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(54) Title: ANTI-CCR8 MONOCLONAL ANTIBODIES AND USES THEREOF

(57) Abstract: Provided are antibodies or fragment thereof having binding specificity to the human chemokine (C-C motif) receptor 8 (CCR8) protein. These antibodies are capable of binding to CCR8 at high affinity and can mediate antibody-dependent cellular cytotoxicity (ADCC).

ANTI-CCR8 MONOCLONAL ANTIBODIES AND USES THEREOF

BACKGROUND

[0001] Chemokine (C-C motif) receptor 8 (CCR8) is a member of the beta chemokine receptor family, and is a seven transmembrane protein similar to G protein-coupled receptors. Chemokines and their receptors are important for the migration of various cell types into the inflammatory sites. This receptor protein preferentially expresses in the thymus. The ligand of the CCR8 is CCL1. CCL8 also functions as a CCR8 agonist.

[0002] CCR8 is expressed principally on regulatory T cells (Treg) and is important for $CCR8^+$ Treg-mediated immunosuppression. Recent studies have demonstrated that CCR8 is uniquely upregulated in human tumor-resident Tregs of cancer patients. It was also demonstrated that $CCR8^+$ myeloid cells were expanded in patients with cancer.

[0003] Antibodies targeting CCR8 have been shown to significantly suppress tumor growth and improve long-term survival in animal models. This antitumor activity correlated with increased tumor specific T cells, and enhanced infiltration of $CD4^+$ and $CD8^+$ T cells. Treatment with the antibodies prevented induction and suppressive function of Tregs without affecting $CD8^+$ T cells. Targeting CCR8, therefore, is a promising cancer immunotherapy approach.

SUMMARY

[0004] Anti-CCR8 antibodies are discovered herein that have high binding affinity to the human CCR8 protein, and are efficient in mediating antibody-dependent cellular cytotoxicity (ADCC).

[0005] In one embodiment, an antibody or fragment thereof is provided, having binding specificity to a human chemokine (C-C motif) receptor 8 (CCR8) protein. The antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 22, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 23, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 24, the CDRL1 comprises the

amino acid sequence of SEQ ID NO: 25 or 28, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27.

[0006] In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 35, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 36, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 37, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25 or 28, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27.

[0007] Also provided, in some embodiments, are compositions comprising the antibody or fragment thereof and a pharmaceutically acceptable carrier. In some embodiments, the composition further comprises a second antibody having specificity to a tumor antigen. In some embodiments, the second antibody is a tumor-opsonizing antibody.

[0008] Methods and uses for the treatment of diseases and conditions are also provided. In one embodiment, provided is a method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. 1 shows the CCR8-binding affinity of the chimeric antibodies with murine VH/VL.

[0010] FIG. 2 shows the ADCC activity of three of the chimeric antibodies.

[0011] FIG. 3 shows that humanized 88D2C6 antibodies have high affinities.

[0012] FIG. 4 shows that humanized 137D1H10 antibodies have high affinities.

[0013] FIG. 5 shows that, compared to the chimeric antibodies, the humanized antibodies have significantly higher ADCC activities.

[0014] FIG. 6 shows the results of cell-based binding of LM-108 and reference antibody to human CCR8 by flow cytometry. Upper panel: FACS binding of LM-108 on human CCR8 expressing HEK293. Lower panel: FACS binding of LM-108 on HEK293 cells.

[0015] FIG. 7 shows cell-based binding of LM-108 to human CCR8 high expressing U2OS cells by flow cytometry.

[0016] FIG. 8 shows cell-based binding of LM-108 to human CCR8 low expressing Jurkat cells by flow cytometry.

[0017] FIG. 9 shows the results of ADCC Reporter Gene Assay of LM-108 with Jurkat/CD16a(158v)/NFAT-luc cells, targeting CCR8 high expressing U2OS cells.

[0018] FIG. 10 shows the results of ADCC Reporter Gene Assay of LM-108 with Jurkat/CD16a(158v)/NFAT cells, targeting CCR8 low expressing Jurkat cells.

[0019] FIG. 11 shows the results of ADCC testing of LM-108 with hPBMCs targeting CCR8-expressing cells (upper panel) or control cells (lower panel).

[0020] FIG. 12 shows ADCP of LM-108 with Monocyte-derived Macrophage by FACS. Upper panel: ADCP of LM-108 LM-108 towards human CCR8 over expressing CHO-K1 cells. Lower panel: ADCP of LM-108 LM-108 towards control CHO-K1 cells.

[0021] FIG. 13 presents imaging results of ADCP of LM-108 with Monocyte-derived Macrophage by Operetta.

[0022] FIG. 14 shows tumor growth curves of CT26 tumor-bearing mice post administration of LM-108m and anti-mPD-1 Ab, in an *in vivo* efficacy study of LM-108m on CT26 syngeneic model with BALB/c mice.

[0023] FIG. 15 shows tumor growth curves of MC38 tumor-bearing mice post administration of LM-108m and vehicle, in an *in vivo* efficacy study of LM-108 on MC38 syngeneic model with hCCR8 ki mice.

[0024] FIG. 16 shows the results of cytotoxicity evaluation of LM-108-vc-MMAE on hCCR8 expressing cells.

DETAILED DESCRIPTION

Definitions

[0025] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "an antibody," is understood to represent one or more antibodies. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0026] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" may be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non- naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

[0027] "Homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present disclosure.

[0028] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %,

98 % or 99 %) of "sequence identity" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences.

[0029] The term "an equivalent nucleic acid or polynucleotide" refers to a nucleic acid having a nucleotide sequence having a certain degree of homology, or sequence identity, with the nucleotide sequence of the nucleic acid or complement thereof. A homolog of a double stranded nucleic acid is intended to include nucleic acids having a nucleotide sequence which has a certain degree of homology with or with the complement thereof. In one aspect, homologs of nucleic acids are capable of hybridizing to the nucleic acid or complement thereof. Likewise, "an equivalent polypeptide" refers to a polypeptide having a certain degree of homology, or sequence identity, with the amino acid sequence of a reference polypeptide. In some aspects, the sequence identity is at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%. In some aspects, the equivalent polypeptide or polynucleotide has one, two, three, four or five addition, deletion, substitution and their combinations thereof as compared to the reference polypeptide or polynucleotide. In some aspects, the equivalent sequence retains the activity (*e.g.*, epitope-binding) or structure (*e.g.*, salt-bridge) of the reference sequence.

[0030] As used herein, an "antibody" or "antigen-binding polypeptide" refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof. Thus the term "antibody" includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

[0031] The terms "antibody fragment" or "antigen-binding fragment", as used herein, is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term "antibody fragment" includes aptamers, spiegelmers, and diabodies. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

[0032] A "single-chain variable fragment" or "scFv" refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins. In some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

[0033] The term antibody encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ l- γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgG_5, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

[0034] Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti- idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to LIGHT antibodies disclosed herein). Immunoglobulin or antibody molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA,

and IgY), class (e.g., IgGl, IgG2, IgG3, IgG4, IgAl and IgA2) or subclass of immunoglobulin molecule.

[0035] Light chains are classified as either kappa or lambda (K, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0036] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VK) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CK) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino- terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CK domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0037] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VK domain and VH domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three-dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and VK chains (*i.e.* CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3). In some instances, *e.g.*, certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. *See, e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993).

[0038] In naturally occurring antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (*see* "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, 196:901-917 (1987)).

[0039] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference in their entireties. The CDR definitions according to Kabat and Chothia include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth in the table below as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can

routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

	Kabat	Chothia
CDR-H1	31-35	26-32
CDR-H2	50-65	52-58
CDR-H3	95-102	95-102
CDR-L1	24-34	26-32
CDR-L2	50-56	50-52
CDR-L3	89-97	91-96

[0040] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983).

[0041] In addition to table above, the Kabat number system describes the CDR regions as follows: CDR-H1 begins at approximately amino acid 31 (*i.e.*, approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tryptophan residue. CDR-H2 begins at the fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino acid. CDR-L1 begins at approximately residue 24 (*i.e.*, following a cysteine residue); includes approximately 10-17 residues; and ends at the next tryptophan residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2 (*i.e.*, following a cysteine residue); includes approximately 7-11 residues and ends at the sequence F or W-G-X-G, where X is any amino acid.

[0042] Antibodies disclosed herein may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be condricthoid in origin (*e.g.*, from sharks).

[0043] As used herein, the term "heavy chain constant region" includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy

chain constant region comprises at least one of: a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, an antibody for use in the disclosure may lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that the heavy chain constant region may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0044] The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a polypeptide may comprise a CH1 domain derived from an IgG_1 molecule and a hinge region derived from an IgG_3 molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG_1 molecule and, in part, from an IgG_3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG_1 molecule and, in part, from an IgG_4 molecule.

[0045] As used herein, the term "light chain constant region" includes amino acid sequences derived from antibody light chain. Preferably, the light chain constant region comprises at least one of a constant kappa domain or constant lambda domain.

[0046] A "light chain-heavy chain pair" refers to the collection of a light chain and heavy chain that can form a dimer through a disulfide bond between the CL domain of the light chain and the CH1 domain of the heavy chain.

[0047] As previously indicated, the subunit structures and three-dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term "VH domain" includes the amino terminal variable domain of an immunoglobulin heavy chain and the term "CH1 domain" includes the first (most amino terminal) constant

region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

[0048] As used herein the term "CH2 domain" includes the portion of a heavy chain molecule that extends, *e.g.*, from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; *see* Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

[0049] As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.*, *J. Immunol* 161:4083 (1998)).

[0050] As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CK regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[0051] As used herein, the term "chimeric antibody" will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0052] As used herein, "percent humanization" is calculated by determining the number of framework amino acid differences (*i.e.*, non-CDR difference) between the humanized domain

and the germline domain, subtracting that number from the total number of amino acids, and then dividing that by the total number of amino acids and multiplying by 100.

[0053] By "specifically binds" or "has specificity to," it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0054] As used herein, the terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0055] By "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on.

[0056] As used herein, phrases such as "to a patient in need of treatment" or "a subject in need of treatment" includes subjects, such as mammalian subjects, that would benefit from

administration of an antibody or composition of the present disclosure used, *e.g.*, for detection, for a diagnostic procedure and/or for treatment.

Anti-CCR8 Antibodies

[0057] The present disclosure provides anti-CCR8 antibodies and fragments that have high affinity to the human CCR8 protein, are potent in mediating ADCC and ADCP, and showed potent *in vivo* tumor inhibitor efficacy. Interesting, when using an anti-CCR8 antibody (from WO2020138489) as a benchmark, the instantly disclosed antibodies exhibited no binding to non-CCR8-expressing cells, while the benchmark antibody bound to those control cells as well. The present antibodies, therefore, can be expected to have less undesired adverse effects in clinical uses. These antibodies, therefore, are suitable agents for treating various diseases featuring overexpressed CCR8, such as cancer.

[0058] In accordance with one embodiment of the present disclosure, therefore, provided are antibodies and antigen-binding fragments thereof that are able to bind to CCR8. Example antibodies include those murine ones listed in **Table 1** (*e.g.*, 137D1H10, 88D2C6, 89B6F8, 132H8E10, 10F11B2, 40H10F3, 53D6A9, 352H11C11, 362G7D3, 362H10A3, 367D10E7, 370D2C10**)**, as well as humanized ones of **Tables 2-3**. Also included are those that include the same CDRs as illustrated herein. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as those illustrated here, and those that compete with the instantly disclosed in binding to CCR8.

[0059] In accordance with one embodiment of the present disclosure, provided is an antibody or fragment thereof that includes the heavy chain and light chain variable domains with the CDR regions disclosed herein, as well as their biological equivalents.

[0060] In one embodiment, the CDRs are those of 88D2C6 or its humanized counterparts, as exemplified in **Tables 2B and 2D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 22 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 23 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 24 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of

SEQ ID NO: 25 or 28 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0061] In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 22 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 23 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 24 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0062] In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 22, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 23, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 24, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25 or 28, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27.

[0063] Also provided, in some embodiments, are those that include the same CDRs as 88D2C6 or its humanized counterparts. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as 88D2C6 or its humanized counterparts, and those that compete with any of them in binding to CCR8.

[0064] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:3 (mouse or chimeric) and 29-31 (humanized), or a peptide having at least 90% sequence identity to an amino acid sequence

selected from the group consisting of SEQ ID NO:3 (mouse or chimeric) and 29-31 (humanized).

[0065] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 (mouse or chimeric), 32-34 and 41-43 (humanized), or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO (mouse or chimeric): 2, 32-34 and 41-43 (humanized).

[0066] In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:29 and the light chain variable region comprises the amino acid sequence of any one of SEQ ID NO: 32-34 and 41-43. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:30 and the light chain variable region comprises the amino acid sequence of any one of SEQ ID NO: 32-34 and 41-43. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:31 and the light chain variable region comprises the amino acid sequence of any one of SEQ ID NO: 32-34 and 41-43. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:29 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:33.

[0067] In one embodiment, the CDRs are those of 137D1H10 or its humanized counterparts, as exemplified in **Tables 3B and 3D**. In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 35 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 36 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 37 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25 or 28 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0068] In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 35 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 36 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 37 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0069] In one embodiment, the CDRH1 comprises the amino acid sequence of SEQ ID NO: 35, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 36, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 37, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25 or 28, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27.

[0070] Also provided, in some embodiments, are those that include the same CDRs as 137D1H10 or its humanized counterparts. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as 137D1H10 or its humanized counterparts, and those that compete with any of them in binding to CCR8.

[0071] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1 (mouse or chimeric) and 38-40 (humanized), or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:3 (mouse or chimeric) and 29-31 (humanized).

[0072] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 (mouse or chimeric), 32-34 and 41-43 (humanized), or a peptide having at least 90% sequence identity to an amino acid

sequence selected from the group consisting of SEQ ID NO (mouse or chimeric): 2, 32-34 and 41-43 (humanized).

[0073] In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:38 and the light chain variable region comprises the amino acid sequence of any one of SEQ ID NO: 32-34 and 41-43. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:39 and the light chain variable region comprises the amino acid sequence of any one of SEQ ID NO: 32-34 and 41-43. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:40 and the light chain variable region comprises the amino acid sequence of any one of SEQ ID NO: 32-34 and 41-43. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:40 and the light chain variable region comprises the amino acid sequence of any one of SEQ ID NO:42.

[0074] The antibodies that contained these CDR regions, whether mouse, humanized or chimeric, had potent CCR8 binding and inhibitory activities. As shown in **Example 4**, certain residues within the CDR can be modified to retain or improve the property or reduce their potential to have post-translational modifications (PTMs). Such modified CDR can be referred to as affinity matured or de-risked CDRs (*e.g.*, SEQ ID NO:25).

[0075] Modified CDRs can include those having one, two or three amino acid addition, deletion and/or substitutions. In some embodiments, the substitutions can be conservative substitutions.

[0076] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another

embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0077] Non-limiting examples of conservative amino acid substitutions are provided in the table below, where a similarity score of 0 or higher indicates conservative substitution between the two amino acids.

Table A. Amino Acid Similarity Matrix

	C	G	P	S	A	T	D	E	N	Q	H	K	R	V	M	I	L	F	Y	W
W	-8	-7	-6	-2	-6	-5	-7	-7	-4	-5	-3	-3	2	-6	-4	-5	-2	0	0	17
Y	0	-5	-5	-3	-3	-3	-4	-4	-2	-4	0	-4	-5	-2	-2	-1	-1	7	10	
F	-4	-5	-5	-3	-4	-3	-6	-5	-4	-5	-2	-5	-4	-1	0	1	2	9		
L	-6	-4	-3	-3	-2	-2	-4	-3	-3	-2	-2	-3	-3	2	4	2	6			
I	-2	-3	-2	-1	-1	0	-2	-2	-2	-2	-2	-2	-2	4	2	5				
M	-5	-3	-2	-2	-1	-1	-3	-2	0	-1	-2	0	0	2	6					
V	-2	-1	-1	-1	0	0	-2	-2	-2	-2	-2	-2	-2	4						
R	-4	-3	0	0	-2	-1	-1	-1	0	1	2	3	6							
K	-5	-2	-1	0	-1	0	0	0	1	1	0	5								
H	-3	-2	0	-1	-1	-1	1	1	2	3	6									
Q	-5	-1	0	-1	0	-1	2	2	1	4										
N	-4	0	-1	1	0	0	2	1	2											
E	-5	0	-1	0	0	0	3	4												
D	-5	1	-1	0	0	0	4													
T	-2	0	0	1	1	3														
A	-2	1	1	1	2															
S	0	1	1	1																
P	-3	-1	6																	
G	-3	5																		
C	12																			

Table B. Conservative Amino Acid Substitutions

For Amino Acid	**Substitution With**
Alanine	D-Ala, Gly, Aib, β-Ala, L-Cys, D-Cys
Arginine	D-Arg, Lys, D-Lys, Orn D-Orn
Asparagine	D-Asn, Asp, D-Asp, Glu, D-Glu Gln, D-Gln
Aspartic Acid	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr, L-Ser, D-Ser
Glutamine	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	Ala, D-Ala, Pro, D-Pro, Aib, β-Ala
Isoleucine	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile
Lysine	D-Lys, Arg, D-Arg, Orn, D-Orn
Methionine	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val

Phenylalanine	D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp
Proline	D-Pro
Serine	D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys
Threonine	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val
Tyrosine	D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp
Valine	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

[0078] It will also be understood by one of ordinary skill in the art that antibodies as disclosed herein may be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein may be similar, *e.g.*, have a certain percent identity to the starting sequence, *e.g.*, it may be 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the starting sequence.

[0079] In certain embodiments, the antibody comprises an amino acid sequence or one or more moieties not normally associated with an antibody. Exemplary modifications are described in more detail below. For example, an antibody of the disclosure may comprise a flexible linker sequence, or may be modified to add a functional moiety (*e.g.*, PEG, a drug, a toxin, or a label).

[0080] Antibodies, variants, or derivatives thereof of the disclosure include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the epitope. For example, but not by way of limitation, the antibodies can be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

[0081] In some embodiments, the antibodies may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

[0082] The antibodies may be conjugated or fused to a therapeutic agent, which may include detectable labels such as radioactive labels, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic or diagnostic agent, a cytotoxic agent, which may be a drug or a toxin, an ultrasound enhancing agent, a non-radioactive label, a combination thereof and other such agents known in the art.

[0083] The antibodies can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antigen-binding polypeptide is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0084] The antibodies can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA). Techniques for conjugating various moieties to an antibody are well known, *see, e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. (1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.*, (eds.), Marcel Dekker, Inc., pp. 623- 53 (1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), Academic Press pp. 303-16 (1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* (52:119-58 (1982)).

Bi-functional Molecules and Combination Therapies

[0085] CCR8 is a chemokine and a tumor antigen. As a tumor antigen targeting molecule, an antibody or antigen-binding fragment specific to CCR8 can be combined with a second antigen-binding fragment specific to an immune cell, or an antigen-binding fragment specific to an immune checkpoint to generate a combination therapy or a bispecific antibody.

[0086] In some embodiments, the immune cell is selected from the group consisting of a T cell, a B cell, a monocyte, a macrophage, a neutrophil, a dendritic cell, a phagocyte, a natural killer cell, an eosinophil, a basophil, and a mast cell. Molecules on the immune cell which can be targeted include, for example, CCL1, CD3, CD16, CD19, CD28, and CD64. Other examples include PD-1,PD-L1, CTLA-4, LAG-3 (also known as CD223), CD28, CD122, 4-1BB (also known as CD137), TIM3, OX-40 or OX40L, CD40 or CD40L, LIGHT, ICOS/ICOSL, GITR/GITRL, TIGIT, CD27, VISTA, B7H3, B7H4, HEVM or BTLA (also known as CD272), killer-cell immunoglobulin-like receptors (KIRs), and CD47.

[0087] As an immune checkpoint inhibitor, an antibody or antigen-binding fragment specific to CCR8 can be combined with a second antigen-binding fragment specific to a tumor antigen to generate a bispecific antibody. A "tumor antigen" is an antigenic substance produced in tumor cells, *i.e.*, it triggers an immune response in the host. Tumor antigens are useful in identifying tumor cells and are potential candidates for use in cancer therapy. Normal proteins in the body are not antigenic. Certain proteins, however, are produced or overexpressed during tumorigenesis and thus appear "foreign" to the body. This may include normal proteins that are well sequestered from the immune system, proteins that are normally produced in extremely small quantities, proteins that are normally produced only in certain stages of development, or proteins whose structure is modified due to mutation.

[0088] An abundance of tumor antigens are known in the art and new tumor antigens can be readily identified by screening. Non-limiting examples of tumor antigens include EGFR, Her2, EpCAM, CD20, CD30, CD33, CD47, CD52, CD133, CD73, CEA, gpA33, Mucins, TAG-72, CIX, PSMA, folate-binding protein, GD2, GD3, GM2, VEGF, VEGFR, Integrin, $\alpha V\beta 3$, $\alpha 5\beta 1$, ERBB2, ERBB3, MET, IGF1R, EPHA3, TRAILR1, TRAILR2, RANKL, FAP and Tenascin.

[0089] In some aspects, the monovalent unit has specificity to a protein that is overexpressed on a tumor cell as compared to a corresponding non-tumor cell. A "corresponding non-tumor cell" as used here, refers to a non-tumor cell that is of the same cell type as the origin of the tumor cell. It is noted that such proteins are not necessarily different from tumor antigens. Non-limiting examples include carcinoembryonic antigen (CEA), which is overexpressed in most colon, rectum, breast, lung, pancreas and gastrointestinal tract carcinomas; heregulin receptors (HER-2, *neu* or c-*erbB*-2), which is frequently overexpressed in breast, ovarian, colon, lung, prostate and cervical cancers; epidermal growth factor receptor (EGFR), which is

highly expressed in a range of solid tumors including those of the breast, head and neck, non-small cell lung and prostate; asialoglycoprotein receptor; transferrin receptor; serpin enzyme complex receptor, which is expressed on hepatocytes; fibroblast growth factor receptor (FGFR), which is overexpressed on pancreatic ductal adenocarcinoma cells; vascular endothelial growth factor receptor (VEGFR), for anti-angiogenesis gene therapy; folate receptor, which is selectively overexpressed in 90% of nonmucinous ovarian carcinomas; cell surface glycocalyx; carbohydrate receptors; and polymeric immunoglobulin receptor, which is useful for gene delivery to respiratory epithelial cells and attractive for treatment of lung diseases such as Cystic Fibrosis.

[0090] Different format of bispecific antibodies are also provided. In some embodiments, each of the anti-PD-L1 fragment and the second fragment each is independently selected from a Fab fragment, a single-chain variable fragment (scFv), or a single-domain antibody. In some embodiments, the bispecific antibody further includes a Fc fragment.

[0091] Bifunctional molecules that include not just antibody or antigen binding fragment are also provided. As a tumor antigen targeting molecule, an antibody or antigen-binding fragment specific to CCR8, such as those described here, can be combined with an immune cytokine or ligand optionally through a peptide linker. The linked immune cytokines or ligands include, but not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, GM-CSF, TNF-α, CD40L, OX40L, CD27L, CD30L, 4-1BBL, LIGHT and GITRL. Such bi-functional molecules can combine the immune checkpoint blocking effect with tumor site local immune modulation.

Antibody-Drug Conjugates

[0092] In some embodiments, the antibodies or fragments may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

[0093] In one embodiment, the antibodies or fragments of the disclosure are covalently attached to a drug moiety. The drug moiety may be, or be modified to include, a group reactive with a conjugation point on the antibody. For example, a drug moiety can be attached by alkylation (e.g., at the epsilon-amino group lysines or the N-terminus of antibodies), reductive amination of oxidized carbohydrate, transesterification between

hydroxyl and carboxyl groups, amidation at amino groups or carboxyl groups, and conjugation to thiols.

[0094] In some embodiments, the number of drug moieties, p, conjugated per antibody molecule ranges from an average of 1 to 8; 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2. In some embodiments, p ranges from an average of 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4 or 2 to 3. In other embodiments, p is an average of 1, 2, 3, 4, 5, 6, 7 or 8. In some embodiments, p ranges from an average of about 1 to about 20, about 1 to about 10, about 2 to about 10, about 2 to about 9, about 1 to about 8, about 1 to about 7, about 1 to about 6, about 1 to about 5, about 1 to about 4, about 1 to about 3, or about 1 to about 2. In some embodiments, p ranges from about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4 or about 2 to about 3.

[0095] For example, when chemical activation of the protein results in formation of free thiol groups, the protein may be conjugated with a sulfhydryl reactive agent. In one aspect, the agent is one which is substantially specific for free thiol groups. Such agents include, for example, malemide, haloacetamides (e.g., iodo, bromo or chloro), haloesters (e.g., iodo, bromo or chloro), halomethyl ketones (e.g., iodo, bromo or chloro), benzylic halides (e.g., iodide, bromide or chloride), vinyl sulfone and pyridylthio.

[0096] The drug can be linked to the antibody or fragment by a linker. Suitable linkers include, for example, cleavable and non-cleavable linkers. A cleavable linker is typically susceptible to cleavage under intracellular conditions. Suitable cleavable linkers include, for example, a peptide linker cleavable by an intracellular protease, such as lysosomal protease or an endosomal protease. In exemplary embodiments, the linker can be a dipeptide linker, such as a valine-citrulline (val-cit), a phenylalanine-lysine (phe-lys) linker, or maleimidocapronic-valine-citruline-p-aminobenzyloxycarbonyl (mc-Val-Cit-PABA) linker. Another linker is Sulfosuccinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate (smcc). Sulfo-smcc conjugation occurs via a maleimide group which reacts with sulfhydryls (thiols, —SH), while its Sulfo-NHS ester is reactive toward primary amines (as found in Lysine and the protein or peptide N-terminus). Yet another linker is maleimidocaproyl (mc). Other suitable linkers include linkers hydrolyzable at a specific pH or a pH range, such as a hydrazone linker. Additional suitable cleavable linkers include disulfide linkers. The linker may be covalently bound to the antibody to such an extent that the antibody must be degraded intracellularly in order for the drug to be released e.g. the mc linker and the like.

[0097] A linker can include a group for linkage to the antibody. For example, linker can include an amino, hydroxyl, carboxyl or sulfhydryl reactive groups (e.g., malemide, haloacetamides (e.g., iodo, bromo or chloro), haloesters (e.g., iodo, bromo or chloro), halomethyl ketones (e.g., iodo, bromo or chloro), benzylic halides (e.g., iodide, bromide or chloride), vinyl sulfone and pyridylthio).

[0098] In some embodiments, the drug moiety is a cytotoxic or cytostatic agent, an immunosuppressive agent, a radioisotope, a toxin, or the like. The conjugate can be used for inhibiting the multiplication of a tumor cell or cancer cell, causing apoptosis in a tumor or cancer cell, or for treating cancer in a patient. The conjugate can be used accordingly in a variety of settings for the treatment of animal cancers. The conjugate can be used to deliver a drug to a tumor cell or cancer cell. Without being bound by theory, in some embodiments, the conjugate binds to or associates with a cancer cell expressing claudin 18.2, and the conjugate and/or drug can be taken up inside a tumor cell or cancer cell through receptor-mediated endocytosis.

[0099] Once inside the cell, one or more specific peptide sequences within the conjugate (e.g., in a linker) are hydrolytically cleaved by one or more tumor-cell or cancer-cell-associated proteases, resulting in release of the drug. The released drug is then free to migrate within the cell and induce cytotoxic or cytostatic or other activities. In some embodiments, the drug is cleaved from the antibody outside the tumor cell or cancer cell, and the drug subsequently penetrates the cell, or acts at the cell surface.

[0100] Examples of drug moieties or payloads are selected from the group consisting of DM1 (maytansine, N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)- or N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)-maytansine), mc-MMAD (6-maleimidocaproyl-monomethylauristatin-D or N-methyl-L-valyl-N-[(1S,2R)-2-methoxy-4-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[[(1S)-2-phenyl-1-(2-thiazolyl)ethyl]amino]propyl]-1-pyrrolidinyl]-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-(9Cl)-L-valinamide), mc-MMAF (maleimidocaproyl-monomethylauristatin F or N-[6-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-1-oxohexyl]-N-methyl-L-valyl-L-valyl-(3R,4S,5S)-3-methoxy-5-methyl-4-(methylamino)heptanoyl-(αR, βR,2S)-β-methoxy-α-methyl-2-pyrrolidinepropanoyl-L-phenylalanine) and mc-Val-Cit-PABA-MMAE (6-maleimidocaproyl-ValcCit-(p-aminobenzyloxycarbonyl)-monomethylauristatin E or N-[[[4-[[N-[6-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-1-oxohexyl]-L-valyl-N5-(aminocarbonyl)-L-

ornithyl]amino]phenyl]methoxy]carbonyl]-N-meth yl-L-valyl-N-[(1S,2R)-4-[(2S)-2-[(1R,2R)-3-[[(1R,2S)-2-hydroxy-1-methyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]-1-pyrrolidinyl] -2-methoxy-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-L-valinamide). DM1 is a derivative of the tubulin inhibitor maytansine while MMAD, MMAE, and MMAF are auristatin derivatives. In some embodiments, the drug moiety is selected from the group consisting of mc-MMAF and mc-Val-Cit-PABA-MMAE. In some embodiments, the drug moiety is a maytansinoid or an auristatin.

[0101] The antibodies or fragments may be conjugated or fused to a therapeutic agent, which may include detectable labels such as radioactive labels, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic or diagnostic agent, a cytotoxic agent, which may be a drug or a toxin, an ultrasound enhancing agent, a non-radioactive label, a combination thereof and other such agents known in the art.

[0102] The antibodies can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antigen-binding polypeptide is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0103] The antibodies can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA). Techniques for conjugating various moieties to an antibody are well known, *see, e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. (1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.*, (eds.), Marcel Dekker, Inc., pp. 623- 53 (1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.),

Academic Press pp. 303-16 (1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* (52:119-58 (1982)).

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

[0104] The present disclosure also provides isolated polynucleotides or nucleic acid molecules encoding the antibodies, variants or derivatives thereof of the disclosure. The polynucleotides of the present disclosure may encode the entire heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules. Additionally, the polynucleotides of the present disclosure may encode portions of the heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules.

[0105] Methods of making antibodies are well known in the art and described herein. In certain embodiments, both the variable and constant regions of the antigen-binding polypeptides of the present disclosure are fully human. Fully human antibodies can be made using techniques described in the art and as described herein. For example, fully human antibodies against a specific antigen can be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Exemplary techniques that can be used to make such antibodies are described in U.S. patents: 6,150,584; 6,458,592; 6,420,140 which are incorporated by reference in their entireties.

Treatment Methods

[0106] As described herein, the antibodies, variants or derivatives of the present disclosure may be used in certain treatment and diagnostic methods.

[0107] The present disclosure is further directed to antibody-based therapies which involve administering the antibodies of the disclosure to a patient such as an animal, a mammal, and a human for treating one or more of the disorders or conditions described herein. Therapeutic compounds of the disclosure include, but are not limited to, antibodies of the disclosure (including variants and derivatives thereof as described herein) and nucleic acids or polynucleotides encoding antibodies of the disclosure (including variants and derivatives thereof as described herein).

[0108] The antibodies of the disclosure can also be used to treat or inhibit cancer. In some embodiments, the cancer cells in the patient express or overexpress CCR8. As provided above, CCR8 can be overexpressed in tumor cells, in particular gastric, pancreatic, esophageal, ovarian, and lung tumors. Inhibition of CCR8 has been shown to be useful for treating the tumors.

[0109] Accordingly, in some embodiments, provided are methods for treating a cancer in a patient in need thereof. The method, in one embodiment, entails administering to the patient an effective amount of an antibody of the present disclosure. In some embodiments, at least one of the cancer cells (e.g., stromal cells) in the patient over-express CCR8.

[0110] Cellular therapies, such as chimeric antigen receptor (CAR) T-cell therapies, are also provided in the present disclosure. A suitable cell can be used, that is put in contact with an anti-CCR8 antibody of the present disclosure (or alternatively engineered to express an anti-CCR8 antibody of the present disclosure). In some embodiments, the antibody is presented in a chimeric antigen receptor (CAR). Upon such contact or engineering, the cell can then be introduced to a cancer patient in need of a treatment. The cancer patient may have a cancer of any of the types as disclosed herein. The cell (e.g., T cell) can be, for instance, a tumor-infiltrating T lymphocyte, a CD4+ T cell, a CD8+ T cell, or the combination thereof, without limitation.

[0111] In some embodiments, the cell was isolated from the cancer patient him- or her-self. In some embodiments, the cell was provided by a donor or from a cell bank. When the cell is isolated from the cancer patient, undesired immune reactions can be minimized.

[0112] Non-limiting examples of cancers include bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer. In some embodiments, the cancer is one or more of gastric, pancreatic, esophageal, ovarian, and lung cancers.

[0113] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the antibodies or variants, or derivatives thereof of the disclosure include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (*e.g.*, acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic,

myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (*e.g.*, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma.

[0114] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular antibodies, variant or derivative thereof used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0115] Methods of administration of the antibodies, variants or include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be

administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray.

[0116] The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0117] Administration can be systemic or local. In addition, it may be desirable to introduce the antibodies of the disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0118] It may be desirable to administer the antigen-binding polypeptides or compositions of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the disclosure, care must be taken to use materials to which the protein does not absorb.

[0119] The amount of the antibodies of the disclosure which will be effective in the treatment, inhibition and prevention of an inflammatory, immune or malignant disease, disorder or condition can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, disorder or condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0120] As a general proposition, the dosage administered to a patient of the antigen-binding polypeptides of the present disclosure is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight, between 0.1 mg/kg and 20 mg/kg of the patient's body weight, or 1 mg/kg to 10

mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the disclosure may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the brain) of the antibodies by modifications such as, for example, lipidation.

[0121] In an additional embodiment, the compositions of the disclosure are administered in combination with cytokines. Cytokines that may be administered with the compositions of the disclosure include, but are not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, anti-CD40, CD40L, and TNF-α.

[0122] In additional embodiments, the compositions of the disclosure are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Diagnostic Methods

[0123] Over-expression of CCR8 is observed in certain tumor samples, and patients having CCR8-over-expressing cells are likely responsive to treatments with the anti-CCR8 antibodies of the present disclosure. Accordingly, the antibodies of the present disclosure can also be used for diagnostic and prognostic purposes.

[0124] A sample that preferably includes a cell can be obtained from a patient, which can be a cancer patient or a patient desiring diagnosis. The cell be a cell of a tumor tissue or a tumor block, a blood sample, a urine sample or any sample from the patient. Upon optional pre-treatment of the sample, the sample can be incubated with an antibody of the present disclosure under conditions allowing the antibody to interact with a CCR8 protein potentially present in the sample. Methods such as ELISA can be used, taking advantage of the anti-CCR8 antibody, to detect the presence of the CCR8 protein in the sample.

[0125] Presence of the CCR8 protein in the sample (optionally with the amount or concentration) can be used for diagnosis of cancer, as an indication that the patient is suitable for a treatment with the antibody, or as an indication that the patient has (or has not) responded to a cancer treatment. For a prognostic method, the detection can be done at once,

twice or more, at certain stages, upon initiation of a cancer treatment to indicate the progress of the treatment.

Compositions

[0126] The present disclosure also provides pharmaceutical compositions. Such compositions comprise an effective amount of an antibody, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (e.g., an immune checkpoint inhibitor).

[0127] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Further, a "pharmaceutically acceptable carrier" will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

[0128] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable

pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0129] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0130] The compounds of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

EXAMPLES

Example 1: Generation of murine monoclonal antibodies against human CCR8

[0131] The human CCR8 protein was used to immunize different strains of mice, and hybridomas were generated accordingly. CCR8 positive binders were selected and subcloned. Subsequently, *in vitro* binding and functional screening were carried out and lead antibodies with highest binding affinity and strongest functional potency were identified.

[0132] The VH/VL sequences of the lead murine antibodies are provided in the table below.

Table 1. VH/VL sequence of the lead murine antibodies

Name	Sequence (CDRs are underlined)	SEQ ID NO:
137D1H10 VH	EVQLVESGGGLVQPKGSLKLSCTASGFTFN**TYAMN**WVRQAPGKGL EWVA**RIRSKANNYATYYADSVKD**RFTISRDDSQRILYLQMNNLKA EDTAMYYCVR**DRSRGEDYAMDY**WGQGTSVTVSS	1
137D1H10 VL	DIVMTQAAPSVPVTPGESVSISC**RSSKSLLHSNGNTYLY**WFLQRP GQSPQLLIY**RMSNLAS**GVPDRFSGSGSGTAFTLRISRVEAEDVGV YYC**MQHLEYPFT**FGAGTKLELK	2
88D2C6 VH	EVQLVETGGGLVQPKGSLKLSCAASGFTFN**PNAMN**WVRQAPGKGL EWVA**RIRSKSNNYATYYADSVKD**RFTISRDDSQSMLYLQMNNLKT EDTAMYYCVR**GKDDGYRHYAMDY**WGQGTSVTVSS	3
88D2C6 VL	DIVMTQAAPSVPVTPGESVSISC**RSSKSLLHSNGNTYLY**WFLQRP GQSPQLLIY**RMSNLAS**GVPDRFSGSGSGTAFTLRISRVEAEDVGV YYC**MQHLEYPFT**FGAGTKLELK	2
89B6F8 VH	DVKLVESGGGLVKPGGSLKLSCAASGFTFS**SYTMS**WVRQTPEKRL EWVA**TISSGDSYTYYPDSVKG**RFTISRDNAKNTLYLRMSSLKSED TAMYYCTR**DHYRYDVYAMDY**WGQGTSVTVSS	4
89B6F8 VL	DIVMTQSPSSLTVTAGEKVTMSC**KSSQSLLNSGNQKNYLT**WYQQK PGQPPKLLIY**WASTRES**GVPDRFTGSGSGTDFTLTISSVQAEDLA VYYC**QNDYSYPLT**FGAGTKLELK	5
132H8E10 VH	QVQLKESGPGLVAPSQSLSITCTVSGFSLT**GYAVN**WVRQPTGKGL EWLG**MIWGDGSTDYNSALKS**RLSISKDNSKSQVFLKMNSLQTDDT ARYYCAR**DSLYGNYPAY**WGQGTLVTVSA	6
132H8E10 VL	DIQMTQSPSSLSASLGGKVTITC**KASQDINKYMA**WYQHKPGKGPR LLIH**STSTLQP**GIPSRFSGSGSGRDYSFSISNLEPEDIATYYC**LQ YDNLLT**FGGGTKLEIK	7
10F11B2 VH	QVQLKESGPGLVQPSQTLSLTCTVSGFSLT**SYSVH**WVRQHSGKTL VWMG**RLWSDGDTSYNSAFTS**RLSISRDTSKSQVFLKMNSLQTEDT GTYYCAR**KAPNGGAFDY**WGQGVMVTVSS	8
10F11B2 VL	QVVLTQPKSVSTSLESTVKLSC**KLNSGNIGSYYVH**WYQQHEGRSP TNMIY**RDDKRPD**GVPDRFSGSIDSSSNSAFLTINNVQTEDEAIYF C**HSSDSSIKCI**FGGGTKLTVL	9
40H10F3 VH	QVQLKESGPGLVQPSQTLSLTCTVSGFSLT**SYNVH**WVRQPPGKGL EWMG**RMRYYGDTSFSSALKS**RLSISRDTSKNQVFLKMSSLQTDDT GTYYCTR**GPPSYYRGDFFDY**WGQGVMVAVSS	10
40H10F3 VL	DIQMTQSPSLLSASVGDRVTINC**KASLNINNYLN**WYQQKLGEAPK LLID**NTNNLQT**GIPSRFSGSGSGTDYTLTISNLQPEDFGTYFC**FQ HNGWPLT**FGSGTKLEIK	11
53D6A9 VH	EVQLVESGGSLVQPGRSLKLSCAASGFTYN**NYVMA**WVRRAPTKGL EWVA**SISTDGVSTQYRDSVKG**RFTISRDNAKTSLFLHMDSLRSED TATYYCAK**DAARGLYGQGGYFDF**WGQGVMVTVSS	12
53D6A9 VL	DIQMTQSPASLSASLGETVSIEC**LASEGISNDLA**WYQQKSGKSPQ LLIY**AATRLEG**GVPSRFSGSGSGTRFSLKISGMQFEDEADYFC**QQ SYKYPWT**FGGGTKLELK	13
352H11C11 VH	EVQLVETGGGLVQPKGSLKLSCAASGFTFN**TNAMN**WVRQAPGKGL EWVA**RIRSKSNNYATYYADSVKD**RFTISRDDSQSILYLQMNNLKT EDTAMYYCVR**GGPIYHMDC**WGQGTSVTVSS	14
352H11C11 VL	DIVMTQAAPSVPVTPGESVSISC**RSSKSLLHSNGNTYLY**WFLQRP GQSPQLLIY**RMSNLAS**GVPDRFSGSGSGTAFTLRISRVEAEDVGV YYC**MQHLEYPFT**FGSGTKLEIK	15
362G7D3 VH	EVQLVETGGGLVQPKGSLKLSCAASGFTFN**TNAMN**WVRQAPGKGL EWVA**RIRSKSNNYATYYADSVKD**RFTISRDDSQSMLYLQMNNLKT	16

	EDTAMYYCVR**DPGLRQGMDY**WGQGTSVTVSS	
362G7D3 VL	DIVMTQAAPSVPVTPGESVSISC**RSSKSLLHSNGNTYLY**WFLQRP GQSPQLLIY**RMSNLAS**GVPDRFSGSGSGTAFTLRISRVEAEDVGV YYC**MQHLEYPFT**FGSGTKLEIK	15
362H10A3 VH	EVQLVESGGGLVQPKGSLKLSCAASGFTFN**TYAMN**WVRQAPGKGL EWVA**RIRSKSNNYATYYADSVKD**RFTISRDDSQSMLYLQMNNLKT EDTAMYYCVR**GGGNYRGDYFDY**WGQGTTLTVSS	17
362H10A3 VL	DIVMTQAAPSVPVTPGESVSISC**RSSKSLLHSNGNTYLY**WFLQRP GQSPQLLIY**RMSNLAS**GVPDRFSGSGSGTAFTLRISRVEAEDVGV YYC**MQHLEYPFT**FGSGTKLEIK	15
367D10E7 VH	EVQLVESGGGLVQPKGSLKLSCAASGFTFN**TYAMN**WVRQAPGKGL EWVA**RIRSKSNNYATYYADSVKD**RFTISRDDSQSMLYLQMNNLKT EDTAMYYCVR**YDRSYAMDY**WGQGTSVTVSS	18
367D10E7 VL v1	QIVLTQSPAIMSASPGEKVTITC**SASSSVSYMH**WFQQKPGTSPKL WIY**STSNLAS**GVPARFSGSGSGTSYSLTISRMEAEDAATYYC**QQR SSYPLT**FGAGTKLELK	19
367D10E7 VL v2	DIVMTQAAPSVPVTPGESVSISC**RSSKSLLHSNGNTYLY**WFLQRP GQSPQLLIY**RMSNLAS**GVPDRFSGSGSGTAFTLRISRVEAEDVGV YYC**MQHLEYPFT**FGAGTKLELK	2
370D2C10 VH	EVQLIETGGGLVQPKGSLKLSCAASGFTFN**TNAMN**WVRQAPGKDL EWVS**RIRTKSNNYATYYADSVKD**RFTISRDDSQSMLYLQMNNLKT EDTAMYYCVT**GTTVVAKEFAY**WGQGTLVTVSA	20
370D2C10 VL	DIVMTQAAPSVPVTPGESVSISC**RSSKSLLHSNGNTYLY**WFLQRP GQSPHLLIY**RMSNLAS**GVPDRFSGSGSGTAFTLRISRVEAEDVGV YYC**MQHLEYPFT**FGSGTKLEIK	21

Example 2. Binding to CCR8 on U2OS Cells

[0133] This example tested the antibodies' activity in binding to human CCR8 protein expressed on U2OS cells.

[0134] Chimeric antibodies (fused to human IgG1 constant regions) of a few of the above identified murine antibodies were expressed from cDNA. The antibodies were incubated with U2OS cells expressing the human CCR8. Among the antibodies tested, 88D2C6 and 137D1H10 exhibited considerably higher affinity than the rest, in a dose-dependent manner (**FIG. 1**).

Example 3. ADCC Measurement

[0135] The potency of 88D2C6 and 137D1H10, along with 370D2C10 (all fused to human IgG1 constant regions, with ADCC-activating mutations in Fc), in mediating ADCC was measured with CCR8-overexpressing CHO K1 and U2OS cells. All three antibodies exhibited high ADCC activities (**FIG. 2**).

[0136] All three antibodies exhibited potent ADCC activities. Among them, however, 88D2C6 had considerably higher potency (EC50: 0.01743 nM with CHO cells and 0.1108 for U2OS cells).

Example 4. Humanization of the mouse mAbs

[0137] The murine antibody variable region genes of 88D2C6 and 352H11C11were employed to create humanized mAbs. The amino acid sequences of the VH and VL of mAb were compared against the available database of human Ig gene sequences to find the overall best-matching human germline Ig gene sequences. The CDRs of the murine antibodies were then grafted into the matched human sequences. The cDNA was synthesized and used to produce the humanized antibodies. Certain back mutations from the murine antibodies were then introduced back to the humanized antibodies. Certain amino acids were mutated to reduce the chance of post-translational modification.

[0138] The amino acid sequences of the humanized antibodies are provided below.

Humanized sequences

A. 88D2C6

Table 2A. Humanization of 88D2C6 – VH

Name	Sequence	SEQ ID NO:
88D2C6 VH	EVQLVETGGGLVQPKGSLKLSCAASGFTFNPNAMNWVRQAPGKGL EWVARIRSKSNNYATYYADSVKDRFTISRDDSQSMLYLQMNNLKT EDTAMYYCVRGKDDGYRHYAMDYWGQGTSVTVSS	3
V1 (CDR grafting)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNPNAMNWVRQASGKGL EWVGRIRSKSNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKT EDTAVYYCTRGKDDGYRHYAMDYWGQGTTVTVSS	29
V2 (with back mutations)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNPNAMNWVRQASGKGL EWVGRIRSKSNNYATYYADSVKDRFTISRDDSKNTAYLQMN**N**LKT EDTAVYYC**V**RGKDDGYRHYAMDYWGQGTTVTVSS	30
V3 (with back mutations)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNPNAMNWVRQASGKGL EWV**A**RIRSKSNNYATYYADSVKDRFTISRDDSKNT**L**YLQMN**N**LKT EDTAVYYC**V**RGKDDGYRHYAMDYWGQGTTVTVSS	31

Table 2B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	PNAMN	22
CDR-H2	RIRSKSNNYATYYADSVKD	23
CDR-H3	GKDDGYRHYAMDY	24

Table 2C. Humanization of 88D2C6 – VL

Name	Sequence	SEQ ID NO:
88D2C6 VL	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRP GQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGV YYCMQHLEYPFTFGAGTKLELK	2
V1 (CDR grafting)	DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSN**A**NTYLYWYLQKP GQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQHLEYPFTFGGGTKVEIK	32
V2 (with back mutations)	DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSN**A**NTYLYW**F**LQKP GQSPQLLIYRMSNLASGVPDRFSGSGSGT**A**FTLKISRVEAEDVGV YYCMQHLEYPFTFGGGTKVEIK	33
V3 (with back mutations)	DIVMTQ**A**PLSLPVTPGEP**V**SISCRSSKSLLHSN**A**NTYLYW**F**LQKP GQSPQLLIYRMSNLASGVPDRFSGSGSGT**A**FTLKISRVEAEDVGV YYCMQHLEYPFTFGGGTK**L**EIK	34

Table 2D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	RSSKSLLHSNGNTYLY	28
CDR-L1 (PM site mutated)	RSSKSLLHSN**A**NTYLY	25
CDR-L2	RMSNLAS	26
CDR-L3	MQHLEYPFT	27

Table 2E. Humanized antibodies

	VL	VL v1	VL v2	VL v3
VH	88-xi			
VH v1			88-H1L2	88-H1L3
VH v2			88-H2L2	88-H2L3
VH v3			88-H3L2	88-H2L3

B. 137D1H10

Table 3A. Humanization of 137D1H10 – VH

Name	Sequence	SEQ ID NO:
137D1H10 VH	EVQLVESGGGLVQPKGSLKLSCTASGFTFNTYAMNWVRQAPGKGL EWVARIRSKANNYATYYADSVKDRFTISRDDSQRILYLQMNNLKA EDTAMYYCVRDRSRGEDYAMDYWGQGTSVTVSS	1
V1 (CDR grafting)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGL EWVGRIRSKANNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKT EDTAVYYCTSDRSRGEDYAMDYWGQGTLVTVSS	38
V2 (with back mutations)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGL EWVGRIRSKANNYATYYADSVKDRFTISRDDSKNTAYLQMN**N**LKT EDTAVYYC**VR**DRSRGEDYAMDYWGQGTLVTVSS	39
V3 (with back mutations)	EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQASGKGL EWV**A**RIRSKANNYATYYADSVKDRFTISRDDSKNT**L**YLQMN**N**LKT EDTAVYYC**VR**DRSRGEDYAMDYWGQGTLVTVSS	40

Table 3B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	TYAMN	35
CDR-H2	RIRSKANNYATYYADSVKD	36
CDR-H3	DRSRGEDYAMDY	37

Table 3C. Humanization of 137D1H10 – VL

Name	Sequence	SEQ ID NO:
137D1H10 VL	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRP GQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGV YYCMQHLEYPFTFGAGTKLELK	2
V1 (CDR grafting)	DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSN**A**NTYLYWYLQKP GQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQHLEYPFTFGQGTKLEIK	41
V2 (with back mutations)	DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSN**A**NTYLYW**F**LQKP GQSPQLLIYRMSNLASGVPDRFSGSGSGT**A**FTLKISRVEAEDVGV YYCMQHLEYPFTFGQGTKLEIK	42
V3 (with back mutations)	DIVMTQSPLSLPVTPGEP**V**SISCRSSKSLLHSN**A**NTYLYW**F**LQKP GQSPQLLIYRMSNLASGVPDRFSGSGSGT**A**FTLKISRVEAEDVGV YYCMQHLEYPFTFGQGTKLEIK	43

Table 3D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	RSSKSLLHSNGNTYLY	28
CDR-L1 (PM site mutated)	RSSKSLLHSN**A**NTYLY	25
CDR-L2	RMSNLAS	26
CDR-L3	MQHLEYPFT	27

Table 3E. Humanized antibodies

	VL	VL v1	VL v2	VL v3
VH	137-xi			
VH v1				
VH v2			137-H2L2	137-H2L3
VH v3			137-H3L2	137-H3L3

Example 5. Testing of Humanized Antibodies

[0139] This example tested some of the humanized antibodies for their binding affinity to CCR8 and their ability to induce ADCC.

[0140] The experimental procedures are similar to Examples 2-3. **FIG. 3** shows the affinities of humanized antibodies of 88D2C6. Apparently, all of them had high affinity (see also Table 4).

Table 4A. Binding to CCR8 expressed in CHO-K1 cells

	88-H1L2	88-H1L3	88-H2L2	88-H2L3	88-H3L2	88-H3L3	88-xi
Bottom	2669	2143	2494	2276	3937	3474	3631
Top	32782	31947	32830	31610	32495	32214	33947
LogEC50	-0.7515	-0.5189	-0.6391	-0.5461	-0.6861	-0.5826	-0.6548
HillSlope	1.502	1.377	1.404	1.408	1.382	1.345	1.480
EC50	0.1772	0.3028	0.2296	0.2844	0.2060	0.2615	0.2214
Span	30113	29804	30336	29334	28557	28740	30316

Table 4B. Binding to CCR8 expressed in U2OS cells

	88-H1L2	88-H1L3	88-H2L2	88-H2L3	88-H3L2	88-H3L3	88-xi
Bottom	2106	1542	2756	1530	2826	1623	1027
Top	28891	27635	28329	25780	27435	27421	29659
LogEC50	-0.6779	-0.5797	-0.7241	-0.5119	-0.6841	-0.6289	-0.6921
HillSlope	1.409	1.387	1.524	1.450	1.521	1.378	1.097
EC50	0.2099	0.2632	0.1888	0.3077	0.2070	0.2350	0.2032
Span	26785	26092	25574	24250	24609	25798	28632

[0141] FIG. 4 shows the affinities of humanized antibodies of 137D1H10 (data in Table 5).

Table 5A. Binding to CCR8 expressed in CHO-K1 cells

	137-H2L2	137-H2L3	137-H3L2	137-H3L3	137-xi
Bottom	2548	2244	4264	3820	3032
Top	29347	30182	33198	33389	34947
LogEC50	-0.7893	-0.5055	-0.6843	-0.7538	-0.7792
HillSlope	1.141	1.272	1.592	1.449	1.581
EC50	0.1625	0.3123	0.2069	0.1763	0.1663
Span	26799	27937	28934	29569	31915

Table 5B. Binding to CCR8 expressed in U2OS cells

	137-H2L2	137-H2L3	137-H3L2	137-H3L3	137-xi
Bottom	915.4	1322	2065	2098	2172
Top	26251	27254	32257	31927	33430
LogEC50	-0.8422	-0.4841	-0.6897	-0.7169	-0.6052
HillSlope	1.171	1.122	1.351	1.492	1.422
EC50	0.1438	0.3280	0.2043	0.1919	0.2482
Span	25336	25932	30192	29829	31258

[0142] In terms of ADCC (**FIG. 5**), surprisingly, all humanized antibodies exhibited greatly higher (about 5 to 10-fold) ADCC inducing activity than the chimeric counterparts.

Example 6. Cell-Based Antibody Binding

[0143] This example compared 137-H3L2 (LM-108) with reference antibodies with respect to cell-based binding

[0144] Cell based binding of LM-108 and reference Ab (synthesized according to WO2020138489) to human CCR8 overexpressing cells (HEK293/H_CCR8) were assessed using flow cytometry. The human CCR8 overexpressing HEK293 (HEK293/H_CCR8) and parental HEK293 cells were incubated with titrated LM-108, reference Ab and its isotype control (from 200 nM, 4 folds dilutions, 12 points) at 4°C for 60 minutes. The cells were washed with FACS buffer twice, then stained with fluorescent conjugated secondary antibody (Alexa Fluor® 647 Goat Anti-Human IgG, Jackson, 109-605-098) at 4°C for 60 minutes. After the cells were washed twice and they were analyzed by flow cytometry.

[0145] Both LM-108 and the reference Ab effectively bound to HEK293/H_CCR8 cells in a dose dependent manner, at EC_{50} of 1.03 nM and 0.99nM, respectively (**FIG. 6a** and **Table 6A**). There was no non-specific binding observed for LM-108 but reference Ab showed slight nonspecific binding on parental HEK293 cells (**FIG. 6b** and **Table 6B**).

Table 6A. Binding to CCR8-expressing HEK293 cells

Antibody	EC50 (nM)	Max (MFI)
LM-108	1.028	76795
Reference Ab	0.9938	86817
Isotype	N.A.	192

Table 6B. Binding to Non-CCR8-expressing HEK293 cells

Antibody	EC50 (nM)	Max (MFI)
LM-108	N.A.	108
Reference Ab	N.A.	472
Isotype	N.A.	145

[0146] Cell-based binding of LM-108 on cells with high levels of CCR8 expression was assessed using flow cytometry. The cells used were U2OS (U2OS/H_CCR8). As shown in **FIG. 7** and **Table 7**, LM-108 could effectively bind to U2OS/H_CCR8 cells in a dose dependent manner with an EC_{50} of 0.25 nM.

Table 7. Binding to High-CCR8-expressing U2OS cells

Antibody	EC50 (nM)	Max (MFI)
LM-108	0.2503	43087
Isotype	N.A.	215

[0147] Cell-based binding of LM-108 on cells with low levels of CCR8 expression was also assessed using flow cytometry. The cells used were Jurkat engineering cells (Jurkat/H_CCR8). As shown in **FIG. 8** and **Table 8**, LM-108 could effectively bind to Jurkat/H_CCR8 cells in a dose dependent manner with an EC50 of 0.21 nM.

Table 8. Binding to Low-CCR8-expressing Jurkat cells

Antibody	EC50 (nM)	Max (MFI)
LM-108	0.2124	1423
Isotype	N.A.	134

Example 7. ADCC Report Gene Assay

[0148] This example shows the results of an ADCC reporter gene assay targeting different CCR8-expressing cells.

ADCC reporter gene assay targeting U2OS/H_CCR8

[0149] The ADCC activity of LM-108 was assessed with a reporter gene assay with CD16a (158v) expressing Jurkat/NFAT-luc reporter cells as effector cells. Human CCR8 overexpressing U2OS cells were used as target cells. U2OS/H_CCR8 cells were planted at the density of 1E4 cells per well in 96-well plate overnight, then co-cultured with $1.5x10^5$ effecter cells each well and the titrated LM-108 and the isotype control to final concentration from 10 μg/ml, 5 times dilution, and 10 points. After incubation under 37°C for 6 hours, One-Glo (Promega) was added for luminescence detection.

[0150] The results are shown in **FIG. 9**. LM-108 exhibited potent ADCC effect (EC_{50}=0.00605nM) with Jurkat/CD16a/NFAT-luc reporter cells targeting U2OS/H_CCR8 cells.

ADCC reporter gene assay targeting Jurkat/H_CCR8

[0151] ADCC activity of LM-108 as assessed by reporter gene assay with CD16a (158v) expressing Jurkat/NFAT-luc reporter cells as effector cells. Human CCR8 low expressing

Jurkat cells were generated as target cells to mimic the expression level on tumor associated Tregs.

[0152] Effector cells (Jurkat reporter cell) and target cells (Jurkat/H_CCR8) at ratio of 3:1 were mixed with the titrated LM-108 and the isotype control (final concentration from 200 nM, 5 times dilution, 11 points). After incubation under 37 °C for 6 hours, One-Glo(Promega) was added for luminescence detection. As shown in **FIG. 10**, LM-108 had dose-dependent ADCC effect with an EC_{50}=0.14 nM.

ADCC effect of LM-108 towards human CCR8 over-expressing HEK293 (HEK293/H_CCR8) and HEK293 cells with primary human PBMCs

[0153] Effector cells (hPBMCs) and target cells (HEK293/H_CCR8 or HEK293) at a ratio of 50:1 were mixed with the titrated LM-108 and the isotype control (final concentration from 10 nM, 7 folds dilution, 12 points). After incubation under 37 °C for 6 hours, 50 µl supernatant from each well was analyzed by Cytotoxicity Detection Kit (Roche, 04744934001)

[0154] As shown in **FIG. 11A**, LM-108 exhibited specific and strong ADCC effect towards CCR8-expressing HEK293 cells in a dose-dependent manner with an EC_{50} of 0.002 nM(Fig.6a). No nonspecific killing observed on parental HEK293 cells (**FIG. 11B**).

Example 8. Testing of Antibody-Dependent Cellular Phagocytosis (ADCP)

[0155] In this example, the antibody-dependent cellular phagocytosis (ADCP) effect of LM-108 towards human CCR8-overexpressed CHO-K1 cells was evaluated with monocyte-derived macrophages (MDMs) by FACS.

[0156] The effector cells, MDMs, were induced from monocytes isolated from human PBMC with the presence of 100 ng/mL of human M-CSF for 7 days. The target cells were CHO-K1 (negative control) and CHO-K1 cells overexpressing human CCR8 which labeled CFSE. The effector cells, CFSE-labeled target cells, and titrated test antibody LM-108 or its isotype control were incubated in a 37°C, 5% CO2 incubator for 2 hours. Cells were washed with FACS buffer (1 x DPBS + 2% FBS), then stained with APC-conjugated human CD14 antibody to identify the macrophages. After wash twice with FACS buffer, the samples were

analyzed by flow cytometry. Phagocytosis index was determined as the percentage of CD14-APC+/CFSE+ double positive cells in total CD14+ positive cells.

[0157] As shown in **FIG. 12A-B**, LM-108 had specific and dose dependent phagocytosis toward human CCR8 overexpressing CHO-K1 cells, but not control cells.

[0158] ADCP of LM-108 with Monocyte-derived Macrophage (MDM) was also measured by Operetta. The effector cells, MDMs, were induced from monocytes isolated from human PBMC with the presence of 100 ng/mL of human M-CSF for 6 days. Then the effector cells were seeded into a black 96-well plate with clear bottom at $2x10^4$ count/well and cultured for another 2 days for adherence. The target cells were Jurkat (negative control) and human CCR8 over expressing Jurkat cells (Jurkat/H_CCR8).The CFSE-labeled target cells and test antibody LM-108 or its isotype control were added into the plate with macrophages, and incubated in a 37 °C, 5% CO_2 incubator for 2 hours. The effector cell: target cell ratio is 1:2. Cells were washed and stained with APC-conjugated human CD14 antibody to identify the macrophages. After washed twice, cells were fixed using 4% paraformaldehyde (PFA) dilution and stored in a 4 °C freezer at dark before taking photos by Operetta.

[0159] As shown in **FIG. 13**, LM-108 induced ADCP towards human CCR8-overexpressed Jurkat cells, while its isotype control did not. Neither LM-108 nor the isotype control induced obvious phagocytosis towards Jurkat cells.

Example 9. *In vivo* Efficacy

[0160] In this example, *in vivo* efficacy studies of LM-108 or its mouse surrogate were performed.

[0161] The first experiment was performed on a CT26 syngeneic model with LM-108m, a surrogate antibody for LM-108. In this study, each mouse was inoculated subcutaneously at the right axillary (lateral) with CT26 tumor cell (5×10^5) in 0.1 ml of PBS for tumor development. The animals were randomly grouped into 4 groups (n=7) on the third day after cell inoculation, then treatment started. PBS group as vehicle, LM-108m and mPD-1 antibody at dose of 10mg/kg was administrated via intraperitoneal (i.p.) as single agent or combo at day 0, day 3, day 7, day 10, day 14 and day 17. The experiment was terminated at day18 due to the average tumor volume of control group reached more than 2000 mm^3.

[0162] Tumor sizes were measured three times weekly in two dimensions using a caliper and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where *a* and *b* were the long and short diameters of the tumor, respectively. The tumor sizes were then used for the calculations of T/C (%) values. T/C (%) was calculated using the formula: $T/C\ \% = (T_i-T_0)/(V_i-V_0) \times 100$, T_i was the average tumor volume of a treatment group on a given day, T_0 was the average tumor volume of the treatment group on the first day of treatment, Vi was the average tumor volume of the vehicle control group on the same day with T_i, and V_0 was the average tumor volume of the vehicle group on the first day of treatment. TGI was calculated for each group using the formula: TGI (%) = [100-T/C].

[0163] FIG. 14 shows the tumor growth curve of CT26 tumor-bearing Balb/c mice post administration of LM-108m and anti-mPD-1 Ab. LM-108m as single agent or in combination of mPD-1 antibody showed strong anti-tumor activity (**Table 9**). Data points represent group (n=7) mean, error bars represent standard error of the mean (SEM). p value was calculated based on tumor size by t-test, compared with vehicle control using PBS.

Table 9. In vivo Efficacy (CT26 syngeneic model)

Days after treatment T/C(%)	**0**	**3**	**6**	**9**	**12**	**15**	**18**
G2: LM-108m 10mpk	-	72.161	86.504	54.453	47.024	35.639	28.219
G3: Anti-mPD-1 Ab 10mpk	-	59.926	70.322	44.463	38.057	35.773	38.326
G4: LM-108m+mPD-1 Ab	-	70.506	60.774	35.973	27.413	20.386	15.292

Days after treatment TGI(%)	**0**	**3**	**6**	**9**	**12**	**15**	**18**
G2: LM-108m 10mpk	-	27.839	13.496	45.547	52.976	64.361	71.781
G3: Anti- mPD-1 Ab 10mpk	-	40.074	29.678	55.537	61.943	64.227	61.674
G4: LM-108m+mPD-1 Ab	-	29.494	39.226	64.027	72.587	79.614	84.708

Days after treatment P value	**0**	**3**	**6**	**9**	**12**	**15**	**18**
G2: LM-108m 10mpk	-	0.053	0.385	0.024	0.026	0.025	0.023
G3: Anti-mPD-1 Ab 10mpk	-	0.024	0.073	0.007	0.008	0.026	0.044
G4: LM-108m+mPD-1 Ab	-	0.037	0.017	0.002	0.006	0.01	0.012

[0164] The second experiment used human CCR8 ki mice which were purchased from BIOCYTOGEN. Each hCCR8 ki mouse was inoculated subcutaneously at the right axillary (lateral) with MC38 tumor cell (1×10^6) in 0.1 ml of PBS for tumor development. The animals were randomly grouped on the third day after cell inoculation, then treatment started for the efficacy study. LM-108 at dose of 10 mg/kg was administrated via intraperitoneal (i.p.) at

day 0, day 3, day 7, day 10. The experiment was terminated when average tumor volume of control group reached more than 2000 mm^3.

[0165] Tumor sizes were measured three times weekly in two dimensions using a caliper and the volume was expressed in mm^3 using the formula: $V = 0.5\ a \times b^2$ where a and b were the long and short diameters of the tumor, respectively. The tumor sizes were then used for the calculations of T/C (%) values. T/C (%) was calculated using the formula: T/C % = $(T_i-T_0)/(V_i-V_0) \times 100$, T_i was the average tumor volume of a treatment group on a given day, T_0 was the average tumor volume of the treatment group on the first day of treatment, Vi was the average tumor volume of the vehicle control group on the same day with T_i, and V_0 was the average tumor volume of the vehicle group on the first day of treatment. TGI was calculated for each group using the formula: TGI (%) = [100-T/C]. Data points represent group (n=8) mean, error bars represent standard error of the mean (SEM). p value was calculated based on tumor size by t-test, compared with Vehicle Control.

[0166] FIG. 15 shows the tumor growth curve of MC38 tumor-bearing mice post administration of LM-108, and the data are summarized in **Table 10**. LM-108 showed potent tumor inhibitory effects.

Table 10. In vivo Efficacy (MC38 syngeneic model)

P Value	Days after treatment						
	0	2	4	7	9	11	14
LM-108 vs. Vehicle	-	0.985	0.503	0.042	0.002	0	0.001

T/C(%)	Days after treatment						
	0	3	5	7	10	12	14
LM-108 vs. Vehicle	-	99.68	95.326	73.673	47.349	34.464	31.23

TGI(%)	Days after treatment						
	0	3	5	7	10	12	14
LM-108 vs. Vehicle	-	0.32	4.674	26.327	52.651	65.536	68.77

Example 10. Cytotoxicity Assessment

[0167] This example evaluated the cytotoxicity of an antibody-drug conjugate, LM-108-vc-MMAE (Monomethyl auristatin E), on human CCR8 expressing HEK293 cells.

[0168] To evaluate if LM-108 can be used for ADC development, cytotoxicity of MMAE conjugated LM-108 was assessed by co-culture the human CCR8 expressing HEK293 cells and the titrated ADC of LM-108-vc-MMAE.

[0169] HEK293/H_CCR8 cells were planted at the density of 1.5 x 10^4 cells per well in 96-well plate overnight, then co-cultured with titrated LM-108-vc-MMAE to final concentration from 100 nm, 3 times dilution, 9 points. After incubation under 37°C for 96 hours, Cell-Titer-Glo (Promega) was added for cell viability evaluation.

[0170] As shown in **FIG. 16**, MMAE conjugated LM-108 had potent cytotoxicity (IC50=0.5285nM) on human CCR8-expressing HEK293 cells.

* * *

[0171] The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

[0172] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. An antibody or fragment thereof having binding specificity to a human chemokine (C-C motif) receptor 8 (CCR8) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region light chain comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein:

(a) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 35,
the CDRH2 comprises the amino acid sequence of SEQ ID NO: 36,
the CDRH3 comprises the amino acid sequence of SEQ ID NO: 37,
the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25 or 28,
the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26, and
the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27, or

(b) the CDRH1 comprises the amino acid sequence of SEQ ID NO: 22,
the CDRH2 comprises the amino acid sequence of SEQ ID NO: 23,
the CDRH3 comprises the amino acid sequence of SEQ ID NO: 24,
the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25 or 28,
the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26, and
the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27.

2. The antibody or fragment thereof of claim 1, wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 35, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 36, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 37, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27.

3. The antibody or fragment thereof of claim 2, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 38-40, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 32-34 and 41-43.

4. The antibody or fragment thereof of claim 2, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:40, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:42.

5. The antibody or fragment thereof of claim 1, wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO: 22, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 23, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 24, the CDRL1 comprises the amino acid sequence of SEQ ID NO: 25, the CDRL2 comprises the amino acid sequence of SEQ ID NO: 26, and the CDRL3 comprises the amino acid sequence of SEQ ID NO: 27.

6. The antibody or fragment thereof of claim 5, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 29-31, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 32-34 and 41-43.

7. The antibody or fragment thereof of claim 5, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:29, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:33.

8. The antibody or fragment thereof of any one of claims 1-7, which is humanized.

9. An antibody or fragment thereof, which has binding specificity to a human chemokine (C-C motif) receptor 8 (CCR8) protein and binds to the same epitope on the CCR8 protein as the antibody or fragment thereof of claim 1, or competes with the antibody or fragment thereof of claim 1 in binding to the CCR8 protein.

10. The antibody or fragment of any one of claims 1-9, which is capable of mediating antibody-dependent cellular cytotoxicity (ADCC).

11. The antibody or fragment of claim 10, which is not afucosylated.

12. The antibody or fragment thereof of any one of claims 1-11, which further has a binding specificity to a second target protein.

13. A composition comprising the antibody or fragment thereof of any one of claims 1-12 and a pharmaceutically acceptable carrier.

14. The composition of claim 13, further comprising a second antibody having specificity to a tumor antigen.

15. A method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of any one of claims 1-12.

16. Use of the antibody or fragment thereof of any one of claims 1-12 for the preparation of a medicament for treating cancer.

17. The method of claim 15 or the use of claim 16, wherein the cancer is selected from the group consisting of bladder cancer, liver cancer, colon cancer, rectal cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, small cell lung cancer, non-small cell lung

cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.

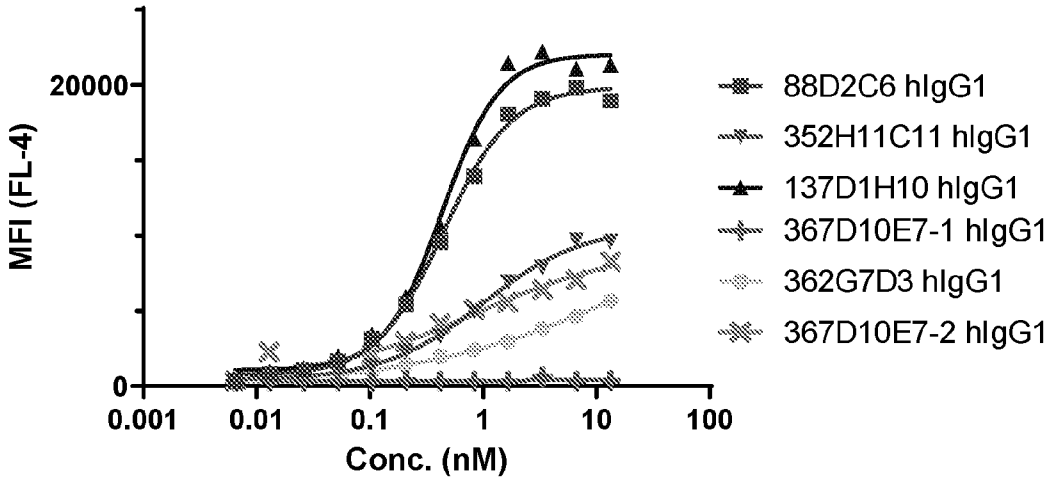

20000
0
MFI (FL-4)
0.001
0.01
0.1
1
10
100
Conc. (nM)
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352H11C11 hIgG1
137D1H10 hIgG1
367D10E7-1 hIgG1
362G7D3 hIgG1
367D10E7-2 hIgG1

FIG. 1

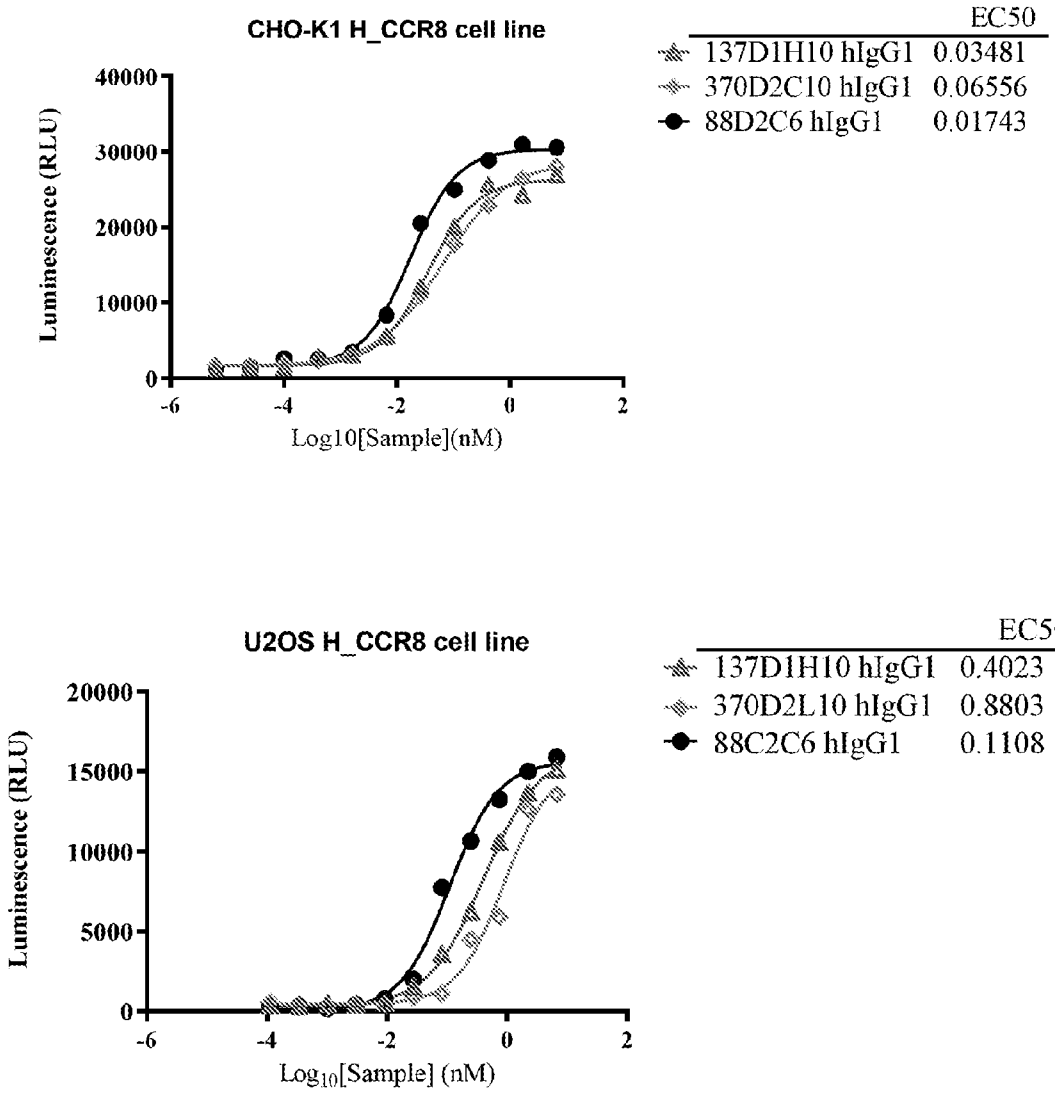

CHO-K1 H_CCR8 cell line
Luminescence (RLU)
40000
30000
20000
10000
0
-6
-4
-2
0
2
Log10[Sample](nM)
EC50
137D1H10 hIgG1 0.03481
370D2C10 hIgG1 0.06556
88D2C6 hIgG1 0.01743
U2OS H_CCR8 cell line
Luminescence (RLU)
20000
15000
10000
5000
0
-6
-4
-2
0
2
Log10[Sample] (nM)
EC50
137D1H10 hIgG1 0.4023
370D2L10 hIgG1 0.8803
88C2C6 hIgG1 0.1108

FIG. 2

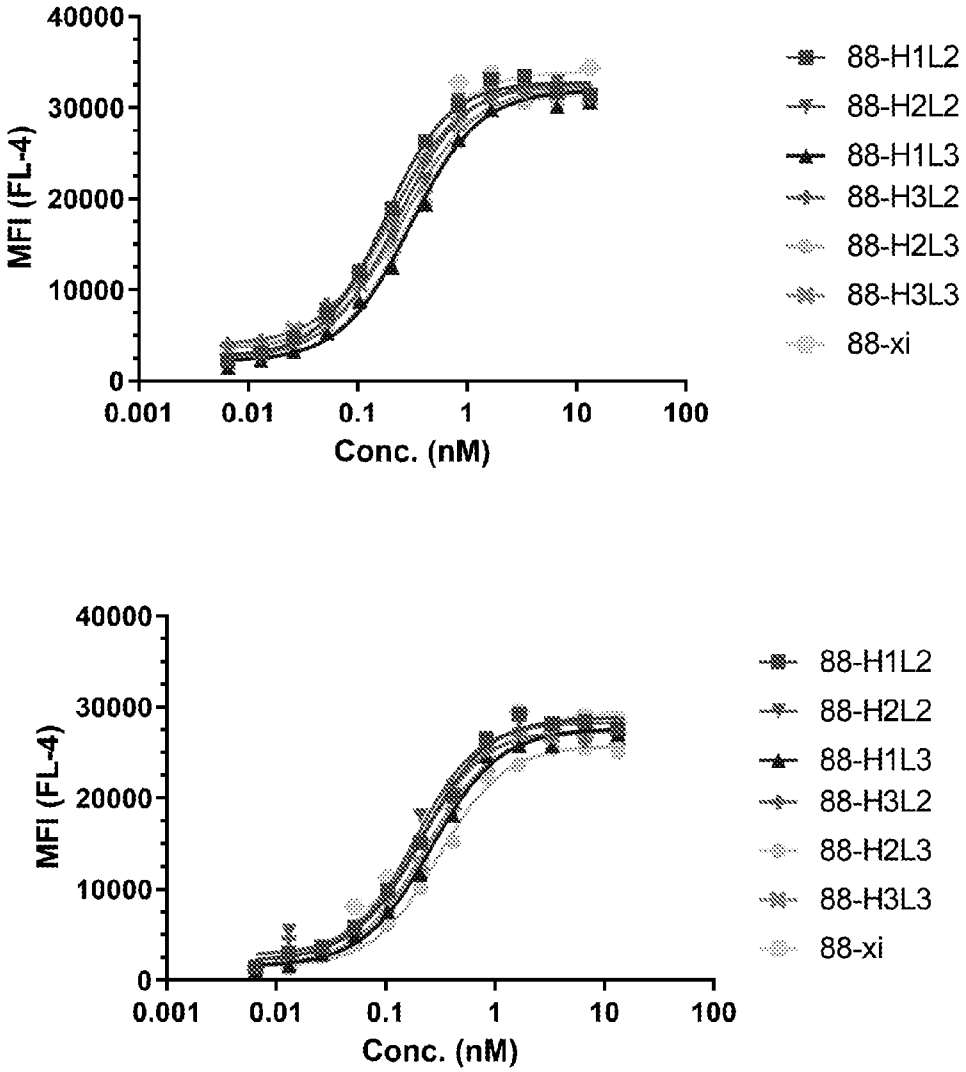

40000
30000
20000
10000
0
MFI (FL-4)
0.001
0.01
0.1
1
10
100
Conc. (nM)
88-H1L2
88-H2L2
88-H1L3
88-H3L2
88-H2L3
88-H3L3
88-xi
40000
30000
20000
10000
0
MFI (FL-4)
0.001
0.01
0.1
1
10
100
Conc. (nM)
88-H1L2
88-H2L2
88-H1L3
88-H3L2
88-H2L3
88-H3L3
88-xi

FIG. 3

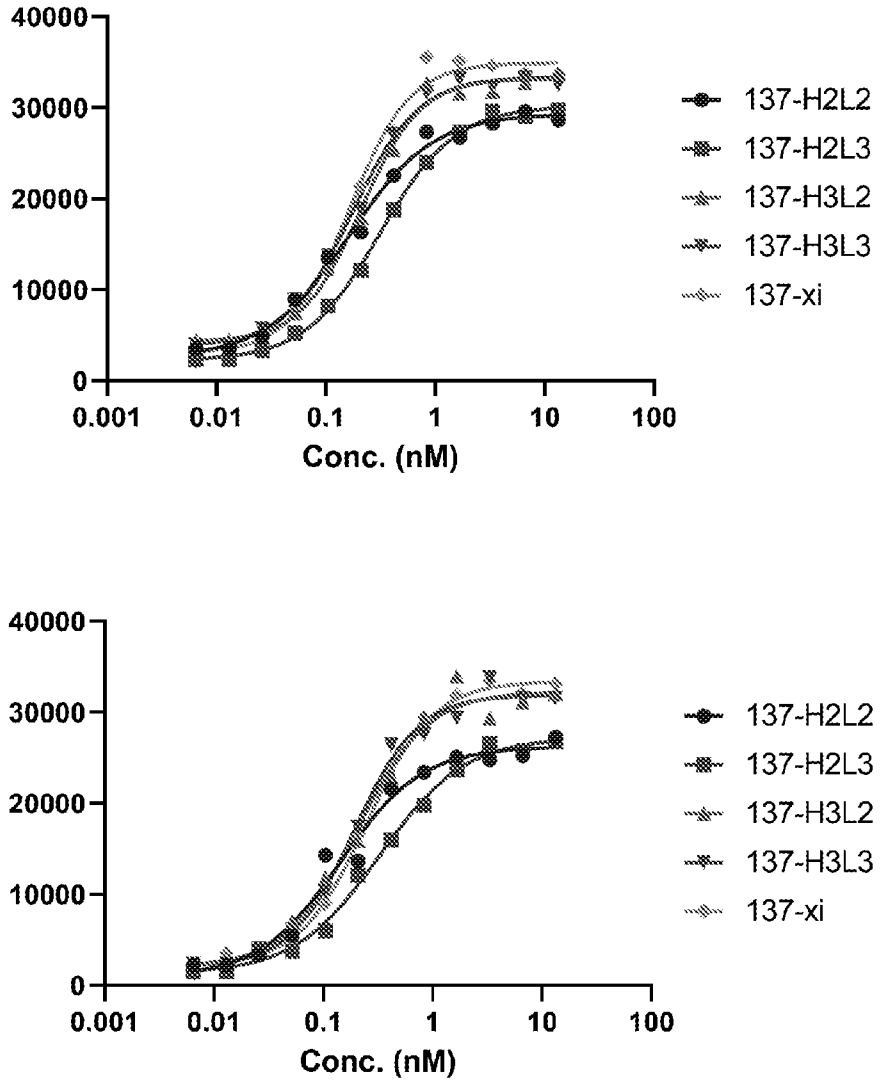

40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
Conc. (nM)
137-H2L2
137-H2L3
137-H3L2
137-H3L3
137-xi
40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
Conc. (nM)
137-H2L2
137-H2L3
137-H3L2
137-H3L3
137-xi

FIG. 4

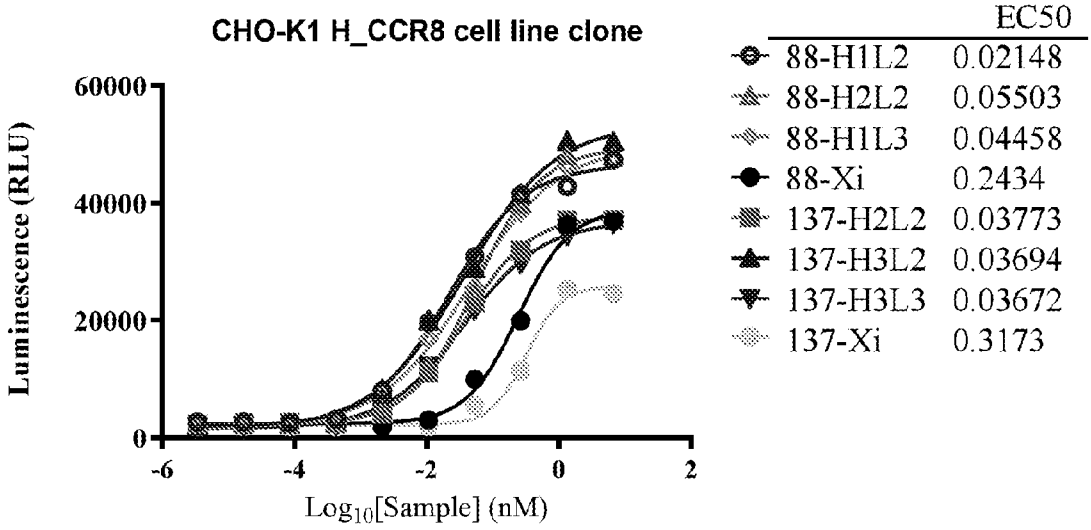

CHO-K1 H_CCR8 cell line clone
Luminescence (RLU)
60000
40000
20000
0
-6
-4
-2
0
2
Log10[Sample] (nM)
EC50
88-H1L2 0.02148
88-H2L2 0.05503
88-H1L3 0.04458
88-Xi 0.2434
137-H2L2 0.03773
137-H3L2 0.03694
137-H3L3 0.03672
137-Xi 0.3173

FIG. 5

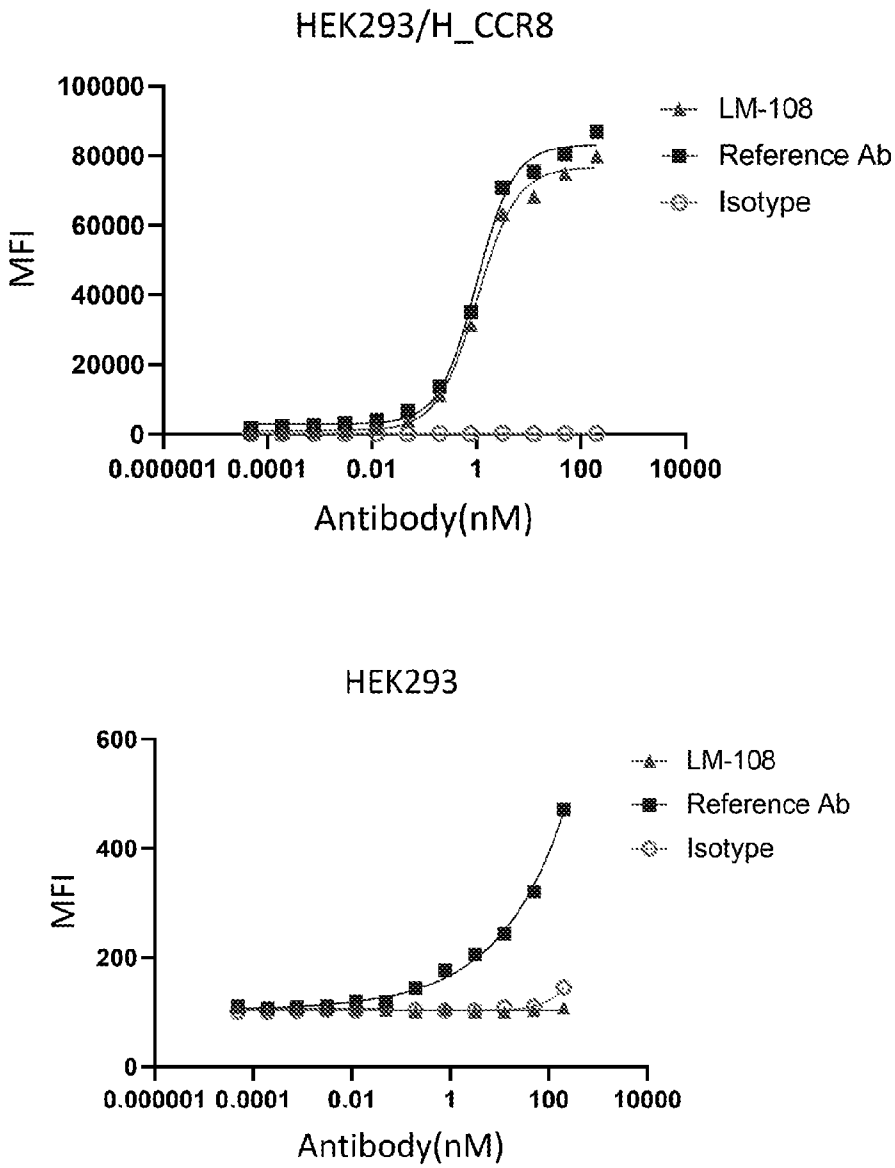

HEK293/H_CCR8
MFI
100000
80000
60000
40000
20000
0
0.000001 0.0001 0.01 1 100 10000
Antibody(nM)
LM-108
Reference Ab
Isotype
HEK293
MFI
600
400
200
0
0.000001 0.0001 0.01 1 100 10000
Antibody(nM)
LM-108
Reference Ab
Isotype

FIG. 6

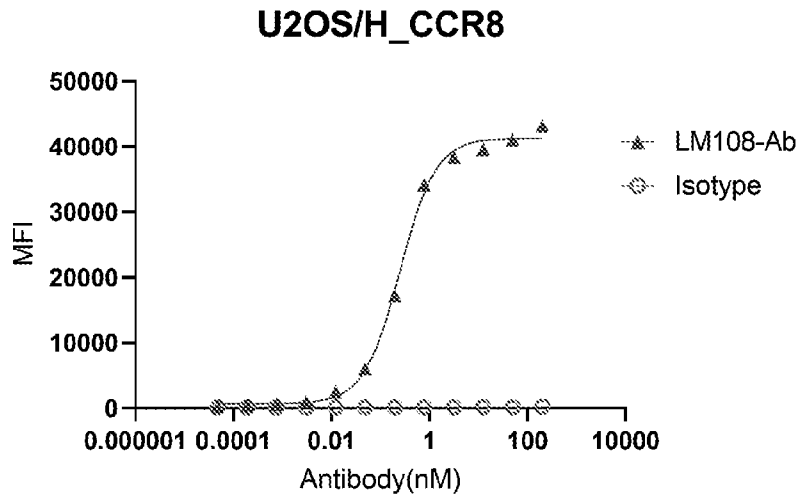

U2OS/H_CCR8
50000
40000
30000
20000
10000
0
MFI
0.000001
0.0001
0.01
1
100
10000
Antibody(nM)
LM108-Ab
Isotype

FIG. 7

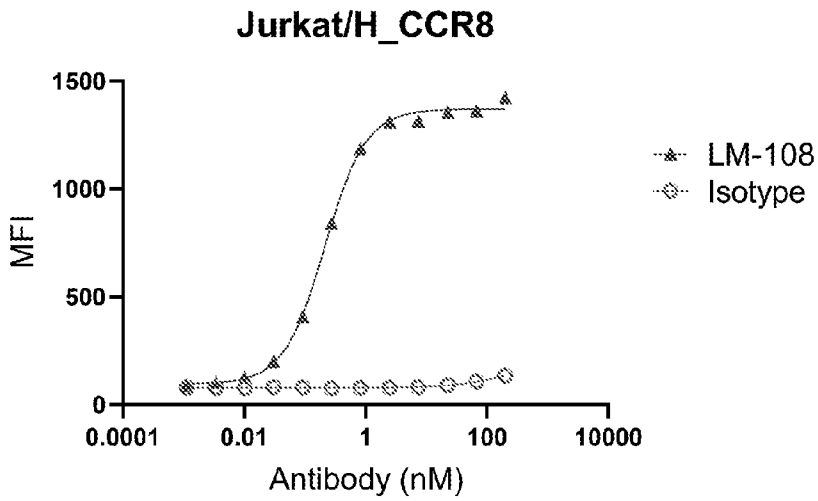

Jurkat/H_CCR8
1500
1000
500
0
MFI
0.0001
0.01
1
100
10000
Antibody (nM)
LM-108
Isotype

FIG. 8

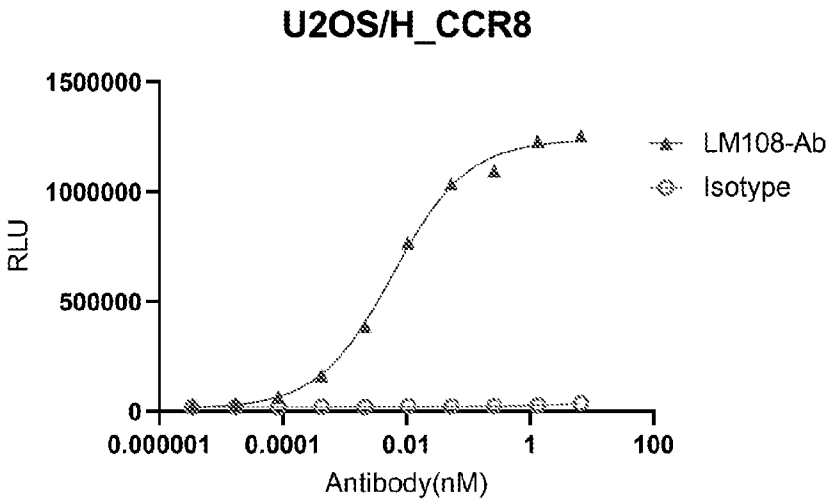

U2OS/H_CCR8
1500000
1000000
500000
0
RLU
0.000001
0.0001
0.01
1
100
Antibody(nM)
LM108-Ab
Isotype

FIG. 9

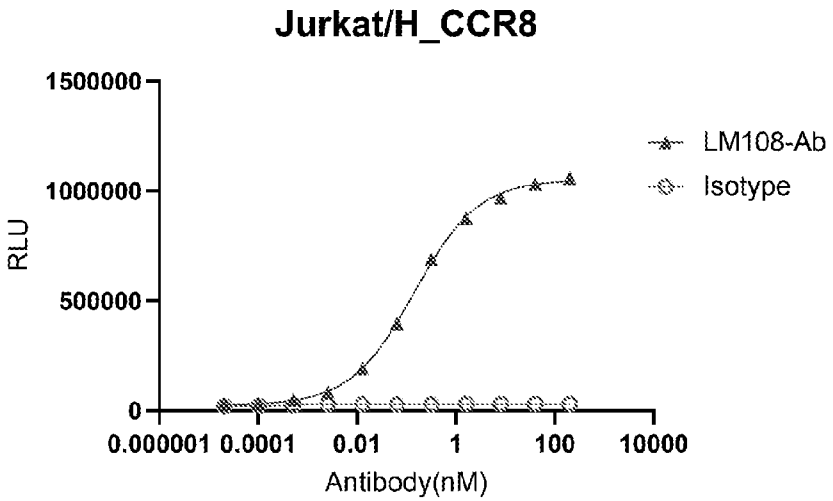

Jurkat/H_CCR8
1500000
1000000
500000
0
RLU
0.000001
0.0001
0.01
1
100
10000
Antibody(nM)
LM108-Ab
Isotype

FIG. 10

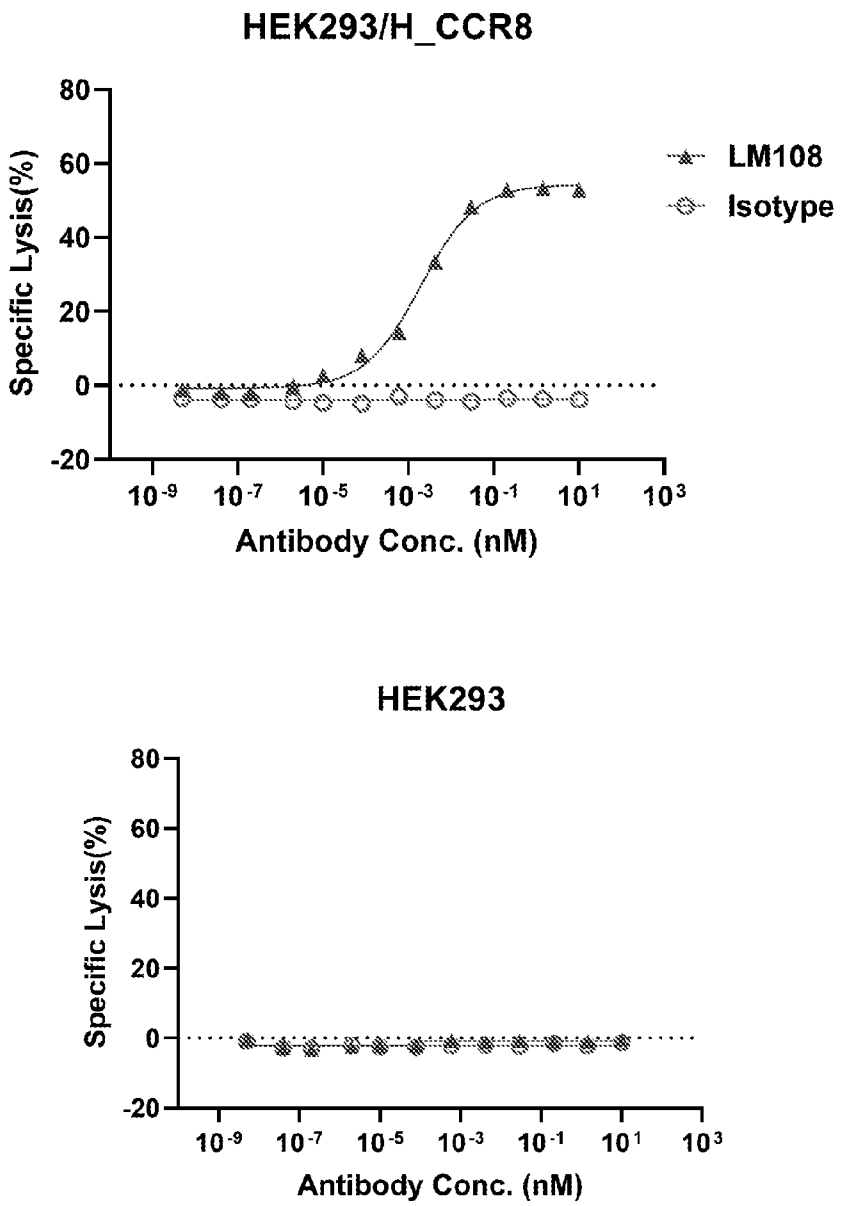

HEK293/H_CCR8
Specific Lysis(%)
80
60
40
20
0
-20
LM108
Isotype
10-9
10-7
10-5
10-3
10-1
101
103
Antibody Conc. (nM)
HEK293
Specific Lysis(%)
80
60
40
20
0
-20
10-9
10-7
10-5
10-3
10-1
101
103
Antibody Conc. (nM)

FIG. 11

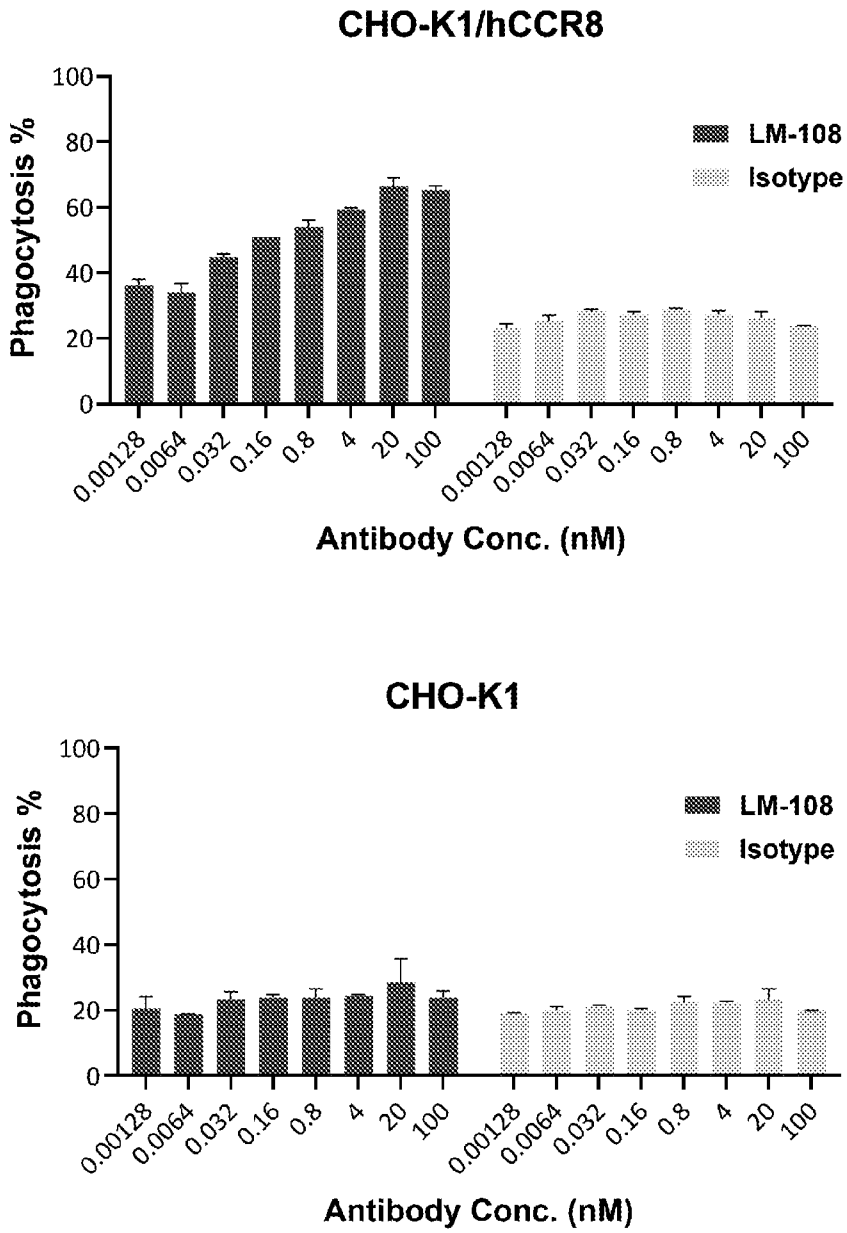
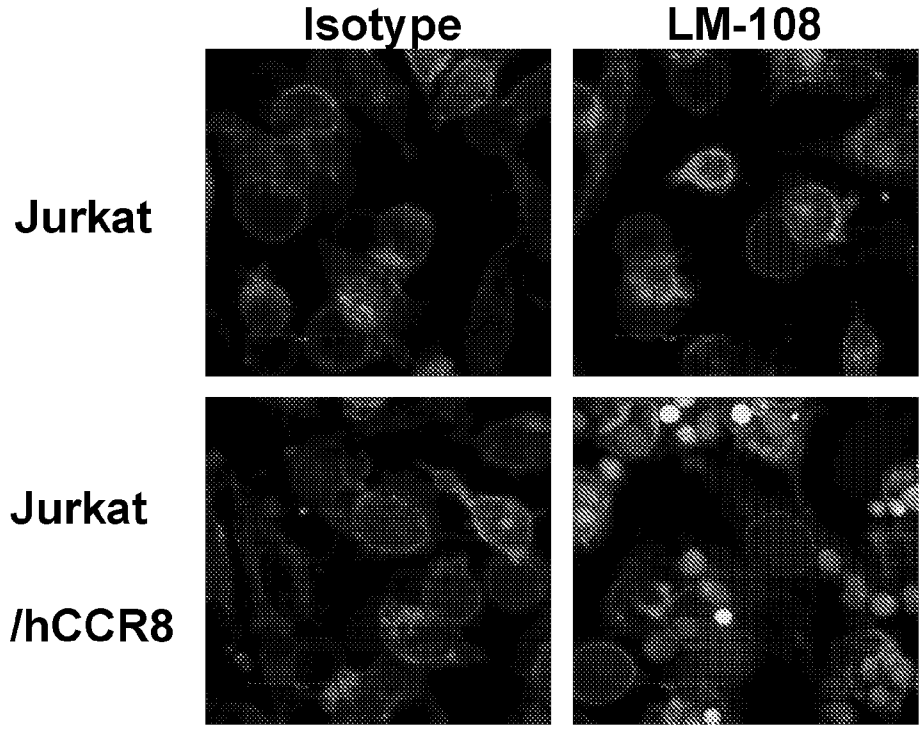

CHO-K1/hCCR8
Phagocytosis %
100
80
60
40
20
0
LM-108
Isotype
0.00128
0.0064
0.032
0.16
0.8
4
20
100
Antibody Conc. (nM)
CHO-K1
Phagocytosis %
100
80
60
40
20
0
LM-108
Isotype
0.00128
0.0064
0.032
0.16
0.8
4
20
100
Antibody Conc. (nM)

FIG. 12



Red: APC conjugated anti-CD14

Green: CFSE staining target cell

Equipment: Operetta, 40x

FIG. 13

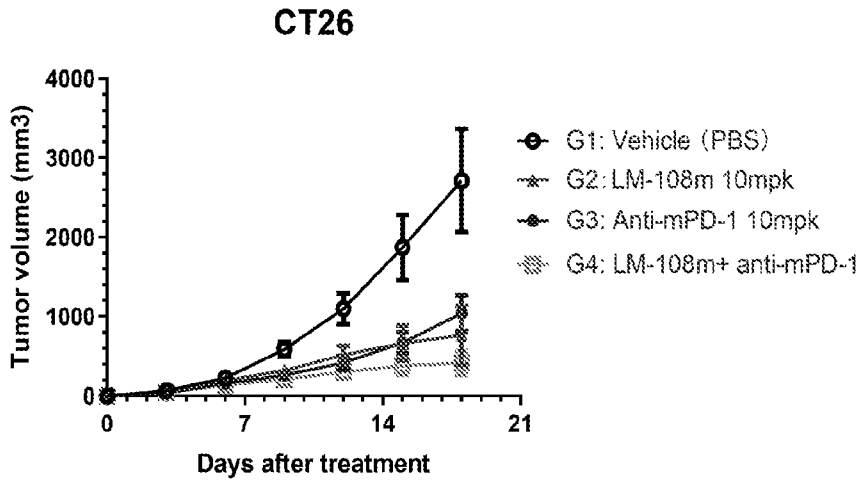

CT26
Tumor volume (mm3)
4000
3000
2000
1000
0
0
7
14
21
Days after treatment
G1: Vehicle (PBS)
G2: LM-108m 10mpk
G3: Anti-mPD-1 10mpk
G4: LM-108m+ anti-mPD-1

FIG. 14

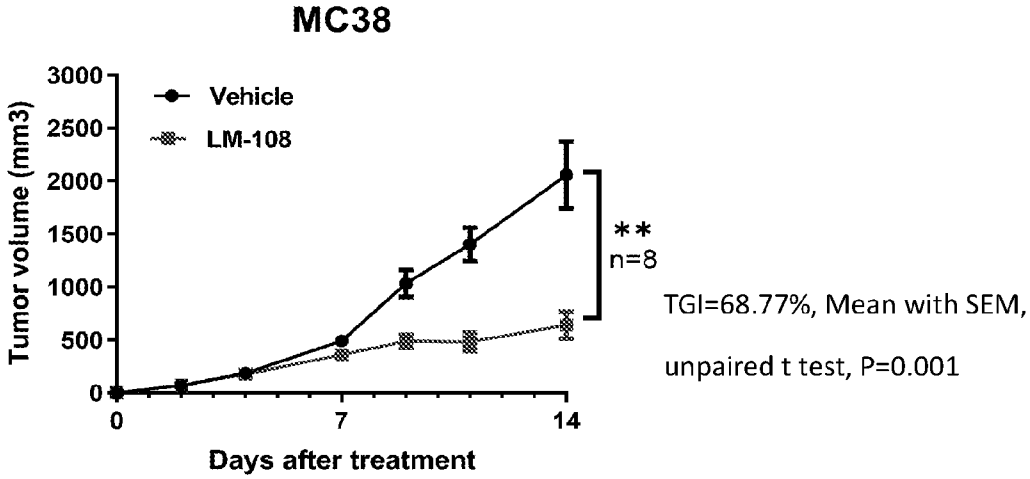

MC38
Tumor volume (mm3)
3000
2500
2000
1500
1000
500
0
0
7
14
Days after treatment
Vehicle
LM-108
**
n=8
TGI=68.77%, Mean with SEM,
unpaired t test, P=0.001

FIG. 15

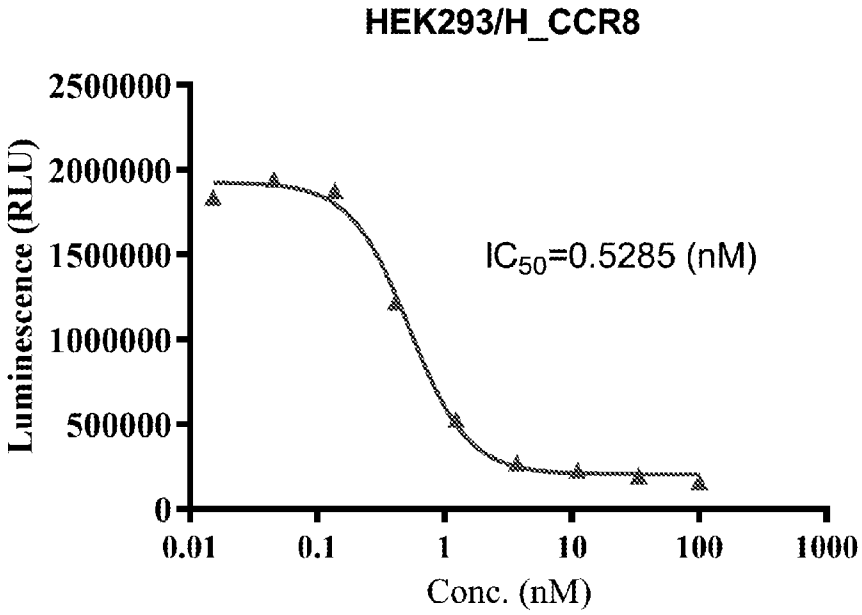

HEK293/H_CCR8
Luminescence (RLU)
2500000
2000000
1500000
1000000
500000
0
IC50=0.5285 (nM)
0.01
0.1
1
10
100
1000
Conc. (nM)

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/122994

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i; A61P 35/00(2006.01)i; A61K 39/395(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; A61P; A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI; CNABS; CNTXT; CNKI; EPTXT; USTXT; WOTXT; Pubmed; ISI Web of knowledge and keywords:chemokine, C-C motif, CCR8, chemokine receptor 8, anti-CCR8, CCL1, CCL8, antibody, etc.;GenBank; Retrieving System for Biological Sequence of Chinese Patent and searched sequences: SEQ ID NOs: 1-43.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 110835371 A (PUMISI BIO TECH SUZHOU CO LTD) 25 February 2020 (2020-02-25) see the whole document	1-14, 16
A	WO 2020138489 A1 (SHIONOGI) 02 July 2020 (2020-07-02) see the whole document	1-14, 16
A	CN 110835374 A (PUMISI BIO TECH SUZHOU CO LTD) 25 February 2020 (2020-02-25) see the whole document	1-14, 16
A	WO 2020177627 A1 (SHANGHAI YICHEN BIOMED CO LTD) 10 September 2020 (2020-09-10) see the whole document	1-14, 16
A	CN 102782148 A (VLST CORP) 14 November 2012 (2012-11-14) see the whole document	1-14, 16
A	WO 9906561 A2 (US HEALTH) 11 February 1999 (1999-02-11) see the whole document	1-14, 16

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 December 2021	**21 January 2022**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China** Facsimile No. **(86-10)62019451**	Authorized officer **WANG,Ying** Telephone No. **86-(10)-62412197**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/122994

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2021/122994

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **15,17**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] PCT Rule 39.1(iv) - Method for treatment of the human or animal body by surgery or therapy.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2021/122994

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	110835371	A	25 February 2020	None			
WO	2020138489	A1	02 July 2020	JP	6894086	B2	23 June 2021
				JP	2021101710	A	15 July 2021
				AU	2019415395	A1	15 July 2021
				TW	202039575	A	01 November 2020
				JP	WO2020138489	A1	18 February 2021
CN	110835374	A	25 February 2020	None			
WO	2020177627	A1	10 September 2020	None			
CN	102782148	A	14 November 2012	US	2011059107	A1	10 March 2011
				WO	2011025962	A1	03 March 2011
				CA	2771781	C	15 January 2019
				US	2015166647	A1	18 June 2015
				SI	2470671	T1	30 April 2015
				CA	2771781	A1	03 March 2011
				KR	20120091013	A	17 August 2012
				EP	2470671	B1	24 December 2014
				HR	P20150162	T1	08 May 2015
				US	9005617	B2	14 April 2015
				SM	T201500011	B	05 March 2015
				US	8313747	B2	20 November 2012
				ES	2531561	T3	17 March 2015
				PT	2470671	E	05 February 2015
				MX	2012002427	A	03 August 2012
				KR	101778813	B1	14 September 2017
				AU	2010286516	A2	14 June 2012
				AU	2010286516	A1	08 March 2012
				EP	2470671	A1	04 July 2012
				RU	2012111820	A	10 October 2013
				CN	102782148	B	20 April 2016
				JP	2013503192	A	31 January 2013
				SI	EP2470671	T1	30 April 2015
				PL	2470671	T3	29 May 2015
				AU	2010286516	B2	06 October 2016
				EP	2470671	A4	03 April 2013
				JP	2016135783	A	28 July 2016
				BR	112012004387	A2	08 September 2015
				IN	1769DEN2012	A	05 June 2015
				DK	2470671	T3	09 February 2015
				JP	6109571	B2	05 April 2017
				US	2013108640	A1	02 May 2013
WO	9906561	A2	11 February 1999	WO	9906561	A3	22 April 1999
				AU	8669598	A	22 February 1999