof from the host cell.
- 10 31. A method for treating a subject afflicted with a cancer, comprising administering to the subject a therapeutically effective amount of the monoclonal antibody or antigen-binding portion thereof of any one of claims 1-23, the immunoconjugate of claim 24, the bispecific molecule of claim 25, or the pharmaceutical composition of claim 26, such that the subject is treated.
- 15 32. A method for inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of the monoclonal antibody or antigen-binding portion thereof of any one of claims 1-23, the immunoconjugate of claim 24, the bispecific molecule of claim 25, or the pharmaceutical composition of claim 26, such that growth of tumor cells in the subject is inhibited.
- 20 33. The method of claim 31 or 32, wherein the method further comprises administering to the subject a therapeutically effective amount of an additional therapeutic agent for treating a cancer, optionally wherein the additional therapeutic agent is a compound that reduces inhibition, or increases stimulation, of the immune system.
- 25 34. The method of any one of claims 31-33, wherein the subject is a human.
35. The method of any one of claims 31-34, wherein:
- (a) the cancer is a solid tumor, optionally wherein the solid tumor is:
- (i) a cancer chosen from squamous cell carcinoma, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), squamous NSCLC,

- non-squamous NSCLC, head and neck cancer, breast cancer, cancer of the esophagus, gastric cancer, gastrointestinal cancer, cancer of the small intestine, liver cancer, hepatocellular carcinoma (HCC), pancreatic cancer (PAC), kidney cancer, renal cell carcinoma (RCC), bladder cancer, cancer of the urethra, cancer of the ureter, colorectal cancer (CRC), colon cancer, colon carcinoma, cancer of the anal region, endometrial cancer, prostate cancer, a fibrosarcoma, neuroblastoma, glioma, glioblastoma, germ cell tumor, pediatric sarcoma, sinonasal natural killer, melanoma, skin cancer, bone cancer, cervical cancer, uterine cancer, carcinoma of the endometrium, carcinoma of the fallopian tubes, ovarian cancer, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, testicular cancer, cancer of the endocrine system, thyroid cancer, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the penis, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain cancer, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, solid tumors of childhood, environmentally-induced cancers, virus-related cancers, cancers of viral origin, advanced cancer, unresectable cancer, metastatic cancer, refractory cancer, recurrent cancer, and any combination thereof;
- or
- (ii) a cancer chosen from non-small cell lung cancer (NSCLC), squamous cell carcinoma of the head and neck (SCCHN), colorectal cancer (CRC), gastric cancer, gastroesophageal (GE) junction cancer, and cervical cancer; or
- (iii) a cancer chosen from head and neck squamous cell carcinoma (HNSC), lung adenocarcinoma (LUAD), stomach adenocarcinoma (STAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), esophageal carcinoma (ESCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD) and cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC); or
- (iv) a cancer chosen from squamous cell carcinoma of the head and

- neck (SCCHN), cervical cancer, colorectal cancer (CRC), non-small cell lung cancer-squamous cell carcinoma (NSCLC-SCC), NSCLC-adenocarcinoma (NSCLC-ADC), pancreatic cancer, gastric cancer, bladder cancer and breast cancer; or
- 5 (v) a cancer chosen from colon adenocarcinoma, bladder carcinoma, mammary carcinoma, and fibrosarcoma; or
- (b) the cancer is a hematological malignancy or the tumor cells are cells of a hematological malignancy, optionally wherein the hematological malignancy is selected from acute lymphoblastic leukemia (ALL), acute
- 10 myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), a T cell lymphoma, Hodgkin's lymphoma (HL), non-Hodgkin's lymphomas (NHLs), multiple myeloma, smoldering myeloma, monoclonal gammopathy of undetermined significance (MGUS), advanced, metastatic, refractory and/or recurrent
- 15 hematological malignancies, and any combinations of said hematological malignancies.
36. The method of claim 31, wherein the additional therapeutic agent is:
- (a) an antagonistic agent that binds specifically to Programmed Death-1 (PD-1), Programmed Death Ligand-1 (PD-L1), Cytotoxic T-Lymphocyte
- 20 Antigen-4 (CTLA-4), Lymphocyte Activation Gene-3 (LAG-3), B and T lymphocyte Attenuator (BTLA), T cell Immunoglobulin and Mucin domain-3 (TIM-3), Killer Immunoglobulin-like Receptor (KIR), Killer cell Lectin-like Receptor G1 (KLRG-1), Adenosine A2a Receptor (A2aR), T Cell Immunoreceptor with Ig and ITIM Domains (TIGIT), V-domain Ig
- 25 Suppressor of T cell activation (VISTA), proto-oncogene tyrosine-protein kinase MER (MerTK), Natural Killer Cell Receptor 2B4 (CD244), or CD160; or
- (b) an agonistic agent that binds specifically to Inducible T cell Co-Stimulator (ICOS), CD137 (4-1BB), CD134 (OX40), CD27, Glucocorticoid-Induced
- 30 TNFR-Related protein (GITR), or HerpesVirus Entry Mediator (HVEM); and optionally wherein:
- (i) the antagonistic agent that binds specifically to PD-1 is chosen from nivolumab, pembrolizumab, cemiplimab, spartalizumab,

camrelizumab, sintilimab, tislelizumab and toripalimab; or

(ii) the antagonistic agent that binds specifically to PD-L1 is chosen from atezolizumab, durvalumab, avelumab, envafolelimab, BMS-936559, CK-301, CS-1001, SHR-1316, CBT-502, and BGB-A333; or

5 (iii) the antagonistic agent that binds specifically to CTLA-4 is ipilimumab or tremelimumab.

37. A method for potentiating an anti-tumor immune response elicited by a therapeutic agent in a subject afflicted with a cancer, comprising administering to the subject a therapeutically effective amount of the monoclonal antibody or antigen-binding portion thereof of any one of claims 1-23, the immunoconjugate of claim 24, the bispecific molecule of claim 25, or the pharmaceutical composition of claim 26, such that the subject experiences a stronger immune response against the cancer compared to the immune response elicited by the therapeutic agent alone.

15 38. The method of claim 37, wherein the therapeutic agent is an immune checkpoint inhibitor or radiotherapy, optionally wherein the checkpoint inhibitor is an anti-PD-1, anti-PD-L1 or anti-CTLA-4 antibody.

39. A kit for treating a subject afflicted with a cancer, the kit comprising:

20 (a) one or more dosages ranging from about 0.01 to about 20 mg/kg body weight of a monoclonal antibody or an antigen-binding portion thereof that binds specifically to C-C Motif Chemokine Receptor 8 (CCR8) and mediates depletion of the CCR8-expressing cell by ADCC;

(b) optionally one or more dosages ranging from about 200 to about 1600 mg of a monoclonal antibody or an antigen-binding portion thereof that binds specifically to PD-1, PD-L1 or CTLA-4; and

25 (c) instructions for using the monoclonal antibody or portion thereof that binds specifically to CCR8, and optionally the monoclonal antibody or portion thereof that binds specifically to PD-1, PD-L1 or CTLA-4, in the method of any one of claims 31-36.

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