

ANTI-GPRC5D MONOCLONAL ANTIBODIES AND USES THEREOF**BACKGROUND**

[0001] G-protein-coupled receptor family C group 5 member D (GPRC5D) is member of the G protein-coupled receptor family. GPRC5D is a transmembrane protein. Overexpression of GPRC5D was observed in patients with multiple myeloma. In particular, its high expression had a significant correlation with poor outcome of the disease and treatment.

[0002] Given its specific high expression on malignant cells, it has been proposed that antibodies that are specific to GPRC5D can be useful for treating the malignancy, such as via a bispecific T-cell-redirecting antibody, or through antibody-dependent cellular cytotoxicity (ADCC).

SUMMARY

[0003] Anti-GPRC5D antibodies, including their humanized derivatives, are discovered herein that have high affinity to the human GPRC5D protein. The antibodies or fragment thereof are capable of targeting cancer cells expressing GPRC5D, and thus can be used to treat the cancer, in particular hematological cancer.

[0004] One embodiment of the present disclosure provides an antibody or antigen-binding fragment thereof having binding specificity to a human G-protein-coupled receptor family C group 5 member D (GPRC5D) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region (VH) comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region (VL) comprising complementarity determining regions CDRL1, CDRL2, and CDRL3. In some embodiments, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise: (a) the amino acid sequences of SEQ ID NO:29-34; (b) the amino acid sequences of SEQ ID NO:42-47; (c) the amino acid sequences of SEQ ID NO:54-59; or (d) the amino acid sequences of SEQ ID NO:68-73.

[0005] In some embodiments, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise the amino acid sequences of SEQ ID NO:29-34. In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:7 and 35-37, and the VL comprises an amino acid sequence selected from the

group consisting of SEQ ID NO:8 and 38-41. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO:35, and the VL comprises the amino acid sequence of SEQ ID NO:38.

[0006] In some embodiments, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise the amino acid sequences of SEQ ID NO:42-47. In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:9 and 48-50, and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NO:10 and 51-53. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO:48, and the VL comprises the amino acid sequence of SEQ ID NO:51.

[0007] In some embodiments, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise the amino acid sequences of SEQ ID NO:54-59. In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:61-64, and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NO:16 and 65-67. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO:61, and the VL comprises the amino acid sequence of SEQ ID NO:65. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO:63, and the VL comprises the amino acid sequence of SEQ ID NO:65.

[0008] In some embodiments, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise the amino acid sequences of SEQ ID NO:68-73. In some embodiments, the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 74-79, and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 80-86. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO:76, and the VL comprises the amino acid sequence of SEQ ID NO:82. In some embodiments, the VH comprises the amino acid sequence of SEQ ID NO:77, and the VL comprises the amino acid sequence of SEQ ID NO:82.

[0009] In some embodiments, the antibody or fragment thereof is humanized. In some embodiments, the antibody or fragment thereof is ADCC-competent.

[0010] Also provided, in some embodiments, the antibody or fragment thereof further comprises a cytotoxic drug conjugated to the antibody or fragment thereof.

[0011] Also provided is a bispecific antibody, comprising the antigen-binding fragment of the present disclosure, and a second antigen-binding fragment having specificity to a second target protein. In some embodiments, the second target protein is selected from the group consisting of CD3, CD16, CD19, CD28, CD64 and 4-1BB. In some embodiments, the second target protein is CD3. In some embodiments, the second target protein is 4-1BB.

[0012] Also 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. Also provided is a method of treating cancer in a patient in need thereof, comprising (a) treating a T cell, a natural killer (NK) cell, or a macrophage, *in vitro*, with the antibody or fragment thereof of the present disclosure, and (b) administering the treated cell to the patient.

[0013] In some embodiments, the cancer is a hematological cancer, such as a GPRC5D-expressing B cell cancer (e.g., multiple myeloma).

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1 shows binding of murine antibodies collected from hybridoma supernatant to GPRC5D expressed on CHO-K1 cells.

[0015] FIG. 2 shows binding of chimeric antibodies to GPRC5D expressed on CHO-K1 cells.

[0016] FIG. 3 shows that antibody binding induced endocytosis.

[0017] FIG. 4 shows that the antibodies were ADCC-competent.

[0018] FIG. 5-8 show the affinity testing results of the humanized antibodies derived from 6G10D9, 58F9G10, 34D3H1, and 37B9C4, respectively.

[0019] FIG. 9 confirms the binding of selected humanized antibodies to GPRC5D expressed on CHO-K1 cells.

[0020] FIG. 10 confirms the binding of selected humanized antibodies to GPRC5D expressed on NCI-H929 cells.

[0021] FIG. 11 shows the ADCC efficacy of tested humanized antibodies.

[0022] FIG. 12 shows the internalization induction activity of the tested humanized antibodies.

[0023] FIG. 13 shows the cell killing activities of the tested antibody-drug conjugates.

[0024] FIG. 14 shows the tumor inhibition activities of the tested antibody.

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.*, $\gamma 1$ - $\gamma 4$). 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.*, IgG₁, IgG₂, IgG₃, IgG₄, IgG₅, 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.*, IgG1, IgG2, IgG3, IgG4, IgA1 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 chondrichthoid 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 IgG₁ molecule and a hinge region derived from an IgG₃ molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG₁ molecule and, in part, from an IgG₃ molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG₁ molecule and, in part, from an IgG₄ 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-GPRC5D Antibodies

[0057] Using the hybridoma technology, the accompanying experimental examples showed that a number of murine antibodies were obtained. Fourteen of the murine antibodies were sequenced, and humanized antibodies were made. These humanized antibodies exhibited potent GPRC5D-binding activities, and were able to induce receptor-mediated endocytosis. *In vivo* testing showed that these antibodies were active in inducing ADCC and inhibiting tumor development.

[0058] Four of the murine antibodies, 34D3H1, 37B9C4, 58F9G10 and 6G10D9, went through the humanization process. Some of the humanized antibodies, including 6-H3L3, 6-H4L3 (both derived from 6G10D9), 58-H1L1, 58-H3L1 (both derived from 58F9G10), 34-H1L1 (derived from 34D3H1) and 37-H1L1 (derived from 37B9C4) further showed promise to continued clinical development.

[0059] In accordance with one embodiment of the present disclosure, therefore, provided is an antibody or antigen-binding fragment thereof having binding specificity to a human G-protein-coupled receptor family C group 5 member D (GPRC5D) protein. The antibody or fragment thereof includes a heavy chain variable region (VH) comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region (VL) comprising complementarity determining regions CDRL1, CDRL2, and CDRL3.

[0060] In some embodiments, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, include (a) the amino acid sequences of SEQ ID NO: 29-34; (b) the amino acid

sequences of SEQ ID NO: 42-47; (c) the amino acid sequences of SEQ ID NO:54-59, or SEQ ID NO:54, 60, and 56-59; or (d) the amino acid sequences of SEQ ID NO:68-73.

[0061] In one embodiment, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, include the amino acid sequences of SEQ ID NO:29-34. Example sequences for the VH include SEQ ID NO:7 and 35-37 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO:7 and 35-37. Example sequences for the VL include SEQ ID NO:8 and 38-41 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO:8 and 38-41.

[0062] In one embodiment, the VH includes the amino acid sequence of SEQ ID NO:35 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:35. In one embodiment, the VL includes the amino acid sequence of SEQ ID NO:38 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:38.

[0063] Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that bind to the same epitope on FAP α as any one of these antibodies. Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that compete with any one of these antibodies in binding to FAP α , such as one that has a VH that includes the amino acid sequence of SEQ ID NO:35 and a VL that includes the amino acid sequence of SEQ ID NO:38.

[0064] In one embodiment, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, include the amino acid sequences of SEQ ID NO:42-47. Example sequences for the VH include SEQ ID NO: 9 and 48-50 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO: 9 and 48-50. Example sequences for the VL include SEQ ID NO:10 and 51-53 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO:10 and 51-53.

[0065] In one embodiment, the VH includes the amino acid sequence of SEQ ID NO:48 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:48. In one embodiment, the VL includes the amino acid sequence of SEQ ID NO:51 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:51.

[0066] Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that bind to the same epitope on FAP α as any one of these antibodies. Also provided,

in some embodiments, are antibodies and antigen-binding fragments thereof that compete with any one of these antibodies in binding to FAP α , such as one that has a VH that includes the amino acid sequence of SEQ ID NO:48 and a VL that includes the amino acid sequence of SEQ ID NO:51.

[0067] In one embodiment, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, include the amino acid sequences of SEQ ID NO:54-59. Example sequences for the VH include SEQ ID NO: 61-64 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO: 61-64. Example sequences for the VL include SEQ ID NO: 16 and 65-67 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO: 16 and 65-67.

[0068] In one embodiment, the VH includes the amino acid sequence of SEQ ID NO:61 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:61. In one embodiment, the VL includes the amino acid sequence of SEQ ID NO:65 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:65.

[0069] Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that bind to the same epitope on FAP α as any one of these antibodies. Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that compete with any one of these antibodies in binding to FAP α , such as one that has a VH that includes the amino acid sequence of SEQ ID NO:61 and a VL that includes the amino acid sequence of SEQ ID NO:65.

[0070] In one embodiment, the VH includes the amino acid sequence of SEQ ID NO:63 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:63. In one embodiment, the VL includes the amino acid sequence of SEQ ID NO:65 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:65.

[0071] Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that bind to the same epitope on FAP α as any one of these antibodies. Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that compete with any one of these antibodies in binding to FAP α , such as one that has a VH that includes the amino acid sequence of SEQ ID NO:63 and a VL that includes the amino acid sequence of SEQ ID NO:65.

[0072] In one embodiment, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, include the amino acid sequences of SEQ ID NO:54, 60, and 56-59. Example sequences for the VH include SEQ ID NO:15 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO:15. Example sequences for the VL include SEQ ID NO: 16 and 65-67 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO: 16 and 65-67.

[0073] In one embodiment, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, include the amino acid sequences of SEQ ID NO: 68-73. Example sequences for the VH include SEQ ID NO: 1 and 74-79 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO: 1 and 74-79. Example sequences for the VL include SEQ ID NO: 2 and 80-86 or a sequence having at least 85%, 90%, or 95% sequence identity to any of SEQ ID NO: 2 and 80-86.

[0074] In one embodiment, the VH includes the amino acid sequence of SEQ ID NO:76 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:76. In one embodiment, the VL includes the amino acid sequence of SEQ ID NO:82 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:82.

[0075] Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that bind to the same epitope on FAP α as any one of these antibodies. Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that compete with any one of these antibodies in binding to FAP α , such as one that has a VH that includes the amino acid sequence of SEQ ID NO:76 and a VL that includes the amino acid sequence of SEQ ID NO:82.

[0076] In one embodiment, the VH includes the amino acid sequence of SEQ ID NO:77 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:77. In one embodiment, the VL includes the amino acid sequence of SEQ ID NO:82 or a sequence having at least 85%, 90%, or 95% sequence identity to SEQ ID NO:82.

[0077] Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that bind to the same epitope on FAP α as any one of these antibodies. Also provided, in some embodiments, are antibodies and antigen-binding fragments thereof that compete with any one of these antibodies in binding to FAP α , such as one that has a VH that includes

the amino acid sequence of SEQ ID NO:77 and a VL that includes the amino acid sequence of SEQ ID NO:82.

[0078] In some embodiments, the antibody or fragment thereof is ADCC-competent. Methods and materials suitable for making an antibody ADCC-competent are known in the art, such as by using the appropriate Fc fragments or decreasing/removing fucosylation.

[0079] In some embodiments, the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, include (a) the amino acid sequences of SEQ ID NO:29-34; (b) the amino acid sequences of SEQ ID NO:42-47; (c) the amino acid sequences of SEQ ID NO:54-59, or SEQ ID NO:54, 60, and 56-59; or (d) the amino acid sequences of SEQ ID NO:68-73, wherein each of the recited CDR sequence includes one, two or three conservative amino acid substitution.

[0080] 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.

[0081] 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

[0082] 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.

[0083] In some embodiments, the anti-CCR8 antibodies are modified mAbs comprising a modified heavy chain constant region, such as an afucosylated heavy chain, that binds with higher affinity to activating Fcγ receptor that mediated enhanced ADCC compared to an unmodified mAb. In some embodiments, the anti-CCR8 antibodies comprises a heavy chain which is of a human IgG1 variant that include the single or combination of L234Y, L235Q, G236W, S239D/M, F243L, H268D, D270E, R292P, S298A, Y300L, V305I, K326D, A330L/M, I332E, K334A/E, P396L that enhance ADCC function (all EU numbering).

[0084] 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).

[0085] 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, 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.

Antibody-Drug Conjugates

[0086] GPRC5D is overexpressed on certain malignant hematological cells, such as multiple myeloma cells. The antibodies and fragments of the instant disclosure, therefore, can be used to target those malignant cells for inhibition, inactivation or destruction. In one example, the antibody or fragment is conjugated to an agent that helps to inhibit, inactivate or destruct the malignant cell. It is shown herein that the antibodies are able to induce GPRC5D-mediated endocytosis.

[0087] 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. In some embodiments, the conjugated agent may be short interfering RNA (siRNA) or an innate modulator, such as a Stimulator of Interferon Genes (STING) agonist, or a TLR7/8 agonist.

[0088] 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.

[0089] 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.

[0090] 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, maleimide, 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.

[0091] 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-citrulline-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.

[0092] 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., maleimide, 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).

[0093] 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 GPRC5D, and the conjugate

and/or drug can be taken up inside a tumor cell or cancer cell through receptor-mediated endocytosis.

[0094] 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.

[0095] 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-(9CI)-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-(α R, β R,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-methyl-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.

[0096] 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.

[0097] 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, thionin acridinium ester, imidazole, acridinium salt and oxalate ester.

[0098] 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 diethylenetriaminepentaacetic 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

[0099] GPRC5D is overexpressed on certain malignant hematological cells, such as multiple myeloma cells. The antibodies and fragments of the instant disclosure, therefore, can be used to target those malignant cells for inhibition, inactivation or destruction. In some embodiments, a bi-functional or bispecific molecule/antibody is provided that targets both the GPRC5D protein and an immune cell.

[0100] 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, CD3, CD16, CD19, CD28, CD64 and 4-1BB (also

known as CD137). Other examples include PD-1, CTLA-4, LAG-3 (also known as CD223), CD28, CD122, 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. Specific examples of bispecificity include, without limitation, GPRC5D/CD3.

[0101] 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.

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

[0102] 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.

[0103] 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

[0104] As described herein, the antibodies, variants or derivatives of the present disclosure may be used in certain treatment and diagnostic methods.

[0105] 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).

[0106] The antibodies of the disclosure can also be used to treat or inhibit cancer. As provided above, GPRC5D can be overexpressed in cancer cells, in particular multiple myeloma. Inhibition of GPRC5D has been shown to be useful for treating the cancers.

[0107] 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 in the patient over-express GPRC5D. In some embodiments, the antibody or fragment is ADCC-competent. In some embodiments, the antibody or fragment further comprises a cytotoxic agent. In some embodiments, the antibody is bispecific that further targets an immune cell, such as a cytotoxic T cell.

[0108] Cellular therapies, such as chimeric antigen receptor (CAR) T-cell (or NK cell, macrophage) therapies, are also provided in the present disclosure. A suitable cell can be used, that is put in contact with an anti-GPRC5D antibody of the present disclosure (or alternatively engineered to express an anti-GPRC5D antibody of the present disclosure). 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, a natural killer (NK) cell, a macrophage, or the combination thereof, without limitation.

[0109] 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.

[0110] Non-limiting examples of cancers include hematological cancers such as multiple myeloma. Other examples include 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)), and lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), and multiple myeloma.

[0111] 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.

[0112] 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, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray.

[0113] The term “parenteral” as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0114] 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.

[0115] 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.

[0116] 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.

[0117] 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.

[0118] 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 .

[0119] 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

[0120] Over-expression of GPRC5D is observed in certain tumor samples, and patients having GPRC5D-over-expressing cells are likely responsive to treatments with the anti-GPRC5D antibodies of the present disclosure. Accordingly, the antibodies of the present disclosure can also be used for diagnostic and prognostic purposes.

[0121] 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 GPRC5D protein potentially present in the sample. Methods such as ELISA can be used, taking advantage of the anti-GPRC5D antibody, to detect the presence of the GPRC5D protein in the sample.

[0122] Presence of the GPRC5D 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

[0123] 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).

[0124] 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.

[0125] 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.

[0126] 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.

[0127] 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 GPRC5D

[0128] The human GPRC5D protein was used to immunize different strains of mice and hybridomas were generated accordingly. More than twenty hybridoma clones were collected for further analysis.

[0129] Antibodies harvested from the supernatants of the hybridomas were tested for their binding to the human GPRC5D protein expressed on CHO K1 cells was tested. CHO-K1 cells stably expressed human GPRC5D, were harvested from flasks. 100 μ l of 1×10^6 cells/ml of cells were incubated with murine antibodies in 3-fold serial dilutions for 30 minutes on ice. After being washed with 200 μ l of FACS buffer twice, cells were incubated with secondary antibody for 30 minutes on ice. Cells were washed with 200 μ l of FACS buffer twice and transferred to BD Falcon 5 ml tube and analyzed by FACS. The results of the study showed that the murine antibodies can bind to human GPRC5D with high EC50 (**FIG.1**). The results are shown in the table below. All of them exhibited good binding activity.

	Bottom	Top	LogEC50	HillSlope	EC50	Span
2D3C12	46110	835137	0.4535	1.132	2.841	789027
6G10D9	6870	1429638	0.9116	0.9535	8.159	1422768

11C1H10	40235	859162	0.5274	1.101	3.368	818927
14C2A11C6	5573	728662	0.9934	0.8801	9.849	723089
14C2F7C6	15616	751279	0.9634	0.9615	9.193	735663
14F3H2	20428	872785	0.8744	1.146	7.489	852357
21C3C11B3	57013	1508205	0.6538	1.1	4.506	1451192
26E1D12	52742	677713	0.2246	1.303	1.677	624970
29A7H11B9D3	71394	1385928	0.4219	1.259	2.642	1314534
29A7H11H4C12	43439	1461931	0.7377	1.109	5.466	1418492
29B2C10	68634	1607305	0.6201	1.305	4.17	1538671
31H11C10H7	16791	961550	0.9534	1.134	8.983	944759
34D3F8B5	-16109	1407656	1.084	0.7475	12.12	1423765
34D3H1	-13314	1412131	0.8843	0.8042	7.662	1425446
34D4A10	2737	921851	1.019	0.8496	10.44	919114
36F11E9H3	13852	766682	0.8732	1.087	7.468	752830
37B9C4	10880	1758861	0.5917	0.9933	3.906	1747981
38A10E9A11	24016	1785041	0.9472	1.099	8.854	1761025
39D2B6	7833	1774093	0.5469	0.98	3.523	1766260
40A5B2	25394	1196144	0.5108	0.9236	3.242	1170750
57G11D4A10	-22363	2089505	0.9703	0.8141	9.34	2111868
58F9G10	-14744	2052463	0.9635	0.8313	9.194	2067207

[0130] Fourteen hybridomas were subcloned and the VH/VL sequences were determined (see Table 1).

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

Name	Sequence (CDRs are underlined)	SEQ ID NO:
6G10D9 VH	QVQLQQSGAELARPGASVKMSCKASGYTFT <u>TYTMH</u> WVKQRPQGGLWLG <u>YINPSSGYTNYNQKFKDK</u> KATLTAGKSSSTAYMQLSSLTSEDSAVYYCAS <u>LR</u> <u>SRGYFDY</u> WGQGTTLTVSS	1
6G10D9 VL	DIVMTQSQTFMSTSVGDRVRITC <u>KASONVGTAVV</u> WYQQKTGQSPRLIY <u>SASNRYT</u> GVPDRFTGSGSGDFTLTISNMQSEDLADFFC <u>QQYSSYPY</u> TFGG GTKLEIK	2
21C3C11B3 VH	QVQLQQSGAELVRPGTSVKVSKASGYAF <u>INYLIE</u> WIKQRPQGGLWIG <u>MINPGSGGTNYNEKFKDK</u> KATLTADKSSSTAYMQLSSLTSDDSAVYFCARN <u>NW</u> <u>DV</u> WGQGTTLTVSS	3
21C3C11B3 VL	DVMTQSPSLPVS LGDQASVSC <u>RSSQSLVHSTGNTYLH</u> WYLQKPGQSPK LLIY <u>KVSNRF</u> SGVPDRFTGSGSGDFTLKISRVEAEDLGVYFC <u>SQSTHVP</u> <u>WT</u> FGGGTKLEIK	4
29B2C10 VH	EVQLQQSGPELVKPGASKMLCKASGYSFT <u>GYTMH</u> WVKQSHGENLEWIG <u>LINPYNGGTNYNQKFKG</u> KATLTVDKSSSTAYMELLTSEDSAVYYCSR <u>WG</u> <u>LRRAMDY</u> WGQGTSTVTVSS	5
29B2C10 VL	DIVMTQSQKFMSTSVGDRVSVTC <u>KASONVGSNVA</u> WYQQKPGQSPKALIY <u>S</u> <u>ASYRYS</u> GVPDRFTGNGSGDFTLTISNVQSEDLAEYFC <u>QQYNSPWT</u> TFGG GTKLEIK	6

34D3H1 VH	EVHVESGGDLVKPGGSLKLSAASGFTFS <u>SYGMS</u> WVRQTPDKRLEWVAT <u>ISSGGSYTYPPDSVKGR</u> FTISRDNAKNTLNLQMSSLSKSEDTAMYYCAR <u>QG</u> <u>GDAMDY</u> WGQGTSTVTVSS	7
34D3H1 VL	DIVLTQSPATLSVTPGDSVLS <u>CRASQSINN</u> LHWYQQKSHEPRLLIKY <u>ASQSI</u> SGIPSRFSGSGSGTDFTLSINSVETEDFGMYFC <u>QOQNSRL</u> TFGAG TKLELK	8
37B9C4 VH	EVNLEESGGGLVQPGGSMKLSVASGFTFS <u>DYWMN</u> WVRQSPEKGLEWVAE <u>IRLKSNNYATHYAESVKGR</u> FTISRDDS KSSVYLQMNRLAEDTGIYYCTR <u>PLLWFRRYYAMDY</u> WGQGTSTVTVSS	9
37B9C4 VL	DIQMTQTSSLSASLGDRITIS <u>SASQGISNYL</u> NWYQQKPDGTVKLLIYY <u>TSSLH</u> SGVPSRFSGSGSGTDYSLTISNLEPADIATYYC <u>QOQYSKL</u> PFTFGS GTKLEIK	10
38A10E9A11 VH	QVQLQQSGAELARPGASVKMSCKASGYTFT <u>TYTMH</u> WVKQRPQGQLEWIGY <u>INPSSGYTNYNQKFKD</u> KATLTAGKSSSTAYMQLSSLTSEDSAVYYCAS <u>LR</u> <u>SRGYFDY</u> WGRGTTLTVSS	11
38A10E9A11 VL	DIVMTQSQKFLSTSVGDRVSITC <u>KASONVGTAVA</u> WYQQKPGQSPKLLIYS <u>ASNRYT</u> GVPDRFTGSGSGTDFTLTISNMQSEDLAGYFC <u>QOQYSSPY</u> TFGG GTKLEIK	12
40A5B2 VH	EVQLQQSGPELVKPGASVKMSCKASGYTFT <u>RNIMH</u> WVKQKPGQGLEWIGY <u>INPYNAGSKYNEKFKG</u> KATLTDISSSTAYMELSSLTSEDSAVYYCARE <u>EE</u> <u>VYYRYGAWFAY</u> WGHGTLTVSA	13
40A5B2 VL	DIVLTQSPASLAVSLGQRATIS <u>CRASQSVSTSSYSYMH</u> WYQQKPGQPPKL LIK <u>YASNLES</u> GVPARFSGSGSGTDFTLNHPVEEEDATYYC <u>QHSWEI</u> PR TFGGGTKLEIK	14
58F9G10 VH	EVQLQQSGPELVKTGASVKISCKASGYSFT <u>GYTIH</u> WVKQSHGKSLEWIGY <u>ISCYNGATSFNQKFKG</u> KATFTVDTSSSTAYMQFNSLTSEDSAVYYCARTE <u>LRGPWFAY</u> WGQGTTLTVSA	15
58F9G10 VL	QTVLTQSPAIMASAPGEKVMTMC <u>SASSSVSYM</u> NWYQQKSGTSPKRWIYDT <u>SKLAS</u> GVPARFSGSGSGTSYSLTISMEAEADAATYYC <u>QOWSN</u> NPLTFGAG TKLELK	16
2D3C12 VH	QVQLQQSGAELVRPGTSVKVSCASGYAF <u>INYLIE</u> WIKQRPQGQLEWIGM <u>INPGSGGTNYNEKFKD</u> KATLTADKSSSTAYMQLSSLTSDSAVYFCARNW <u>DV</u> WGQGTTLTVSS	17
2D3C12 VL	DVMTQSPLSLPVSLGDAQSVSC <u>RSSQSLVHSTGNTYLH</u> WYLQKPGQSPK LLIY <u>KVSNRFS</u> GVDPDRFSGSGSGTDFTLKISRVEAEDLGVYFC <u>SQSTHVP</u> <u>WT</u> FGGGTKLEIK	18
14C2A11C6 VH	QVHLQQSGAELARPGASVKMSCKASGYTFT <u>TYTMH</u> WVKQRPQGQLEWIGY <u>INPN</u> SAYTNYNQKFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCARRV <u>LLLRVLDFDY</u> WGQGTTLTVSS	19
14C2A11C6 VL	DVQITQSPSYLAASPGETITINC <u>RASKSINKYL</u> TWYQEKPGKTNKLLIYS <u>GSTLQS</u> GIPIPSRFSGSGSGTDFTLTISNLEPEDFAMYYC <u>QQHNEYPL</u> TFGT GTKLELK	20
14F3H2 VH	EVQLQQSGPELVKPGASMKISCKASGYSFT <u>GYTMN</u> WVKQSHGKNLEWIGL <u>INPYNGGITRYNQKFKG</u> KATLTVDKSSSTAYMELLSLTSEDSAVYYCARW <u>G</u> <u>LRRAMDY</u> WGQGTSTVTVSS	21
14F3H2 VL	DIVMTQSQKFMSTSVGDRVSVTC <u>KASONVGTNVA</u> WYQQKPGQSPKALIYS <u>ASYRYS</u> GVDPDRFTGSGSGTDFTLTISNVQSEDLAEYFC <u>QOYNSSPW</u> TFGG GTKLEIK	22
26E1D12 VH	EVQLQQSGPELVKPGASMKISCKASGYSFT <u>GYTMN</u> WVKQSHGKNLEWIGL <u>INPYNGGTNYNQKFKG</u> KATLAVDKSSSTAYMDLLSLTSEDSAVYYCSRW <u>G</u> <u>LRRAMDY</u> WGQGTSTVTVSS	23
26E1D12 VL	DIVMTQSQKFMSTSVGDRVSVTC <u>KASONVGSNVA</u> WYQQKPGQSPKALIYS <u>ASYRYS</u> GVDPDRFTGSGSGTDFTLTISNVQSEDLAEYFC <u>QOYNNSPW</u> TFGG GTKLEIK	24

29A7H11H4C12 VH	EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMHWVKQSHGENLEWIGL INPYNGGTNYNQKFKGKATLTVDKSSSTAYMELLSTSEDSAVYYCSRWG LRRAMDYWGQGTSTVTVSS	25
29A7H11H4C12 VL	DIVMTQSQKFMSTSIGDRSVTCASQNVGSNVAWYQQKPGQSPKALIYS ASYRYSGVDPDRFTGNGSGTDFTLTISNVQSEDLAEYFCQQYYNSPWTFGG GTKLEIK	26
34D4A10 VH	QVQLQQSGAELVRPGTSVKVSCASGYAFISYLTIEWIKQRPQGQLEWIGM INPGSGGTNYNEKFKDKATLTADKSSSTAYMQLSSLTSDDSAVYFCARNW DVWGQGTTLTVSS	27
34D4A10 VL	DVVMTQSPLSLPVSLGDAQSVSCRSSQSLVHSTGNTYLHWYLPKPGQSPK LLIYKVSNNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSSQSTHVP WTFGGGTGLEIK	28

Example 2. Binding of Chimeric Antibodies to GPRC5D

[0131] The murine VH and VK genes were produced synthetically and then respectively cloned into vectors containing the human gamma 1 and human kappa constant domains. The purified chimeric antibodies were produced from transfected CHO cells.

[0132]

CHO-K1 cells stably expressed human GPRC5D, were harvested from flasks. 100 µl of 1×10^6 cells/ml of cells were incubated with primary chimeric antibodies in 3-fold serial dilutions starting from 300 nM to 0.001 nM for 30 minutes on ice. After being washed with 200 µl of FACS buffer twice, cells were incubated with secondary antibody for 30 minutes on ice. Cells were washed with 200 µl of FACS buffer twice and transferred to BD Falcon 5 ml tube and analyzed by FACS. The results of the study showed that the chimeric antibodies can bind to human GPRC5D with high EC₅₀ (FIG. 2).

[0133] The results are shown in the table below.

	Bottom	Top	LogEC50	HillSlope	EC50	Span
58F9G10	-521.8	414579	0.3596	0.8258	2.289	415101
40A5B2	1487	440269	0.3436	0.8189	2.206	438781
38A9E10A11	3016	386984	0.2453	1.037	1.759	383968
37B9C4	2078	405062	0.249	1.045	1.774	402985
34D3H1	3448	507844	0.5214	1.012	3.322	504396
29B2C10	4326	430443	0.342	1.041	2.198	426116
21C3C11B3	5774	403938	0.2293	1.286	1.696	398164
6G10D9	6886	458124	0.1012	1.343	1.262	451238

Example 3. EC50 of internalization of chimeric GPRC5D antibodies on CHO-GPRC5D cells

[0134] pHAb Dyes are pH sensor dyes that have very low fluorescence at pH > 7 and a dramatic increase in fluorescence as the pH of the solution becomes acidic. pHAb Dyes have excitation maxima (Ex) at 532nm and emission maxima (Em) at 560nm. pHAb Dye-conjugated antibodies can be used for monitoring receptor-mediated antibody internalization. When an antibody-pHAb Dye conjugate binds to its receptor on the cell membrane, it exhibits minimal fluorescence. However, upon receptor-mediated internalization, antibody-pHAb Dye conjugates traffic to the endosome and lysosomal vesicles where pH is acidic, causing the pHAb Dye to fluoresce. This fluorescence can be detected using various techniques, including cell imaging, flow cytometry and fluorescent plate-based readers with appropriate filters.

[0135] Stably transfected human GPRC5D CHO cells were harvested with 0.05% Trypsin/EDTA (Gibco, 25300-054) and plated in a 96-well black plate (Thermo Scientific #165305) at the density of 10 K per 90 μ l per well. Plates were incubated for 20–24 h before treatment with pHAb labeled antibodies.

[0136] For internalization, pHAb conjugated chimeric GPRC5D antibodies were added to the cells at different and mixed gently for 1–2 min on a plate mixer and then incubated overnight to allow internalization (internalization can be detected in a few hours). Plates were read on a fluorescent plate reader at Ex/Em: 532 nm/560 nm on a Tecan Infinity M1000 Pro. To achieve higher sensitivity, media was replaced by PBS before reading the plate.

[0137] The results normalized with DAR are shown in the **FIG. 3**. As shown in **FIG. 3**, the tested chimeric antibodies have potent internalization activity.

Example 4. Testing of Antibody-Dependent Cellular Cytotoxicity (ADCC)

[0138] The ADCC Reporter Bioassay uses an alternative readout at an earlier point in ADCC MOA pathway activation: the activation of gene transcription through the NFAT (nuclear factor of activated T-cells) pathway in the effector cell. In addition, the ADCC Reporter Bioassay uses engineered Jurkat cells stably expressing the Fc γ RIIIa receptor, V158 (high affinity) variant, and an NFAT response element driving expression of firefly luciferase as effector cells. Antibody biological activity in ADCC MOA is quantified through the

luciferase produced as a result of NFAT pathway activation; luciferase activity in the effector cell is quantified with luminescence readout. Signal is high, and assay background is low.

[0139] Serial dilutions of GPRC5D chimeric monoclonal antibody were incubated for 6 hours of induction at 37°C with engineered Jurkat effector cells (ADCC Bioassay Effector Cells), with or without ADCC Bioassay Target Cells (GPRC5D). Luciferase activity was quantified using Bio-Glo™ Reagent (in **FIG. 4**). All of the tested antibodies exhibited potent capability in inducing ADCC.

Example 5. Humanization of the mouse mAbs

[0140] The murine antibody variable region genes were employed to create humanized mAbs. In the first step of this process, 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.

[0141] The amino acid sequences of the humanized antibody are provided below.

Humanized sequences

A. 34D3H1

Table 2A. Humanization of 34D3H1 – VH

Name	Sequence	SEQ ID NO:
34D3H1 VH	EVHLVESGGDLVKPGGSLKLSAASGFTFSSYGMSSWVRQTPDKRLEWVAT ISSGGSYTYYPDSVKGRFTISRDNAKNTLNLMSSSLKSEDTAMYYCARQG GDAMDYWGQGTSTVTVSS	7
V1 (CDR grafting)	EVQLVESGGGLVKPGGSLRLSAAAGFTFSSYGMSSWVRQAPGKLEWVST ISSGGSYTYYPDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQG GDAMDYWGQGTSTVTVSS	35
V2 (with back mutations)	EVQLVESGGGLVKPGGSLRLSAAAGFTFSSYGMSSWVRQTPGKLEWVAT ISSGGSYTYYPDSVKGRFTISRDNAKNSLYLQMSSSLRAEDTAVYYCARQG GDAMDYWGQGTSTVTVSS	36
V3 (with back mutations)	EVHLESVGGGLVKPGGSLRLSAAAGFTFSSYGMSSWVRQTPGKRLEWVAT ISSGGSYTYYPDSVKGRFTISRDNAKNSLYLQMSSSLRAEDTAVYYCARQG GDAMDYWGQGTSTVTVSS	37

Table 2B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	SYGMS	29
CDR-H2	TISSGGSYTYYPDSVKG	30
CDR-H3	QGGDAMDY	31

Table 2C. Humanization of 34D3H1 – VL

Name	Sequence	SEQ ID NO:
34D3H1 VL	DIVLTQSPATLSVTPGDSVSLSC <u>RASQSI</u> NNNLHWYQQKSHESPRLLIKY <u>ASQSI</u> SGIPSRFSGSGSGTDFTLTISNSVETEDFGMYFC <u>QQSNSRLT</u> FGAG TKLEIK	8
V1 (CDR grafting)	DIQMTQSPSSLSASVGDRTITC <u>RASQSI</u> NNNLHWYQQKPGKAPKLLIYY <u>ASQSI</u> SGVPSRFSGSGSGTDFTLTISLQPEDFATYYC <u>QQSNSRLT</u> FGGG TKVEIK	38
V2 (with back mutations)	DIQMTQSPSSLSASVGDRTITCRASQSI>NNNLHWYQQKPGKAPKLLIYY ASQSI <u>SGI</u> PSRFSGSGSGTDFTLTIS <u>SV</u> QPEDFATY <u>EC</u> QQSNSRLTFGGG TKVEIK	39
V3 (with back mutations)	DIQ <u>L</u> TQSPSSLSASVGDRTITCRASQSI>NNNLHWYQQKPGK <u>SP</u> KLLIYY ASQSI <u>SGI</u> PSRFSGSGSGTDFTLTIS <u>SV</u> QPEDFATY <u>EC</u> QQSNSRLTFGGG TK <u>L</u> EIK	40
V4 (with back mutations)	DIV <u>L</u> TQSPSSLS <u>V</u> SVGDRT <u>L</u> TCRASQSI>NNNLHWYQQKPGK <u>SP</u> KLLIYY ASQSI <u>SGI</u> PSRFSGSGSGTDFTLTIS <u>SV</u> QPEDFATY <u>EC</u> QQSNSRLTFGGG TK <u>L</u> EIK	41

Table 2D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	RASQSI>NNNLH	32
CDR-L2	YASQSI	33
CDR-L3	QQSNSRLT	34

Table 2E. Humanized antibodies

	VL	VL v1	VL v2	VL v3	VL v4
VH	34-XI				
VH v1		34-H1L1	34-H1L2	34-H1L3	34-H1L4
VH v2		34-H2L1	34-H2L2	34-H2L3	34-H2L4
VH v3		34-H3L1	34-H3L2	34-H3L3	34-H3L4

B. 37B9C4**Table 3A. Humanization of 37B9C4 – VH**

Name	Sequence	SEQ ID NO:
37B9C4 VH	EVNLEESGGGLVQPGGSMKLSVASGFTFSDYWMNWRQSPKGLEWVAE <u>IRLKSNNYATHYAESVKGR</u> FTISRDDSKSSVYLQMNNLRAEDTGIYYCTR <u>PLLWFRRYYAMDYWGQ</u> TSVTSS	9
V1 (CDR grafting)	EVQLVESGGGLVQPGGSLRLSAAAGFTFSDYWMNWRQAPKGLEWVAE <u>IRLKSNNYATHYAESVKGR</u> FTISRDNKNSLYLQMNSLRAEDTAVYYCAR <u>PLLWFRRYYAMDYWGQ</u> TLVTSS	48
V2 (with back mutations)	EVQLVESGGGLVQPGGSLRLSAAAGFTFSDYWMNWRQ <u>SP</u> KGLEWVAE IRLKSNNYATHYAESVKGRFTISRDNK <u>SS</u> LYLQMNSLRAEDTAVYYC <u>TR</u> PLLWFRRYYAMDYWGQTLVTSS	49

V3 (with back mutations)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYWMNWVRQSPGKGLEWVAEIRLKSNYYATHYAESVKGRFTISRDDSKSSVYLQMNSLRAEDTAVYYCTRPLLWFRYYAMDYWGQGLTVTVSS	50
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Table 3B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	DYWMN	42
CDR-H2	EIRLKSNYYATHYAESVKG	43
CDR-H3	PLLWFRYYAMDY	44

Table 3C. Humanization of 37B9C4 – VL

Name	Sequence	SEQ ID NO:
37B9C4 VL	DIQMTQTSSLSASLGDRITISC <u>SASQGISNYLN</u> WYQQKPDGTVKLLIYY <u>TSSLHSGVPSRFS</u> SGSGGTDYSLTISNLEPADATYYC <u>QQYSKLPFT</u> FGSGTKLEIK	10
V1 (CDR grafting)	DIQMTQSPSSLSASVGDRTITC <u>SASQGISNYLN</u> WYQQKPGKAPKLLIYY <u>TSSLHSGVPSRFS</u> SGSGGTDFTLTISLQPEDFATYYC <u>QQYSKLPFT</u> FGQGTKLEIK	51
V2 (with back mutations)	DIQMTQSPSSLSASVGDRTITC <u>SASQGISNYLN</u> WYQQKPGKTVKLLIYYTSSLHSGVPSRFS <u>SGSGGTDY</u> TLTISLQPEDFATYYCQQYSKLPFTFGQGTKLEIK	52
V3 (with back mutations)	DIQMTQSPSSLSASVGDRTITC <u>SASQGISNYLN</u> WYQQKPGKTVKLLIYYTSSLHSGVPSRFS <u>SGSGGTDY</u> TLTISLQPEDFATYYCQQYSKLPFTFGSGTKLEIK	53

Table 3D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	SASQGISNYLN	45
CDR-L2	YTSSLHS	46
CDR-L3	QQYSKLPFT	47

Table 3E. Humanized antibodies

	VL	VL v1	VL v2	VL v3
VH	37B9C4-XI			
VH v1		37-H1L1	37-H1L2	37-H1L3
VH v2		37-H2L1	37-H2L2	37-H2L3
VH v3		37-H3L1	37-H3L2	37-H3L3

C. 58F9G10**Table 4A. Humanization of 58F9G10 – VH**

Name	Sequence	SEQ ID NO:
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58F9G10 VH	EVQLQQSGPELVKLTGASVKISCKASGYSFTGYIHWVKQSHGKSLIEWIGY <u>IS</u> SCYNGATSFNQKF ~~K~~ GKATFTVDTSSSTAYMQFNSLTSEDSAVYYCARTE <u>LRGPWFAY</u> WGQGT LTVSA	15
V1 (CDR grafting)	QVQLVQSGAEVKKPGASVKVSKASGYSFTGYIHWVRQAPGQGLEWMGY <u>IS</u> SYNAATSFNQKF ~~K~~ GRVTMTTRDTSTSTVYMESSLRSEDTAVYYCARTE <u>LRGPWFAY</u> WGQGT LTVSS	61
V2 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGYSFTGYIHWVRQAPGQGLEWIGY ISSYNAATSFNQKF ~~K~~ GRVTEVDTSTSTVYMESSLRSEDTAVYYCARTE LRGPWFAYWGQGT LTVSS	62
V3 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGYSFTGYIHWVKQAPGQGLEWIGY ISSYNAATSFNQKF ~~K~~ GRVTEVDTSTSTVYMESSLRSEDTAVYYCARTE LRGPWFAYWGQGT LTVSS	63
V4 (with back mutations)	QVQLVQSGAEVKKPGASVKISCKASGYSFTGYIHWVKQAPGQGLEWIGY ISSYNAATSFNQKF ~~K~~ GRATEVDTSTSTAYMESSLRSEDTAVYYCARTE LRGPWFAYWGQGT LTVSS	64

Table 4B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	GYIHW	54
CDR-H2	YISCYNGATSFNQKF ~~K~~ G YISSYNAATSFNQKF ~~K~~ G	60 55
CDR-H3	TELRGPWFAY	56

Table 4C. Humanization of 58F9G10 – VL

Name	Sequence	SEQ ID NO:
58F9G10 VL	QTVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDT <u>SKLAS</u> GVPARFSGSGSGTSYSLTISSEAEADAATYYCQQWSNNPLTFGAG TKLEIK	16
V1 (CDR grafting)	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQKPGKAPKLLIYDT <u>SKLAS</u> GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSNNPLTFGQG TKLEIK	65
V2 (with back mutations)	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQKPGKAPKRLLIYDT SKLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQWSNNPLTFGQG TKLEIK	66
V3 (with back mutations)	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQKPGKAPKRLLIYDT SKLASGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQWSNNPLTFGQG TKLEIK	67

Table 4D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	SASSSVSYMN	57
CDR-L2	DTSKLAS	58
CDR-L3	QQWSNNPLT	59

Table 3E. Humanized antibodies

	VL	VL v1	VL v2	VL V3
VH	58F9G10-XI			
VH v1		58-H1L1	58-H1L2	58-H1L3
VH v2		58-H2L1	58-H2L2	58-H2L3
VH v3		58-H3L1	58-H3L2	58-H3L3
VH v4		58-H4L1	58-H4L2	58-H4L3

D. 6G10D9**Table 5A. Humanization of 6G10D9 – VH**

Name	Sequence	SEQ ID NO:
6G10D9 VH	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTMHWWKQRPQGLEWLG YINPSSGYTNYNQKFKDKATLTAGKSSSTAYMQLSSLTSEDSAVYYCASLR SRGYFDYWGQGTTLTVSS	1
V1 (CDR grafting)	QVQLVQSGAEVKKPGASVKVSKASGYTFTTYTMHWWKQRPQGLEWGM YINPSSGYTNYNQKFKDRVTMTDTSTSTVYMESSLRSEDTAVYYCSRLR SRGYFDYWGQGTLLTVSS	74
V2 (with back mutations)	QVQLVQSGAEVKKPGASVKVSKASGYTFTTYTMHWWKQRPQGLEWLG YINPSSGYTNYNQKFKDRVTMTADTSTSTVYMESSLRSEDTAVYYCARLR SRGYFDYWGQGTLLTVSS	75
V3 (with back mutations)	QVQLVQSGAEVAKPGASVKVSKASGYTFTTYTMHWWKQAPQGLEWLG YINPSSGYTNYNQKFKDRVTMTADTSTSTVYMESSLRSEDTAVYYCASLR SRGYFDYWGQGTLLTVSS	76
V4 (with back mutations)	QVQLVQSGAEVAKPGASVKMSCKASGYTFTTYTMHWWKQAPQGLEWLG YINPSSGYTNYNQKFKDRVTLTADTSTSTVYMESSLRSEDTAVYYCASLR SRGYFDYWGQGTLLTVSS	77
V5 (with back mutations)	QVQLQQSGAEVAKPGASVKMSCKASGYTFTTYTMHWWKQRPQGLEWLG YINPSSGYTNYNQKFKDRVTLTADKSTSTVYMESSLRSEDTAVYYCASLR SRGYFDYWGQGTLLTVSS	78
V6 (with back mutations)	QVQLQQSGAEVAKPGASVKMSCKASGYTFTTYTMHWWKQRPQGLEWLG YINPSSGYTNYNQKFKDRATLTAGKSTSTVYMESSLRSEDTAVYYCASLR SRGYFDYWGQGTLLTVSS	79

Table 5B. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-H1	TYTMH	D01
CDR-H2	YINPSSGYTNYNQKFKD	D02
CDR-H3	LRSGYFDY	D03

Table 5C. Humanization of 6G10D9 – VL

Name	Sequence	SEQ ID NO:
6G10D9 VL	DIVMTQSQTFTMSTSVGDRVRITCKASONVGTAVVWYQQKGTGQSPRLLIYS ASNRYTGVPDRFTGSGSGTDFLTISNMQSEDLADFFCQOYSSYPYTFGG GTKLEIK	2

V1 (CDR grafting)	DIQLTQSPSFLSASVGDRVTITCKASQNVGTAVVWYQQKPGKAPKLLIYS ASNRYTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYSSYPYTFGQ GTKLEIK	80
V2 (with back mutations)	DIQLTQSPSFLSISVGDRVTITCKASQNVGTAVVWYQQKPGKSPKLLIYS ASNRYTGVPSRFSGSGSGTEFTLTISSLQPEDFATFYCQQYSSYPYTFGQ GTKLEIK	81
V3 (with back mutations)	DIQMTQSPSFLSISVGDRVTITCKASQNVGTAVVWYQQKPGKSPKLLIYS ASNRYTGVPSRFSGSGSGTEFTLTISSMQPEDFATFFCQQYSSYPYTFGQ GTKLEIK	82
V4 (with back mutations)	DIQMTQSPSFLSISVGDRVTITCKASQNVGTAVVWYQQKPGKSPKLLIYS ASNRYTGVPSRFSGSGSGTEFTLTISSMQPEDFATFFCQQYSSYPYTFGG GTKLEIK	83
V1a (CDR grafting version a)	EIVMTQSPATLSVSPGERATLSCASQNVGTAVVWYQQKPGQAPRLLIYS ASNRYTGIPARFSGSGSGTEFTLTISSLQSEDAVYYCQQYSSYPYTFGQ GTKLEIK	84
V2a (with back mutations)	EIVMTQSPATLSISVSPGERATLSCASQNVGTAVVWYQQKPGQSPRLLIYS ASNRYTGIPDRFSGSGSGTEFTLTISSLQSEDAVFFCQQYSSYPYTFGQ GTKLEIK	85
V3a (with back mutations)	EIVMTQSPATLSISVSPGERVTISCKASQNVGTAVVWYQQKPGQSPRLLIYS ASNRYTGIPDRFTGSGSGTDFTLTISSMQSEDAVFFCQQYSSYPYTFGG GTKLEIK	86

Table 5D. CDR Sequences

CDR	Sequence	SEQ ID NO:
CDR-L1	<u>KASQNVGTAVV</u>	71
CDR-L2	<u>SASNRYT</u>	72
CDR-L3	<u>QQYSSYPYT</u>	73

Table 5E. Humanized antibodies

	VL	VL v1	VL v2	VL v3	VL v4	VL v1a	VL v2a	VL v3a
VH	6G10D9-XI							
VH v1		6-H1L1	6-H1L2	6-H1L3				
VH v2		6-H2L1	6-H2L2	6-H2L3				
VH v3		6-H3L1	6-H3L2	6-H3L3				
VH v4		6-H4L1	6-H4L2	6-H4L3				
VH v5								
VH v6								

Example 6. Testing of Humanized Antibodies

[0142] This example tested some of the humanized antibodies for the ability to bind to GPRC5D expressed on CHO-K1 cells.

[0143] Out of the tested humanized antibodies derived from 6G10D9, 6-H2L1 and 6-H3L3 outperformed others (FIG. 5, Table 6).

Table 6. Activities of humanized antibodies of 6G10D9 to bind GPRC5D

	Bottom	Top	LogEC50	HillSlope	EC50	Span
6G10D9-XI	6858	121725	0.2992	1.525	1.991	114867
6-H1L1	192.6	~ 6397	~ 3.102	1.198	~ 1264	~ 6205
6-H1L2	191.9	898894	5.224	1.19	167639	898702
6-H1L3	226.2	16478302	5.389	1.156	244790	16478076
6-H2L1	189.1	661560	5.039	1.232	109294	661371
6-H2L2	192.5	1271084	5.37	1.191	234364	1270892
6-H2L3	215.7	10592380	5.039	1.23	109279	10592164
6-H3L1	-205.1	74293	2.352	0.8392	225.1	74498
6-H3L2	68.94	47463	2.244	0.9532	175.3	47394
6-H3L3	3994	119304	0.9553	1.13	9.022	115310
6-H4L1	61.5	50293	1.654	0.9277	45.05	50231
6-H4L2	9.56	39449	1.976	0.911	94.63	39440
6-H4L3	2344	126413	0.9049	1.052	8.033	124069

[0144] As shown in **FIG. 6** and **Table 7**, all of the humanized versions of 58F9G10 appeared to have good performance.

Table 7. Activities of humanized antibodies of 58F9G10 to bind GPRC5D

	Bottom	Top	LogEC50	HillSlope	EC50	Span
58F9G10-XI	6697	127349	0.2925	1.292	1.961	120652
58-H1L1	15047	107881	0.2519	1.342	1.786	92834
58-H1L2	5500	114432	0.2632	1.238	1.833	108932
58-H1L3	4227	105346	0.2308	1.14	1.701	101119
58-H2L1	3617	109672	0.3776	1.064	2.386	106055
58-H2L2	4837	107888	0.3448	1.091	2.212	103050
58-H2L3	1229	91713	0.2697	0.9633	1.861	90483
58-H3L1	9887	103663	-0.1918	1.382	0.643	93776
58-H3L2	5981	105458	0.3742	1.195	2.367	99476
58-H3L3	4022	97718	0.3177	1.105	2.078	93695
58-H4L1	4993	104559	0.3655	1.179	2.32	99566
58-H4L2	4600	105051	0.3407	1.143	2.191	100451
58-H4L3	6249	102650	0.2874	1.27	1.938	96401

[0145] Likewise, as shown in **FIG. 7** and **Table 8**, all of the humanized versions of 34D3H1 appeared to have good performance, comparable to the chimeric antibody.

Table 8. Activities of humanized antibodies of 34D3H1 to bind GPRC5D

	Bottom	Top	LogEC50	HillSlope	EC50	Span
34-XI	16525	192054	0.4947	1.454	3.124	175530
34-H1L1	10921	196570	0.5908	1.314	3.898	185650
34-H1L2	13856	182030	0.5836	1.436	3.834	168174
34-H1L3	10620	185840	0.5732	1.167	3.743	175220
34-H1L4	10299	188925	0.5999	1.195	3.98	178626
34-H2L1	7854	187153	0.6188	1.173	4.157	179298
34-H2L2	8736	178873	0.6254	1.118	4.221	170137
34-H2L3	7063	185842	0.5832	1.11	3.83	178779
34-H2L4	4393	192405	0.6092	1.041	4.066	188012
34-H3L1	11139	178248	0.5207	1.228	3.317	167109
34-H3L2	9036	179809	0.6464	1.146	4.43	170773
34-H3L3	9118	192976	0.6508	1.1	4.475	183858
34-H3L4	12751	192533	0.5981	1.263	3.963	179782

[0146] Also, as shown in **FIG. 8** and **Table 9**, all of the humanized versions of 37B9C4 appeared to have good performance, comparable to the chimeric counterpart.

Table 9. Activities of humanized antibodies of 37B9C4 to bind GPRC5D

	Bottom	Top	LogEC50	HillSlope	EC50	Span
37B9C4-XI	11711	188479	0.2364	1.502	1.723	176769
37-H1L1	9596	178413	0.3574	1.613	2.277	168817
37-H1L2	8848	181865	0.3502	1.534	2.24	173017
37-H1L3	10445	173229	0.2332	1.528	1.711	162784
37-H2L1	9184	175072	0.351	1.583	2.244	165888
37-H2L2	9383	175293	0.3455	1.656	2.216	165910
37-H2L3	8049	168032	0.3334	1.564	2.155	159982
37-H3L1	6372	179261	0.2921	1.35	1.96	172889
37-H3L2	9193	179304	0.3153	1.428	2.067	170111
37-H3L3	7780	181053	0.2846	1.305	1.926	173273

[0147] Based on the above data, humanized antibodies 6-H3L3, 6-H4L3, 58-H1L1, 58-H3L1, 34-H1L1 and 37-H1L1 were selected for further confirmative testing.

Example 7. Confirmative Testing of Select Humanized Antibodies

[0148] This example tested some of the humanized antibodies (6-H3L3, 6-H4L3, 58-H1L1, 58-H3L1, 34-H1L1 and 37-H1L1) for their ability to bind to the human GPRC5D protein expressed on CHO-K1 and NCI-H929 cells.

[0149] The binding data to CHO-K1 are charted in **FIG. 9** and summarized in **Table 10** below.

Table 10. Binding to GPRC5D on CHO-K1 cells

	Bottom	Top	LogEC50	HillSlope	EC50	Span
6-XI	523.9	104077	0.4723	1.095	2.967	103553
58-XI	52.25	107000	0.5562	0.9481	3.599	106948
6-H3L3	172	134045	1.412	0.8045	25.82	133873
6-H4L3	109.4	145833	1.421	0.7392	26.34	145724
58-H1L1	275.4	99454	0.579	0.972	3.793	99178
58-H3L1	75.37	104402	0.612	0.9135	4.093	104326
34-XI	688.6	117714	0.5776	1.054	3.781	117025
37-XI	792.6	113379	0.3877	1.175	2.442	112586
34-H1L1	170.8	119038	0.781	0.8745	6.04	118867
37-H1L1	523.2	102496	0.511	1.042	3.244	101973

Table 11. Binding to GPRC5D on NCI-H929 cells

	Bottom	Top	LogEC50	HillSlope	EC50	Span
6-XI	269.4	13785	0.05146	1.313	1.126	13515
58-XI	261.4	14738	0.1596	1.269	1.444	14476
6-H3L3	129.8	21859	1.465	0.8063	29.18	21730
6-H4L3	80.05	28987	1.606	0.7142	40.33	28907
58-H1L1	281	13474	0.1648	1.319	1.462	13193
58-H3L1	275.5	13934	0.3	1.297	1.995	13658
34-XI	181.1	18146	0.7587	0.9911	5.738	17965
34-H1L1	199.3	17911	0.8048	1.011	6.379	17712
37-XI	337.6	13807	-0.05621	1.41	0.8786	13470
37-H1L1	341.8	13534	-0.1991	1.419	0.6323	13192

[0150] These data, therefore, confirm that these selected humanized antibodies are suitable for further clinical development.

Example 8. ADCC of Humanized Antibodies

[0151] The ADCC Reporter Bioassay uses an alternative readout at an earlier point in ADCC MOA pathway activation: the activation of gene transcription through the NFAT (nuclear factor of activated T-cells) pathway in the effector cell. In addition, the ADCC Reporter Bioassay uses engineered Jurkat cells stably expressing the FcγRIIIa receptor, V158 (high affinity) variant, and an NFAT response element driving expression of firefly luciferase as effector cells. Antibody biological activity in ADCC MOA is quantified through the luciferase produced as a result of NFAT pathway activation; luciferase activity in the effector cell is quantified with luminescence readout. Signal is high, and assay background is low.

[0152] Serial dilutions of GPRC5D hu Abs were incubated for 6 hours of induction at 37°C with engineered Jurkat effector cells (ADCC Bioassay Effector Cells), with ADCC Bioassay Target Cells (expressing GPRC5D). Luciferase activity was quantified using Bio-Glo™ Reagent.

[0153] The results are presented in **FIG. 11**, which shows that these humanized antibodies induced strong ADCC activities on GPRC5D-overexpressing cell lines and endogenous MM (multiple myeloma) cell lines.

Example 9. Internalization of Humanized Antibodies

[0154] This example tested the humanized antibodies for their internalization induction activities. The example used a new hydrophilic and bright pH sensor dye (pHAb dye), which is not fluorescent at neutral pH but becomes highly fluorescent at acidic pH with internalization. It can be used to detect the process of internalization. NCI-H929 and MM.1R cells endogenously expressed human GPRC5D as the target cells, the detection antibody labeled with pHAb dye was added to evaluate the internalization of GPRC5D hu antibody in vitro.

[0155] Serial dilutions of GPRC5D hu antibodies were incubated for 24 hours at 37°C. Luciferase activity was detected. The results, in **FIG. 12**, show that these humanized antibodies have very strong internalization activities to both GPRC5D-overexpressing cell lines and endogenous MM cell lines.

Example 10. Killing Activity of GPRC5D ADC

[0156] This example tested two antibody-drug conjugates (ADC) for their killing activities of targeted cells. These ADC included 37B9C4 and 58F9G10, respectively, conjugated to the toxic drug Monomethyl auristatin E (MMAE).

[0157] The human GPRC5D expressing HEK293 and NCI-H929 engineering cells (HEK293/H_GPRC5D and NCI-H929/H_GPRC5D), GPRC5D endogenously expressing NCI-H929 and MM.1R cells were seeded to a 96-well plate at 3000~4000 cells per well. The cells were treated with 37B9C4-MMAE and 58F9G10-MMAE at respective concentrations for 5 days. The cell viability was measured by CellTiteseeded to a 96-well plate at 3000~4000 cells per well with r-Glo reagent. The luciferase activity was detected by Envision. The results, in **FIG. 13** and **Table 12**, show that these humanized antibodies have very strong killing activities.

Table 12. ADC Killing Activities

	Hek-293/H_GPRC5D (IC50, nM)	MM.1R (IC50, nM)	NCI-H929 (IC50, nM)	NCI-H929/GPRC5D (IC50, nM)
37B9C4-MMAE	0.025	0.14	0.23	0.11
58F9G10-MMAE	0.026	0.07	0.99	0.14

Example 11. In vivo Anti-Tumor Activities

[0158] This example used a CDX animal model to test the anti-tumor activities of antibody 37B9C4 (H1L1).

[0159] In this study, 6-8 weeks female NCG mice (Jiangsu Jicui Yaokang Biotechnology Co., Ltd) were used. Each mouse was inoculated subcutaneously at the right axillary (lateral) with MM.1R tumor cell (2×10^6) in 0.1 ml of PBS with matrigel (V:V=1:1) for tumor development. The animals were randomly grouped when tumor volume reach around 60 mm³, then treatment started for the efficacy study. 37B9C4 at dose of 1mg/kg, 3mg/kg and 10 mg/kg were administrated via intravenous (i.v.) at day 0, day 7, day 14. The experiment was terminated at day 19 when the average tumor volume in vehicle group was over 2000 mm³. The average tumor volume of PBS group, 37B9C4 (1 mg/kg) group, 37B9C4 (3 mg/kg) group and 37B9C4 (10 mg/kg) group were 2498.58 mm³, 1196.15 mm³, 0.00 mm³ (6/6 CR), and 0.00 mm³ (6/6 CR) respectively. The tumor size were measured three times weekly in

two dimensions using a caliper and the volume was expressed in mm³ using the formula: $V = 0.5 a \times b^2$ where a and b were the long and short diameters of the tumor, respectively. Data points represent group (n=6) mean, error bars represent standard error of the mean (SEM).

[0160] The body weight of MM.1R tumor bearing mice was monitored regularly as an indirect measure of toxicity. **FIG. 14A** shows the tumor growth curve of MM.1R tumor-bearing mice post administration of 37B9C4. The antibody dose-dependently inhibited the tumor growth in this tumor model, and achieved complete tumor inhibition at or above 3 mg/kg.

[0161] The detailed changes of body weight and relative change of body weight of MM.1R tumor bearing mice after administration are shown in **FIG. 14B**. During the administration period, all groups of mice had no significant body weight loss, and the mice in the administration group had good tolerance, demonstrating the safety of the treatment.

* * *

[0162] 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.

[0163] 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 antigen-binding fragment thereof having binding specificity to a human G-protein-coupled receptor family C group 5 member D (GPRC5D) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region (VH) comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region (VL) comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, and wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise:
 - (a) the amino acid sequences of SEQ ID NO:42-47;
 - (b) the amino acid sequences of SEQ ID NO:29-34;
 - (c) the amino acid sequences of SEQ ID NO:54-59; or
 - (d) the amino acid sequences of SEQ ID NO:68-73.
2. The antibody or antigen-binding fragment thereof of claim 1, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise the amino acid sequences of SEQ ID NO:42-47.
3. The antibody or antigen-binding fragment thereof of claim 2, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:9 and 48-50, and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NO:10 and 51-53.
4. The antibody or antigen-binding fragment thereof of claim 2, wherein the VH comprises the amino acid sequence of SEQ ID NO:48, and the VL comprises the amino acid sequence of SEQ ID NO:51.

5. The antibody or antigen-binding fragment thereof of claim 1, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise the amino acid sequences of SEQ ID NO:29-34.
6. The antibody or antigen-binding fragment thereof of claim 5, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:7 and 35-37, and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NO:8 and 38-41.
7. The antibody or antigen-binding fragment thereof of claim 5, wherein the VH comprises the amino acid sequence of SEQ ID NO:35, and the VL comprises the amino acid sequence of SEQ ID NO:38.
8. The antibody or antigen-binding fragment thereof of claim 1, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise the amino acid sequences of SEQ ID NO:54-59.
9. The antibody or antigen-binding fragment thereof of claim 8, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:61-64, and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NO:16 and 65-67.
10. The antibody or antigen-binding fragment thereof of claim 8, wherein the VH comprises the amino acid sequence of SEQ ID NO:61, and the VL comprises the amino acid sequence of SEQ ID NO:65.

11. The antibody or antigen-binding fragment thereof of claim 8, wherein the VH comprises the amino acid sequence of SEQ ID NO:63, and the VL comprises the amino acid sequence of SEQ ID NO:65.
12. The antibody or antigen-binding fragment thereof of claim 1, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise the amino acid sequences of SEQ ID NO:68-73.
13. The antibody or antigen-binding fragment thereof of claim 12, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1 and 74-79, and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and 80-86.
14. The antibody or antigen-binding fragment thereof of claim 12, wherein the VH comprises the amino acid sequence of SEQ ID NO:76, and the VL comprises the amino acid sequence of SEQ ID NO:82.
15. The antibody or antigen-binding fragment thereof of claim 12, wherein the VH comprises the amino acid sequence of SEQ ID NO:77, and the VL comprises the amino acid sequence of SEQ ID NO:82.
16. The antibody or fragment thereof of any one of claims 1-15, which is humanized.
17. The antibody or fragment thereof of any one of claims 1-16, which is ADCC-competent.
18. The antibody or fragment thereof of any one of claims 1-16, further comprising a cytotoxic drug conjugated to the antibody or fragment thereof.

19. A bispecific antibody, comprising the antigen-binding fragment of any one of claims 1-16, and a second antigen-binding fragment having specificity to a second target protein.
20. The bispecific antibody of claim 19, wherein the second target protein is selected from the group consisting of CD3, CD16, CD19, CD28, CD64 and 4-1BB.
21. The bispecific antibody of claim 19, wherein the second target protein is CD3.
22. 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 1-21.
23. A method of treating cancer in a patient in need thereof, comprising (a) treating a T cell, a natural killer (NK) cell, or a macrophage, *in vitro*, with the antibody or fragment thereof of any one of claims 1-21, and (b) administering the treated cell to the patient.
24. The method of claim 23, further comprising, prior to step (a), isolating the T cell, the NK cell, or the macrophage, from an individual.
25. The method of claim 24, wherein the T cell, the NK cell, or the macrophage, is isolated from the patient.
26. The method of claim 23, wherein the T cell is a tumor-infiltrating T lymphocyte, a CD4⁺ T cell, a CD8⁺ T cell, or the combination thereof.
27. The method of any one of claims 22-26, wherein the cancer is a hematological cancer.

28. The method of claim 27, wherein the hematological cancer is a GPRC5D-expressing B cell cancer.

29. The method of claim 28, wherein the GPRC5D-expressing B cell cancer is multiple myeloma.