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(54) Title: ANTI-SLC34A2/ANTI-4-1BB ANTIBODIES AND USES THEREOF

(57) Abstract: Provided are bispecific and multi-specific antibodies that target a tumor antigen and 4-1BB, particular multi-specific antibodies having binding specificity to the human SLC34A2 protein and human 4-1BB protein. Methods of using the antibodies or antigen-binding fragments thereof for treating cancer are also provided.

ANTI-SLC34A2/ANTI-4-1BB ANTIBODIES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of International Application No. PCT/CN2023/140639, filed December 21, 2023, the content of which is hereby incorporated by reference in its entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The content of the electronic sequence listing (371465.xml; Size: 16,805 bytes; and Date of Creation: December 19, 2024) is herein incorporated by reference in its entirety.

BACKGROUND

[0003] Cell membrane transporter proteins such as transporters belonging to glucose transporter GLUT, ATP-binding cassette transporter ABC, and solute carrier transporter SLC families are frequently upregulated on cancer cells, compared to adjacent normal cells. High levels of transporters are found in a wide range of solid tumors, correlating with poor survival. One of the potential molecular tumor markers may be the sodium-dependent phosphate transporter NaPi2b encoded by the SLC34A2 (Solute carrier 34 A2) gene.

[0004] SLC34A2 encodes the type II Na/Pi co-transporter (NaPi2b) which is a multi-transmembrane sodium-dependent phosphate transporter responsible for transcellular inorganic phosphate absorption. NaPi2b is highly abundant in the brush-border membrane of the small intestine, where it is involved in the transcellular flux of inorganic phosphates via the apical membrane of epithelial cells. An altered expression of sodium-dependent phosphate transporter NaPi2b has been reported in ovarian cancer, lung cancer, gastric cancer, thyroid cancer, and other cancers.

[0005] 4-1BB (CD137 and TNFRSF9), which was first identified as an inducible costimulatory receptor expressed on activated T cells, is a membrane spanning glycoprotein of the Tumor Necrosis Factor (TNF) receptor superfamily. Current understanding of 4-1BB indicates that expression is generally activation dependent and encompasses a broad subset of immune cells including activated NK and NKT cells, regulatory T cells, dendritic cells (DC) including follicular DC; stimulated mast cells, differentiating myeloid cells, monocytes, neutrophils, eosinophils, and activated B cells. 4-1BB expression has also been demonstrated on tumor

vasculature and atherosclerotic endothelium. The ligand that stimulates 4-1BB (4-1BBL) is expressed on activated antigen-presenting cells (APCs), myeloid progenitor cells and hematopoeitic stem cells.

[0006] Interaction of 4-1BB on activated normal human B cells with its ligand at the time of B cell receptor engagement stimulates proliferation and enhances survival. 4-1BB is undetectable on the surface of naive T cells but expression increases upon activation. Upon 4-1BB activation, TRAF 1 and TRAF 2, which are pro-survival members of the TNFR-associated factor (TRAF) family, are recruited to the 4-1BB cytoplasmic tail, resulting in downstream activation of NFkB and the Mitogen Activated Protein (MAP) Kinase cascade including Erk, ink, and p38 MAP kinases.

[0007] The cancer cell-specific expression of SLC34A2 and immune cell expression of 4-1BB indicates that these two targets can be a potential cancer target for antibody therapy.

SUMMARY

[0008] The present disclosure provides multi-specific antibodies having binding specificity to the human SLC34A2 protein and 4-1BB protein. These antibodies and fragments are useful in the treatment of diseases and conditions such as cancers. In certain embodiments, the multi-specific antibody further comprises a Fc domain with reduced or eliminated effector function. In certain embodiments, the second antibody moiety is a sdAb.

[0009] One embodiment of the present disclosure provides a multi-specific antibody comprising: (1) a first antibody moiety specifically binds to a human Solute carrier 34 A2 (SLC34A2) protein, and (2) a second antibody moiety specifically binds to a human 4-1BB protein. In some embodiments, the multi-specific antibody further comprises a Fc domain. In some embodiments, Fc is modified to reduce or eliminate effector function.

[0010] In some embodiments, the first antibody moiety or the second antibody moiety is a $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv or sdAb. In some embodiments, the first antibody moiety is Fab'. In some embodiments, the second antibody moiety is a sdAb. In some embodiments, the Fc domain is derived from any one selected from the group consisting of IgG1, IgG2, IgG3, and IgG4. In some embodiments, the Fc domain is derived from IgG1.

[0011] In some embodiments, the first antibody moiety is a Fab' fused to N-terminus of the Fc domain and the second antibody moiety is a sdAb fused to C-terminus of the Fc domain. In some embodiments, the second antibody moiety is fused to the Fc domain via a linker.

[0012] In some embodiments, the second antibody moiety comprises a HCDR1 comprising the amino acid of SSAMS (SEQ ID NO: 34), a HCDR2 comprising the amino acid of GIYSGGSTYYTESVKD (SEQ ID NO: 35), and a HCDR3 comprising the amino acid of WGTLRFGVWAEYDH (SEQ ID NO: 36). In some embodiments, the second antibody moiety comprises the amino acid sequence of SEQ ID NO: 9 or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 9.

[0013] In some embodiments, the first antibody moiety comprises a heavy chain variable region comprising heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, and a light chain variable region comprising heavy chain complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, respectively, comprise amino acid sequences selected from the group consisting of: (a) HCDR1: TNNMH (SEQ ID NO: 10), HCDR2: AIYPGSGATAYNQKFKG (SEQ ID NO: 11), HCDR3: GMYGHGAMDY (SEQ ID NO: 12), LCDR1: KASQDVGTAVA (SEQ ID NO: 13), LCDR2: WATTRHS (SEQ ID NO: 14), and LCDR3: QQYSSNPLT (SEQ ID NO: 15); (b) HCDR1: SYITH (SEQ ID NO: 16), HCDR2: AIYPGNADTSYIQKFKG (SEQ ID NO: 17), HCDR3: GTYGTSAWFTY (SEQ ID NO: 18), LCDR1: RARQNIGNNLY (SEQ ID NO: 19), LCDR2: YASQSIS (SEQ ID NO: 20), and LCDR3: QQSFSWPLT (SEQ ID NO: 21); (c) HCDR1: TYNMH (SEQ ID NO: 22), HCDR2: AIYPGSGDTSYNQKFKG (SEQ ID NO: 23), HCDR3: STIITTGAVDY (SEQ ID NO: 24), LCDR1: KASQDVGTAVA (SEQ ID NO: 25), LCDR2: WTSTRHT (SEQ ID NO: 26), and LCDR3: QQYSRIPLT (SEQ ID NO: 27); and (d) HCDR1: EYIIH (SEQ ID NO: 28), HCDR2: GIIPNNDVTNYKQNFRG (SEQ ID NO: 29), HCDR3: WRNAYYSAMDS (SEQ ID NO: 30), LCDR1: TASQDVGTAVA (SEQ ID NO: 31), LCDR2: WASTRHT (SEQ ID NO: 32), and LCDR3: QQYRTSPLT (SEQ ID NO: 33).

[0014] In some embodiments, the first antibody moiety comprises: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 1, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 2; (b) a heavy chain variable

region comprising the amino acid sequence of SEQ ID NO: 3, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 3, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 4, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 4; (c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 5, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 6, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 6; or (d) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 7, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8, or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 8.

[0015] Also provided, in some embodiments, are polynucleotides encoding the antibody or fragment, and compositions comprising the antibody or fragment thereof and a pharmaceutically acceptable carrier.

[0016] Methods and uses for the treatment of diseases and conditions are also provided. In one embodiment, provided is a method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 shows the schematic of anti-SLC34A2-4-1BB bispecific antibody.

[0018] FIG. 2 shows that all the bispecific antibodies bound to human 4-1BB overexpressing CHO-K1 cells in a concentration-dependent manner.

[0019] FIG. 3 shows that all the bispecific antibodies bound to SLC34A2 overexpressing HEK293 cells in a concentration-dependent manner.

[0020] FIG. 4 shows that all the bispecific antibodies activated SLC34A2-dependent 4-1BB signaling in a concentration dependent manner in the presence of HEK293 with SLC34A2 overexpression.

[0021] FIG. 5 shows the lead bispecific antibody 30H9-263-1-3-1 exhibited concentration-dependent binding activity to human and cynomolgus 4-1BB while had no cross-reactivity to mouse 4-1BB.

[0022] FIG. 6 shows the lead bispecific antibody 30H9-263-1-3-1 exhibited similar binding activity to SLC34A2 from different species including human, cynomolgus, rhesus, rat and mouse.

[0023] FIG. 7 shows that bispecific antibody 30H9-263-1-3-1 efficiently bound to OVCAR3 and HCC4006 with endogenous SLC34A2 expression.

[0024] FIG. 8 shows that 30H9-263-1-3-1 induced significant 4-1BB activation in the presence of target cells with SLC34A2 expression.

[0025] FIG. 9 shows that the bispecific antibody 30H9-263-1-3-1 induced significant IL-2 and IFN-γ production by primary PBMCs in the presence of target cells with SLC34A2 expression.

[0026] FIG.10 shows that the bispecific antibody 30H9-263-1-3-1 exhibited strong anti-tumor efficacy in human 4-1BB transgenic mice tumor model.

DETAILED DESCRIPTION

Definitions

[0027] 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.

[0028] 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.

[0029] "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.

[0030] 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.

[0031] 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.

[0032] 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.

[0033] The expression "single domain antibodies" (sdAbs) or "single variable domain (SVD) antibodies" generally refers to antibodies in which a single variable domain (VH or VL) can confer antigen binding. In other words, the single variable domain does not need to interact with another variable domain in order to recognize the target antigen. Examples of single domain antibodies include those derived from camelids (lamas and camels) and cartilaginous fish (e.g., nurse sharks) and those derived from recombinant methods from humans and mouse antibodies (Nature (1989) 341:544-546; Dev Comp Immunol (2006) 30:43-56; Trend Biochem Sci (2001) 26:230-235; Trends Biotechnol (2003):21:484-490; WO 2005/035572; WO 03/035694; Febs Lett (1994) 339:285-290; WO00/29004; WO 02/051870). When the sdAb contains only a heavy chain, it can be exchangable used with "VHH" or "single heavy chain variable domain antibody" or "nanobody".

[0034] 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.

[0035] A "Fab" with regard to an antibody refers to a monovalent antigen-binding fragment of the antibody consisting of a single light chain (both variable and constant regions) bound to the variable region and first constant region of a single heavy chain by a disulfide bond. Fab can be obtained by papain digestion of an antibody at the residues proximal to the N-terminus of the disulfide bond between the heavy chains of the hinge region.

[0036] A "Fab'" refers to a Fab fragment that includes a portion of the hinge region, which can be obtained by pepsin digestion of an antibody at the residues proximal to the C-terminus of the disulfide bond between the heavy chains of the hinge region and thus is different from Fab in a small number of residues (including one or more cysteines) in the hinge region.

[0037] A "$F(ab)_2$" refers to a dimer of Fab' that comprises two light chains and part of the two heavy chains.

[0038] 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.

[0039] A full-length antibody comprises two heavy chains and two light chains. The variable regions of the light and heavy chains are responsible for antigen binding. The variable domains of the heavy chain and light chain may be referred to as "VH" and "VL", respectively. The variable regions in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain (LC) CDRs including LC-CDR1, LC-CDR2, and LC-CDR3, heavy chain (HC) CDRs including HC-CDR1, HC-CDR2, and HC-CDR3). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined or identified by the conventions of Kabat, Chothia, or Al-Lazikani (Al-Lazikani 1997; Chothia 1985; Chothia 1987; Chothia 1989; Kabat 1987; Kabat 1991). The three CDRs of the heavy or light chains are interposed between flanking stretches known as framework regions (FRs), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable loops. The constant regions of the heavy and light chains are not involved in antigen binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequence of the constant region of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of α, δ, ε, γ, and μ heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as lgG1 (γ1 heavy chain), lgG2 (γ2 heavy chain), lgG3 (γ3 heavy chain), lgG4 (γ4 heavy chain), lgA1 (α1 heavy chain), or lgA2 (α2 heavy chain).

[0040] 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.

[0041] Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti- idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to LIGHT antibodies disclosed herein). Immunoglobulin or antibody molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgGl, IgG2, IgG3, IgG4, IgAl and IgA2) or subclass of immunoglobulin molecule.

[0042] 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.

[0043] 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.

[0044] 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).

[0045] 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)).

[0046] 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

[0047] 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).

[0048] 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.

[0049] Antibodies disclosed herein may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be condricthoid in origin (*e.g.*, from sharks).

[0050] 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.

[0051] The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a polypeptide may comprise a CH1 domain derived from an IgG_1 molecule and a hinge region derived from an IgG_3 molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG_1 molecule and, in part, from an IgG_3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG_1 molecule and, in part, from an IgG_4 molecule.

[0052] 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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)).

[0057] The term "Fc region", "Fc domain" or "fragment crystallizable region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the

carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies described herein include human IgG1, IgG2 (IgG2A, IgG2B), IgG3 and IgG4.

[0058] 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).

[0059] 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.

[0060] "Humanized antibody" is used herein to describe an antibody that comprises heavy and light chain variable region sequences from a non-human species (e.g. a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like," i.e., more similar to human germline variable sequences. A "humanized antibody" is an antibody or a variant, derivative, analog, or fragment thereof, which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having the amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human

immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In an embodiment, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

[0061] The term "epitope" as used herein refers to the specific group of atoms or amino acids on an antigen to which an antibody or antibody moiety binds. Two antibodies or antibody moieties may bind the same epitope within an antigen if they exhibit competitive binding for the antigen.

[0062] 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.

[0063] 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."

[0064] 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.

[0065] 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.

[0066] 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-SLC34A2 Antibody Moiety

[0067] The present disclosure provides multi-specific antibodies containing one or more antigen-binding moieties that have binding specificity to the human SLC34A2 protein. The clones, 30H9, 62G10, 63A11 and 91C9 were selected for construction of the multi-specific antibodies.

[0068] In accordance with one embodiment of the present disclosure, provided are antigen-binding moieties that include the heavy chain and light chain variable domains with the CDR regions. The CDRs are summarized in **Table A1** below (Kabat numbering).

Table A1. CDR sequences of the anti-SLC34A2 antibody moieties

Antibody chain	Sequences		SEQ ID NO:
30H9-VH	CDR1	TNNMH	10
	CDR2	AIYPGSGATAYNQKFKG	11
	CDR3	GMYGHGAMDY	12
30H9-VL	CDR1	KASQDVGTAVA	13
	CDR2	WATTRHS	14
	CDR3	QQYSSNPLT	15
62G10-VH	CDR1	SYITH	16

	CDR2	AIYPGNADTSYIQKFKG	17
	CDR3	GTYGTSAWFTY	18
62G10-VL	CDR1	RARQNIGNNLY	19
	CDR2	YASQSIS	20
	CDR3	QQSFSWPLT	21
63A11-VH	CDR1	TYNMH	22
	CDR2	AIYPGSGDTSYNQKFKG	23
	CDR3	STIITTGAVDY	24
63A11-VL	CDR1	KASQDVGTAVA	25
	CDR2	WTSTRHT	26
	CDR3	QQYSRIPLT	27
91C9-VH	CDR1	EYIIH	28
	CDR2	GIIPNNDVTNYKQNFRG	29
	CDR3	WRNAYYSAMDS	30
91C9-VL	CDR1	TASQDVGTAVA	31
	CDR2	WASTRHT	32
	CDR3	QQYRTSPLT	33

[0069] The variable regions are summarized in **Table A2** below.

Table A2. Variable region sequences of the anti-SLC34A2 antigen-binding moieties

Antibody	Chain	Sequence	SEQ ID NO:
30H9	**VH**	QVQLVQSGAEVKKPGASVKVSCKTSGYTFTTNNMHWVRQAPGQG LEWIGAIYPGSGATAYNQKFKGRVTMTADTSTSTVYMELSSLRS EDTAVYYCARGMYGHGAMDYWGQGTLVTVSS	1
	VL	DIQLTQSPSFLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAP KLLIYWATTRHSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYC QQYSSNPLTFGQGTKLEIK	2
62G10	**VH**	QVQLVQSGAEVVKPGASVKMSCKASGYTFPSYITHWVKQAPGQG LEWIGAIYPGNADTSYIQKFKGRATLTADKSTSTAYMELSSLRS EDTAVYYCARGTYGTSAWFTYWGQGTLVTVSS	3
	VL	EIVLTQSPATLSVSPGERATLSCRARQNIGNNLYWYQQKPGQSP RLLIKYASQSISGIPSRFSGSGSGTDFTLTISSLQSEDFAVYFC QQSFSWPLTFGQGTKLEIK	4
63A11	**VH**	QVQLVQSGAEVVKPGASVKMSCKTSGYTFITYNMHWVRQAPGQG LEWIGAIYPGSGDTSYNQKFKGRVTLTADKSTSTAYMELSSLRS EDTAVYYCSISTIITTGAVDYWGQGTLVTVSS	5
	VL	DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKVP KLLIYWTSTRHTGVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QQYSRIPLTFGGGTKVEIK	6

91C9F1	**VH**	QVQLVQSGAEVKKPGSSVKVSCKTSGFFFTEYIIHWVRQAPGQG LEWIGGIIPNNDVTNYKQNFRGRVTLTADKSTSTAYMELSSLRS EDTAVYYCARWRNAYYSAMDSWGQGTTVTVSS	7
	VL	DIQVTQSPSSLSTSVGDRVTITCTASQDVGTAVAWYQQKPGKSP KLLIYWASTRHTGVPERFTGSGSGTDFTLTISSVQPEDVATYFC QQYRTSPLTFGVGTKVEIK	8

[0070] In some embodiments, the VH CDR1, CDR2, and CDR3 are selected from any set of VH CDR1, CDR2, and CDR3 shown in Table 1, and the VL CDR1, CDR2, and CDR3 are selected from any set of VL CDR1, CDR2, and CDR3 shown in Table 1. In some embodiments, the VH CDR1, CDR2, and CDR3 and the VL CDR1, CDR2, and CDR3 are selected from those derived from the same antigen-binding moieties.

[0071] In some embodiments, at least one, or two, or three, or four, or five, or six of the VH CDR1, CDR2, and CDR3 and the VL CDR1, CDR2, and CDR3 of the above are modified by one, two or three amino acid additions, deletions, substitutions, or the combinations thereof.

[0072] According to specific embodiments, the antibody are a humanized. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., Nature, 321: 522-525 (1986); Riechmann et al., Nature, 332: 323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2: 593-596 (1992)).

[0073] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321: 522-525 (1986); Riechmann et al., Nature 332: 323-327 (1988); Verhoeyen et al., Science, 239: 1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0074] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1, or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 1.

[0075] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a light chain variable region comprising the amino acid sequence of SEQ ID NO: 3 or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 3.

[0076] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 5.

[0077] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7 or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 7.

[0078] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a light chain variable region comprising the amino acid sequence of

SEQ ID NO: 2 or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 2.

[0079] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a light chain variable region comprising the amino acid sequence of SEQ ID NO: 4 or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 4.

[0080] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a light chain variable region comprising the amino acid sequence of SEQ ID NO: 6 or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 6.

[0081] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8 or a peptide having at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 8.

[0082] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 2.

[0083] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 4.

[0084] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 6.

[0085] In certain embodiments, the antibodies and the antigen-binding fragment thereof are humanized and comprise a heavy chain variable region comprising the amino acid sequence of

SEQ ID NO: 7, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

Anti-4-1BB Antibody Moieties

[0086] The present disclosure provides multi-specific antibodies containing one or more antigen-binding moieties that have binding specificity to the human 4-1BB protein.

[0087] In accordance with one embodiment of the present disclosure, provided are antigen-binding moieties that include a single domain antibody (sdAb) with the CDR regions. The CDRs are summarized in **Table B1** below (Kabat numbering). Exemplary single heavy chain variable domain are shown in **Table B2**.

Table B1. CDR sequences of the anti-4-1BB antibody moieties

Antibody moiety	Sequences		SEQ ID NO:
263-1-3-1	CDR1	SSAMS	34
	CDR2	GIYSGGSTYYTESVKD	35
	CDR3	WGTLRFGVWAEYDH	36

Table B2. Exemplary single heavy chain variable domain of the anti-4-1BB antibody moiety

Antibody	Chain	Sequence	SEQ ID NO:
263-1-3-1	sdAb	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSAMSWARQAPGKGFEWVSGIYSGGSTYYTESVKDRFTISRDNSKNTVYLQMNSLKPEDTAVYYCATWGTLRFGVWAEYDHWGQGTQVTVSS	9

[0088] It is appreciated that CDRs can be modified to include those having one, two or three amino acid addition, deletion and/or substitutions. In some embodiments, the substitutions can be conservative substitutions.

[0089] In one embodiments, the HCDR1 includes the amino acid sequence of SEQ ID NO: 34, the HCDR2 includes the amino acid sequence of SEQ ID NO: 35, the HCDR3 includes the amino acid sequence of SEQ ID NO: 36. In some embodiments, the VHH/sdAb comprises the amino acid sequence of SEQ ID NO: 9 or a peptide having at least 90% (or at least 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of SEQ ID NO: 9.

[0090] The CDRs, heavy chain variable regions, light chain variable regions or single heavy chain variable domains of the present disclosure can be further modified. In some embodiments, the modified heavy chain variable region, light chain variable region or single heavy chain variable domain retains at least about 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity and is still capable of binding to the target site.

[0091] In some embodiments, the modification is substitution at no more than one hot spot position from each of the CDRs. In some embodiments, the modification is substitution at one, two or three such hot spot positions. In one embodiment, the modification is substitution at one of the hot spot positions. Such substitutions, in some embodiments, are conservative substitutions.

[0092] 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.

[0093] 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 C1. 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 C2. 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

[0094] 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.

Multi-specific antibodies

[0095] An antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. The term "multispecific antibody" as used herein refers to an antibody comprising an antigen-binding domain that has polyepitopic specificity (i.e., is capable of binding to two, or more, different epitopes on one molecule or is capable of binding to epitopes on two, or more, different molecules). In some embodiments, multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigen binding sites (such as a bispecific antibody). In some embodiments, the first antibody domain and the second antibody domain of the multispecific antibody may bind epitopes located within two distinct molecules (intermolecular binding).

[0096] In certain embodiments, a multispecific antibody provided herein may be a bispecific antibody. The term "bispecific antibody" as used herein refers to a multispecific antibody comprising an antibody domain that is capable of binding to two different epitopes on one molecule or is capable of binding to epitopes on two different molecules. A bispecific antibody may also be referred to herein as having "dual specificity" or as being "dual specific." Exemplary bispecific antibodies may bind both SLC34A2 and any other antigen. In certain embodiments, one of the binding specificities is for SLC34A2 and the other is for 4-1BB. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express SLC34A2.

[0097] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, Nature 305: 537 (1983), WO 93/08829, and Traunecker et al, EMBO J. 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731, 168, WO2009/089004, US2009/0182127, US2011/0287009, Marvin and

Zhu, Acta Pharmacol. Sin. (2005) 26(6):649-658, and Kontermann (2005) Acta Pharmacol. Sin., 26: 1-9).

[0098] The present disclosure provides the anti-SLC34A2 antigen-binding moieties and the anti-4-1BB antigen-binding moieties as bispecific or multi-specific antibodies. The bispecific antibodies provided herein show comparable binding activity to human SLC34A2 protein and human 4-1BB protein, respectively, as compared with the monospecific anti-SLC34A2 (**FIG. 3**) and anti-4-1BB antibodies (**FIG. 2**), and SLC34A2-dependent 4-1BB signaling (**FIG. 4**). The bispecific antibodies provided herein also show specific affinity to monkey 4-1BB protein, but not to mouse 4-1BB protein (**FIG. 5**), while exhibiting specific affinity to monkey, rat and mouse SLC34A2 protein (**FIG. 6**). In addition, the 4-1BB pathway activation (**FIG. 8**) and T cell activation (**FIG. 9**) can be induced by targeting the SLC34A2 antigen simultaneously using the bispecific antibodies, which also showed potent *in vivo* anti-tumor effects (**FIG. 10**).

[0099] In certain embodiments, the anti-SLC34A2 antibody moiety provided herein has a format of full-length antibody/IgG format, $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv or sdAb/VHH/nanobody.

[0100] In certain embodiments, the anti-4-1BB antibody moiety provided herein has a format of full-length antibody/IgG format, $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv or sdAb/VHH/nanobody.

[0101] In certain embodiments, the anti-SLC34A2 antibody moiety provided herein has a Fab' format and the anti-4-1BB antibody moiety provided herein has a single domain antibody (sdAb).

[0102] In certain embodiments, the bispecific antibody provided herein further comprises a Fc domain. In certain embodiments, the anti-SLC34A2 antibody moiety is fused to the N-terminus of the Fc domain, and the anti-4-1BB antibody moiety is fused to the N-terminus of the anti-SLC34A2 antibody moiety. In certain embodiments, the anti-SLC34A2 antibody moiety is fused to the N-terminus of the Fc domain, and the anti-4-1BB antibody moiety is fused to the C-terminus of the Fc domain.

[0103] The Fc region can be engineered to enhance or eliminate effector function. IgG antibodies can induce direct anti-tumor effects by way of indirect anti-tumor effects via the Fc-mediated effector functions that engage other immune cells or killer mechanisms. "Effector

functions" or "antibody effector functions" as used herein refer to biological activities attributable to the binding of Fc region of an antibody to its effectors such as C1 complex and Fc receptor (FcγRIIa or FcγRIIIa). Exemplary effector functions include: complement dependent cytotoxicity (CDC) induced by interaction of antibodies and C1q on the C1 complex; antibody-dependent cell-mediated cytotoxicity (ADCC) induced by binding of Fc region of an antibody to Fc receptor on an effector cell; and antibody dependent cell mediated phagocytosis (ADCP) , where nonspecific cytotoxic cells that express Fcγ receptors (FcγRs) recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[0104] Among the four IgG subclasses, IgG1 and IgG3 induce the strongest Fc-effector functions. However, since IgG1 has the longest half-life and is more stable than IgG3, most therapeutic antibodies with Fc-mediated functions are of IgG1 isotype.

[0105] IgG2 and IgG4 isotypes have significantly lower binding affinity to FcγRs. Recent evidence suggests that the IgG2 isotype is not completely devoid of effector function, whereas the IgG4 isotype can undergo in vivo Fab arm exchange leading to bispecific antibody and off-target effects.

[0106] In certain embodiments, the antibody or antigen-binding fragment thereof provided herein is of an isotype of IgG1, IgG2, IgG3 or IgG4. In certain embodiments, the antibody or antigen-binding fragment thereof is of an isotype of IgG1. In certain embodiments, the antibody or antigen-binding fragment thereof is of human IgG1. In certain embodiments, the antibody or antigen-binding fragment thereof is modified IgG1 that have reduced or eliminated effector function.

[0107] In certain embodiments, the modified IgG1 comprises an N297A modification. In certain embodiments, the modified IgG1 comprises the amino acid sequence of SEQ ID NO: 37, or a peptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 37 (**Table D**).

Table D. Sequence of IgG1 N297A Fc domain

Name	Sequence	SEQ ID NO:
IgG1 N297A	CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQY**A**STYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSP	37

[0108] In certain embodiments, the anti-4-1BB antibody moiety is fused to the Fc domain via a linker.

[0109] Linkers within the scope of the present disclosure are characterized in terms of amino acid content, length, rigidity and secondary structure. Linkers within the scope of the present disclosure separate a function polypeptide and another functional polypeptide and allow proper folding and functioning of each domain. In this manner, a linker can be tailored to the particular functional polypeptide and the other functional polypeptide. According to one aspect, functional independence of the structural and fused (heterologous) domains is maximized by a suitable linker to limit steric interference between domains during the export and assembly processes of the bacterial cell. According to one aspect, longer and more flexible linkers of the type (GGGGS)n (SEQ ID NO: 38), (GGGS)n (SEQ ID NO: 39), or (GGS)n, wherein n is an integer from 1 to 20 are exemplary. According to an additional aspect, cell stress is minimized by limiting the overall length of the fusion protein. Longer linker sequences and higher induction levels stress the biosynthetic machinery of the cells, inhibiting cell growth and leading to cell lysis in extreme cases.

[0110] Linkers within the scope of the present disclosure facilitate functioning of the antibody domains. Linkers within the scope of the present disclosure allow efficient protein processing and export through the bacterial curli secretion machinery as well as provide the proper spatial and physicochemical separation of the respective antibody domains to retain their respective functions.

[0111] Linkers within the scope of the present disclosure include amino acid residues. The amino acid residues may be any of the naturally occurring amino acid residues. Amino acid residues may also be synthetic amino acids known to those of skill in the art. Representative amino acids which may be used in linkers include Glycine, Alanine, Valine, Leucine, Isoleucine, Serine, Cysteine, Selenocysteine, Threonine, Methionine, Proline, Phenylalanine, Tyrosine, Tryptophan, Histidine, Lysine, Arginine, Aspartate, Glutamate, Asparagine, and Glutamine.

[0112] According to one aspect, the linker length can be any length which may be expressed from a cell, such as a bacterial cell when linking the antibody domains. According to one aspect, a linker sequence is a polypeptide sequence of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 24, 48 or more amino acids.

[0113] In certain embodiments, the antibody comprises the 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).

[0114] Antibodies, variants, or derivatives thereof of the disclosure include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the epitope. For example, but not by way of limitation, the antibodies can be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

[0115] The antibodies can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antigen-binding polypeptide is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0116] The antibodies can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA). Techniques for conjugating various moieties to an antibody are well known, *see, e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. (1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.*, (eds.), Marcel Dekker, Inc., pp. 623-53 (1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of

Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), and Academic Press pp. 303-16 (1985).

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

[0117] 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.

[0118] 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

[0119] As described herein, the antibodies, variants or derivatives of the present disclosure may be used in certain treatment and diagnostic methods.

[0120] 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).

[0121] The antibodies of the disclosure can also be used to treat or inhibit cancer. In some embodiments, the cancer cells in the patient express or overexpress SLC34A2. As provided above, SLC34A2 can be overexpressed in tumor cells, in particular gastric, pancreatic, esophageal, ovarian, and lung tumors. Inhibition of SLC34A2 has been shown to be useful for treating the tumors.

[0122] Accordingly, in some embodiments, provided are methods for treating a cancer in a patient in need thereof. The method, in one embodiment, entails administering to the patient an effective amount of an antibody of the present disclosure. In some embodiments, at least one of the cancer cells (e.g., stromal cells) in the patient over-express SLC34A2.

[0123] 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.

[0124] Non-limiting examples of cancers include bladder cancer, liver cancer, colon cancer, rectal cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.

[0125] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the antibodies or variants, or derivatives thereof of the disclosure include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (*e.g.*, acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (*e.g.*, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma,

adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma.

[0126] 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.

[0127] Methods of administration of the antibodies, variants or include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray.

[0128] The term “parenteral” as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0129] 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.

[0130] 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.

[0131] The amount of the antibodies of the disclosure which will be effective in the treatment, inhibition and prevention of an 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.

[0132] 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.

[0133] 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.

[0134] In additional embodiments, the compositions of the disclosure are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Compositions

[0135] 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).

[0136] 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.

[0137] 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.

[0138] 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.

[0139] The antibodies 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 Anti-SLC34A2-4-1BB Bispecific Antibody

[0140] Anti-SLC34A2-4-1BB bispecific antibodies were designed in a tetravalent IgG (H)-VHH fusion format, in which the Fc domain was an IgG1 backbone with N297A mutation to disable Fcγ function. The anti-SLC34A2 portion constituted a full IgG unit, while the anti-4-1BB was a VHH placed at the C-terminal side of the Fc fragment via a G4S linker. The format of bispecific antibody is shown in **FIG. 1**. The sequences of previous identified anti-SLC34A2 arm and anti-4-1BB nanobody used to construct bispecific antibody are shown in **Table 1**.

Table 1. VH/VL sequences of the lead bispecific antibodies

Sequences of Example anti-SLC34A2 Unit		SEQ ID NO:
30-H9	**VH:** QVQLVQSGAEVKKPGASVKVSCKTSGYTFTTNNMHWVRQAPGQGLEWIGAIYPGSGATAYN QKFKGRVTMTADTSTSTVYMELSSLRSEDTAVYYCARGMYGHGAMDYWGQGTLVTVSS	1
	VL: DIQLTQSPSFLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAPKLLIYWATTRHSGVPSR FSGSGSGTEFTLTISSLQPEDFATYYCQQYSSNPLTFGQGTKLEIK	2
62-G10	**VH:** QVQLVQSGAEVVKPGASVKMSCKASGYTFPSYITHWVKQAPGQGLEWIGAIYPGNADTSYI QKFKGRATLTADKSTSTAYMELSSLRSEDTAVYYCARGTYGTSAWFTYWGQGTLVTVSS	3
	VL: EIVLTQSPATLSVSPGERATLSCRARQNIGNNLYWYQQKPGQSPRLLIKYASQSISGIPSR FSGSGSGTDFTLTISSLQSEDFAVYFCQQSFSWPLTFGQGTKLEIK	4
63-A11	**VH:** QVQLVQSGAEVVKPGASVKMSCKTSGYTFITYNMHWVRQAPGQGLEWIGAIYPGSGDTSYN QKFKGRVTLTADKSTSTAYMELSSLRSEDTAVYYCSISTIIITGAVDYWGQGTLVTVSS	5
	VL: DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKVPKLLIYWTSTRHTGVPSR FSGSGSGTDFTLTISSLQPEDVATYYCQQYSRIPLTFGGGTKVEIK	6
91-C9F1	**VH:** QVQLVQSGAEVKKPGSSVKVSCKTSGFFFTEYIIHWVRQAPGQGLEWIGGIIPNNDVTNYK QNFRGRVTLTADKSTSTAYMELSSLRSEDTAVYYCARWRNAYYSAMDSWGQGTTVTVSS	7
	VL: DIQVTQSPSSLSTSVGDRVTITCTASQDVGTAVAWYQQKPGKSPKLLIYWASTRHTGVPER FTGSGSGTDFTLTISSVQPEDVATYFCQQYRTSPLTFGVGTKVEIK	8
Sequences of Example anti-4-1BB Unit		
263-1-3-1	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSSAMSWARQAPGKGFEWVSGIYSGGSTYYTE SVKDRFTISRDNSKNTVYLQMNSLKPEDTAVYYCATWGTLRFGVWAEYDHWGQGTQVTVSS	9

[0141] The resulting bispecific antibodies were produced transiently in CHO-K1 cells and purified by protein A affinity chromatography. The well qualified bispecific antibodies were applied to *in vitro* characterization including cell-based 4-1BB binding, SLC34A2 binding and SLC34A2-dependent 4-1BB activation reporter assay.

Cell-based 4-1BB binding

[0142] To evaluate 4-1BB binding activity of bispecific antibodies, CHO-K1 cells-overexpressing human 4-1BB were incubated with different concentrations of anti-SLC34A2-4-1BB bispecific antibodies at 4 °C for 30 minutes. Then, the cells were washed twice with FACS buffer and stained with PE conjugated secondary antibody at 4 °C for 30 minutes. After washing twice with FACS buffer, MFI of PE was analyzed by a NovoCyte flow cytometer.

[0143] As shown in **FIG. 2 and Table 2**, all the bispecific antibodies bound to human 4-1BB-overexpressing CHO-K1 cells in a concentration-dependent manner, which was comparable to the parental anti-41BB antibody 263-1-3-1.

Table 2. Binding affinity to 4-1BB overexpressing CHO cell

Antibody	EC50 (nM)	Top (MFI)
30H9-263-1-3-1	1.04	50942
62G10-263-1-3-1	1.06	51725
63A11-263-1-3-1	1.34	49440
91C9-263-1-3-1	1.69	52852
263-huNb 1-3-1 sdAb	0.75	50125

Cell-based SLC34A2 binding

[0144] To evaluate SLC34A2 binding potency of bispecific antibodies, HEK293 cells overexpressing SLC34A2 were incubated with different concentrations of anti-SLC34A2-4-1BB bispecific antibodies at 4 °C for 30 minutes. Then, the cells were washed twice with FACS buffer and stained with PE conjugated secondary antibody at 4 °C for 30 minutes. After wash twice with FACS buffer, MFI of PE was analyzed by a NovoCyte flow cytometer.

[0145] As shown in **FIG. 3** and **Table 3**, all the bispecific antibodies bound to the engineered HEK293 cells in a concentration-dependent manner.

Table 3. Binding affinity to SLC34A2 overexpressing HEK293 cell

Antibody	EC50 (nM)	Top (MFI)
30H9-263-1-3-1	2.467	234948
62G10-263-1-3-1	2.87	229440
63A11-263-1-3-1	5.036	235037
91C9-263-1-3-1	4.533	258968
30-H9	0.7288	243225
62-G10	2.218	242152

63-A11	3.631	246215
91-C9F1	1.745	253151

SLC34A2-dependent 4-1BB activation

[0146] To evaluate the ability of anti-SLC34A2-4-1BB bispecific antibody to activate 4-1BB signal, a reporter gene assay was used. In this assay, Jurkat cells engineered to have surface 4-1BB expressing and an NF-κB luciferase reporter construct were used as effector cells. HEK293 cells overexpressing SLC34A2 were used as target cells. In brief, effector cells at a density of 1E5 cells per well were co-incubated with 1E4 target cells in the presence of 4-fold serially diluted anti-SLC34A2-4-1BB bispecific antibodies at 37 °C in 5% CO_2 incubator. After overnight incubation, luminescence was obtained by adding the substrate of luciferase and measured by a microplate reader.

[0147] As shown in **FIG. 4** and **Table 4**, all the bispecific antibodies activated SLC34A2-dependent 4-1BB signaling in the presence of the target cells.

Table 4. SLC34A2-dependent 4-1BB signaling

Antibody	EC50 (nM)	Top (RLU)
30H9-263-1-3-1	0.24	657050
62G10-263-1-3-1	0.50	1091761
63A11-263-1-3-1	0.42	759454
91C9-263-1-3-1	0.41	758376
263-huNb 1-3-1 sdAb	NA	NA

Example 2. Antigen binding of the lead anti-SLC34A2-4-1BB bispecific antibody

[0148] This example evaluated the binding activity of the lead bispecific antibody of 30H9-263-1-3-1 in **Example 1** to SLC34A2 and 4-1BB compared with reference antibodies, using cell-based assays.

3.1 Cell-based binding to 4-1BB

[0149] The anti-SLC34A2-4-1BB bispecific antibody 30H9-263-1-3-1 was tested for *in vitro* binding with different species derived 4-1BB expressed on the surface of CHO-K1 cell line with the parental anti-4-1BB antibody 263-1-3-1 as reference.

[0150] As shown in **FIGs. 5A-5B**, the bispecific antibody efficiently bound to human and cynomolgus/rhesus 4-1BB over-expressing CHO-K1 cells in a concentration-dependent

manner. The mean EC_{50} of the bispecific antibody was estimated to be 0.56 nM for human 4-1BB and 1.05 nM for cynomolgus/rhesus 4-1BB. Notably, all the tested antibodies showed no cross-reactivity to mouse 4-1BB and no non-specific binding activity to the blank CHO-K1 cells as shown in **FIGs. 5C-5D**.

3.2 Cell-based binding to SLC34A2

[0151] To evaluate the antigen binding property in a cell-based setting, 30H9-263-1-3-1 was analyzed for their binding to different species derived SLC34A2 overexpressed on HEK293 by flow cytometry as described above. The parental SLC34A2 monoclonal antibody 30H9 was included in parallel as reference. As shown in **FIGs. 6A-6F**, all the tested antibodies showed typical sigmoidal binding behavior against engineered HEK293 cells overexpressing human, cynomolgus, rhesus, rat and mouse SLC34A2, while showed no non-specific binding activity to the blank HEK-293 cells.

[0152] The binding activity to tumor cells with endogenous SLC34A2 expression was also evaluated by flow cytometry. As shown in **FIGs. 7A-7B**, 30H9-263-1-3-1 efficiently bound to OVCAR3 and HCC4006 in a concentration-dependent manner with EC50 of 1.04 nM and 1.49 nM, respectively.

Example 3. SLC34A2-dependent 4-1BB signal activation in 4-1BB reporter assay

[0153] This example used a classical reporter assay to investigate the ability of the anti-SLC34A2-4-1BB bispecific antibody 30H9-263-1-3-1 to activate 4-1BB signaling. In this assay, the engineered Jurkat cells which stably express 4-1BB and have an NK-κB luciferase reporter construct integrated into the genome were used as effector cells. HEK-293 cells engineered to overexpress SLC34A2 and tumor cell line OVCAR3 endogenously expressing SLC34A2 were used as target cells. Following 4-1BB activation, endogenous NF-κB transcription factors bind to the DNA response elements to induce transcription of the luciferase gene, whose protein product is then quantified by measuring the luminescence signal after adding the substrate. In this study, the effector cells and target cells were co-incubated overnight with different concentrations of 30H9-263-1-3-1 or reference antibodies at 37 °C in 5% CO2 incubator. Then, the substrate of luciferase was added, and the luminescence intensity was determined by a microplate reader. The parental anti-4-1BB antibody 263-1-3-1 were used as a control.

[0154] As shown in **FIGs. 8A-8C**, the bispecific antibody 30H9-263-1-3-1 induced significant 4-1BB activation in the presence of target cells with SLC34A2 expression. The EC50 of 30H9-263-1-3-1 was estimated to be 0.25 nM with human SLC34A2_HEK-293 as target cells and 0.11 nM with OVCAR3 as target cells. Notably, 30H9-263-1-3-1 failed to activate 4-1BB signaling in the presence of target cells that did not express SLC34A2, indicating that 30H9-263-1-3-1-mediated 4-1BB activation was dependent on SLC34A2 engagement. Briefly, the Rhesus SLC34A2 expressing HEK293 engineering cells (HEK293/ Rhesus _SLC34A2) were incubated with chimeric Abs (from 200 nM, 4 folds dilution, 8 points). After incubation at 4°C for 60 minutes, the cells were washed with FACS buffer twice, then stained with fluorescent conjugated secondary antibody at 4°C for 30 minutes. After that the cells were washed twice and analyzed by flow cytometry.

Example 4. SLC34A2-dependent primary T cell activation

[0155] To test the ability of anti-SLC34A2-4-1BB bispecific antibody 30H9-263-1-3-1 to activate human T cells, human PBMCs (4E5 cells per well) were co-cultured with HEK-293 cells overexpressing SLC34A2 (2E4 cells per well) or OVCAR3 cells in the presence of human anti-CD3 antibody (OKT3 at 0.25μg/mL) and serially diluted bispecific antibody. The level of IL-2 in the culture supernatant was determined by Human IL-2 ELISA KIT (CAT#Mabtech-3445-1H-20) after 24 hours incubation. Meanwhile, the level of IFN-γ was determined by Human IFN-γ ELISA KIT (CAT#Mabtech-3420-1H-20) after 48 hours incubation.

[0156] As shown in **FIGs. 9A-9D**, as compared with the parental 4-1BB antibody, SLC34A2-4-1BB bispecific antibody induced significant cytokines production by primary PBMCs in the presence of engineered HEK293 cells. The EC_{50} for 30H9-263-1-3-1 was estimated to be 0.55 nM for IL-2 and 0.36 nM for IFN-γ. Similar results were observed when PBMCs were co-cultured with OVCAR3 cells. The EC_{50} for 30H9-263-1-3-1 in the presence of OVCAR3 was estimated to be 0.24 nM for IL-2 and 0.14 nM for IFN-γ.

Example 5. Tumor growth inhibition by Anti-SLC34A2-4-1BB Bispecific Antibody (*in vivo*)

[0157] To evaluate anti-tumor efficacy of the anti-SLC34A2-4-1BB bispecific antibody 30H9-263-1-3-1, a syngeneic mouse tumor model using human 4-1BB transgenic mice inoculated with MC38 cells ectopically expressing human SLC34A2 was employed. Humanized-4-1BB

mice were subcutaneously implanted with 1×10^6 hSLC34A2-MC38 cells. When the average tumor volume grew to 56 mm^3, tumor-bearing mice were randomized into two groups (N=6/group) and were intraperitoneally administered vehicle (PBS) and 30H9-263-1-3-1, respectively. Antibody was given twice a week. Tumor volume was monitored by caliper measurement three times per week for the duration of the experiment.

[0158] As shown in **FIG. 10**, injection of 30H9-263-1-3-1 led to significant tumor inhibition and all the mice in the treated group were tumor free by around one month post treatment initiation. When rechallenged with a second dose of the same tumor cells, these tumor-free mice were resistant to tumor re-challenge and were deemed tumor free while the tumor cells continued to grow in naïve mice, suggesting that the anti-SLC34A2-4-1BB bispecific antibody 30H9-263-1-3-1 exhibited strong anti-tumor efficacy and induced long-term protective immunological memory.

* * *

[0159] 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.

[0160] 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. A multi-specific antibody comprising:

(1) a first antibody moiety specifically binds to a human Solute carrier 34 A2 (SLC34A2) protein, and

(2) a second antibody moiety specifically binds to a human 4-1BB protein.

2. The multi-specific antibody of claim 1, further comprising a Fc domain.

3. The multi-specific antibody of claim 1, wherein Fc is modified to reduce or eliminate effector function.

4. The multi-specific antibody of any one of claims 1-3, wherein the first antibody moiety or the second antibody moiety is a $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv or sdAb.

5. The multi-specific antibody of claim 4, wherein the first antibody moiety is Fab'.

6. The multi-specific antibody of any one of claims 1-5, wherein the second antibody moiety is a sdAb.

7. The multi-specific antibody of any one of the preceding claims, wherein the Fc domain is derived from any one selected from the group consisting of IgG1, IgG2, IgG3, and IgG4.

8. The multi-specific antibody of claim 7, wherein the Fc domain is derived from IgG1.

9. The multi-specific antibody of any one of the preceding claims, wherein the first antibody moiety is a Fab' fused to N-terminus of the Fc domain and the second antibody moiety is a sdAb fused to C-terminus of the Fc domain.

10. The multi-specific antibody of claim 9, wherein the second antibody moiety is fused to the Fc domain via a linker.

11. The multi-specific antibody of any one of claims 1-10, wherein the second antibody moiety comprises a HCDR1 comprising the amino acid of SSAMS (SEQ ID NO: 34), a HCDR2 comprising the amino acid of GIYSGGSTYYTESVKD (SEQ ID NO: 35), and a HCDR3 comprising the amino acid of WGTLRFGVWAEYDH (SEQ ID NO: 36).

12. The multi-specific antibody of claim 11, wherein the second antibody moiety comprises the amino acid sequence of SEQ ID NO: 9 or a peptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 9.

13. The multi-specific antibody of any one of the preceding claims, wherein the first antibody moiety comprises a heavy chain variable region (VH) comprising heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, and a light chain variable region (VL) comprising heavy chain complementarity determining regions LCDR1, LCDR2, and LCDR3, wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, respectively, comprise the amino acid sequences of:

(a) SEQ ID NO:10-15;

(b) SEQ ID NO:16-21;

(c) SEQ ID NO:22-27, or

(d) SEQ ID NO:28-33.

14. The multi-specific antibody of claim 13, wherein the VH and the VL of the first antibody moiety comprises, respectively, the amino acid sequences of:

(a) SEQ ID NO:1 and 2;

(b) SEQ ID NO:3 and 4;

(c) SEQ ID NO:5 and 6; or

(d) SEQ ID NO:7 and 8.

15. The multi-specific antibody of any one of claims 2-14, wherein the Fc domain comprises the amino acid sequence having at least 80% identity to SEQ ID NO: 37.

16. A composition comprising the multi-specific antibody of any one of claims 1-15, and a pharmaceutically acceptable carrier.

17. An isolated cell comprising one or more polynucleotide encoding the multi-specific antibody of any one of claims 1-15.

18. A polynucleotide encoding one or more chains of the multi-specific antibody of any one of claims 1-15.

19. A method of treating a cancer in a patient in need thereof, comprising administering to the patient the multi-specific antibody of any one of claims 1-15 or the composition of claim 16.

20. The method of claim 19, further comprising administering to the patient a therapy for treating said cancer.

21. The method of claim 20, wherein said therapy is selected from the group consisting of immunotherapy, chemotherapy and radiotherapy.

22. Use of the multi-specific antibody of any one of claims 1-15 or the composition of claim 16 in the manufacture of a medicament for treating a cancer in a patient in need thereof.

23. The multi-specific antibody of any one of claims 1-15 or the composition of claim 16 for use in the treatment of cancer in a patient in need thereof.

24. The method of any one of claims 19-21 or the use of claim 22 or the multi-specific antibody or composition for use of claim 23, wherein the cancer is selected from the group consisting of bladder cancer, liver cancer, colon cancer, rectal cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.

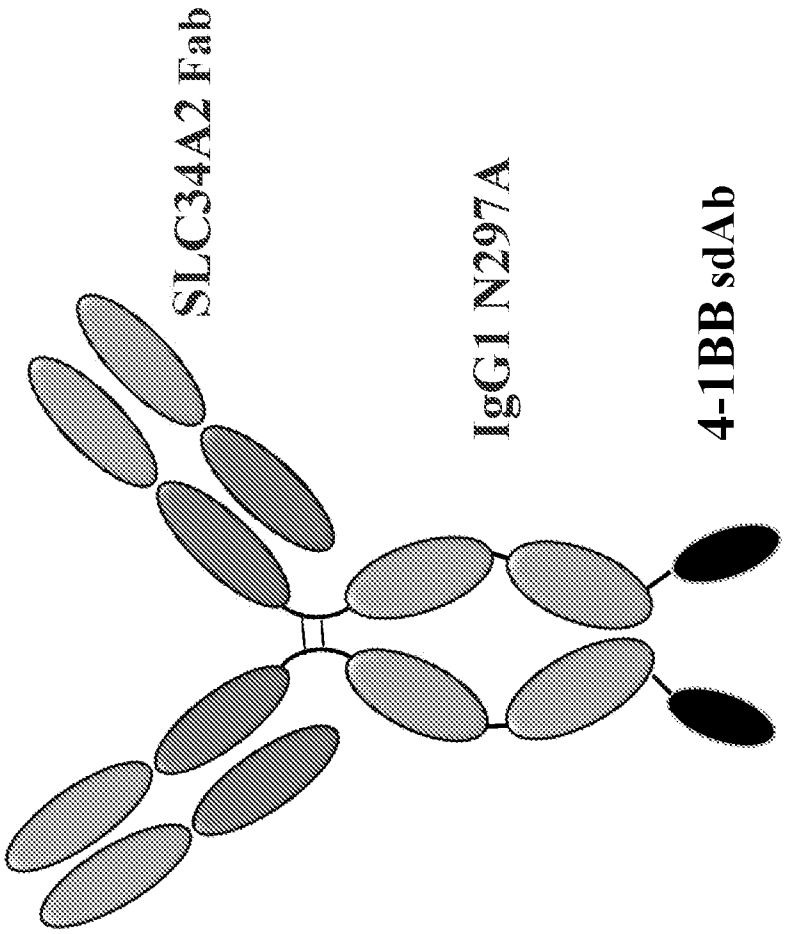


FIG. 1

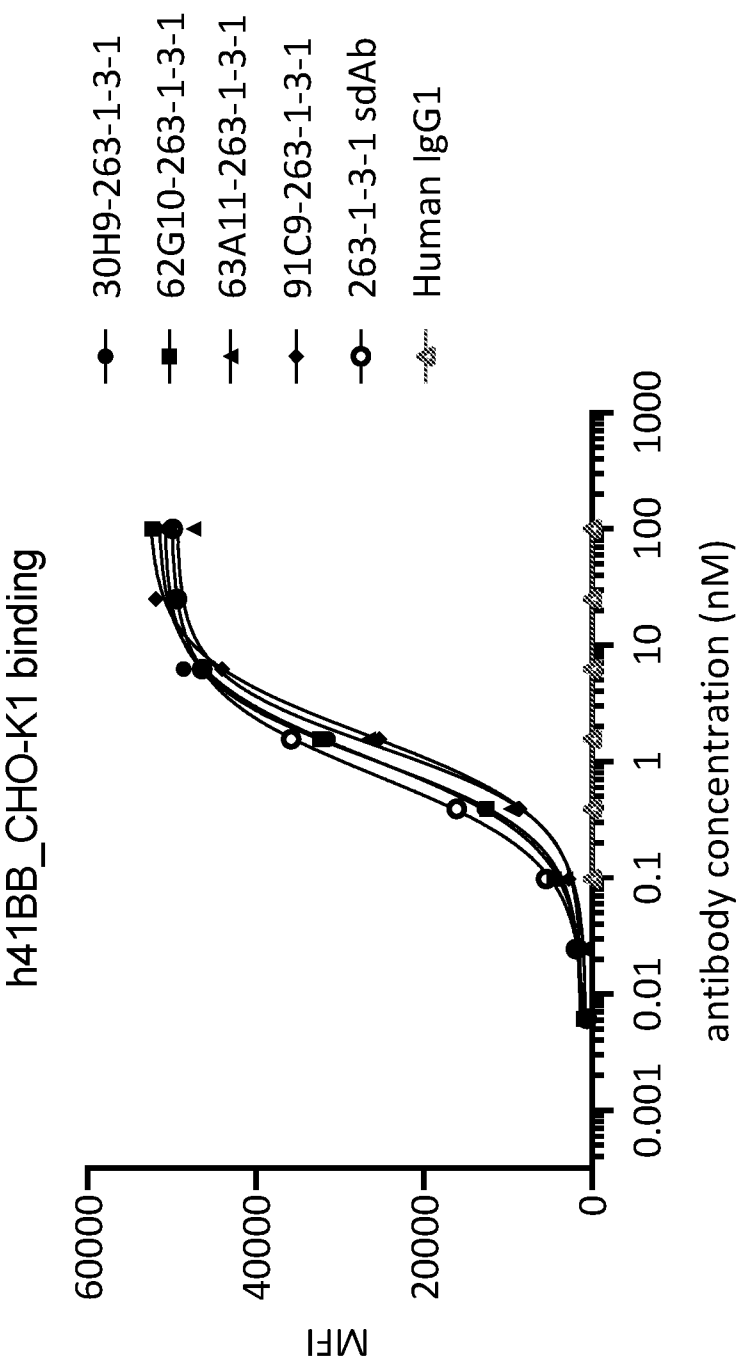

h41BB_CHO-K1 binding
30H9-263-1-3-1
62G10-263-1-3-1
63A11-263-1-3-1
91C9-263-1-3-1
263-1-3-1 sdAb
Human IgG1
MFI
60000
40000
20000
0
0.001
0.01
0.1
1
10
100
1000
antibody concentration (nM)

FIG. 2

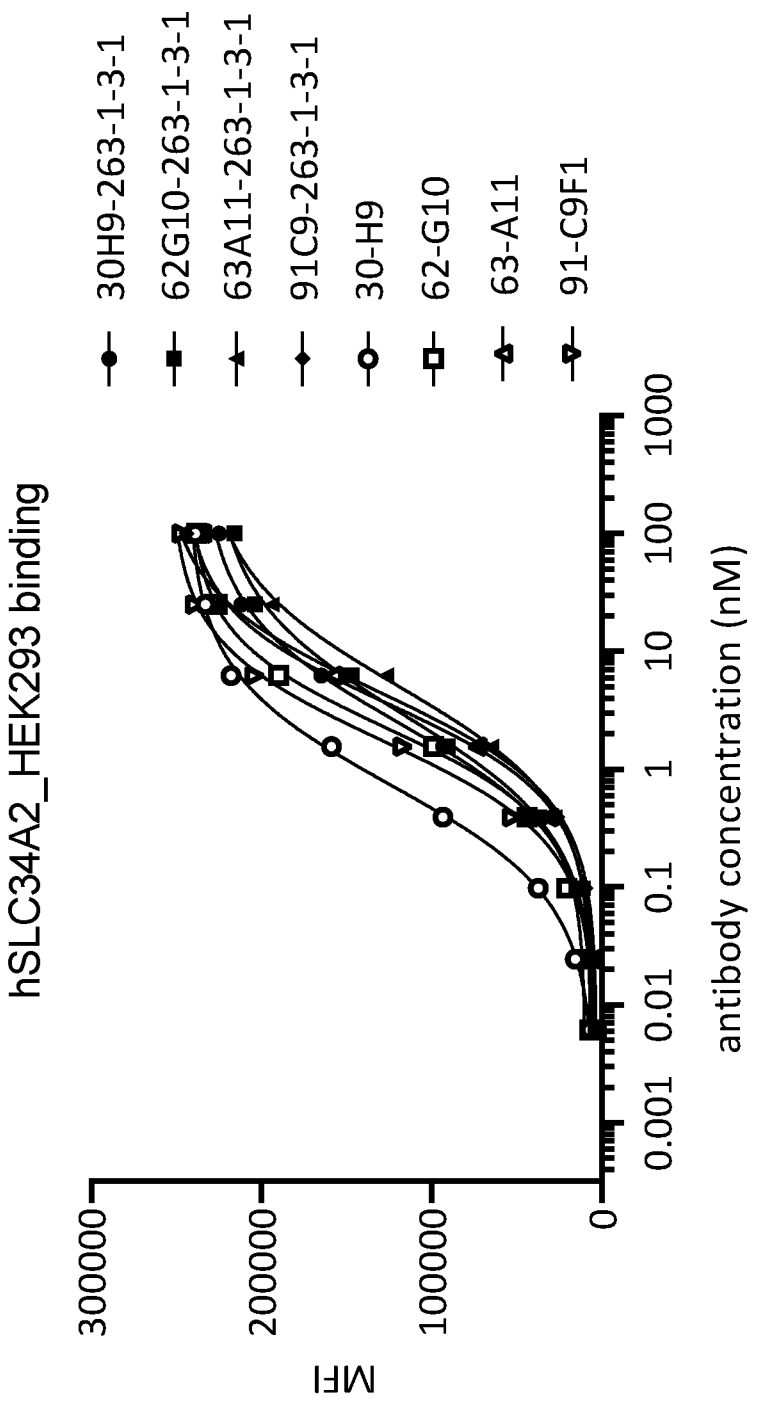

hSLC34A2_HEK293 binding
30H9-263-1-3-1
62G10-263-1-3-1
63A11-263-1-3-1
91C9-263-1-3-1
30-H9
62-G10
63-A11
91-C9F1
MFI
300000
200000
100000
0
0.001
0.01
0.1
1
10
100
1000
antibody concentration (nM)

FIG. 3

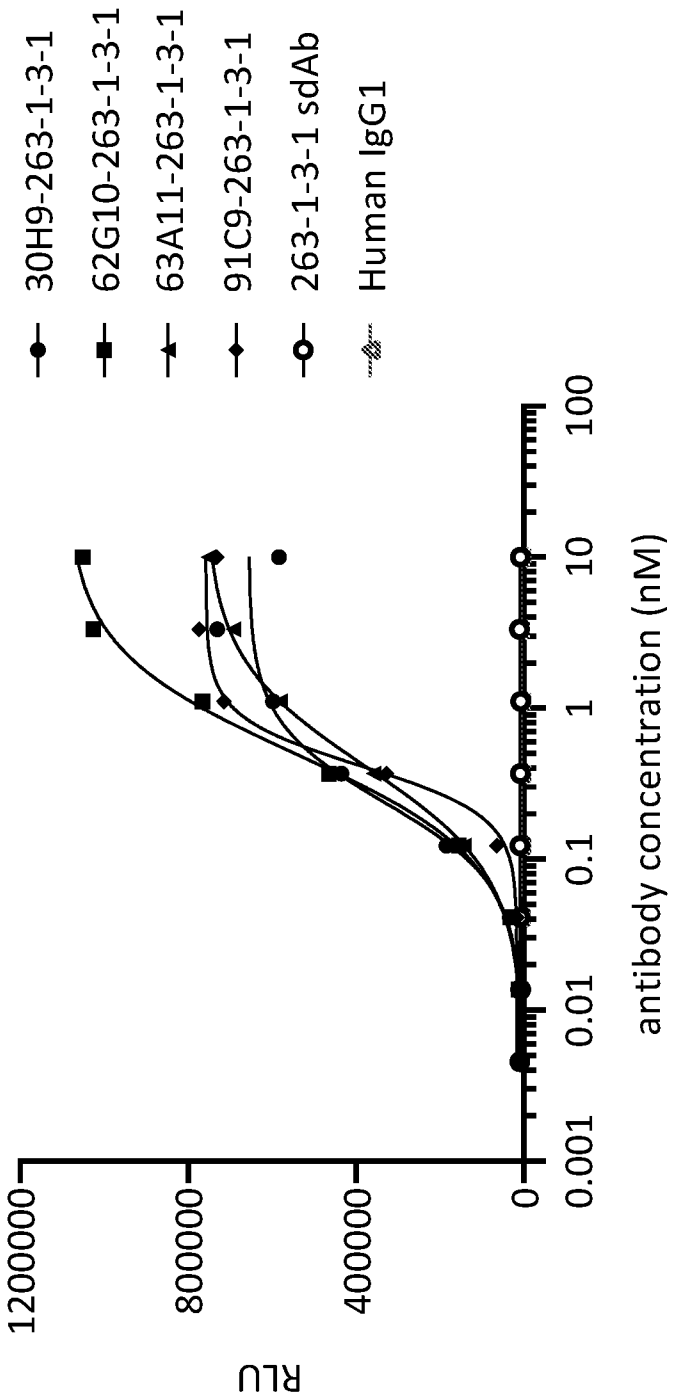

Human SLC34A2-HEK-293 + Human 4-1BB-Jurkat
30H9-263-1-3-1
62G10-263-1-3-1
63A11-263-1-3-1
91C9-263-1-3-1
263-1-3-1 sdAb
Human IgG1
RLU
1200000
800000
400000
0
0.001
0.01
0.1
1
10
100
antibody concentration (nM)

FIG. 4

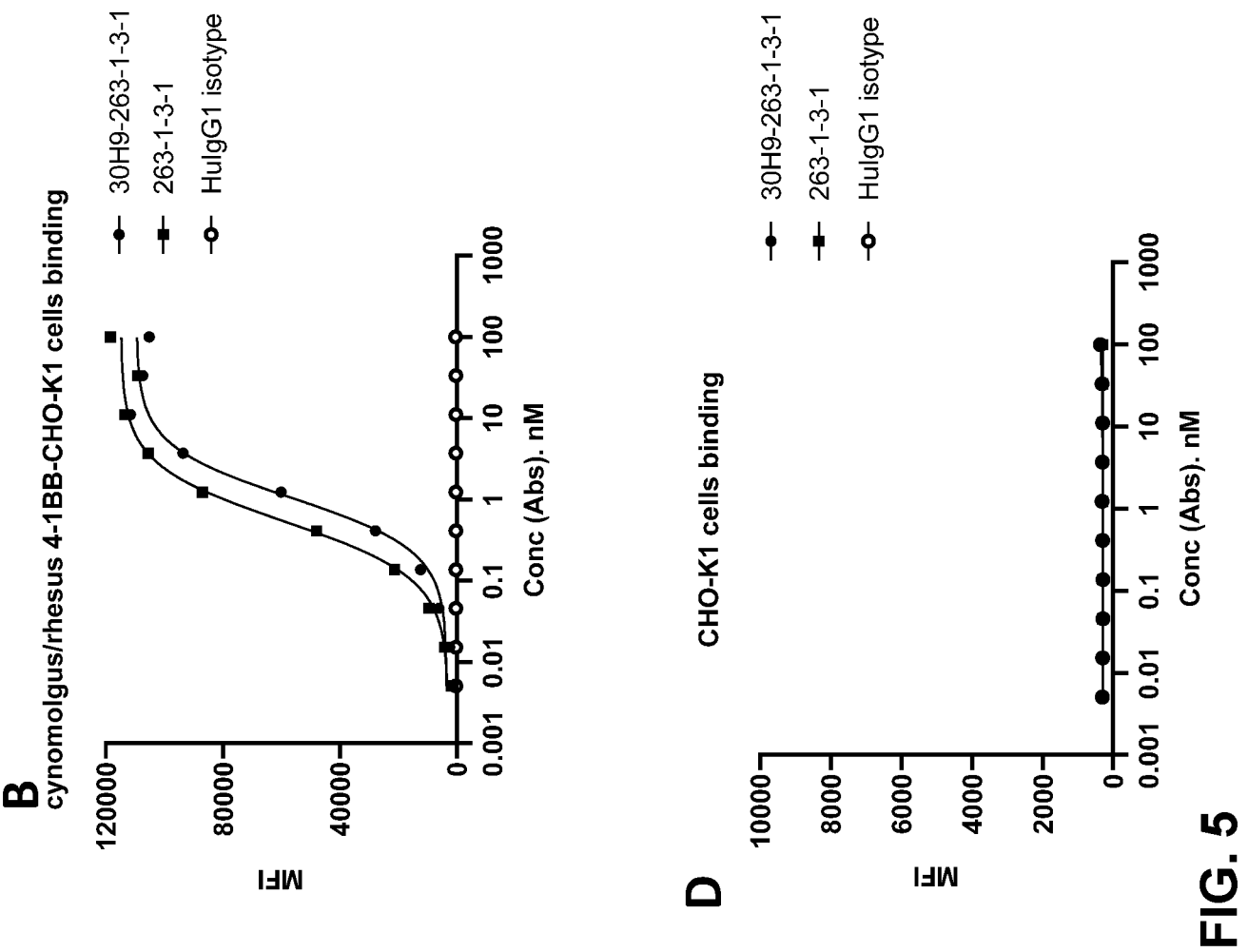

A
human 4-1BB-CHO-K1 cells binding
30H9-263-1-3-1
263-1-3-1
HulgG1 isotype
MFI
80000
60000
40000
20000
0
0.001
0.01
0.1
1
10
100
1000
Conc (Abs). nM
B
cynomolgus/rhesus 4-1BB-CHO-K1 cells binding
30H9-263-1-3-1
263-1-3-1
HulgG1 isotype
MFI
120000
80000
40000
0
0.001
0.01
0.1
1
10
100
1000
Conc (Abs). nM
C
mouse 4-1BB-CHO-K1 cells binding
30H9-263-1-3-1
263-1-3-1
HulgG1 isotype
MFI
10000
8000
6000
4000
2000
0
0.001
0.01
0.1
1
10
100
1000
Conc (Abs). nM
D
CHO-K1 cells binding
30H9-263-1-3-1
263-1-3-1
HulgG1 isotype
MFI
10000
8000
6000
4000
2000
0
0.001
0.01
0.1
1
10
100
1000
Conc (Abs). nM

FIG. 5

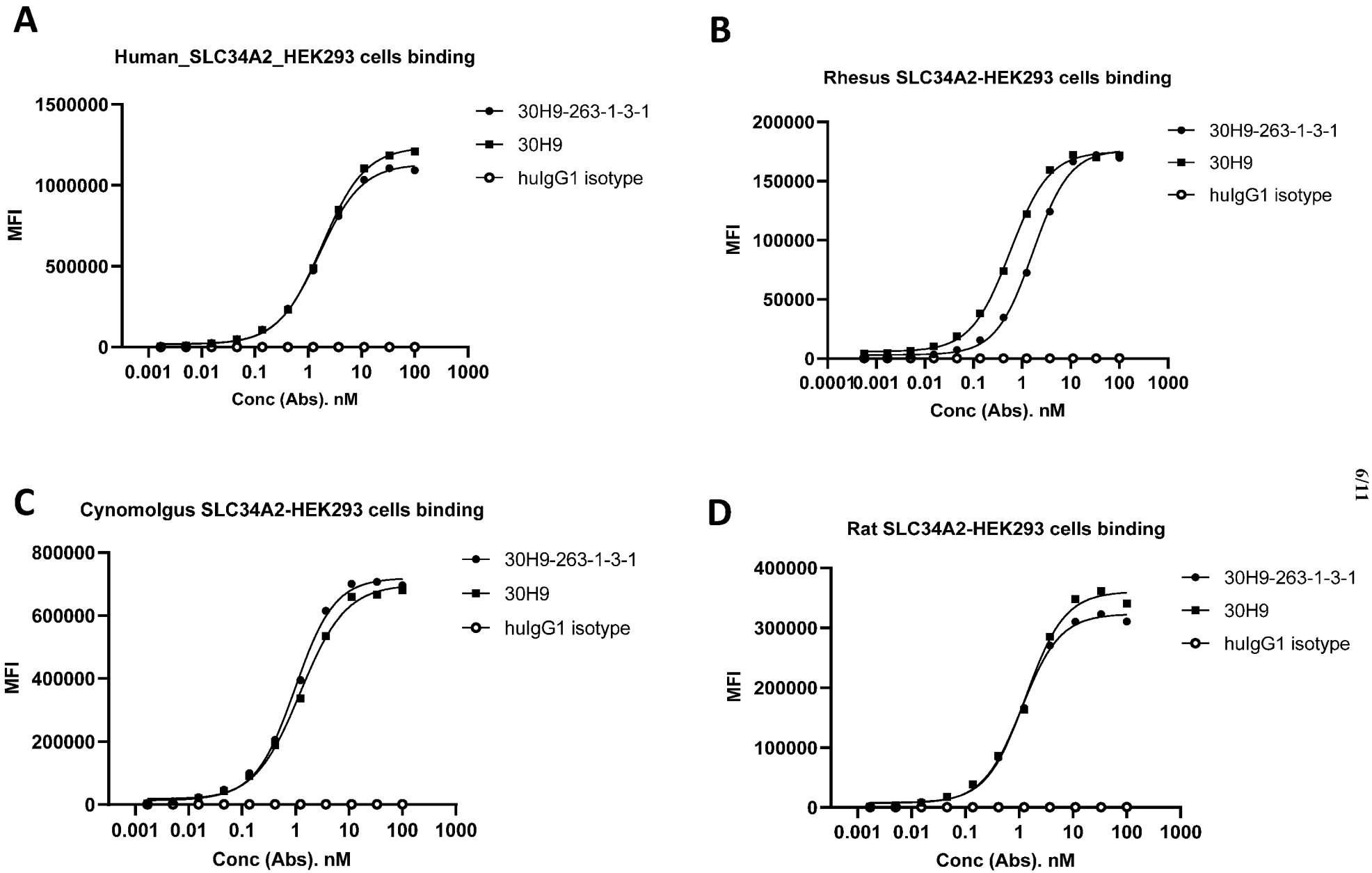
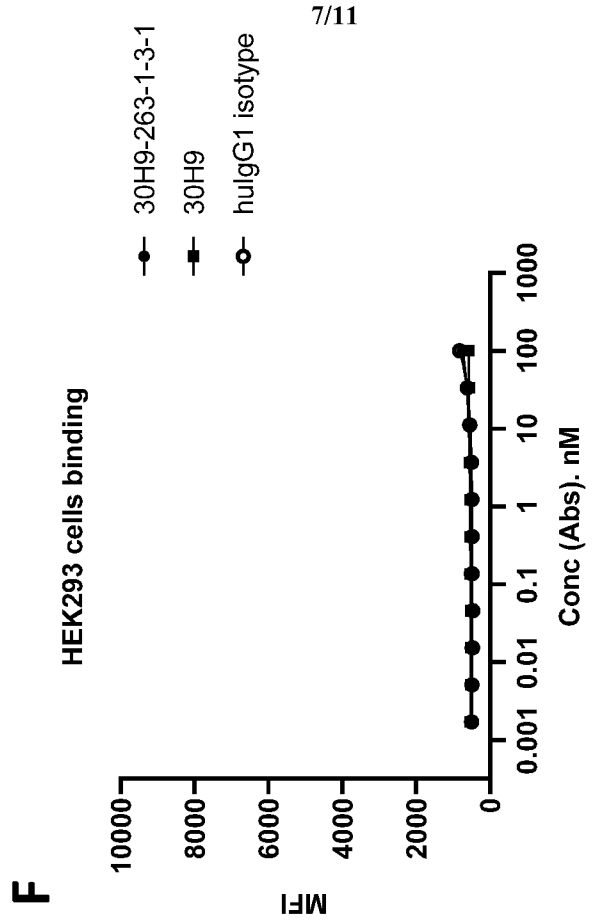

A
Human_SLC34A2_HEK293 cells binding
MFI
1500000
1000000
500000
0
0.001 0.01 0.1 1 10 100 1000
Conc (Abs). nM
30H9-263-1-3-1
30H9
huIgG1 isotype
B
Rhesus SLC34A2-HEK293 cells binding
MFI
200000
150000
100000
50000
0
0.0001 0.001 0.01 0.1 1 10 100 1000
Conc (Abs). nM
30H9-263-1-3-1
30H9
huIgG1 isotype
C
Cynomolgus SLC34A2-HEK293 cells binding
MFI
800000
600000
400000
200000
0
0.001 0.01 0.1 1 10 100 1000
Conc (Abs). nM
30H9-263-1-3-1
30H9
huIgG1 isotype
D
Rat SLC34A2-HEK293 cells binding
MFI
400000
300000
200000
100000
0
0.001 0.01 0.1 1 10 100 1000
Conc (Abs). nM
30H9-263-1-3-1
30H9
huIgG1 isotype

FIG. 6


F
HEK293 cells binding
MFI
10000
8000
6000
4000
2000
0
0.001 0.01 0.1 1 10 100 1000
Conc (Abs). nM
30H9-263-1-3-1
30H9
hulgG1 isotype

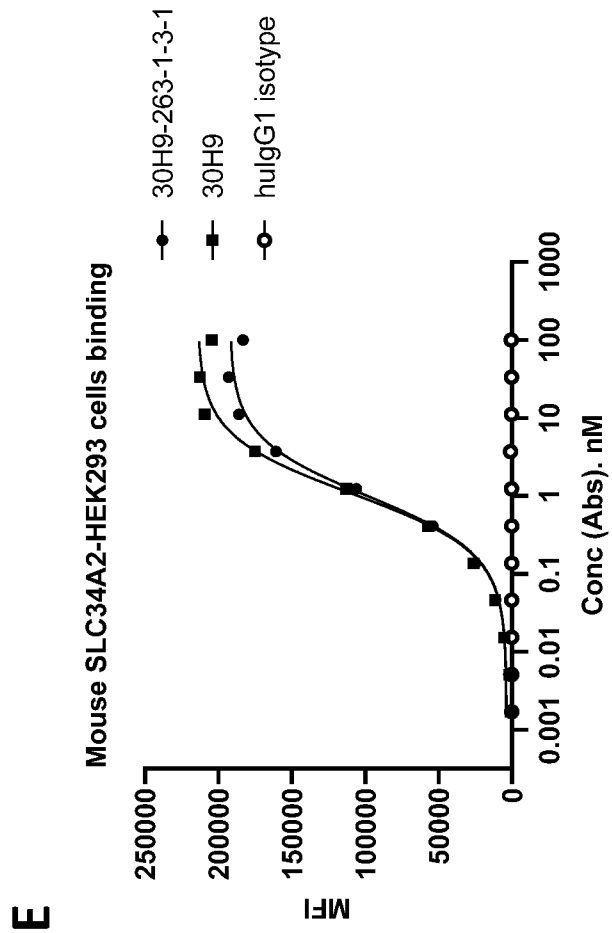

E
Mouse SLC34A2-HEK293 cells binding
MFI
250000
200000
150000
100000
50000
0
0.001 0.01 0.1 1 10 100 1000
Conc (Abs). nM
30H9-263-1-3-1
30H9
hulgG1 isotype

FIG. 6 (cont'd)

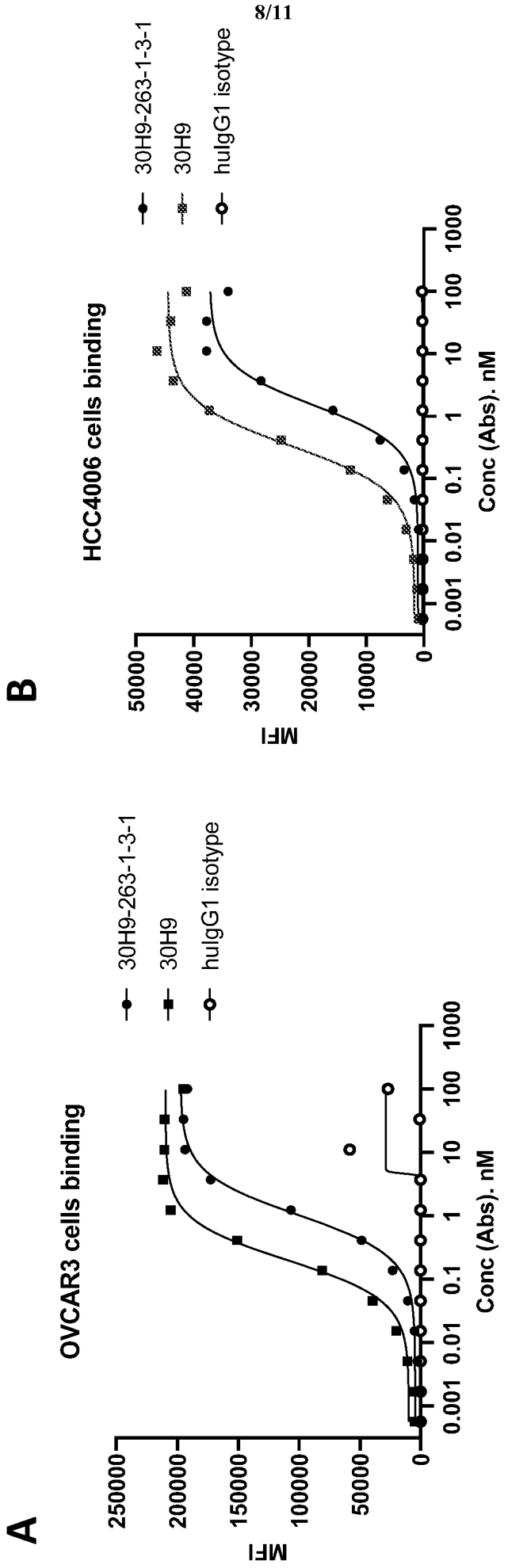

A
OVCAR3 cells binding
MFI
250000
200000
150000
100000
50000
0
0.001
0.01
0.1
1
10
100
1000
Conc (Abs). nM
30H9-263-1-3-1
30H9
huIgG1 isotype
B
HCC4006 cells binding
MFI
50000
40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
1000
Conc (Abs). nM
30H9-263-1-3-1
30H9
huIgG1 isotype

FIG. 7

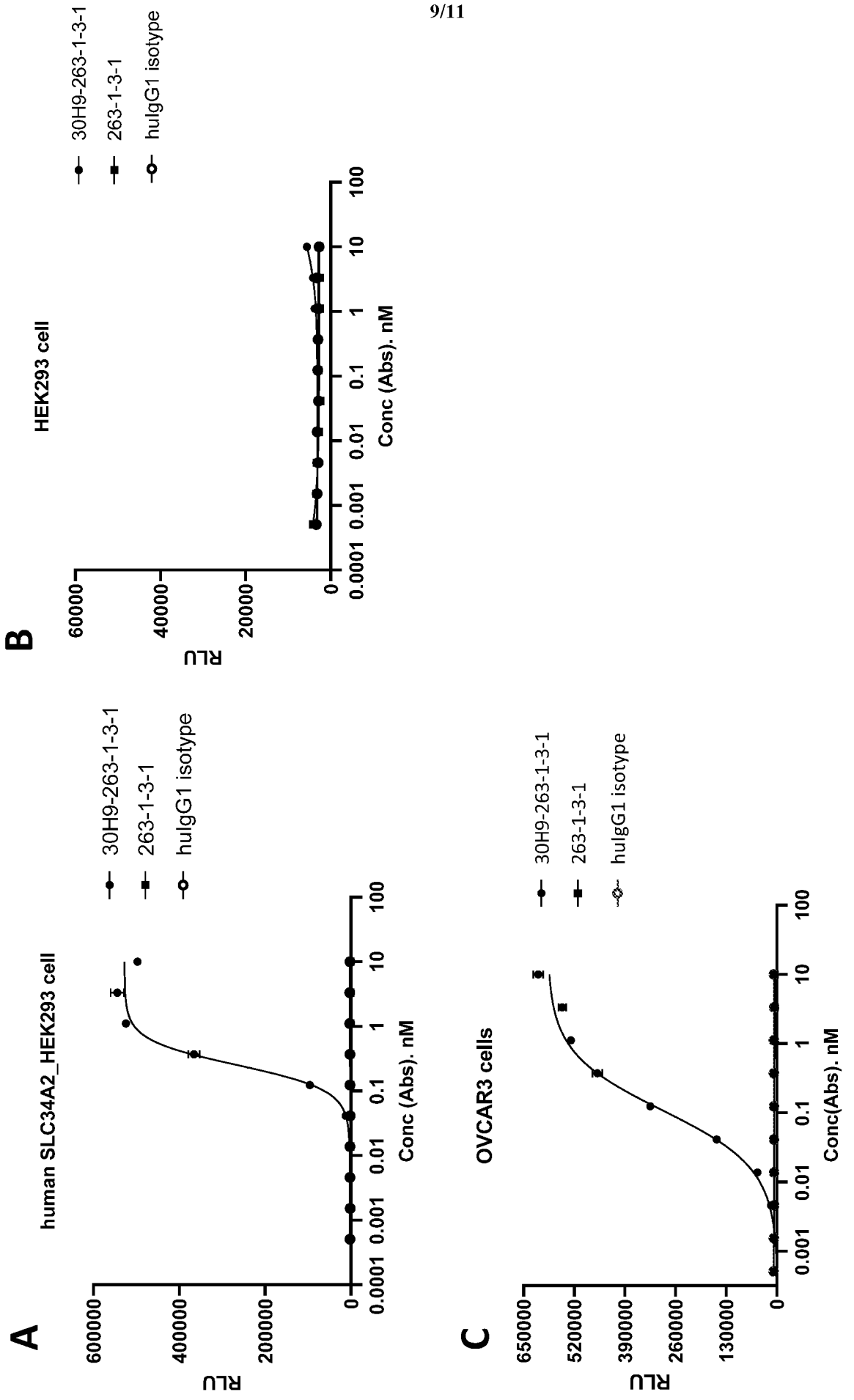

A
human SLC34A2_HEK293 cell
30H9-263-1-3-1
263-1-3-1
hulgG1 isotype
RLU
600000
400000
200000
0
0.0001 0.001 0.01 0.1 1 10 100
Conc (Abs). nM
B
HEK293 cell
30H9-263-1-3-1
263-1-3-1
hulgG1 isotype
RLU
60000
40000
20000
0
0.0001 0.001 0.01 0.1 1 10 100
Conc (Abs). nM
C
OVCAR3 cells
30H9-263-1-3-1
263-1-3-1
hulgG1 isotype
RLU
650000
520000
390000
260000
130000
0
0.001 0.01 0.1 1 10 100
Conc(Abs). nM

FIG. 8

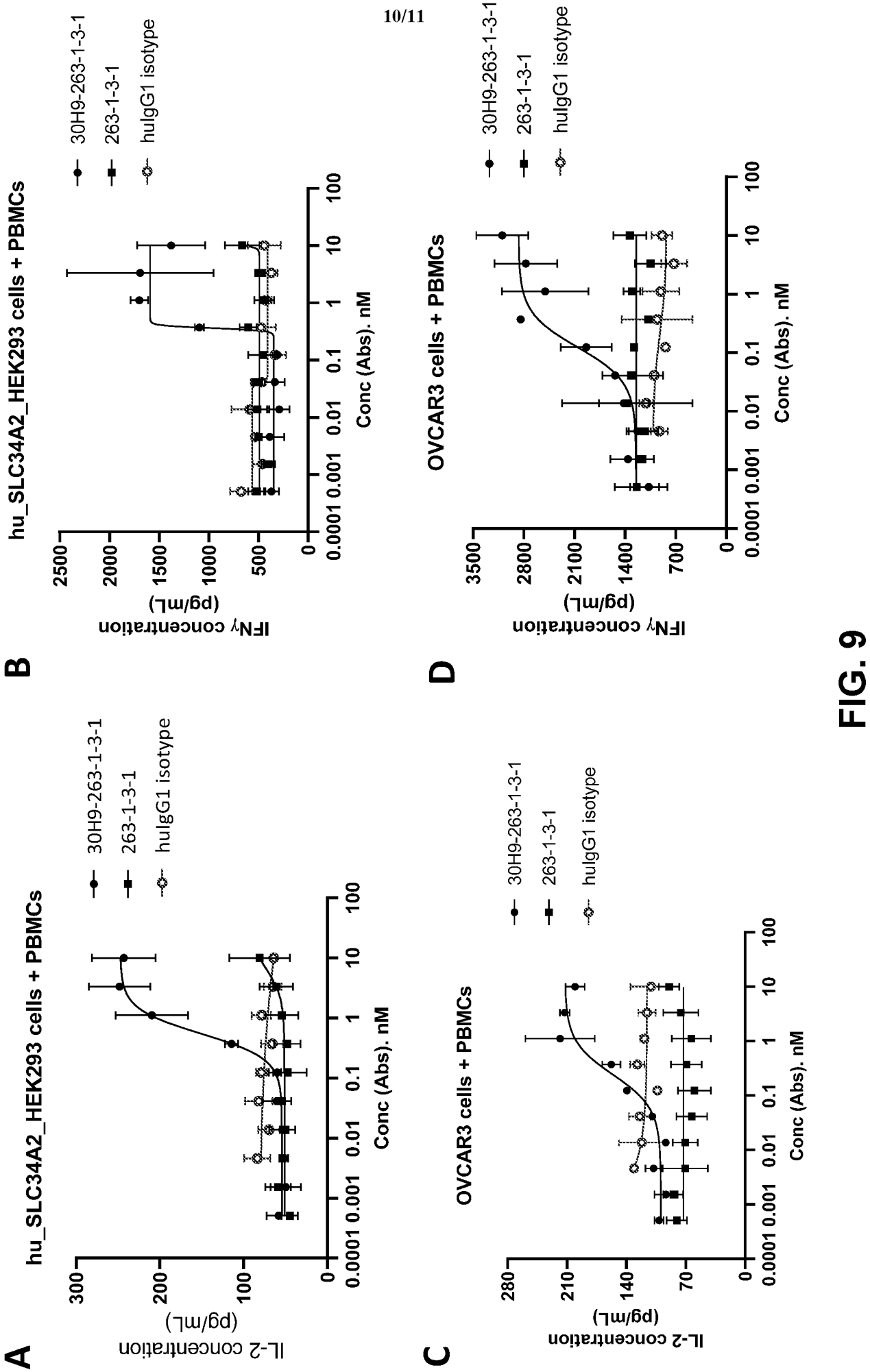

A
hu_SLC34A2_HEK293 cells + PBMCs
IL-2 concentration (pg/mL)
300
200
100
0
0.0001 0.001 0.01 0.1 1 10 100
Conc (Abs). nM
30H9-263-1-3-1
263-1-3-1
hulgG1 isotype
B
hu_SLC34A2_HEK293 cells + PBMCs
IFNγ concentration (pg/mL)
2500
2000
1500
1000
500
0
0.0001 0.001 0.01 0.1 1 10 100
Conc (Abs). nM
30H9-263-1-3-1
263-1-3-1
hulgG1 isotype
C
OVCAR3 cells + PBMCs
IL-2 concentration (pg/mL)
280
210
140
70
0
0.0001 0.001 0.01 0.1 1 10 100
Conc (Abs). nM
30H9-263-1-3-1
263-1-3-1
hulgG1 isotype
D
OVCAR3 cells + PBMCs
IFNγ concentration (pg/mL)
3500
2800
2100
1400
700
0
0.0001 0.001 0.01 0.1 1 10 100
Conc (Abs). nM
30H9-263-1-3-1
263-1-3-1
hulgG1 isotype

FIG. 9

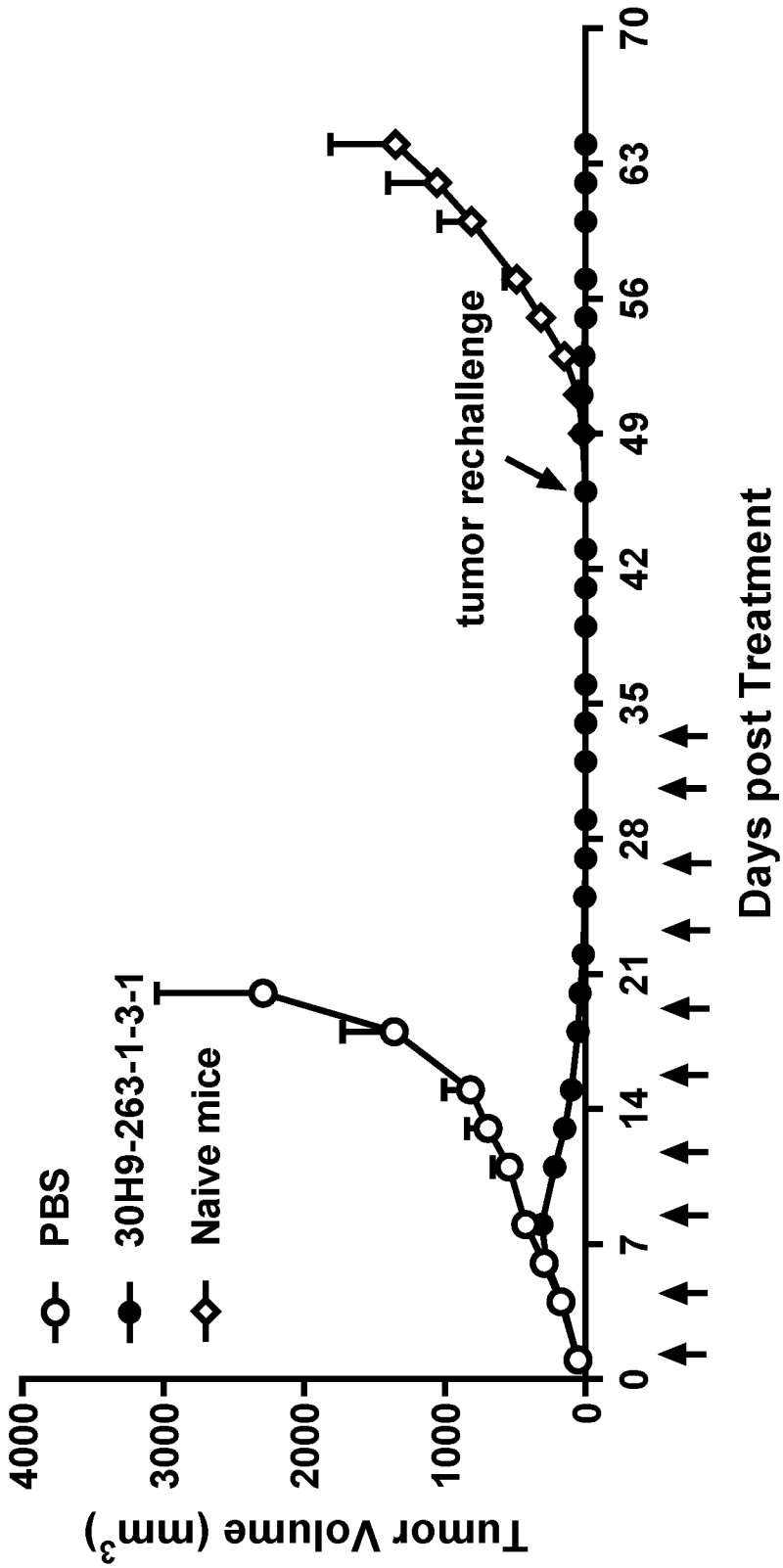

Tumor Volume (mm³)
4000
3000
2000
1000
0
PBS
30H9-263-1-3-1
Naive mice
0
7
14
21
28
35
42
49
56
63
70
tumor rechallenge
Days post Treatment

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/140840

A. CLASSIFICATION OF SUBJECT MATTER

C07K16/28(2006.01)i; C07K16/46(2006.01)i; C07K16/30(2006.01)i; C07K16/32(2006.01)i; A61K39/395(2006.01)i; A61P35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC:C07K,A61K,A61P

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

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

CNTXT,ENTXTC,VCN,VEN,PUBMED,NCBI,CNKI,BAIDU,BING,STN:SLC34A2,NaPi2b,4-1BB,CD137,TNFRSF9,antibody,bispecific,multispecific,CDR,SEQ ID NOs.:1-36

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2023052369 A1 (ZYMEWORKS INC.) 16 February 2023 (2023-02-16) Claims 1-83; Paragraphs 56, 91, 95, 101, 109, 123, 125, 141 and 164 in the description	1-10,15-24
X	CN 109476747 A (ABBVIE BIOTHERAPEUTICS INC.) 15 March 2019 (2019-03-15) Claims 1-82; Paragraph 239 in the description	1-10,15-24
X	CN 116406380 A (SHANGHAI LINXIZHI ENTERPRISE MANAGEMENT CO., LTD.) 07 July 2023 (2023-07-07) Claims 1-28; Paragraph 55 in the description	1-10,15-24
A	CN 113286825 A (ABL BIO INC. et al.) 20 August 2021 (2021-08-20) Claims 1-18	1-24
A	CN 109310885 A (MERSANA THERAPEUTICS INC.) 05 February 2019 (2019-02-05) Claims 1-35	1-24
A	US 2011129483 A1 (RITTER,G. et al.) 02 June 2011 (2011-06-02) Claims 1-41	1-24

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
26 March 2025	**03 April 2025**

Name and mailing address of the ISA/CN	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/140840

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/140840

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

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

1. ☑ Claims Nos.: **19-21,24(partial)**
because they relate to subject matter not required to be searched by this Authority, namely:

The subject matter of claims 19-21, 24(partial) relates to a method of treating a cancer in a patient in need. Thus, claims 19-21, 24(partial) do not warrant an international search according to the criteria set out in PCT Rule 39.1(iv). However, the search has been carried out and based on the manufacturing of a medicament.

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

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

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2024/140840

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