

(19) **DANMARK**

(10) **DK/EP 4214240 T3**



(12)

Oversættelse af europæisk patentskrift

Patent- og
Varemærkestyrelsen

-
- (51) Int.Cl.: **C 07 K 16/28 (2006.01)** **A 61 P 35/00 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2024-11-18**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2024-10-09**
- (86) Europæisk ansøgning nr.: **22754752.8**
- (86) Europæisk indleveringsdag: **2022-07-27**
- (87) Den europæiske ansøgnings publiceringsdag: **2023-07-26**
- (86) International ansøgning nr.: **US2022074214**
- (87) Internationalt publikationsnr.: **WO2023010054**
- (30) Prioritet: **2021-07-27 US 202163226118 P**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
- (73) Patenthaver: **AbbVie Inc., 1 N. Waukegan Road, North Chicago, IL 60064, USA**
- (72) Opfinder: **MCCLUSKEY, Andrew, J., , Chicago, IL 60064, USA**
SCHMIDT PAUSTIAN, Amanda, M., , Chicago, IL 60064, USA
SEAGAL, Jane, , Chicago, IL 60064, USA
WILSBACHER, Julie, L., , Chicago, IL 60064, USA
- (74) Fuldmægtig i Danmark: **Novagraaf Brevets, Bâtiment O2, 2 rue Sarah Bernhardt CS90017, F-92665 Asnières-sur-Seine cedex, Frankrig**
- (54) Benævnelse: **ANTI-CCR8-ANTISTOFFER**
- (56) Fremdragne publikationer:
EP-A1- 3 431 105
WO-A1-2020/138489
WO-A1-2021/142002
LINE BARINGTON: "Role of Conserved Disulfide Bridges and Aromatic Residues in Extracellular Loop 2 of Chemokine Receptor CCR8 for Chemokine and Small Molecule Binding", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 291, no. 31, 19 May 2016 (2016-05-19), US, pages 16208 - 16220, XP093167668, ISSN: 0021-9258, Retrieved from the Internet
<URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4965569/pdf/zbc16208.pdf> DOI: 10.1074/jbc.M115.706747
DIETMAR REUSCH ET AL: "Comparison of methods for the analysis of therapeutic immunoglobulin G Fc-glycosylation profiles-Part 1: Separation-based methods", MABS, vol. 7, no. 1, 2 January 2015 (2015-01-02), US, pages 167 - 179, XP055694176, ISSN: 1942-0862, DOI: 10.4161/19420862.2014.986000
GUTIERREZ JULIO ET AL: "Analysis of post-translational CCR8 modifications and their influence on receptor activity", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 279, no. 15, 1 April 2004 (2004-04-01), pages 14726 - 14733, XP002459212, ISSN: 0021-9258, DOI: 10.1074/JBC.M309689200

Fortsættes ...

DESCRIPTION

Description

3. TECHNICAL FIELD

[0001] The present application pertains to novel anti-CCR8 antibodies which comprise (i) a VH chain comprising three CDRs; and (ii) a VL chain comprising three CDRs, wherein:

VH CDR#1 is GFIFSNVAMY (SEQ ID NO: 1);

VH CDR#2 is RIKTKFNYYATYYADAVKG (SEQ ID NO:2);

VH CDR#3 is GDRNKPFAY (SEQ ID NO:3);

VL CDR#1 is RASTSVITLLH (SEQ ID NO:4);

VL CDR#2 is GASNLES (SEQ ID NO:5); and

VL CDR#3 is QQSWNDPYT (SEQ ID NO:6);

wherein the antibody comprises an IgG1 Fc constant region that is afucosylated.

[0002] The invention further provides a composition comprising a plurality of anti-CCR8 antibodies which comprise (i) a VH chain comprising three CDRs; and (ii) a VL chain comprising three CDRs, wherein:

VH CDR#1 is GFIFSNVAMY (SEQ ID NO: 1);

VH CDR#2 is RIKTKFNYYATYYADAVKG (SEQ ID NO:2);

VH CDR#3 is GDRNKPFAY (SEQ ID NO:3);

VL CDR#1 is RASTSVITLLH (SEQ ID NO:4);

VL CDR#2 is GASNLES (SEQ ID NO:5); and

VL CDR#3 is QQSWNDPYT (SEQ ID NO:6); and

wherein greater than 80% of the anti-CCR8 antibodies in the composition are afucosylated.

4. BACKGROUND

[0003] Within tumors, regulatory T cells (Tregs) are a key suppressive population known to prevent anti-tumor immune responses. An increased presence of intratumoral Tregs has been associated with poorer patient outcomes in several cancers (Shang et al, Nature Sci. Reports, 2015; Fridman et al., Nat Rev Clin Oncol. 2017; Bruni et al., Nat Rev Cancer. 2020). Chemokine receptor 8 (CCR8) is a cell surface protein uniquely expressed by intratumoral Tregs in several human cancers (Plitas et al, Immunity 2016; De Simone et al, Immunity 2016). This makes CCR8 an attractive target to mediate selective intratumoral Treg depletion via antibody-dependent cellular cytotoxicity (ADCC) to enhance anti-tumor immunity.

WO2021/142002 antibodies or antigen-binding portions thereof that specifically bind human CCR8, polynucleotides and vectors encoding the same, and pharmaceutical compositions comprising the same. WO2020/138489 also discloses an anti-CCR8 antibody and the use of said antibody for treating or preventing cancers. EP3431105 discloses pharmaceutical composition for cancer treatment comprising an antibody against CCR8.

[0004] While Treg depletion has long been investigated, most of these treatments have limited efficacy owing to target expression on tumor-infiltrating effector T cell populations and/or Tregs outside the tumor environment. There remains a need in the art for a monoclonal antibody therapeutic that triggers the death of intratumoral immunosuppressive Tregs without depleting other key effector T cell populations in the tumor microenvironment or peripheral Tregs.

5. SUMMARY OF INVENTION

[0005] Anti-CCR8 monoclonal antibodies have been shown to mediate selective intratumoral Treg depletion via antibody-dependent cellular cytotoxicity (ADCC). Because CCR8 is preferentially expressed by tumor infiltrating Tregs and is not highly expressed in peripheral blood Tregs or by beneficial effector T cell populations, an anti-CCR8 antibody would deplete intratumoral Tregs and enhance anti-tumor immunity. While anti-CCR8 antibody monotherapy is independently effective, the specific removal of Tregs within a tumor provides an amenable environment for appropriate combination therapy approaches aimed at concurrently stimulating the anti-tumor immune response. For instance, combination with a checkpoint inhibitor like anti-PD-1 may be required to fully drive potent anti-tumor immunity upon Treg depletion.

[0006] Accordingly, the amino acid sequence for a monoclonal, humanized antibody that specifically binds CCR8 and mediates ADCC of CCR8 expressing Tregs is provided. The antibody structurally consists of a variable heavy and variable light chain comprising complementary determining regions (CDRs) that specifically bind CCR8. The antibody also includes a human heavy chain constant region comprising a fragment crystallizable region (Fc) and a light chain constant region. These structural elements, as coded by the antibody's amino acid sequence, comprise a pharmaceutical composition effective at treating solid tumors in a patient, either as a monotherapy or in combination with other therapeutics.

6. BRIEF DESCRIPTION OF THE DRAWINGS

[0007]

FIG. 1 shows binding of chimeric rat/hlgG1 human CCR8 specific chimeric mAbs to Jurkat cells expressing human or cynomolgus CCR8.

FIG. 2 shows results of epitope binning experiments.

FIG. 3 shows heavy and light chain sequences of ABBV-514.

FIG. 4A shows binding dose-response curves for afucosylated ABBV-514 to TALL-1 cells expressing endogenous CCR8 or TALL-1 CCR8 knockout cells and to Jurkat parent cells or cells overexpressing human or cyno CCR8. FIG. 4B shows binding of fucosylated (WT PR-1925514) vs afucosylated ABBV-514 to TALL-1 cells.

FIGS. 5A-D show ADCC reporter bioassay data for fucosylated (WT PR-1925514) vs afucosylated ABBV-514.

FIG. 6 shows results of an ADCC assay with purified NK effector cells and TALL-1 target cells.

FIG. 7 show results of a CCR8 beta-arrestin reporter assay showing effect of ABBV-514 on CCL1 binding to CCR8.

7. DETAILED DESCRIPTION

[0008] Tregs are a subset of CD4⁺ T cells with intratumoral immunosuppressive effects. Tregs suppress the activation, proliferation, and cytokine production of CD4⁺ T cells and CD8⁺ T cells, preventing deleterious autoimmune responses. However, Tregs also suppress tumor immunity, and high intratumoral Treg levels have been associated with negative outcomes in several cancers.

[0009] CCR8 is a C-C motif chemokine receptor consisting of a seven-pass transmembrane protein that mediates chemotaxis and cell/cell interactions in the context of T-helper type 2 (Th2) lymphocyte immune responses and T cell trafficking to the skin. CCR8-deficient mice are viable, fertile, and largely normal, except that they cannot mount robust Th2 responses in certain Th2-associated preclinical models (Chensue et al., J Exp Med 5, 2001). The ligand primarily associated with CCR8 is CCL1, although CCL18 (human) and CCL8 (murine) are also ligands for this receptor.

[0010] CCR8-expressing Tregs that infiltrate tumors show a highly activated and immunosuppressive phenotype. Tumor studies in CCR8 knockout mice indicate that the loss of CCR8 expression does not influence Treg recruitment to the tumor microenvironment,

activation status, or suppressive capacity (Van Damme et al., J Immunother Cancer. 9(2), 2021). Rather, CCR8 expression is a marker of highly suppressive Tregs. Therefore, depletion of CCR8-expressing Tregs provides anti-tumor benefits.

[0011] CCR8-specific surrogate antibodies mediate selective intratumoral Treg depletion via ADCC. Additionally, anti-CCR8 surrogate antibodies significantly enhance the frequency of circulating tumor-specific CD8⁺ effector T lymphocytes. These effects correlate with efficacy in mouse syngeneic tumor models. (Campbell et al., Cancer Research 81, 2021).

[0012] The present inventors have developed therapeutic monoclonal antibodies, as defined in the claims, that specifically bind CCR8 expressed on the surface of cells, for example, intratumoral Tregs. The antibody is composed of two variable chains, one heavy and one light, as defined in the claims. On each variable chain, there are three CDRs that allow the antibody to bind to CCR8, as defined in the claims. On both variable chains, there are a total of six different CDRs, as defined in the claims. Additionally, the antibody contains a human heavy chain constant region comprising a human Fc of the immunoglobulin class G1 (IgG1), as defined in the claims. The anti-CCR8 antibodies described comprise an IgG1 Fc constant region that is afucosylated and demonstrate *in vitro* functionality, immunosafety, and drug-like properties.

[0013] The antibody comprises an IgG1 Fc constant region that is afucosylated. Afucosylation may be carried out by techniques known in the art. See, e.g., Mol Cancer Ther (2020) 19 (5): 1102-1109) and PNAS (2013)110(14) 5404-5409. For example, production of antibodies in cell lines defective in GDP-fucose formation due to, for example, a deficiency in GDP-mannose 4,6-dehydratase; production of antibodies in cells that have decreased levels of fucosyltransferase; production of antibodies in cells that have decreased levels of GDP-fucose transporter; production of antibodies in cells that overexpress β -1,4-mannosyl-glycoprotein 4- β -N-acetylglucosaminyltransferase (GnT-III); or production of antibodies in cells that express a bacterial GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD). The cells used to produce the afucosylated anti-CCR8 antibodies of the invention may be CHO cells engineered to express Pseudomonas RMD. The degree of afucosylation of antibodies can be determined through techniques known in the art.

[0014] To obtain anti-CCR8 antibodies that cross-react with human and cynomolgus CCR8, the ability of mouse-human (rat/hIgG1) chimeric antibodies to bind Jurkat cells overexpressing either human or cynomolgus CCR8 was evaluated. Results were analyzed via flow cytometry as carried out by techniques known in the art. As a result, the final anti-CCR8 antibody cross-reacts with human and cynomolgus CCR8 and mediates ADCC in both species, but does not bind mouse, rat, or rabbit CCR8.

[0015] In certain embodiments, an afucosylated antibody of the invention has higher affinity for activating receptors for IgG, as well as enhanced activity in purified natural killer cell or peripheral blood mononuclear cell (PBMC) ADCC assays compared to a fucosylated form of that antibody. The ADCC activity of anti-CCR8 antibodies can be demonstrated using ADCC

bioassay techniques known in the art. For example, in human FcγRIIIa V158 or F158 allelic variant reporter lines cocultured with human or cynomolgus CCR8-expressing Jurkat cells, anti-CCR8 antibodies triggered ADCC as measured via luminescence induction using techniques known in the art.

[0016] Additionally, the antibodies described herein interfere with the efficacy of other naturally occurring ligands, like CCL1, that also bind CCR8, but only at substantially higher EC₅₀s than for binding or ADCC activity. This testing was carried out by techniques known in the art, for example via beta-arrestin reporter assays.

[0017] As used herein, the term "antibody" (Ab) refers to an immunoglobulin molecule that specifically binds to CCR8, as defined in the claims. The anti-CCR8 antibodies of the disclosure, as defined in the claims, bind to human CCR8 on Tregs and thereby modulate the immune system. Anti-CCR8 antibodies of the disclosure, as defined in the claims, comprise complementarity determining regions (CDRs), also known as hypervariable regions, in both the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). As is known in the art, the amino acid position/boundary delineating a hypervariable region of an antibody can vary, depending on the context and the various definitions known in the art. Some positions within a variable domain may be viewed as hybrid hypervariable positions in that these positions can be deemed to be within a hypervariable region under one set of criteria while being deemed to be outside a hypervariable region under a different set of criteria. One or more of these positions can also be found in extended hypervariable regions. The disclosure provides antibodies comprising modifications in these hybrid hypervariable positions. The variable domains of native heavy and light chains each comprise four FR regions, largely by adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the target binding site of antibodies. See Kabat et al., *Sequences of Proteins of Immunological Interest* (National Institute of Health, Bethesda, Md. 1987).

[0018] The antibodies as defined in the claims may be polyclonal, monoclonal, genetically engineered, and/or otherwise modified in nature, including but not limited to chimeric antibodies, humanized antibodies, human antibodies, single chain antibodies, etc. In various embodiments, the antibodies comprise all or a portion of a constant region of an antibody. The anti-CCR8 antibodies described herein comprise an IgG1 as defined in the claims. As used herein, the "constant region" of an antibody includes the natural constant region, allotypes or variants.

[0019] The light constant region of an anti-CCR8 antibody may be a kappa (κ) light region or a lambda (λ) region. A λ light region can be any one of the known subtypes, e.g., λ1, λ2, λ3, or λ4. In some embodiments, an anti-CCR8 antibody comprises a kappa (κ) light region.

[0020] The term "monoclonal antibody" as used herein is not limited to antibodies produced

through hybridoma technology. A monoclonal antibody is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, by any means available or known in the art. Monoclonal antibodies useful with the present disclosure can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof.

[0021] The term "chimeric" antibody as used herein refers to an antibody having variable sequences derived from a non-human immunoglobulin, such as a rat or a mouse antibody, and human immunoglobulin constant regions, typically chosen from a human immunoglobulin template.

[0022] "Humanized" forms of non-human (e.g., murine) antibodies comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody can also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin consensus sequence.

[0023] "Human antibodies" include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous functional immunoglobulins. Human antibodies can be made by a variety of methods known in the art including phage display methods using antibody libraries derived from human immunoglobulin sequences.

[0024] Anti-CCR8 antibodies of the disclosure include full-length (intact) antibody molecules.

[0025] The anti-CCR8 antibodies as defined in the claims may be antibodies whose sequences have been modified to alter at least one constant region-mediated biological effector function. For example, the anti-CCR8 antibodies described herein include antibodies that have been modified to acquire or improve at least one constant region-mediated biological effector function relative to an unmodified antibody, e.g., to enhance FcγR interactions (See, e.g., US Patent Appl. No. 2006/0134709) or to enhance the antibody's ability to mediate ADCC. For example, an anti-CCR8 antibody of the disclosure as defined in the claims can have a constant region that binds FcγRt, FcγRIIA, FcγRIIB, FcγRIIIA and/or FcγRIIIB with greater affinity than the corresponding unmodified constant region. An anti-CCR8 antibody of the disclosure as defined in the claims can be one that has a modified Fc region and mediates an enhanced ADCC response, wherein the ADCC response is enhanced with respect to an antibody having the same variable regions (i.e., VH and VL) and a wild type IgG1 Fc region (i.e., wild type CL, CH1, CH2, and CH3). Fc modifications able to enhance ADCC, such as amino acid sequence mutations, are known in the art, and can include the following sets of mutations: S239D/I332E; F243L/R292P/Y300L/V305I/P396L; S239D/I332E/A330L; and S298A/E333A/K334A.

[0026] Anti-CCR8 antibodies that comprise a human IgG4 constant region can comprise the

S228P mutation, which has been reported to prevent Fab arm exchange. See, e.g., Silva, JP et al. *Journal of Biological Chemistry*, 290(9), 5462-5469 (2015).

[0027] In some embodiments, the anti-CCR8 antibodies as defined in the claims include modifications that increase or decrease their binding affinities to the fetal Fc receptor, FcRn, for example, by mutating the immunoglobulin constant region segment at particular regions involved in FcRn interactions. In particular embodiments, an anti-CCR8 antibody of the IgG class is mutated such that at least one of amino acid residues 250, 314, and 428 of the heavy chain constant region is substituted alone, or in any combinations thereof. For position 250, the substituting amino acid residue can be any amino acid residue other than threonine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, glutamine, arginine, serine, valine, tryptophan, or tyrosine. For position 314, the substituting amino acid residue can be any amino acid residue other than leucine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, methionine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, or tyrosine. For position 428, the substituting amino acid residues can be any amino acid residue other than methionine, including, but not limited to, alanine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, asparagine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, or tyrosine. An exemplary substitution known to modify Fc effector function is the Fc substitution M428L, which can occur in combination with the Fc substitution T250Q. Additional specific combinations of suitable amino acid substitutions are identified in Table 1 of U.S. Patent No. 7,217,797. Such mutations increase binding to FcRn, which protects the antibody from degradation and increases its half-life.

[0028] Anti-CCR8 antibodies with high affinity for human CCR8 may be desirable for therapeutic and diagnostic uses. Accordingly, the present disclosure contemplates antibodies having a high binding affinity to human CCR8. In specific embodiments, the anti-CCR8 antibodies binds to human CCR8 with an affinity of at least about 100 nM, but may exhibit higher affinity, for example, at least about 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.1 nM, 0.01 nM, or even higher. In some embodiments, the antibodies bind human CCR8 with an affinity in the range of about 1 pM to about 10 nM, of about 100 pM to about 10 nM, about 100 pM to about 1 nM, or an affinity ranging between any of the foregoing values.

[0029] In some embodiments, the invention provides a monoclonal anti-CCR8 antibody, as defined in the claims, comprising two sets of six different complementarity-determining regions (CDRs), two sets of two different variable regions, two full heavy chains, two full light chains, and a human heavy chain constant region.

[0030] In some embodiments, the antibody as defined in the claims is a recombinant, afucosylated, humanized, IgG1 kappa monoclonal antibody that binds to chemokine receptor 8.

[0031] The antibody comprises six CDRs comprising the following sequences:

CDR-H1:	GFIFSNVAMY (SEQ ID NO: 1)
CDR-H2:	RIKTKFNYYATYYADAVKG (SEQ ID NO: 2)
CDR-H3:	GDRNKPFAY (SEQ ID NO: 3)
CDR-L1:	RASTSVITLLH (SEQ ID NO: 4)
CDR-L2:	GASNLES (SEQ ID NO: 5)
CDR-L3:	QQSWNDPYT (SEQ ID NO: 6)

[0032] The antibody of this disclosure comprises a CDR-H1 having the amino acid sequence shown as SEQ ID NO: 1, a CDR-H2 having the amino acid sequence shown as SEQ ID NO: 2; a CDR-H3 having the amino acid sequence shown as SEQ ID NO: 3, a CDR-L1 having the amino acid sequence shown as SEQ ID NO: 4, a CDR-L2 having the amino acid sequence shown as SEQ ID NO: 5; and a CDR-L3 having the amino acid sequence shown as SEQ ID NO: 6; and comprises an IgG Fc constant region that is afucosylated.

[0033] In some embodiments, the antibody of this disclosure comprises a heavy chain variable region comprising the amino acid sequence shown as SEQ ID NO: 7:

EVQLVESGGGLVQPGGSLKL SCAASGFIFSNVAMYWRQA SGKGLEWARIKTKFNYYAT
YYADAVKGRFTISRDDSKNM VYLQMNSLKTEDTAVYYCTA GDRNKPFAYWGQGTLLTVSS
(SEQ ID NO: 7); and a light chain variable region comprising the amino acid sequence shown
as SEQ ID NO: 8: ETVVTQSPATLSLSPGERAT LSCRASTSVITLLHWFQQKP
GQAPRLLIHGASNLESRVPA RFSGSGSGTDFTLTSSLEP EDFATYFCQQSWNDPYTFGQ
GTKLEIK (SEQ ID NO: 8).

[0034] In some embodiments, the antibody of this disclosure comprises a heavy chain comprising the amino acid sequence shown as SEQ ID NO: 9 (constant regions are **bold**; the variable heavy domain is underlined; CDRs are **underlined bold italic** (disclosed as SEQ ID NOS: 1-3, respectively, in order of appearance)):

EVQLVESGGGLVQPGGSLKSCAAS**GFIFSNVAMYWRQA**SGKGLEWARIKTKFNYYATY
YADAVKGRFTISRDDSKNMVYLQMNSLKTEDTAVYYCTAGDRNKPFAYWGQGTLLTVSSAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (full-length sequence disclosed as SEQ ID
NO: 9) and a light chain comprising the amino acid sequence shown as SEQ ID NO: 10
(constant regions are **bold**; the variable light domain is underlined; CDRs are **underlined bold italic**
italic (CDR sequences disclosed as SEQ ID NOS: 4-6, respectively, in order of appearance)):
ETVVTQSPATLSLSPGERATLSCRASTSVITLLHWFQQKPGQAPRLLIH**GASNLES**RVPARFSG
SGSGTDFTLTSSLEPEDFATYFCQQSWNDPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSG
TASVCLLNFPYFPAKVKVQKVDNALQSGNSQESVTEQDSKDYSLSSITLTLSKADYE

KHKVYACEVTHQGLSSPVTKSFNRGEC (full-length sequence disclosed as SEQ ID NO: 10).

[0035] In an embodiment, the antibody of the disclosure comprises a light chain according to SEQ ID NO: 10, and a heavy chain having the C-terminal lysine truncated, for example, a heavy chain according to SEQ ID NO: 9 with the C-terminal lysine truncated:

EVQLVESGGGLVQPGGSLKLSCAASGFIFSN**AVMYWVRQASGKGLEWARIKTKFN****NYATY**
YADAVKGRFTISRDDSKNMVYLQMNSLKTEDTAVYYCTAGDRNKPFAYWGQGT**LVTVSSAS**
 TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
 YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
 EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKS
 RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (terminal lysine truncated sequence disclosed
 as SEQ ID NO: 11)

[0036] The heavy chain of the antibody of this disclosure may be encoded by the following nucleotide sequence (full-length sequence disclosed as SEQ ID NO: 12):

ATGGAATTCGGCCTGAGCTGGCTGTTCTTGGTGGCCATCCTGAAGGGCGTGCAGTGCGAAGT
 CCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATCTCTGAAGCTGTCTTG
 TGCCGCCAGCGGCTTCATCTTCAGCAACGCCGTGATGTACTGGGTCCGACAGGCCCTCTGG
 CAAAGGCCTGGAATGGGTCGCCAGAATCAAGACCAAGTTCAACAACCTACGCCACCTACT
 ACGCCGACGCCGTGAAGGGCAGATTCACCATCAGCAGGGACGACAGCAAGAACATGGT
 GTACCTGCAGATGAACAGCCTGAAAACCGAGGACACCGCCGTGTAATACTGCACAGCCG
 GCGACAGAAACAAGCCCTTTGCCTATTGGGGCCAGGGCACCCCTGGTTACCGTTAGCTCTG
 CCTCCACCAAGGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCT
 GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGA
 CGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCT
 CTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG
 CTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG
 TGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTGC
 CCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCCTAAACCCAA
 GGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA
 GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT
 AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCA
 GCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
 GTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGG
 GCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCA
 AGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCC
 GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCG
 TGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGC
 AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA
 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGCAAAATGA

Secretion signal peptide in *italics*; includes final stop codon (TGA); constant region is **bold**;

CDRs are underlined.

[0037] The light chain of the antibody of this disclosure may be encoded by the following nucleotide sequence (full-length sequence disclosed as SEQ ID NO: 13):

*ATGGACATGCGGGT*GCCCCGCCAGCTGCTGGGACTTC*TGCTGCTGIGGTTCCCCGGCAGCAG*
ATGCGAGACAGTGGTCACACAGTCTCCCGCCACACTGTCACTGTCTCCAGGCGAAAGAGC
*CACACTGAGCTGT*AGAGCCAGCACACGCGTGATCACACTGCTGCACTGGTTCCAGCAGA
AGCCTGGACAGGCTCCAGACTGCTGATTCACGGCGCCAGCAACCTGGAAAGCAGAGTG
CCTGCCAGATTTTCCGGCAGCGGCTCTGGCACCGATTTCACCCTGACCATAAGCAGCCTG
*GAACCTGAGGACTTCGCCACCTACTTTTGCC**CAGCAGAGCTGGAACGACCCCTACACCTTT*
GGCCAGGGCACCAAGCTGGAAATCAAGCGAACTGTGGCTGCACCATCTGTCTTTCATC
*TTCCCCGCCATCTGATGAGCAGTTGAAATCTGGA**ACTGCCTCTGTTGTGTGCCTGCTG*
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCCA
ATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACA
GCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAG
GGGAGAGTGTTAG

Secretion signal peptide in *italics*; includes final stop codon (TAG); constant region is **bold**; CDRs are underlined.

[0038] In some embodiments, the antibody as defined in the claims comprises a human heavy chain constant region comprising human CH1, human hinge, human CH2, and human CH3 domain. In some embodiments, the encoded heavy chain constant region comprises an Fc portion, wherein the Fc portion is a human IgG1, IgG2, IgG3, IgG4, or IgM isotype. In an embodiment, the Fc is an IgG1, and the allotype is z non a. In an embodiment, the light chain is a kappa light chain.

[0039] In some embodiments, the antibody as defined in the claims comprises an IgG1 Fc constant region that is afucosylated. Afucosylation may be carried out by techniques known in the art. For example, production of antibodies in cell lines defective in GDP-fucose formation due to, for example, a deficiency in GDP-mannose 4,6-dehydratase; production of antibodies in cells that have decreased levels of fucosyltransferase; production of antibodies in cells that have decreased levels of GDP-fucose transporter; production of antibodies in cells that overexpress β -1,4-mannosylglycoprotein 4- β -N-acetylglucosaminyltransferase (GnT-III); or production of antibodies in cells that express a bacterial GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD). The cells used to produce the afucosylated anti-CCR8 antibodies of the invention as defined in the claims are CHO cells engineered to express Pseudomonas RMD. The degree of afucosylation of antibodies can be determined through techniques known in the art. Typically, the antibody is 70% or more, 80% or more, 90% or more, or about 99%, or about 100% afucosylated. Preferably, the degree of afucosylation is equal to or greater than 80%. In some embodiments, the antibody is 70% or more, 80% or more, 90% or more, or about 100% afucosylated at position ASN-300 (EU: ASN-297). Afucosylation can be determined via hydrophilic interaction chromatography (HILIC) assay techniques, in which the degree of afucosylation is determined by polarity-dependent separation of the fragmented antibodies.

[0040] In an embodiment, the total afucosylated glycan species is determined by analysis of released N-linked glycans by HILIC with fluorescent detection. The glycans are released using peptide N-glycosidase F (PNGaseF) and subsequently labeled with a fluorescent tag. Fluorescently labeled N-linked glycans are analyzed by HILIC with fluorescence detection. The percent afucosylated glycan species is determined based on the sum of the peak areas of all afucosylated glycan peaks relative to the total peak area of all glycan peaks in the chromatogram. All peaks with a relative abundance of 0.5% or greater are included in the determination of percent afucosylated glycan species.

7.1. Polynucleotides Encoding the Anti-CCR8 Antibodies, Expression Systems and Methods of Making the Antibodies

[0041] The present disclosure encompasses polynucleotide molecules encoding immunoglobulin light and heavy chain genes for anti-CCR8 antibodies, vectors comprising such polynucleotides, and host cells capable of producing the anti-CCR8 antibodies as defined in the claims.

[0042] An anti-CCR8 antibody as defined in the claims can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, optionally, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered.

[0043] To generate polynucleotides encoding such anti-CCR8 antibodies, DNA fragments encoding the light and heavy chain variable regions are first obtained. These DNAs can be obtained by amplification and modification of germline DNA or cDNA encoding light and heavy chain variable sequences, for example using the polymerase chain reaction (PCR).

[0044] Once DNA fragments encoding anti-CCR8 antibody-related VH and VL segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked," as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0045] The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2, CH3 and, optionally, CH4). The sequences of human heavy

chain constant region genes are known in the art (See, e.g., Kabat, E.A., et al., 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but in certain embodiments is an IgG1 or IgG4. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

[0046] The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (See, e.g., Kabat, et al., 1991, Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but in certain embodiments is a kappa constant region.

[0047] To express the anti-CCR8 antibodies as defined in the claims, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors or, more typically, both genes are inserted into the same expression vector.

[0048] The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the anti-CCR8 antibody-related light or heavy chain sequences, the expression vector can already carry antibody constant region sequences. For example, one approach to converting the anti-CCR8 monoclonal antibody-related VH and VL sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[0049] In addition to the antibody chain genes, the recombinant expression vectors of the disclosure carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes.

[0050] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the disclosure can carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced. For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, lipofection, calcium-phosphate precipitation, DEAE-dextran transfection and the like.

[0051] It is possible to express the antibodies of the disclosure in either prokaryotic or eukaryotic host cells. In certain embodiments, expression of antibodies is performed in eukaryotic cells, e.g., mammalian host cells, of optimal secretion of a properly folded and immunologically active antibody. Exemplary mammalian host cells for expressing the recombinant antibodies of the disclosure include Chinese Hamster Ovary (CHO cells) (including DHFR- CHO cells, described in Urlaub and Chasin, 1980, Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, 1982, Mol. Biol. 159:601-621), NSO myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It is understood that variations on the above procedure are within the scope of the present disclosure. For example, it can be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an anti-CCR8 antibody of this disclosure.

[0052] Recombinant DNA technology can also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to human CCR8. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the disclosure.

[0053] For recombinant expression of an anti-CCR8 antibody of the disclosure, the host cell can be co-transfected with two expression vectors of the disclosure, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors can contain identical selectable markers, or they can each

contain a separate selectable marker. Alternatively, a single vector can be used which encodes both heavy and light chain polypeptides.

[0054] Once a polynucleotide encoding one or more portions of an anti-CCR8 antibody has been obtained, further alterations or mutations can be introduced into the coding sequence, for example to generate polynucleotides encoding antibodies with different CDR sequences, antibodies with reduced affinity to the Fc receptor, or antibodies of different subclasses.

[0055] The anti-CCR8 antibodies as defined in the claims can also be produced by chemical synthesis or by using a cell-free platform.

7.2. Purification of Anti-CCR8Antibodies

[0056] Once a polypeptide of the disclosure has been produced by recombinant expression, it can be purified by any method known in the art for purification of a protein. Once isolated, an anti-CCR8 antibody can be further purified.

7.3. Compositions

[0057] The antibodies as defined in the claims may be provided as a composition suitable for administration to a subject. In some embodiments, the antibody composition is a pharmaceutical composition, comprising an antibody of this disclosure and a pharmaceutically acceptable carrier.

[0058] In an embodiment, a pharmaceutical composition comprising a plurality of anti-CCR8 antibodies and a pharmaceutically acceptable carrier is provided, as defined in the claims. The plurality of anti-CCR8 antibody of the pharmaceutical composition is afucosylated. Typically, the plurality of antibodies is 80% or more, 90% or more, or about 99%, or about 100% afucosylated. Preferably, the degree of afucosylation is equal to or greater than 90%. In some embodiments, the plurality of antibodies is 80% or more, 90% or more, or about 100% afucosylated at position ASN-300 (EU: ASN-297). The degree of afucosylation of antibodies can be determined through techniques known in the art. Afucosylation can be determined via hydrophilic interaction chromatography (HILIC) assay techniques, in which the degree of afucosylation is determined by polarity-dependent separation of the fragmented antibodies. In an embodiment, the total afucosylated glycan species is determined by analysis of released N-linked glycans by HILIC with fluorescent detection. The glycans are released using peptide N-glycosidase F (PNGaseF) and subsequently labeled with a fluorescent tag. Fluorescently labeled N-linked glycans are analyzed by HILIC with fluorescence detection. The percent afucosylated glycan species is determined based on the sum of the peak areas of all afucosylated glycan peaks relative to the total peak area of all glycan peaks in the chromatogram. All peaks with a relative abundance of 0.5% or greater are included in the

determination of percent afucosylated glycan species.

7.4. Summary of Properties of Subject Antibodies

[0059] Properties of subject antibodies, exemplified by but not limited to ABBV-514, include the following:

High affinity binding to CCR8, e.g., FACS binding mean EC_{50} of 2 $\mu\text{g/mL}$ or lower, 1 $\mu\text{g/mL}$ or lower, from 0.3 through 0.7 $\mu\text{g/mL}$, 0.4 through 0.6 $\mu\text{g/mL}$, about 0.5 $\mu\text{g/mL}$, or 0.55 $\mu\text{g/mL}$ determined with endogenously expressed CCR8 on TALL-1 cells (Human adult T-ALL; RRID: CVCL 1736); or FACS binding mean EC_{50} to human CCR8 overexpressed on Jurkat cells of 2 $\mu\text{g/mL}$ or lower, 1 $\mu\text{g/mL}$ or lower, from 0.3 through 0.8 $\mu\text{g/mL}$, 0.4 through 0.7 $\mu\text{g/mL}$, about 0.5 $\mu\text{g/mL}$, or 0.6 $\mu\text{g/mL}$; or FACS binding mean EC_{50} to CCR8 expressed on CD45RA low Tregs in human blood of 2 $\mu\text{g/mL}$ or lower, 1 $\mu\text{g/mL}$ or lower, from 0.2 through 0.6 $\mu\text{g/mL}$, 0.3 through 0.5 $\mu\text{g/mL}$, about 0.5 $\mu\text{g/mL}$, or 0.48 $\mu\text{g/mL}$.

[0060] High specificity binding to human CCR8, e.g., no specific FACS binding to TALL-1 CCR8 knockout cells or parental Jurkat cells.

[0061] Cross reactivity with cynomolgus CCR8, e.g., FACS binding mean EC_{50} to cyno CCR8 overexpressed on Jurkat cells of 5 $\mu\text{g/mL}$ or lower, 3 $\mu\text{g/mL}$ or lower, 2 $\mu\text{g/mL}$ or lower, approximately 1.5 $\mu\text{g/mL}$, or 1.82 $\mu\text{g/mL}$; or to CCR8 expressed on CD45RA low Tregs in cyno blood of 5 $\mu\text{g/mL}$ or lower, 3 $\mu\text{g/mL}$ or lower, 2 $\mu\text{g/mL}$ or lower, approximately 1.5 $\mu\text{g/mL}$, or 1.62 $\mu\text{g/mL}$.

[0062] Poor ability to block CCL1/CCR8 interaction, e.g., EC_{50} of CCL1/CCR8 blocking activity at least 30x higher, at least 40x higher, between 30x and 70x higher, at least 50x higher, or at least about 50x higher than EC_{50} of binding to human CCR8.

[0063] Enhanced ability to induce ADCC with respect to an antibody with the same variable regions and a wild type, fucosylated IgG1.

[0064] Enhanced binding to Fc γ receptors with respect to an antibody with the same variable regions and a wild type, fucosylated IgG1.

[0065] Good immunosafety determined by cytokine release assay.

7.5. Methods of Use

[0066] The antibody as defined in the claims may be used for treating patients who have solid tumors. A composition comprising anti-CCR8 antibodies may be administered to a subject in need thereof.

[0067] In the PD-1 refractory Pan02 mouse model, a combination of CCR8 and PD-1 targeting antibodies showed improved in vivo efficacy over monotherapy with either antibody. Depleting immunosuppressive CCR8+ Tregs may synergize with PD-1/PD ligand 1 (PD-L1) blockade to promote a stronger CD8+ effector T-cell response and enhance antitumor immunity. In an embodiment, the composition comprising anti-CCR8 antibodies is administered as part of combination therapy comprising administration of a PD-1 or PD-L1 targeting antibody. In an embodiment, the composition comprising anti-CCR8 antibodies is administered as a combination therapy with pembrolizumab, budigalimab, nivolumab, cemiplimab, or dostarlimab. In an embodiment, the composition comprising anti-CCR8 antibodies is administered as a combination therapy with atezolizumab, avelumab, durvalumab.

8. EXAMPLES

[0068] The following examples, which highlight certain features and properties of the exemplary embodiments of the antibodies and binding fragments described herein are provided for purpose of illustration.

8.1. Example 1: Production of Rat Hybridomas

[0069] Rats were immunized with 2 different full-length human CCR8 cDNA vectors (6 rats for each vector). Lymph node cells were isolated and fused to NS0 to generate hybridomas. Following expansion, hybridoma supernatants were screened for binding to human or cynomolgus ("cyno" or "cy") CCR8 overexpressed on HEK293 cells. 46 rat/hlgG1 chimeric mAbs were expressed via high-throughput antibody production, and 21 were confirmed to bind to human CCR8 (FIG. 1). Four chimeric mAbs AC-254290, AC-254532, AC-254546, and AC-254259 were selected for full humanization based on highest cyno cross-reactivity in Jurkat CCR8 overexpressing lines.

8.2. Example 2: Epitope Mapping by Competition Binding to huCCR8

[0070] The selected chimeric antibodies were tested for binding to human CCR8 on Jurkat cells through cellular epitope mapping assays along with human CCR8 antibodies from BD Biosciences (clone 433H) and Biolegend (clone L263G8). Both commercial antibodies were reported as being generated by immunizing mice with human CCR8-transfectants, and neither are cross reactive with cyno CCR8. The antibodies were tested pairwise with cells stained with saturating concentrations of antibody 1, washed, and then stained with antibody 2 or vice versa.

[0071] mAbs AC-254290 and AC-254532 interfered with binding in either order suggesting

they bind to the same epitope (Epitope A) (FIG. 2). AC-254546 shows moderate epitope binding interference with AC-254290 and AC-254532 but no evidence of binding to the same epitope; therefore AC-254546 binds to Epitope B which is unique but close to Epitope A. AC-254259 had no binding interference with any other antibody and is likely a unique epitope (Epitope C). BD Biosciences clone 433H had the same epitope binding as AC-254290 and some interference binding with AC-254532, AC-254546, and Biolegend clone L263G8, and is therefore binned with Epitope A. Based on interference data with BD Biosciences clone 433H and the other antibodies, Biolegend clone L263G8 is binned as Epitope D, which is unique but close to Epitope A but not Epitope B.

8.3. Example 3: Humanization of Rat Variable Domain/ Human IgG1 Fc chimeras

[0072] The four chimeric antibodies AC-254290, AC-254532, AC-254546, and AC-254259 were humanized by (i) identifying the rodent antibody sequence; (ii) identifying the CDRs and antibody frameworks; (iii) creating a VH-VL structural model; (iv) identifying a framework residue for back mutation, to maintain function of rodent antibody; (v) selecting human germlines with high identity, most similar CDR canonical structures, and least back mutations needed; and (iv) generating a VH/VL sequence by CDR grafting and incorporating the selected back mutations.

[0073] Four humanized antibodies were created for each selected antibody. AC-254290 required both sequence-based and structure-based approaches for humanization. Straight CDR grafting resulted in an antibody having poor binding to human CCR8 expressing Jurkat cells. Back-mutations based on a predicted rodent VH/VL structure/interface were tested. The residue triplet prior to HCDR3 was CTA, which is atypical. Position 94 (the A in CTA) is almost always an arginine (R). Two humanized versions with CTR exhibited poor binding to human CCR8 expressing Jurkat cells, with EC₅₀s of 3.96 and 5.61 µg/mL. In contrast, two humanized versions with CTA prior to HCDR3 (CTAGDRNKPFAY (SEQ ID NO: 14)), one of which being AC-264700, demonstrated strong binding, with EC₅₀s of 0.2559 and 0.2302 µg/mL, comparing favorably to the AC-254290 parent antibody (EC₅₀ of 0.4538 µg/mL).

[0074] The humanized antibodies were assessed for their ability to bind human or cynomolgus CCR8. EC₅₀s were determined by testing 8 concentrations of antibody starting at 30 µg/mL with 5x dilutions for binding to the human or cynomolgus CCR8 overexpressing Jurkat cells. Following incubation with test CCR8 antibodies, cells were washed and incubated with fluorochrome labeled secondary antibodies, washed and analyzed via flow cytometry.

[0075] AC-254259 and AC-254532 exhibited decreased binding to cynoCCR8 and high non-specific binding to HEK293 cells, respectively. AC-264711 (humanized AC-254546) and AC-264700 (humanized AC-254290) were advanced because of their superior target binding, absence of non-specific binding, and limited requirement for liability engineering.

8.4. Example 4: Liability Engineering of AC-264700

[0076] AC-264700 had DS motifs in HCDR2 and in LCDR1 at amino acids 61-62 and 27-28, respectively (kabat) which are risks for isomerization and a DP motif at amino acid positions 94-95 (kabat) in LCDR3 that made it subject to potential fragmentation. The HCDR2 DS liability was mutated to DA based on experience with previous antibodies. Removal of the DP motif disrupted binding, but fragmentation of antibodies retaining the DP motif was not manifested under stress testing. Twenty-two LCDR1 DS variant antibodies were created, and their binding properties were assessed using flow cytometry against overexpressed huCCR8 or cyCCR8 Jurkat cells. Non-specific binding to Jurkat parental cells was assessed through flow cytometry. Ten of the variant antibodies were selected for further characterization based on favorable functional and drug-like properties. Two were chosen for advancement based on their overall combination of properties, including binding, reduced self-interaction, lack of non-specificity, cyno cross reactivity, and activity in the hFcγRIIIa V variant ADCC reporter bioassay (Promega).

[0077] The candidate molecules were assessed for binding capacity using flow cytometry through known methods against overexpressed huCCR8 or cyCCR8 on the surface of Jurkat cells. The exemplary antibodies were tested for their ability to self-interact, bind to huCCR8 and cyCCR8, capacity for ADCC using the reporter bioassay, and non-specific binding to HEK293 cells. AC-277357 had superior binding capacity for both huCCR8 and cyCCR8 Jurkat cells when compared to parent and other candidate antibodies (Table 1). The AC-SINS self-interaction scores for each candidate was <1. At 100µg/mL, non-specific binding maximums ranged from 97 to 144 GMFI. At 10µg/mL, non-specific binding maximums ranged from 94-111 GMFI. At 1µg/mL, non-specific binding maximums ranged from 87-102 GMFI. AC-277357 had the lowest non-specific binding maximum to HEK293 cells at 100µg/mL, strongest binding to huCCR8 and cyCCR8 Jurkat cells, and largest fold induction of signal with both huCCR8 and cyCCR8 Jurkat target cells in the ADCC reporter bioassay. Additionally, AC-277357 had one of the lowest self-interaction scores when compared to the parent antibody.

Table 1: Comparison of AC-264700 Variants

Antibody	AC#	AC-264700	AC-277357	AC-277371	AC-277483
	Mutation	DS	D27→T	D27→G	S28→A
Binding to hu/cy CCR8 Jurkats	huCCR8 EC ₅₀ (nM)	2.33	1.51	1.87	2.90
	cyCCR8 EC ₅₀ (nM)	5.27	2.37	4.83	5.93
ADCC reporter hu/cyCCR8 expressing Jurkat target cells	huCCR8 EC ₅₀ (nM)	0.12	0.10	0.05	0.07

Antibody	AC#	AC-264700	AC-277357	AC-277371	AC-277483
	Mutation	DS	D27→T	D27→G	S28→A
	Max Fold Induction	12.8	15.7	12.7	13.6
	cyCCR8 EC ₅₀ (nM)	0.79	0.75	0.57	1.19
	Max Fold Induction	12.0	12.3	11.7	10.0

8.5. Example 5: Liability Engineering of AC-264711

[0078] AC-264711 had a NS motif at amino acids 60-61 (kabat) in the HCDR2 region, which presented a risk for deamination. Additionally, residue M100c in HCDR3 showed high levels of oxidation during stress testing.

[0079] Candidate AC-264711 variants with NS mutations to remove liabilities were tested for their capacity to self-interact, non-specifically bind to HEK293 cells, bind to huCCR8 and cyCCR8 Jurkat cells, and induce signal in the ADCC reporter bioassay with huCCR8 expressing Jurkat cells as targets. Removing the NS motif was tolerated. Twenty-nine NS variants were created, and their binding properties were assessed using flow cytometry against Jurkat parental cells and overexpressed huCCR8 or cyCCR8 Jurkat cells. Five antibodies with the best binding and drug-like property profiles were tested in the ADCC reporter bioassay and the three antibodies with the best combination of properties, AC-275889, AC-275896 and AC-275898, were advanced for M100c mutations to prevent oxidation.

[0080] Variants of AC-275889, AC-275896 and AC-275898 with M100c mutations were generated and tested in the same assays as NS mutants. Data from these assays were used to prioritize eight antibodies for drug-like property testing. AC-291774 and AC-291790 had the highest self-interaction AC-SINS scores at 8.38 and 6.75, respectively. The remaining candidates had comparable AC-SINS scores ranging from 1.73 to 2.79. AC-275896 and AC-275898 had the largest non-specific binding to HEK293 cells at 1780 and 1065, GFMI respectively. AC-291790, AC-275889, and AC-275898 had non-specific binding maximums of 335, 148, and 141, respectively. At 10 and 1µg/mL, all candidate molecules had comparable non-specific binding maximums ranging from 61 to 67 and 57 to 60, respectively. AC-291774 and AC-291790 had the lowest non-specific binding as indicated by binding to Jurkat parental cells and hydrophobic interaction chromatography, while maintaining potent binding to huCCR8 and cyCCR8 expressing Jurkat cells and comparable EC₅₀s and fold induction of ADCC

reporter signal with both huCCR8 and cyCCR8 expressing Jurkat cells when compared to AC-275889, AC-275896 and AC-275898 (Table 2).

Table 2. In vitro assays of AC-264711 Variants

Antibody	AC Number	AC-275889	AC-275896	AC-275898	AC-291774	AC-291790
	Mutation	N60→A	S61→E	S61→Q	N60→A M106→I	S61→Q M106→I
Binding to hu/cy CCR8 Jurkats	huCCR8 EC ₅₀ (nM)	12.19	49.41	8.39	9.57	17.67
	cyCCR8 EC ₅₀ (nM)	7.08	13.70	24.41	9.00	15.28
ADCC huCCR8 expressing Jurkats	huCCR8 EC ₅₀ (nM)	0.07	0.15	0.12	0.19	0.23
	Max Fold Induction	15.6	14.1	17.0	16.8	18.6
	cyCCR8 EC ₅₀ (nM)	0.10	0.41	0.10	0.30	0.30
	Max Fold Induction	8.7	7.7	10.7	10.8	12.7

8.6. Example 6: Final Candidate Antibody Selection

[0081] AC-277357, AC-277371, AC-291774 and AC-291790 were selected to be further evaluated in additional drug-like property and in vitro immunosafety experiments. To generate an afucosylated variant for characterization, cells expressing AC-277357 were transfected with a plasmid for expression of the Pseudomonas enzyme GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD), which converts an intermediate in the de novo synthesis of fucose to a dead-end product, resulting in the production of afucosylated AC-277357.

[0082] Candidate molecules were assessed for Jurkat huCCR8, Jurkat cyCCR8 and TALL-1 binding activity, hPBMC ADCC activity with TALL-1 as targets, ADCC activity in F158 effector cells with huCCR8 overexpressing Jurkat cells as targets, and V158 effector cells with huCCR8 overexpressing Jurkat cells as targets. AC-277357 (also referred to as PR-1925514), showed improved binding to endogenous levels of CCR8 expression on TALL-1 cells and the afucosylated version was more active in primary cell ADCC assays (data not shown). AC-277357/PR-1925514 binds more potently to CCR8 on human Tregs and has a better profile in cytokine release assays than AC-291774 (also referred to as PR-1928444) (data not shown). AC-277357/PR-1925514 with an afucosylated human IgG1 Fc was chosen as the lead antibody because it had the best in vitro functional and in vitro immunosafety (IVIS) profile as well as excellent drug like properties.

8.7. Example 7: Binding and Functional Potency Assays

[0083] Antibodies were screened via single point flow cytometry to assess binding to Jurkat cells lines overexpressing human or cynomolgus monkey CCR8. EC₅₀s were determined by testing eight concentrations of antibody starting at 30 µg/mL with 5x dilutions for binding to the Jurkat human CCR8, Jurkat cyno CCR8 and parental Jurkat cells via flow cytometry. Following incubation with test CCR8 antibodies, cells were washed and incubated with fluorochrome labeled secondary antibodies, washed and analyzed via flow cytometry.

[0084] Human FcγRIIIa V Variant Core Kit or Human FcγRIIIa F Variant Cell Propagation Model were purchased from Promega and used as directed. Jurkat target cell lines overexpressing human or cynomolgus monkey CCR8 or parental Jurkat control cells were washed once in ADCC assay buffer, diluted to 6×10^6 cells per mL in ADCC assay buffer, and plated at 25 µL per well into white walled 96-well assay plates (Costar). Antibodies were diluted at 3x final concentration in ADCC assay buffer for addition at 25 µL per well (1:5 fold 7-point dilutions ranging from 1 µg/mL to 0.000064 µg/mL were tested). Effector cells (stably expressing either the human FcγRIIIa V158 variant, the human FcγRIIIa F158 variant, or mouse FcγRIV and an NFAT response element driving expression of firefly luciferase) were thawed and added at a final effector to target ratio of 1:2.5. After incubation at 37°C, 5% CO₂ for 6 hours BioGlo reagent (Promega) was mixed and added as directed and luminescence signal was measured.

8.8. Example 8: Dose response curves for ABBV-514

[0085] Fucosylated and afucosylated (renamed ABBV-514) versions of AC-277357/PR-1925514 were evaluated for cell binding to Jurkat cell lines overexpressing human or cyno CCR8 and to TALL-1 cells that express endogenous levels of CCR8. Cell binding was assessed through flow cytometry using similar methods discussed above. Additionally, binding was evaluated for parental Jurkat (no CCR8 expression) and CCR8 knock-out (KO) TALL-1 control lines. ABBV-514 showed binding to both human and cyno CCR8 overexpressed in Jurkat cells, but not to the parental Jurkat or CCR8 KO TALL-1 cells (FIG. 4A). ABBV-514 also bound to endogenous CCR8 on TALL-1 cells similar to that of fucosylated (WT) AC-277357/PR-1925514 (FIG. 4B).

[0086] Both fucosylated WT PR-1925514 and ABBV-514 mediate ADCC as measured by luminescence induction in a human FcγRIIIa reporter cell-based bioassay using either FcγRIIIa V158 (FIGS. 5A, 5C) or F158 (FIGS. 5B, 5D) allelic variant reporter lines cocultured with human (FIGS. 5A, 5B) or cyno (FIGS. 5C, 5D) CCR8 overexpressing Jurkat cells. The fold change in luminescence compared to untreated cocultures is presented as the mean ± SEM of 2-5 independent experiments. ABBV-514 exhibited increased ADCC activity as indicated by the ADCC reporter bioassay compared to fucosylated WT PR-1925514. For purposes of

comparison to concentrations expressed in $\mu\text{g/mL}$, a concentration of 1 $\mu\text{g/mL}$ of antibody equals 6.76 nM.

8.9. Example 9: Primary cell ADCC assay data for ABBV-514

[0087] The activity of ABBV-514 was further evaluated in a cell cytotoxicity-based ADCC assay using TALL-1 cells as targets and human primary NK cells as effectors. Six donor sources of NK cells were used to as target cells and cell death of TALL-1 target cells was assessed via flow cytometry using similar methods discussed above. ABBV-514 mediated ADCC of CCR8 expressing TALL-1 cells by all six donor NK cell specimens (FIG. 6). ADCC activity in human whole blood cultures was assessed via flow cytometry using adapted procedures described above. After 48 hours, a culture of human whole blood with ABBV-514 was analyzed to assess the fraction of CD45RA low Tregs of total CD45+ hematopoietic cells. CD45RA low Tregs includes live CD45+, CD3+, CD4+, CD25+, CD127 low, Foxp3+, CD45RA low cells, which are known to be enriched in CCR8 expression. Human whole blood cell data from 11 healthy donors were assessed. In each donor, ABBV-514 decreased the amount percentage of CD45RA low Tregs of live CD45+ cells (data not shown).

8.10. Example 10: Assessment of CCL1 Ligand Blockade by ABBV-514

[0088] A CCR8 beta-arrestin reporter assay was used to determine whether ABBV-514 blocked the interaction of CCR8 with its ligand, CCL1 (FIG. 7). Inhibition of CCL1-mediated reporter activation in the presence of ABBV-514 was observed only at substantially higher concentrations of antibody than those at which binding or ADCC activity was observed, with an EC_{50} of CCL1 blockade observed at 23.1 $\mu\text{g/mL}$.

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- [WO2021142002A](#) [0003]
- [WO2020138489A](#) [0003]
- [EP3431105A](#) [0003]
- [US20060134709A](#) [0025]
- [US7217797B](#) [0027]

Non-patent literature cited in the description

- **SHANG et al.** Nature Sci. Reports, 2015, [0003]
- **FRIDMAN et al.** Nat Rev Clin Oncol., 2017, [0003]
- **BRUNI et al.** Nat Rev Cancer., 2020, [0003]
- **PLITAS et al.** Immunity, 2016, [0003]
- **DE SIMONE et al.** Immunity, 2016, [0003]
- **CHENSUE et al.** J Exp Med, 2001, vol. 5, [0009]
- **VAN DAMME et al.** J Immunother Cancer., vol. 9, 2021- [0010]
- **CAMPBELL et al.** Cancer Research, 2021, vol. 81, [0011]
- Mol Cancer Ther, 2020, vol. 19, 51102-1109 [0013]
- PNAS, 2013, vol. 110, 145404-5409 [0013]
- **SILVA, JP et al.** Journal of Biological Chemistry, 2015, vol. 290, 95462-5469 [0026]
- **KABAT, E.A. et al.** Sequences of Proteins of Immunological Interest U.S. Department of Health and Human Services 19910000 [0045]
- **KABAT et al.** Sequences of Proteins of Immunological Interest U.S. Department of Health and Human Services 19910000 [0046]
- **URLAUBCHASIN** Proc. Natl. Acad. Sci. USA, 1980, vol. 77, 4216-4220 [0051]
- **KAUFMANSHARP** Mol. Biol., 1982, vol. 159, 601-621 [0051]

PATENTKRAV

1. Anti-CCR8-antistof omfattende (i) en VH-kæde, der omfatter tre CDR'er; og (ii) en VL-kæde, der omfatter tre CDR'er, hvor:

VH-CDR nr. 1 er GFIFSNAVMY (SEQ ID NO: 1);

5 VH-CDR nr. 2 er RIKTKFNYYATYYADAVKG (SEQ ID NO:2);

VH-CDR nr. 3 er GDRNKPFAY (SEQ ID NO:3);

VL-CDR nr. 1 er RASTSVITLLH (SEQ ID NO:4);

VL-CDR nr. 2 er GASNLES (SEQ ID NO:5); og

VL-CDR nr. 3 er QQSWNDPYT (SEQ ID NO:6);

10 hvor antistoffet omfatter en konstant IgG1-Fc-region, der er afucosyleret.

2. Anti-CCR8-antistof ifølge krav 1, hvor antistoffet omfatter en variabel tungkædereion, der omfatter aminosyresekvensen, der er fremsat som SEQ ID NO: 7, og en variabel letkædereion, der omfatter aminosyresekvensen, der er fremsat som SEQ ID NO: 8.

15 3. Anti-CCR8-antistof ifølge krav 1, hvor antistoffet omfatter en tung kæde, der omfatter aminosyresekvensen, der er fremsat som SEQ ID NO: 9, og en let kæde, der omfatter aminosyresekvensen, der er fremsat som SEQ ID NO: 10.

4. Sammensætning omfattende en flerhed af anti-CCR8-antistoffer ifølge krav 1, hvor mere end 80 % af anti-CCR8-antistofferne i sammensætningen er
20 afucosylerede.

DRAWINGS

Drawing

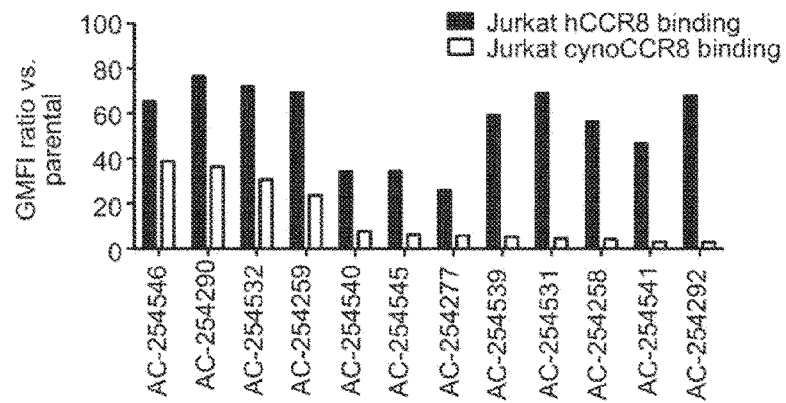


FIG. 1

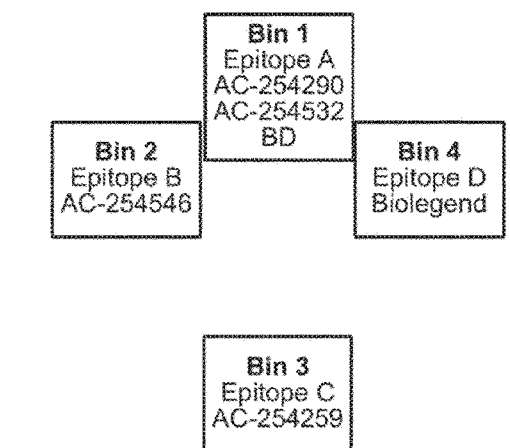


FIG. 2

ABBV-514 sequence information

SPR-1925514 LightChain huCCR8 (AC-277357) [hu IgG1/K] (VL domain underlined, constant region bold)

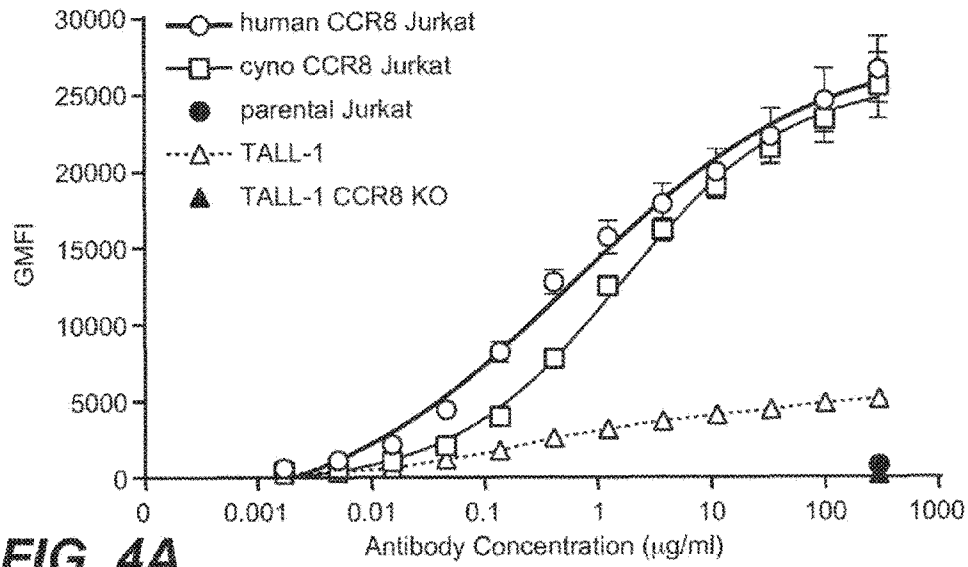
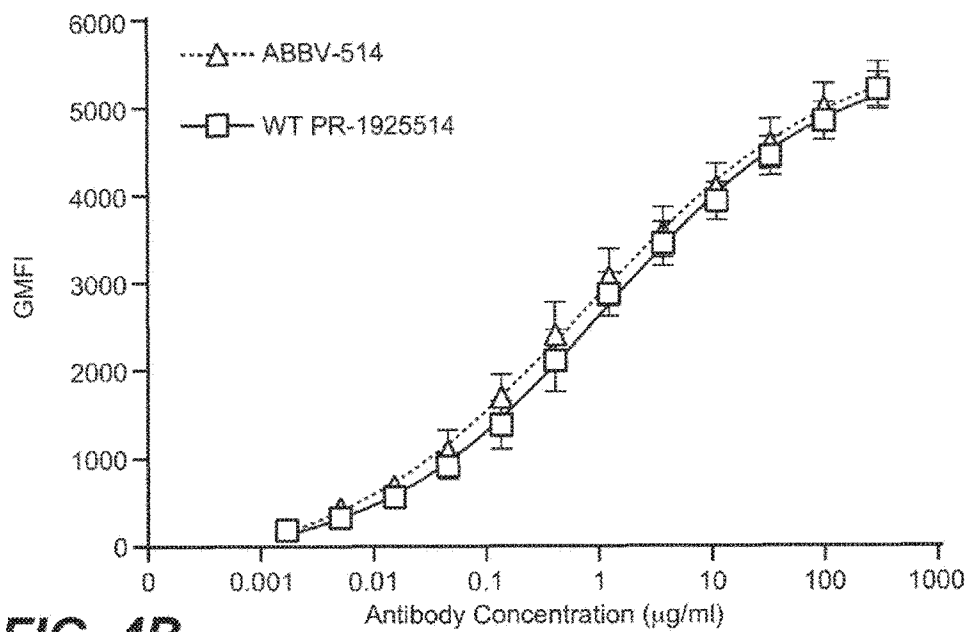
ETV TQSPATLSLSPGERATLS CRASTSVITLH WFQOKPGQAPRLIH ASNIESRVPARFSGSGIDFTLISSLEPEDFATYFOG
SWNDP YFGQGTLEIKRTVAAPSVFIFPPSDEQLKSGTASWCLLNNFYPR AKVQWKVDNALQSGNSQESVTEQDSKDSYSL
SSITLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

>PR-1925514 HeavyChain huCCR8 (AC-277357) [hu IgG1/K] VH domain underlined; **constant region bold**

EVQLVESGGGLVQPGGSLKLSCAASGFISSNAVMYVNRQASCKGLEWVARIKTKENNYATYYADAVKGRFTISRDDSKNMYVYQ
MNSLKTEDIAVYYCTAGDRNKPFAVWGOGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHCTCPCPAPELGGPSVFLFPPKPKDITL
MISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTIPPVLDSDGSSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK

CDR1	CDR2	CDR3	AfucoS
------	------	------	--------

FIG. 3

**FIG. 4A****FIG. 4B**

ADCC Bioassay Jurkat human CCR8 effector cells
with Fc γ R11a V158 Variant Reporter Cells

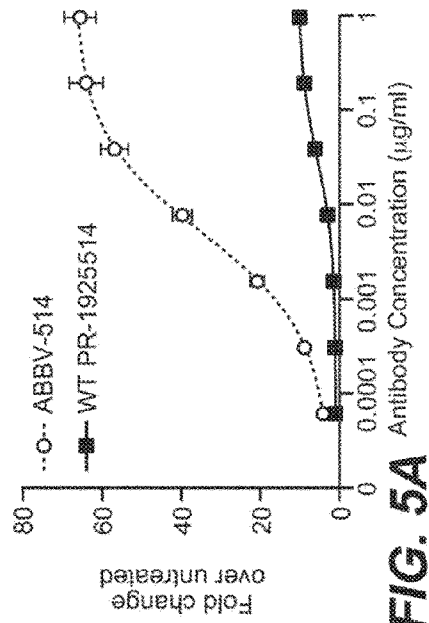


FIG. 5A

ADCC Bioassay Jurkat human CCR8 effector cells
with Fc γ R11a F158 Variant Reporter Cells

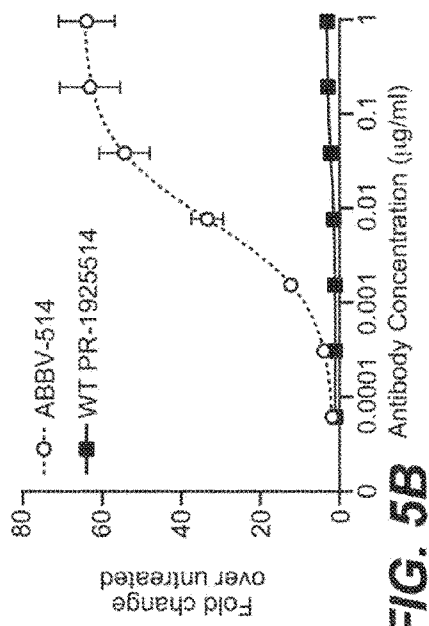


FIG. 5B

ADCC Bioassay Jurkat cyno CCR8 effector cells
with Fc γ R11a V158 Variant Reporter Cells

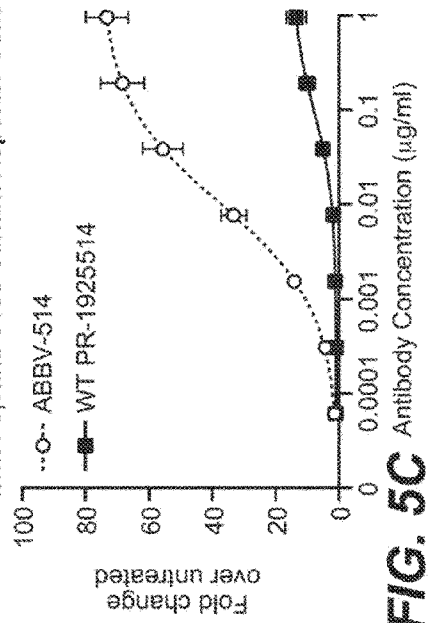


FIG. 5C

ADCC Bioassay Jurkat cyno CCR8 effector cells
with Fc γ R11a F158 Variant Reporter Cells

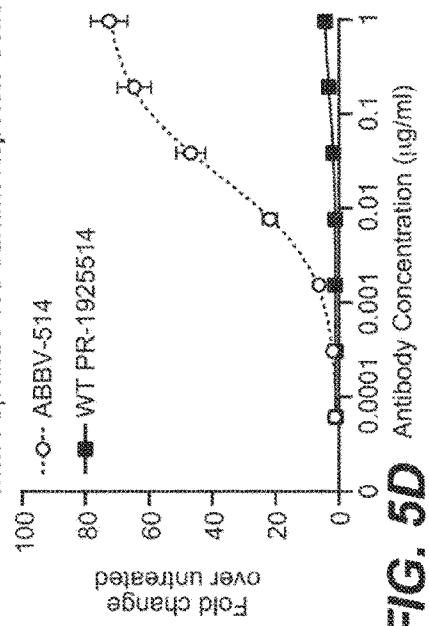
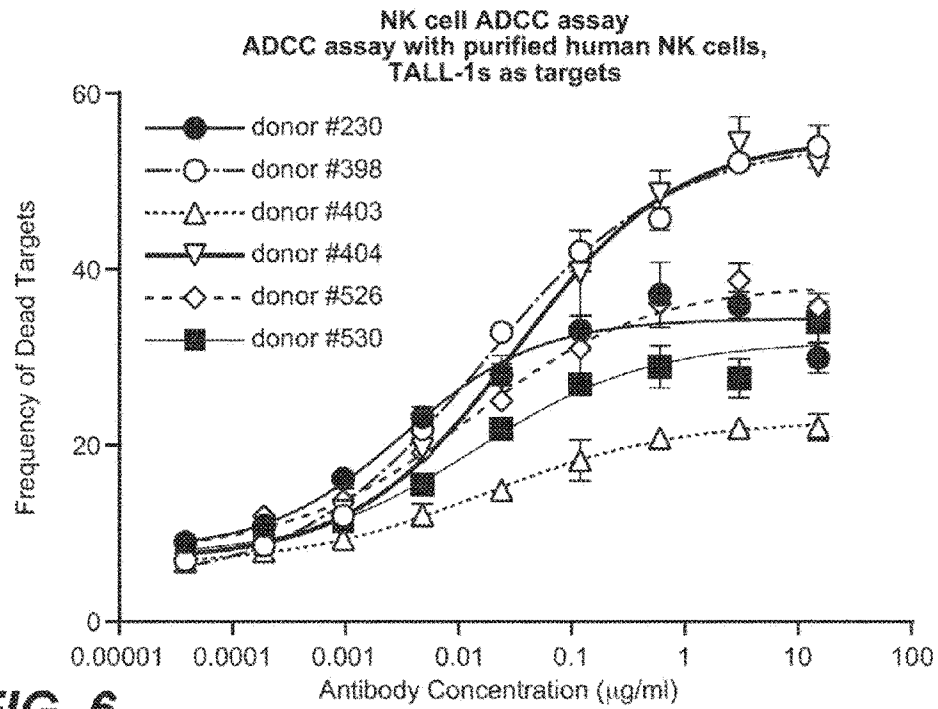
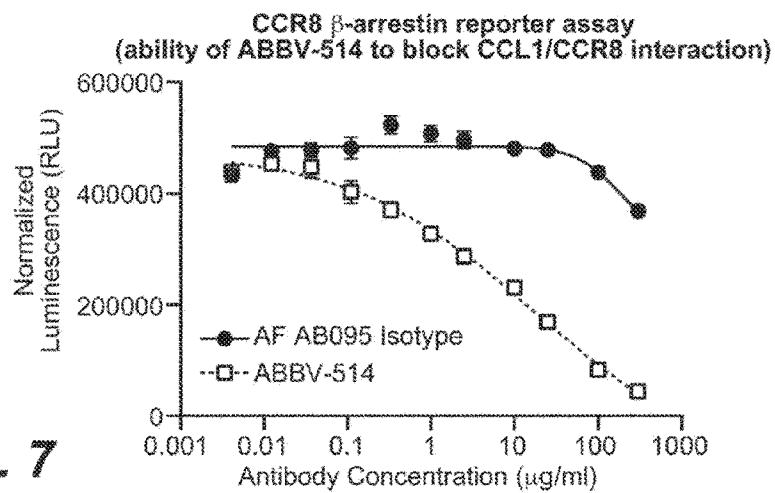


FIG. 5D

**FIG. 6****FIG. 7**

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.

