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(54) Title: HUMANIZED ANTI-CLAUDIN 18.2 CHIMERIC ANTIGEN RECEPTORS AND USES THEREOF

(57) Abstract: Chimeric antigen receptors (CARs) specific to CLDN18.2, vectors encoding the CLDN18.2 CAR, recombinant host cells comprising the CLDN18.2 CAR (CAR-Ts or CAR-NKs), and methods of using the CAR-Ts or CAR-NKs to treat a disease associated with the expression of CLDN18.2 thereof are described.

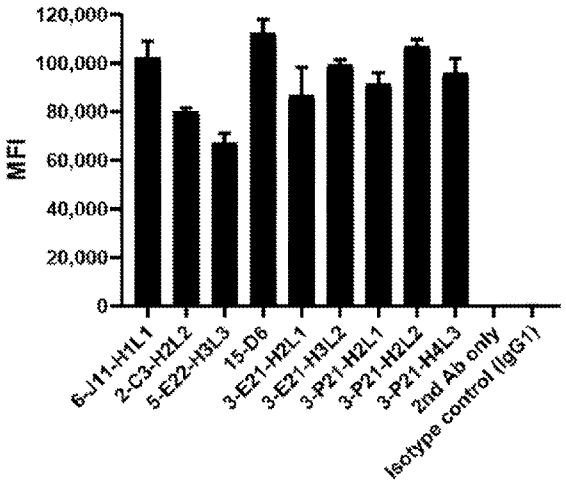

FIG. 1A

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HUMANIZED ANTI-CLAUDIN 18.2 CHIMERIC ANTIGEN RECEPTORS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/896,758, filed on September 6, 2019; U.S. Provisional Application No. 62/859,843, filed on June 11, 2019; and U.S. Provisional Application No. 62/825,955, filed on March 29, 2019. Each disclosure is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to humanized anti-claudin18.2 (CLDN18.2) chimeric antigen receptors (CARs), nucleic acids and expression vectors encoding the CARs, T cells engineered to express the CARs (CAR-T) and NK cells engineered to express the CARs (CAR-NK). Methods of making the CARs, methods of making the CAR-Ts/CAR-NKs, and methods of using the CAR-Ts/CAR-NKs to treat a disease associated with the expression of CLDN18.2, including cancer, are also provided.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "065799.19WO1 Sequence Listing" and a creation date of March 11, 2020 and having a size of 147 kb. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] The standard of care anti-cancer medicines provides significant benefits. Recently, the availability of immuno-oncology drugs, such as anti-PD-1 mAbs, anti-PD-L1 mAbs and anti-CD3 bispecific T-cell engagers, has advanced the concept of leveraging and activating patients' immune system to fight various types of cancer. However, poor response, insufficient efficacy, and/or safety issues remain to be resolved. CAR-T (chimeric antigen receptor-T) cell therapies involve genetically engineering a patient's own immune cells, such as T cells, and redirecting them to a suitable cell surface antigen on cancer cells (Mayor et al., Immunotherapy. 2016; 8:491-494). This approach has demonstrated success in patients suffering from chemorefractory B cell malignancies and other cancers (Pettitt et al., Mol Ther. 2018; 26:342-353). T cells can be engineered to possess specificity to one or more cancer cell surface targets/antigens to recognize

and kill the cancer cell. The process includes transducing T cells with DNA or other genetic material encoding the chimeric antigen receptor (CAR), which comprises an extracellular antigen specific binding domain, such as one or more single chain variable fragments (scFv) of a monoclonal antibody, a hinge and transmembrane region, and an intracellular signaling domain (including one or more costimulatory domains and one or more activating domains) (Kochenderfer et al., Nat Rev Clin Oncol. 2013; 10:267–276). CAR-expressing immune cells, such as T cells and NK cells, can be used to treat various diseases, including liquid and solid tumors. Successful CAR-T cell therapies can specifically recognize and destroy targeted cells and maintain the ability to persist and proliferate over time.

[0005] Claudin 18.2 (CLDN18.2), also known as claudin-18a2.1, is a member of the claudin (CLDN) family transmembrane proteins of at least 27 isoforms in humans. Claudins are the major structural components of tight junction between epithelial cells and function as ion pores to regulate the paracellular permeability of cations and anions (Sahin et al., Physiol Rev. 2013; 93:525-569). The expression of CLDN18 is normally limited to lung and stomach tissues. CLDN18 has two splicing variants. CLDN18.1 is the lung-specific variant whereas CLDN18.2 is the stomach-specific variant. The splicing variants differ at their N-terminal 69 amino acid residues due to alternative splicing of the first exon (Niimi et al., Mol Cell Biol. 2001; 21:7380-7390). Studies with CLDN18.2 knockout mice suggest that CLDN18.2 plays a critical role in preventing gastric acid leakage into the stomach lumen (Hayash et al., Gastroenterology 2012; 142:292-304).

[0006] Dysregulated expression of claudins are detected in many cancers and may contribute to tumorigenesis and cancer invasiveness (Singh et al., J Oncology 2010; 2010: 541957). The expression of CLDN18.2 is elevated in pancreatic ductal adenocarcinomas (PDAC) (Tanaka et al., J Histochem Cytochem. 2011; 59:942-952), esophageal tumors, non-small cell lung cancers (NSCLC), ovarian cancers (Sahin et al., Clin Cancer Res. 2008; 14:7624-7634), bile duct adenocarcinomas (Keira et al., Virchows Arch. 2015; 466:265-277), and cholangiocarcinomas (Shinozaki et al., Virchows Arch. 2011; 459:73-80). CLDN18.2 is an ideal target for CAR-T cell therapies to treat and cure CLDN18.2-positive cancers.

BRIEF SUMMARY OF THE INVENTION

[0007] In one general aspect, the invention relates to a chimeric antigen receptor (CAR) construct that induces T cell mediated cancer killing, wherein the CAR construct comprises at least one antigen binding domain that specifically binds human claudin 18.2 (CLDN18.2), a hinge region, a transmembrane region, and an intracellular signaling domain.

[0008] Provided are isolated polynucleotides comprising a nucleic acid sequence encoding a chimeric antigen receptor (CAR). The CAR can comprise (a) an extracellular domain comprising at least one antigen binding domain that specifically binds claudin 18.2 (CLDN18.2); (b) a hinge region; (c) a transmembrane region; and (d) an intracellular signaling domain.

[0009] In certain embodiments, the antigen binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 21, 22, 23, 51, 52 and 53, respectively;
(2) SEQ ID NOs: 24, 25, 26, 54, 55 and 56, respectively;
(3) SEQ ID NOs: 27, 28, 29, 57, 58 and 59, respectively;
(4) SEQ ID NOs: 30, 31, 32, 60, 61 and 62, respectively;
(5) SEQ ID NOs: 33, 34, 35, 63, 64 and 65, respectively;
(6) SEQ ID NOs: 36, 37, 38, 66, 67 and 68, respectively;
(7) SEQ ID NOs: 39, 40, 41, 69, 70 and 71, respectively;
(8) SEQ ID NOs: 42, 43, 44, 72, 73 and 74, respectively;
(9) SEQ ID NOs: 45, 46, 47, 75, 76 and 77, respectively; or
(10) SEQ ID NOs: 48, 49, 50, 78, 79 and 80, respectively;

wherein the antigen binding domain thereof specifically binds CLDN18.2, preferably human CLDN18.2.

[0010] In certain embodiments, the antigen binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 81, 82, 83, 111, 112 and 113, respectively;
(2) SEQ ID NOs: 84, 85, 86, 114, 115 and 116, respectively;
(3) SEQ ID NOs: 87, 88, 89, 117, 118 and 119, respectively;
(4) SEQ ID NOs: 90, 91, 92, 120, 121 and 122, respectively;
(5) SEQ ID NOs: 93, 94, 95, 123, 124 and 125, respectively;
(6) SEQ ID NOs: 96, 97, 98, 126, 127 and 128, respectively;
(7) SEQ ID NOs: 99, 100, 101, 129, 130 and 131, respectively;
(8) SEQ ID NOs: 102, 103, 104, 132, 133 and 134, respectively;
(9) SEQ ID NOs: 105, 106, 107, 135, 136 and 137, respectively; or
(10) SEQ ID NOs: 108, 109, 110, 138, 139 and 140, respectively;

wherein the antigen binding domain thereof specifically binds CLDN18.2, preferably human CLDN18.2.

[0011] In certain embodiments, the antigen binding domain comprises a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, or a light chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20.

[0012] In certain embodiments, the antigen binding domain comprises:

(1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

(2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;

(3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;

(4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

(5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

(6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;

(7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

(8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

(9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18; or

(10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20.

[0013] In certain embodiments, the antigen binding domain is humanized and comprises a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 142, 143, 146, 147, 151, 152, 154, 155, 156, 159, 160, 161, 162, 166, 167, 170, 171, 172, 175, 176, 177, 178, 179, 180, 186, 187, 191, 192, or 193, or a light chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 144, 145, 148,

149, 150, 153, 157, 158, 163, 164, 165, 168, 169, 173, 174, 181, 182, 183, 184, 185, 188, 189, 190, 194, 195, 196, or 197.

[0014] In certain embodiments, the antigen binding domain is humanized and comprises:

(1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:142, and a light chain variable region having the polypeptide sequence of SEQ ID NO:144;

(2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:142, and a light chain variable region having the polypeptide sequence of SEQ ID NO:145;

(3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:143, and a light chain variable region having the polypeptide sequence of SEQ ID NO:144;

(4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:143, and a light chain variable region having the polypeptide sequence of SEQ ID NO:145;

(5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:148;

(6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:149;

(7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:150;

(8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:148;

(9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:149;

(10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:150;

(11) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:151, and a light chain variable region having the polypeptide sequence of SEQ ID NO:153;

(12) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:152, and a light chain variable region having the polypeptide sequence of SEQ ID NO:153;

(13) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:154, and a light chain variable region having the polypeptide sequence of SEQ ID NO:157;

(14) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:155, and a light chain variable region having the polypeptide sequence of SEQ ID NO:157;

(15) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:156, and a light chain variable region having the polypeptide sequence of SEQ ID NO:158;

(16) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:159, and a light chain variable region having the polypeptide sequence of SEQ ID NO:163;

(17) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:159, and a light chain variable region having the polypeptide sequence of SEQ ID NO:164;

(18) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:160, and a light chain variable region having the polypeptide sequence of SEQ ID NO:163;

(19) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:160, and a light chain variable region having the polypeptide sequence of SEQ ID NO:164;

(20) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:161, and a light chain variable region having the polypeptide sequence of SEQ ID NO:165; or

(21) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:162, and a light chain variable region having the polypeptide sequence of SEQ ID NO:165.

[0015] In certain embodiments, the antigen binding domain is a single chain variable fragment (scFv) that specifically binds CLDN18.2, preferably human CLDN18.2.

[0016] In certain embodiments, the antigen binding domain is a humanized single chain variable fragment (scFv) that specifically binds CLDN18.2, preferably human CLDN18.2. In certain embodiments, the humanized single chain variable fragment (scFv) comprises a polypeptide sequence at least 95% identical to any one of SEQ ID NOs: 198-215.

[0017] In certain embodiments, the chimeric antigen receptor (CAR) comprises one or more antigen binding domains.

[0018] In certain embodiments, the intracellular signaling domain comprises one or more costimulatory domains and one or more activating domains.

[0019] Also provided are chimeric antigen receptors (CARs) encoded by the isolated polynucleotides of the invention.

[0020] Also provided are vectors comprising the isolated polynucleotides comprising nucleic acids encoding the CARs of the invention.

[0021] Also provided are host cells comprising the vectors of the invention.

[0022] In certain embodiments, the host cell is a T cell, preferably a human T cell. In certain embodiments, the host cell is a NK cell, preferably a human NK cell. The T cell or NK cell can, for example, be engineered to express the CAR of the invention to treat diseases such as cancer.

[0023] Also provided are methods of making a host cell expressing a chimeric antigen receptor (CAR) of the invention. The methods comprise transducing a T cell or a NK cell with a vector comprising the isolated nucleic acids encoding the CARs of the invention.

[0024] Also provided are methods of producing a CAR-T cell or CAR-NK cell of the invention. The methods comprise culturing T cells or NK cells comprising the isolated polynucleotide comprising a nucleic acid encoding a chimeric antigen receptor (CAR) of the invention under conditions to produce the CAR-T cell or CAR-NK cell, and recovering the CAR-T cell or CAR-NK cell.

[0025] Also provided are methods of generating a population of RNA-engineered cells comprising a chimeric antigen receptor (CAR) of the invention. The methods comprise contacting a cell with the isolated polynucleotide comprising a nucleic acid encoding a chimeric antigen receptor (CAR) of the invention, wherein the isolated polynucleotide is an *in vitro* transcribed RNA or synthetic RNA.

[0026] Also provided are methods of treating cancer in a subject in need thereof, comprising administering to the subject the CAR-T cells and/or CAR-NK cells of the invention. The cancer can be any liquid or solid cancer, for example, it can be selected from, but not limited to, a lung cancer, a gastric cancer, an esophageal cancer, a bile duct cancer, a cholangiocarcinoma, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[0027] Also provided are methods of treating an inflammatory disease in a subject in need thereof, comprising administering to the subject the CAR-T cells and/or CAR-NK cells of the invention.

[0028] In certain embodiments, the methods of treating cancer or inflammatory disease in a subject in need thereof further comprise administering to the subject in need thereof an agent that increases the efficacy of a cell expressing a CAR molecule.

[0029] In certain embodiments, the methods of treating cancer or inflammatory disease in a subject in need thereof further comprise administering to the subject in need thereof an agent that ameliorates one or more side effects associated with administration of a cell expressing a CAR molecule.

[0030] In certain embodiments, the methods of treating cancer or inflammatory disease in a subject in need thereof further comprise administering to the subject in need thereof an agent that treats the disease associated with Claudin 18.2.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] The foregoing summary, as well as the following detailed description of preferred embodiments of the present application, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the application is not limited to the precise embodiments shown in the drawings.

[0032] FIGs. 1A-1B show the binding of humanized anti-CLDN18.2 mAbs to HEK293-CLDN18.2 and HEK293-CLDN18.1, which express the full-length human CLDN18.2 and CLDN18.1, respectively. The experiment was carried out by FACS analysis.

[0033] FIGs. 2A-2D show the binding of humanized anti-CLDN18.2 mAbs to HEK293-CLDN18.2 cells stably expressing full-length human CLDN18.2. The experiment was carried out by FACS analysis.

[0034] FIGs. 3A-3L show the binding of humanized scFvs to HEK293-CLDN18.2 cells stably expressing full-length human CLDN18.2. The experiment was carried out by FACS analysis.

[0035] FIG. 4 shows the tumor cell killing activity of the CAR T cells assembled with an anti-CLDN18.2 scFv against CLDN18.2-expressing cells (HEK293-CLDN18.2); CLDN18.1-expressing cells (HEK293-CLDN18.1) were used as control.

DETAILED DESCRIPTION OF THE INVENTION

[0036] Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

[0037] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification.

[0038] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

[0039] Unless otherwise stated, any numerical values, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term "about." Thus, a numerical value typically includes ± 10% of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v). As used herein, the

use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0040] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

[0041] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains" or "containing," or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers and are intended to be non-exclusive or open-ended. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those elements but can include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0042] As used herein, the conjunctive term "and/or" between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by "and/or," a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or" as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or."

[0043] As used herein, the term "consists of," or variations such as "consist of" or "consisting of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, but that no additional integer or group of integers can be added to the specified method, structure, or composition.

[0044] As used herein, the term "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, and the optional inclusion of

any recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure or composition. See M.P.E.P. § 2111.03.

[0045] As used herein, "subject" means any animal, preferably a mammal, most preferably a human. The term "mammal" as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys, humans, etc., more preferably a human.

[0046] The words "right," "left," "lower," and "upper" designate directions in the drawings to which reference is made.

[0047] It should also be understood that the terms "about," "approximately," "generally," "substantially," and like terms, used herein when referring to a dimension or characteristic of a component of the preferred invention, indicate that the described dimension/characteristic is not a strict boundary or parameter and does not exclude minor variations therefrom that are functionally the same or similar, as would be understood by one having ordinary skill in the art. At a minimum, such references that include a numerical parameter would include variations that, using mathematical and industrial principles accepted in the art (e.g., rounding, measurement or other systematic errors, manufacturing tolerances, etc.), would not vary the least significant digit.

[0048] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences (e.g., chimeric antigen receptors (CARs) comprising antigen binding domains specific for CLDN18.2 and polynucleotides that encode them, CLDN18.2 polypeptides and CLDN18.2 polynucleotides that encode them), refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

[0049] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0050] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in

the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally, Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

[0051] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased.

[0052] Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)).

[0053] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is

less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0054] A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

[0055] As used herein, the term "isolated" means a biological component (such as a nucleic acid, peptide or protein) has been substantially separated, produced apart from, or purified away from other biological components of the organism in which the component naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins that have been "isolated" thus include nucleic acids and proteins purified by standard purification methods. "Isolated" nucleic acids, peptides and proteins can be part of a composition and still be isolated if the composition is not part of the native environment of the nucleic acid, peptide, or protein. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

[0056] As used herein, the term "polynucleotide," synonymously referred to as "nucleic acid molecule," "nucleotides" or "nucleic acids," refers to any polyribonucleotide or polydeoxyribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short nucleic acid chains, often referred to as oligonucleotides.

[0057] As used herein, the term "vector" is a replicon in which another nucleic acid segment can be operably inserted so as to bring about the replication or expression of the segment.

[0058] As used herein, the term "host cell" refers to a cell comprising a nucleic acid molecule of the invention. The "host cell" can be any type of cell, e.g., a primary cell, a cell in culture, or a cell from a cell line. In one embodiment, a "host cell" is a cell transfected or transduced with a nucleic acid molecule of the invention. In another embodiment, a "host cell" is a progeny or potential progeny of such a transfected or transduced cell. A progeny of a cell may or may not be identical to the parent cell, e.g., due to mutations or environmental influences that can occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0059] The term "expression" as used herein, refers to the biosynthesis of a gene product. The term encompasses the transcription of a gene into RNA. The term also encompasses translation of RNA into one or more polypeptides, and further encompasses all naturally occurring post-transcriptional and post-translational modifications. The expressed CAR can be within the cytoplasm of a host cell, into the extracellular milieu such as the growth medium of a cell culture or anchored to the cell membrane.

[0060] As used herein, the term "immune cell" or "immune effector cell" refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune cells include T cells, B cells, natural killer (NK) cells, mast cells, and myeloid-derived phagocytes. According to particular embodiments, the engineered immune cells are T cells, and are referred to as CAR-T cells because they are engineered to express CARs of the invention.

[0061] As used herein, the term "engineered immune cell" refers to an immune cell, also referred to as an immune effector cell, that has been genetically modified by the addition of extra genetic material in the form of DNA or RNA to the total genetic material of the cell. According to embodiments herein, the engineered immune cells have been genetically modified to express a CAR construct according to the invention.

Chimeric Antigen Receptor (CAR)

[0062] As used herein, the term "chimeric antigen receptor" (CAR) refers to a recombinant polypeptide comprising at least an extracellular domain that binds specifically to an antigen or a target, a transmembrane domain and an intracellular T cell receptor-activating signaling domain. Engagement of the extracellular domain of the CAR with the target antigen on the surface of a target cell results in clustering of the CAR and delivers an activation stimulus to the CAR-containing cell. CARs redirect the specificity of immune effector cells and trigger proliferation,

cytokine production, phagocytosis and/or production of molecules that can mediate cell death of the target antigen-expressing cell in a major histocompatibility (MHC)-independent manner.

[0063] In one aspect, the CAR comprises an antigen binding domain, a hinge region, a costimulatory domain, an activating domain and a transmembrane region. In one aspect, the CAR comprises an antigen binding domain, a hinge region, two costimulatory domains, an activating domain and a transmembrane region. In one aspect, the CAR comprises two antigen binding domains, a hinge region, a costimulatory domain, an activating domain and a transmembrane region. In one aspect, the CAR comprises two antigen binding domains, a hinge region, two costimulatory domains, an activating domain and a transmembrane region.

[0064] As used herein, the term "signal peptide" refers to a leader sequence at the amino-terminus (N-terminus) of a nascent CAR protein, which co-translationally or post-translationally directs the nascent protein to the endoplasmic reticulum and subsequent surface expression.

[0065] As used herein, the term "extracellular antigen binding domain," "extracellular domain," or "extracellular ligand binding domain" refers to the part of a CAR that is located outside of the cell membrane and is capable of binding to an antigen, target or ligand.

[0066] As used herein, the term "hinge region" refers to the part of a CAR that connects two adjacent domains of the CAR protein, e.g., the extracellular domain and the transmembrane domain.

[0067] As used herein, the term "transmembrane domain" refers to the portion of a CAR that extends across the cell membrane and anchors the CAR to cell membrane.

Costimulatory Domains

[0068] As used herein, chimeric antigen receptors can incorporate costimulatory (signaling) domains to increase their potency. A costimulatory (signaling) domain can be derived from a costimulatory molecule. Costimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response. Costimulatory domains can be derived from costimulatory molecules, which can include, but are not limited to CD28, CD28T, OX40, 4-1BB/CD137, CD2, CD3 (alpha, beta, delta, epsilon, gamma, zeta), CD4, CD5, CD7, CD9, CD16, CD22, CD27, CD30, CD33, CD37, CD40, CD45, CD64, CD80, CD86, CD134, CD137, CD154, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1; CD11a and CD18), CD247, CD276 (B7-H3), LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), NKG2C, Ig alpha (CD79a), DAP10, Fc gamma receptor, MHC class I molecule, TNFR, integrin, signaling lymphocytic activation molecule, BTLA, Toll ligand receptor, ICAM-1, CDS, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD8

alpha, CD8 beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, ITGAE, CD103, ITGAL, CD1a, CD1b, CD1c, CD1d, ITGAM, ITGAX, ITGB1, CD29, ITGB2 (CD18), ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD 160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD83 ligand, cytokine receptor, activating NK cell receptors, or fragments or any combination thereof.

Activating Domains

[0069] As used herein, chimeric antigen receptors can comprise activating domains. Activating domains can include, but are not limited to, CD3. CD3 is an element of the T cell receptor on native T cells and has been shown to be an important intracellular activating element in CARs. In a preferred embodiment, the CD3 is CD3 zeta.

Hinge region

[0070] As described herein, the chimeric antigen receptor can comprise a hinge region. This is a portion of the extracellular domain, sometimes referred to as a "spacer" region. A variety of hinges can be employed in accordance with the invention, including costimulatory molecules, as discussed above, immunoglobulin (Ig) sequences, or other suitable molecules to achieve the desired special distance from the target cell. In some embodiments, the entire extracellular region comprises a hinge region.

Transmembrane region

[0071] As used herein, chimeric antigen receptors (CARs) can comprise a transmembrane region/domain. The CAR can be designed to comprise a transmembrane domain that is fused to the extracellular domain of the CAR. It can similarly be fused to the intracellular domain of the CAR. In one embodiment, the transmembrane domain that is naturally associated with one of the domains in a CAR is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this invention can be derived from (i.e. comprise or engineered from), but are not limited to, CD28, CD28T, OX40, 4-1BB/CD137, CD2, CD3 (alpha, beta, delta, epsilon, gamma, zeta), CD4, CD5, CD7, CD9, CD16, CD22, CD27, CD30, CD33, CD37, CD40, CD45, CD64, CD80, CD86,

CD134, CD137, CD154, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1; CD11a and CD18), CD247, CD276 (B7-H3), LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), NKG2C, Ig alpha (CD79a), DAP10, Fc gamma receptor, MHC class I molecule, TNFR, integrin, signaling lymphocytic activation molecule, BTLA, Toll ligand receptor, ICAM-1, CDS, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD8 alpha, CD8 beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, ITGAE, CD103, ITGAL, CD1a, CD1b, CD1c, CD1d, ITGAM, ITGAX, ITGB1, CD29, ITGB2 (CD18), ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD 160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, CD83 ligand, cytokine receptor, activating NK cell receptors, an immunoglobulin protein, or fragments or any combination thereof.

Immune Cells

[0072] According to particular aspects, the invention provides cells that are immune cells that comprise the isolated polynucleotides or vectors comprising the isolated polynucleotides comprising the nucleotide sequence encoding the CAR are provided herein. The immune cells comprising the isolated polynucleotides and/or vectors of the invention can be referred to as "engineered immune cells." Preferably, the engineered immune cells are derived from a human (are of human origin prior to being made recombinant).

[0073] The engineered immune cells can, for example, be cells of the lymphoid lineage. Non-limiting examples of cells of the lymphoid lineage can include T cells and Natural Killer (NK) cells. T cells express the T cell receptor (TCR), with most cells expressing α and β chains and a smaller population expressing γ and δ chains. T cells useful as engineered immune cells of the invention can be $CD4^+$ or $CD8^+$ and can include, but are not limited to, T helper cells ($CD4^+$), cytotoxic T cells (also referred to as cytotoxic T lymphocytes, CTL; $CD8^+$ cells), and memory T cells, including central memory T cells, stem-like memory T cells, and effector memory T cells, natural killer T cells, mucosal associated invariant T cells, and $\gamma\delta$ T cells. Other exemplary immune cells include, but are not limited to, macrophages, antigen presenting cells (APCs), or any immune cell that expresses an inhibitor of a cell-mediated immune response, for example, an immune checkpoint inhibitor pathway receptor (e.g., PD-1). Precursor cells of immune cells that can be used according to the invention, include, hematopoietic stem and/or progenitor cells. Hematopoietic stem and/or progenitor cells can be derived from bone marrow, umbilical cord

blood, adult peripheral blood after cytokine mobilization, and the like, by methods known in the art. The immune cells are engineered to recombinantly express the CARs of the invention.

[0074] Immune cells and precursor cells thereof can be isolated by methods known in the art, including commercially available methods (see, e.g., Rowland Jones et al., Lymphocytes: A Practical Approach, Oxford University Press, NY (1999)). Sources for immune cells or precursors thereof include, but are not limited to, peripheral blood, umbilical cord blood, bone marrow, or other sources of hematopoietic cells. Various techniques can be employed to separate the cells to isolated or enrich desired immune cells. For instance, negative selection methods can be used to remove cells that are not the desired immune cells. Additionally, positive selection methods can be used to isolate or enrich for the desired immune cells or precursors thereof, or a combination of positive and negative selection methods can be employed. If a particular type of cell is to be isolated, e.g., a particular T cell, various cell surface markers or combinations of markers (e.g., CD3, CD4, CD8, CD34) can be used to separate the cells.

[0075] The immune cells or precursor cells thereof can be autologous or non-autologous to the subject to which they are administered in the methods of treatment of the invention. Autologous cells are isolated from the subject to which the engineered immune cells recombinantly expressing the CAR are to be administered. Optionally, the cells can be obtained by leukapheresis, where leukocytes are selectively removed from withdrawn blood, made recombinant, and then retransfused into the donor. Alternatively, allogeneic cells from a non-autologous donor that is not the subject can be used. In the case of a non-autologous donor, the cells are typed and matched for human leukocyte antigen (HLA) to determine the appropriate level of compatibility. For both autologous and non-autologous cells, the cells can optionally be cryopreserved until ready for use.

[0076] Various methods for isolating immune cells that can be used for recombinant expression of the CARs of the invention have been described previously, and can be used, including, but not limited to, using peripheral donor lymphocytes (Sadelain et al., Nat. Rev. Cancer 3:35-45 (2003); Morgan et al., Science 314:126-9 (2006)), using lymphocyte cultures derived from tumor infiltrating lymphocytes (TILs) in tumor biopsies (Panelli et al., J. Immunol. 164:495-504 (2000); Panelli et al., J. Immunol. 164:4382-92 (2000)) and using selectively *in vitro* expanded antigen-specific peripheral blood leukocytes employing artificial antigen-presenting cells (AAPCs) or dendritic cells (Dupont et al., Cancer Res. 65:5417-427 (2005); Papanicolaou et al., Blood 102:2498-505 (2003)). In the case of using stem cells, the cells can be isolated by methods well known in the art (see, e.g., Klug et al., Hematopoietic Stem Cell

Protocols, Humana Press, NJ (2002); Freshney et al., Culture of Human Stem Cells, John Wiley & Sons (2007)).

[0077] According to particular embodiments, the method of making the engineered immune cells comprises transfecting or transducing immune effector cells isolated from an individual such that the immune effector cells express one or more CAR(s) according to embodiments of the invention. Methods of preparing immune cells for immunotherapy are described, e.g., in WO2014/130635, WO2013/176916 and WO2013/176915, which are incorporated herein by reference. Individual steps that can be used for preparing engineered immune cells are disclosed, e.g., in WO2014/039523, WO2014/184741, WO2014/191128, WO2014/184744 and WO2014/184143, which are incorporated herein by reference.

[0078] In a particular embodiment, the immune effector cells, such as T cells, are genetically modified with CARs of the invention (e.g., transduced with a viral vector comprising a nucleic acid encoding a CAR) and then are activated and expanded *in vitro*. In various embodiments, T cells can be activated and expanded before or after genetic modification to express a CAR, using methods as described, for example, in US6352694, US6534055, US6905680, US6692964, US5858358, US6887466, US6905681, US7144575, US7067318, US7172869, US7232566, US7175843, US5883223, US6905874, US6797514, US6867041, US2006/121005, which are incorporated herein by reference. T cells can be expanded *in vitro* or *in vivo*. Generally, the T cells of the invention can be expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex-associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. As non-limiting examples, T cell populations can be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD3 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore, or by activation of the CAR itself. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. Conditions appropriate for T cell culture include, e.g., an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 5 (Lonza)) that can contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), cytokines, such as IL-2, IL-7, IL-15, and/or IL-21, insulin, IFN-g, GM-CSF, TGFβ and/or any other additives for the growth of cells known to the skilled artisan. In other embodiments, the T cells can be activated and stimulated to proliferate with

feeder cells and appropriate antibodies and cytokines using methods such as those described in US6040177, US5827642, and WO2012129514, which are incorporated herein by reference.

Antigen binding domain

[0079] As used herein, the term "antigen binding domain" refers to an antibody fragment such as, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)2, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), a single domain antibody (sdab) an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen binding domain is capable of binding to the same antigen to which the parent antibody binds. According to particular embodiments, the antigen binding domain comprises a single-chain antibody molecule (scFv).

[0080] As used herein, the term "antibody" is used in a broad sense and includes immunoglobulin or antibody molecules including human, humanized, composite and chimeric antibodies and antibody fragments that are monoclonal or polyclonal. In general, antibodies are proteins or peptide chains that exhibit binding specificity to a specific antigen. Antibody structures are well known. Immunoglobulins can be assigned to five major classes (i.e., IgA, IgD, IgE, IgG and IgM), depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Accordingly, the antibodies of the invention can be of any of the five major classes or corresponding sub-classes. Preferably, the antibodies of the invention are IgG1, IgG2, IgG3 or IgG4. Antibody light chains of vertebrate species can be assigned to one of two clearly distinct types, namely kappa and lambda, based on the amino acid sequences of their constant domains. Accordingly, the antibodies of the invention can contain a kappa or lambda light chain constant domain. According to particular embodiments, the antibodies of the invention include heavy and/or light chain constant regions from rat or human antibodies. In addition to the heavy and light constant domains, antibodies contain an antigen-binding region that is made up of a light chain variable region and a heavy chain variable region, each of which contains three domains (i.e., complementarity determining regions 1-3; CDR1, CDR2, and CDR3). The light chain variable region domains are alternatively referred to as LCDR1, LCDR2, and LCDR3, and the heavy chain variable region domains are alternatively referred to as HCDR1, HCDR2, and HCDR3.

[0081] As used herein, the term "single-chain antibody" refers to a conventional single-chain antibody in the field, which comprises a heavy chain variable region and a light chain variable region connected by a short peptide of about 5 to about 20 amino acids. As used herein, the term "single domain antibody" refers to a conventional single domain antibody in the field, which comprises a heavy chain variable region and a heavy chain constant region or which comprises only a heavy chain variable region.

[0082] As used herein, the term "human antibody" refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any technique known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide.

[0083] As used herein, the term "humanized antigen binding domain" refers to a non-human antigen binding domain that is modified to increase the sequence homology to that of a human antibody, such that the antigen-binding properties of the antigen binding domain are retained, but its antigenicity in the human body is reduced.

[0084] As used herein, the term "chimeric antigen binding domain" refers to an antigen binding domain wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. The variable region of both the light and heavy chains often corresponds to the variable region of an antigen binding domain derived from one species of mammal (e.g., mouse, rat, rabbit, etc.) having the desired specificity, affinity, and capability, while the constant regions correspond to the sequences of an antigen binding domain derived from another species of mammal (e.g., human) to avoid eliciting an immune response in that species.

[0085] As used herein, the term "CLDN18.2" refers to claudin 18 variant 2, claudin-18.2 or claudin-18a2.1, which belongs to the claudin family of transmembrane proteins. CLDN18.2 is specifically expressed on the surface of epithelial cells in stomach (Niimi et al., Mol Cell Biol. 2001; 21:7380-7390) and becomes one of the major structural components of the tight junction between the epithelial cells (Sahin et al., Physiol Rev. 2013; 93:525-569). The term "human CLDN18.2" refers to a CLDN18.2 originated from a human. An exemplary amino acid sequence of a human CLDN18.2 is represented in GenBank Accession No. AAL15637.1 (SEQ ID NO:141).

[0086] As used herein, an antigen binding domain that "specifically binds to CLDN18.2" refers to an antigen binding domain that binds to a CLDN18.2, preferably a human CLDN18.2, with a KD of 1×10^{-7} M or less, preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term "KD" refers to the dissociation

constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antigen binding domains can be determined using methods in the art in view of the present disclosure. For example, the KD of an antigen binding domain can be determined by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as an Octet RED96 system.

[0087] The smaller the value of the KD of an antigen binding domain, the higher affinity that the antigen binding domain binds to a target antigen.

[0088] According to a particular aspect, the invention relates to chimeric antigen receptors (CAR)s comprising an antigen binding domain, wherein the antigen binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 21, 22, 23, 51, 52 and 53, respectively;
(2) SEQ ID NOs: 24, 25, 26, 54, 55 and 56, respectively;
(3) SEQ ID NOs: 27, 28, 29, 57, 58 and 59, respectively;
(4) SEQ ID NOs: 30, 31, 32, 60, 61 and 62, respectively;
(5) SEQ ID NOs: 33, 34, 35, 63, 64 and 65, respectively;
(6) SEQ ID NOs: 36, 37, 38, 66, 67 and 68, respectively;
(7) SEQ ID NOs: 39, 40, 41, 69, 70 and 71, respectively;
(8) SEQ ID NOs: 42, 43, 44, 72, 73 and 74, respectively;
(9) SEQ ID NOs: 45, 46, 47, 75, 76 and 77, respectively; or
(10) SEQ ID NOs: 48, 49, 50, 78, 79 and 80, respectively;

wherein the antigen binding domain thereof specifically binds CLDN18.2, preferably human CLDN18.2.

[0089] According to another particular aspect, the invention relates to chimeric antigen receptors (CARs) comprising an antigen binding domain, wherein the antigen binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 81, 82, 83, 111, 112 and 113, respectively;
(2) SEQ ID NOs: 84, 85, 86, 114, 115 and 116, respectively;
(3) SEQ ID NOs: 87, 88, 89, 117, 118 and 119, respectively;
(4) SEQ ID NOs: 90, 91, 92, 120, 121 and 122, respectively;

(5) SEQ ID NOs: 93, 94, 95, 123, 124 and 125, respectively;

(6) SEQ ID NOs: 96, 97, 98, 126, 127 and 128, respectively;

(7) SEQ ID NOs: 99, 100, 101, 129, 130 and 131, respectively;

(8) SEQ ID NOs: 102, 103, 104, 132, 133 and 134, respectively;

(9) SEQ ID NOs: 105, 106, 107, 135, 136 and 137, respectively; or

(10) SEQ ID NOs: 108, 109, 110, 138, 139 and 140, respectively;

wherein the antigen binding domain thereof specifically binds CLDN18.2, preferably human CLDN18.2.

[0090] According to another particular aspect, the invention relates to an antigen binding domain comprising a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, or a light chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20.

[0091] According to another particular aspect, the invention relates to an antigen binding domain, comprising:

(1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

(2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;

(3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;

(4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

(5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

(6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;

(7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

(8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

(9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18; or

(10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20.

[0092] According to another particular aspect, the antigen binding domain is humanized and comprises a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 142, 143, 146, 147, 151, 152, 154, 155, 156, 159, 160, 161, 162, 166, 167, 170, 171, 172, 175, 176, 177, 178, 179, 180, 186, 187, 191, 192, or 193, or a light chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 144, 145, 148, 149, 150, 153, 157, 158, 163, 164, 165, 168, 169, 173, 174, 181, 182, 183, 184, 185, 188, 189, 190, 194, 195, 196, or 197.

[0093] According to another particular aspect, the antigen binding domain is humanized and comprises:

(1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:142, and a light chain variable region having the polypeptide sequence of SEQ ID NO:144;

(2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:142, and a light chain variable region having the polypeptide sequence of SEQ ID NO:145;

(3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:143, and a light chain variable region having the polypeptide sequence of SEQ ID NO:144;

(4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:143, and a light chain variable region having the polypeptide sequence of SEQ ID NO:145;

(5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:148;

(6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:149;

(7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:150;

(8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:148;

(9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:149;

(10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:150;

(11) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:151, and a light chain variable region having the polypeptide sequence of SEQ ID NO:153;

(12) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:152, and a light chain variable region having the polypeptide sequence of SEQ ID NO:153;

(13) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:154, and a light chain variable region having the polypeptide sequence of SEQ ID NO:157;

(14) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:155, and a light chain variable region having the polypeptide sequence of SEQ ID NO:157;

(15) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:156, and a light chain variable region having the polypeptide sequence of SEQ ID NO:158;

(16) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:159, and a light chain variable region having the polypeptide sequence of SEQ ID NO:163;

(17) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:159, and a light chain variable region having the polypeptide sequence of SEQ ID NO:164;

(18) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:160, and a light chain variable region having the polypeptide sequence of SEQ ID NO:163;

(19) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:160, and a light chain variable region having the polypeptide sequence of SEQ ID NO:164;

(20) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:161, and a light chain variable region having the polypeptide sequence of SEQ ID NO:165; or

(21) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:162, and a light chain variable region having the polypeptide sequence of SEQ ID NO:165.

[0094] According to another particular aspect, the antigen binding domain is a single chain variable fragment (scFv) that specifically binds CLDN18.2, preferably human CLDN18.2.

[0095] In certain embodiments, the antigen binding domain is a humanized single chain variable fragment (scFv) that specifically binds CLDN18.2, preferably human CLDN18.2. In certain embodiments, the humanized single chain variable fragment (scFv) comprises a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any one of SEQ ID NOs:198-215. In certain embodiments, the humanized single chain variable fragment (scFv) comprises a polypeptide sequence having an amino acid sequence selected from the group consisting of SEQ ID NOs:198-215.

[0096] According to another particular aspect, the chimeric antigen receptor comprises one or more antigen binding domains.

[0097] According to another particular aspect, the intracellular signaling domain comprises one or more costimulatory domains and one or more activating domains.

[0098] In another general aspect, the invention relates to an isolated polynucleotide comprising a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain thereof of the invention. It will be appreciated by those skilled in the art that the coding sequence of a protein can be changed (e.g., replaced, deleted, inserted, etc.) without changing the amino acid sequence of the protein. Accordingly, it will be understood by those skilled in the art that nucleic acid sequences encoding antigen binding domains thereof of the invention can be altered without changing the amino acid sequences of the proteins.

[0099] In another general aspect, the invention relates to a vector comprising the isolated polynucleotide comprising the nucleic acid encoding the CAR, wherein the CAR comprises an antigen binding domain thereof of the invention. Any vector known to those skilled in the art in view of the present disclosure can be used, such as a plasmid, a cosmid, a phage vector or a viral vector. In some embodiments, the vector is a recombinant expression vector such as a plasmid. The vector can include any element to establish a conventional function of an expression vector, for example, a promoter, ribosome binding element, terminator, enhancer, selection marker, and origin of replication. The promoter can be a constitutive, inducible, or repressible promoter. A number of expression vectors capable of delivering nucleic acids to a cell are known in the art and can be used herein for production of an antigen binding domain thereof in the cell. Conventional cloning techniques or artificial gene synthesis can be used to generate a recombinant expression vector according to embodiments of the invention.

[00100] In another general aspect, the invention relates to a cell transduced with the vector comprising the isolated nucleic acids encoding the CARs of the invention. The term "transduced" or "transduction" refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transduced" cell is one which has been transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny. In certain embodiments, the cell is a human CAR-T cell, wherein the T cell is engineered to express the CAR of the invention to treat diseases such as cancer. In certain embodiments, the cell is a human CAR-NK cell, wherein the NK cell engineered to express the CAR of the invention is used to treat diseases such as cancer.

[00101] In another general aspect, the invention relates to a method of making a CAR-T cell by transducing a T cell with a vector comprising the isolated nucleic acids encoding the CARs of the invention.

[00102] In another general aspect, the invention relates to a method of producing the CAR-T cell thereof of the invention, comprising culturing T-cells comprising a nucleic acid encoding a

chimeric antigen receptor (CAR) of the invention under conditions to produce the CAR-T cell, and recovering the CAR-T cell.

[00103] In another general aspect, the invention relates to a method of making a CAR- NK cell by transducing a NK cell with a vector comprising the isolated nucleic acids encoding the CARs of the invention.

[00104] In another general aspect, the invention relates to a method of producing a CAR-NK cell of the invention, comprising culturing NK cells comprising nucleic acids encoding the chimeric antigen receptor (CAR) thereof under conditions to produce the CAR-NK cell, and recovering the CAR-NK cell.

[00105] In another general aspect, the invention relates to a method of generating a population of RNA-engineered cells comprising a chimeric antigen receptor (CAR) of the invention. The methods comprise contacting a population of cells with isolated polynucleotides comprising a nucleic acid encoding a CAR of the invention, wherein the isolated polynucleotides are *in vitro* transcribed RNA or synthetic RNA.

Pharmaceutical Compositions

[00106] In another general aspect, the invention relates to a pharmaceutical composition comprising an isolated polynucleotide of the invention, an isolated polypeptide of the invention, a host cell of the invention, and/or an engineered immune cell of the invention and a pharmaceutically acceptable carrier. The term "pharmaceutical composition" as used herein means a product comprising an isolated polynucleotide of the invention, an isolated polypeptide of the invention, a host cell of the invention, and/or an engineered immune cell of the invention together with a pharmaceutically acceptable carrier. Polynucleotides, polypeptides, host cells, and/or engineered immune cells of the invention and compositions comprising them are also useful in the manufacture of a medicament for therapeutic applications mentioned herein.

[00107] As used herein, the term "carrier" refers to any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microsphere, liposomal encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. As used herein, the term "pharmaceutically acceptable carrier" refers to a non-toxic material that does not interfere with the effectiveness of a composition according to the invention or the biological activity of a composition according to the invention. According to particular embodiments, in view of the present disclosure, any pharmaceutically acceptable carrier suitable for use in a polynucleotide, polypeptide, host cell, and/or engineered immune cell pharmaceutical composition can be used in the invention.

[00108] The formulation of pharmaceutically active ingredients with pharmaceutically acceptable carriers is known in the art, e.g., Remington: The Science and Practice of Pharmacy (e.g. 21st edition (2005), and any later editions). Non-limiting examples of additional ingredients include: buffers, diluents, solvents, tonicity regulating agents, preservatives, stabilizers, and chelating agents. One or more pharmaceutically acceptable carrier may be used in formulating the pharmaceutical compositions of the invention.

Methods of use

[00109] In another general aspect, the invention relates to a method of treating a cancer in a subject in need thereof, comprising administering to the subject the CAR-T cells and/or CAR-NK cells of the invention. The cancer can, for example, be selected from but not limited to, a lung cancer, a gastric cancer, an esophageal cancer, a bile duct cancer, a cholangiocarcinoma, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[00110] In another general aspect, the invention relates to a method of treating an inflammatory disease in a subject in need thereof, comprising administering to the subject the CAR-T cells and/or CAR-NK cells of the invention.

[00111] According to embodiments of the invention, the CAR-T cell or CAR-NK cell comprises a therapeutically effective amount of the expressed CARs of the invention. As used herein, the term "therapeutically effective amount" refers to an amount of an active ingredient or component that elicits the desired biological or medicinal response in a subject. A therapeutically effective amount can be determined empirically and in a routine manner, in relation to the stated purpose.

[00112] As used herein with reference to CARs, a therapeutically effective amount means an amount of the CAR molecule expressed in the transduced T cell or NK cell that modulates an immune response in a subject in need thereof. Also, as used herein with reference to CARs, a therapeutically effective amount means an amount of the CAR molecule expressed in the transduced T cell or NK cell that results in treatment of a disease, disorder, or condition; prevents or slows the progression of the disease, disorder, or condition; or reduces or completely alleviates symptoms associated with the disease, disorder, or condition.

[00113] As used herein with reference to CAR-T cell or CAR-NK cell, a therapeutically effective amount means an amount of the CAR-T cells or CAR-NK cells that modulates an immune response in a subject in need thereof. Also, as used herein with reference to CAR-T cell or CAR-NK cell, a therapeutically effective amount means an amount of the CAR-T cells or CAR-NK cells that results in treatment of a disease, disorder, or condition; prevents or slows the progression of the disease, disorder, or condition; or reduces or completely alleviates symptoms associated with the disease, disorder, or condition.

[00114] According to particular embodiments, the disease, disorder or condition to be treated is cancer, preferably a cancer selected from the group consisting of a lung cancer, a gastric cancer, an esophageal cancer, a bile duct cancer, a cholangiocarcinoma, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors. According to other particular embodiments, the disease, disorder or condition to be treated is an inflammatory disease.

[00115] According to particular embodiments, a therapeutically effective amount refers to the amount of therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of the disease, disorder or condition to be treated or a symptom associated therewith; (ii) reduce the duration of the disease, disorder or condition to be treated, or a symptom associated therewith; (iii) prevent the progression of the disease, disorder or condition to be treated, or a symptom associated therewith; (iv) cause regression of the disease, disorder or condition to be treated, or a symptom associated therewith; (v) prevent the development or onset of the disease, disorder or condition to be treated, or a symptom associated therewith; (vi) prevent the recurrence of the disease, disorder or condition to be treated, or a symptom associated therewith; (vii) reduce hospitalization of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (viii) reduce hospitalization length of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (ix) increase the survival of a subject with the disease, disorder or condition to be treated, or a symptom associated therewith; (xi) inhibit or reduce the disease, disorder or condition to be treated, or a symptom associated therewith in a subject; and/or (xii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

[00116] The therapeutically effective amount or dosage can vary according to various factors, such as the disease, disorder or condition to be treated, the means of administration, the target site, the physiological state of the subject (including, e.g., age, body weight, health), whether the subject is a human or an animal, other medications administered, and whether the treatment is prophylactic or therapeutic. Treatment dosages are optimally titrated to optimize safety and efficacy.

[00117] According to particular embodiments, the compositions described herein are formulated to be suitable for the intended route of administration to a subject. For example, the compositions described herein can be formulated to be suitable for intravenous, subcutaneous, or intramuscular administration.

[00118] The cells of the invention can be administered in any convenient manner known to those skilled in the art. For example, the cells of the invention can be administered to the subject by aerosol inhalation, injection, ingestion, transfusion, implantation, and/or transplantation. The compositions comprising the cells of the invention can be administered transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, intrapleurally, by intravenous (i.v.) injection, or intraperitoneally. In certain embodiments, the cells of the invention can be administered with or without lymphodepletion of the subject.

[00119] The pharmaceutical compositions comprising cells of the invention expressing CARs of the invention can be provided in sterile liquid preparations, typically isotonic aqueous solutions with cell suspensions, or optionally as emulsions, dispersions, or the like, which are typically buffered to a selected pH. The compositions can comprise carriers, for example, water, saline, phosphate buffered saline, and the like, suitable for the integrity and viability of the cells, and for administration of a cell composition.

[00120] Sterile injectable solutions can be prepared by incorporating cells of the invention in a suitable amount of the appropriate solvent with various other ingredients, as desired. Such compositions can include a pharmaceutically acceptable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like, that are suitable for use with a cell composition and for administration to a subject, such as a human. Suitable buffers for providing a cell composition are well known in the art. Any vehicle, diluent, or additive used is compatible with preserving the integrity and viability of the cells of the invention.

[00121] The cells of the invention can be administered in any physiologically acceptable vehicle. A cell population comprising cells of the invention can comprise a purified population of cells. Those skilled in the art can readily determine the cells in a cell population using various well known methods. The ranges in purity in cell populations comprising genetically modified

cells of the invention can be from about 50% to about 55%, from about 55% to about 60%, from about 60% to about 65%, from about 65% to about 70%, from about 70% to about 75%, from about 75% to about 80%, from about 80% to about 85%, from about 85% to about 90%, from about 90% to about 95%, or from about 95% to about 100%. Dosages can be readily adjusted by those skilled in the art, for example, a decrease in purity could require an increase in dosage.

[00122] The cells of the invention are generally administered as a dose based on cells per kilogram (cells/kg) of body weight of the subject to which the cells are administered. Generally, the cell doses are in the range of about 10^4 to about 10^{10} cells/kg of body weight, for example, about 10^5 to about 10^9, about 10^5 to about 10^8, about 10^5 to about 10^7, or about 10^5 to about 10^6, depending on the mode and location of administration. In general, in the case of systemic administration, a higher dose is used than in regional administration, where the immune cells of the invention are administered in the region of a tumor and/or cancer. Exemplary dose ranges include, but are not limited to, 1×10^4 to 1×10^8, 2×10^4 to 1×10^8, 3×10^4 to 1×10^8, 4×10^4 to 1×10^8, 5×10^4 to 6×10^8, 7×10^4 to 1×10^8, 8×10^4 to 1×10^8, 9×10^4 to 1×10^8, 1×10^5 to 1×10^8, 1×10^5 to 9×10^7, 1×10^5 to 8×10^7, 1×10^5 to 7×10^7, 1×10^5 to 6×10^7, 1×10^5 to 5×10^7, 1×10^5 to 4×10^7, 1×10^5 to 4×10^7, 1×10^5 to 3×10^7, 1×10^5 to 2×10^7, 1×10^5 to 1×10^7, 1×10^5 to 9×10^6, 1×10^5 to 8×10^6, 1×10^5 to 7×10^6, 1×10^5 to 6×10^6, 1×10^5 to 5×10^6, 1×10^5 to 4×10^6, 1×10^5 to 4×10^6, 1×10^5 to 3×10^6, 1×10^5 to 2×10^6, 1×10^5 to 1×10^6, 2×10^5 to 9×10^7, 2×10^5 to 8×10^7, 2×10^5 to 7×10^7, 2×10^5 to 6×10^7, 2×10^5 to 5×10^7, 2×10^5 to 4×10^7, 2×10^5 to 4×10^7, 2×10^5 to 3×10^7, 2×10^5 to 2×10^7, 2×10^5 to 1×10^7, 2×10^5 to 9×10^6, 2×10^5 to 8×10^6, 2×10^5 to 7×10^6, 2×10^5 to 6×10^6, 2×10^5 to 5×10^6, 2×10^5 to 4×10^6, 2×10^5 to 4×10^6, 2×10^5 to 3×10^6, 2×10^5 to 2×10^6, 2×10^5 to 1×10^6, 3×10^5 to 3×10^6 cells/kg, and the like. Additionally, the dose can be adjusted to account for whether a single dose is being administered or whether multiple doses are being administered. The precise determination of what would be considered an effective dose can be based on factors individual to each subject.

[00123] As used herein, the terms "treat," "treating," and "treatment" are all intended to refer to an amelioration or reversal of at least one measurable physical parameter related to a cancer and/or an inflammatory disease, disorder or condition, which is not necessarily discernible in the subject, but can be discernible in the subject. The terms "treat," "treating," and "treatment," can also refer to causing regression, preventing the progression, or at least slowing down the progression of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an alleviation, prevention of the development or onset, or reduction in the duration of one or more symptoms associated with the disease, disorder, or condition, such as a tumor or more preferably a cancer. In a particular embodiment, "treat," "treating," and

"treatment" refer to prevention of the recurrence of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an increase in the survival of a subject having the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to elimination of the disease, disorder, or condition in the subject.

[00124] According to particular embodiments, provided are compositions used in the treatment of a cancer and/or an inflammatory disease, disorder or condition. For cancer therapy, the provided compositions can be used in combination with another treatment including, but not limited to, a chemotherapy, an anti-CD20 mAb, an anti-TIM-3 mAb, an anti-LAG-3 mAb, an anti-EGFR mAb, an anti-HER-2 mAb, an anti-CD19 mAb, an anti-CD33 mAb, an anti-CD47 mAb, an anti-CD73 mAb, an anti-DLL-3 mAb, an anti-apelin mAb, an anti-TIP-1 mAb, an anti-FOLR1 mAb, an anti-CTLA-4 mAb, an anti-PD-L1 mAb, an anti-PD-1 mAb, other immuno-oncology drugs, an antiangiogenic agent, a radiation therapy, an antibody-drug conjugate (ADC), a targeted therapy, or other anticancer drugs.

[00125] According to particular embodiments, the methods of treating cancer and/or inflammatory disease in a subject in need thereof comprise administering to the subject the CAR-T cells and/or CAR-NK cells of the invention in combination with an agent that increases the efficacy of a cell expressing a CAR molecule. Such agents include, but are not limited to, an antibody fragment that binds to CD73, CD39, PD1, PD-L1, PD-L2, CTLA4, TIM3 or LAG3, or an adenosine A2a receptor antagonist.

[00126] According to particular embodiments, the methods of treating cancer and/or inflammatory disease in a subject in need thereof comprise administering to the subject the CAR-T cells and/or CAR-NK cells of the invention in combination with an agent that ameliorates one or more side effects associated with administration of a cell expressing a CAR molecule. Such agents include, but are not limited to, a steroid, an inhibitor of TNFα, or an inhibitor of IL-6.

[00127] According to particular embodiments, the methods of treating cancer and/or inflammatory disease in a subject in need thereof comprise administering to the subject the CAR-T cells and/or CAR-NK cells of the invention in combination with an agent that treats the disease associated with Claudin 18.2. Such agents include, but are not limited to, an anti-Claudin 18.2 monoclonal antibody or bispecific antibody.

[00128] As used herein, the term "in combination," in the context of the administration of two or more therapies to a subject, refers to the use of more than one therapy. The use of the term "in combination" does not restrict the order in which therapies are administered to a subject. For example, a first therapy (e.g., a composition described herein) can be administered prior to (e.g.,

5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject.

EMBODIMENTS

[00129] The invention provides also the following non-limiting embodiments.

[00130] Embodiment 1 is an isolated polynucleotide comprising a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises: (a) an extracellular domain comprising at least one antigen binding domain that specifically binds claudin 18.2 (CLDN18.2); (b) a hinge region; (c) a transmembrane region; and (d) an intracellular signaling domain.

[00131] Embodiment 2 is the isolated polynucleotide of embodiment 1, wherein the antigen binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 21, 22, 23, 51, 52 and 53, respectively;
(2) SEQ ID NOs: 24, 25, 26, 54, 55 and 56, respectively;
(3) SEQ ID NOs: 27, 28, 29, 57, 58 and 59, respectively;
(4) SEQ ID NOs: 30, 31, 32, 60, 61 and 62, respectively;
(5) SEQ ID NOs: 33, 34, 35, 63, 64 and 65, respectively;
(6) SEQ ID NOs: 36, 37, 38, 66, 67 and 68, respectively;
(7) SEQ ID NOs: 39, 40, 41, 69, 70 and 71, respectively;
(8) SEQ ID NOs: 42, 43, 44, 72, 73 and 74, respectively;
(9) SEQ ID NOs: 45, 46, 47, 75, 76 and 77, respectively; or
(10) SEQ ID NOs: 48, 49, 50, 78, 79 and 80, respectively;

wherein the antigen binding domain thereof specifically binds CLDN18.2, preferably human CLDN18.2.

[00132] Embodiment 3 is the isolated polynucleotide of embodiment 1, wherein the antigen binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 81, 82, 83, 111, 112 and 113, respectively;

(2) SEQ ID NOs: 84, 85, 86, 114, 115 and 116, respectively;

(3) SEQ ID NOs: 87, 88, 89, 117, 118 and 119, respectively;

(4) SEQ ID NOs: 90, 91, 92, 120, 121 and 122, respectively;

(5) SEQ ID NOs: 93, 94, 95, 123, 124 and 125, respectively;

(6) SEQ ID NOs: 96, 97, 98, 126, 127 and 128, respectively;

(7) SEQ ID NOs: 99, 100, 101, 129, 130 and 131, respectively;

(8) SEQ ID NOs: 102, 103, 104, 132, 133 and 134, respectively;

(9) SEQ ID NOs: 105, 106, 107, 135, 136 and 137, respectively; or

(10) SEQ ID NOs: 108, 109, 110, 138, 139 and 140, respectively;

wherein the antigen binding domain thereof specifically binds CLDN18.2, preferably human CLDN18.2.

[00133] Embodiment 4 is the isolated polynucleotide of any one of embodiments 1-3, wherein the antigen binding domain comprises a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, or a light chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20.

[00134] Embodiment 5 is the isolated polynucleotide of any one of embodiments 1-4, wherein the antigen binding domain comprises:

(1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

(2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;

(3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;

(4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

(5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

(6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;

(7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

(8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

(9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18; or

(10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20.

[00135] Embodiment 6 is the isolated polynucleotide of any one of embodiments 1-3, wherein the antigen binding domain is humanized and comprises a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 142, 143, 146, 147, 151, 152, 154, 155, 156, 159, 160, 161, 162, 166, 167, 170, 171, 172, 175, 176, 177, 178, 179, 180, 186, 187, 191, 192, or 193, or a light chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 144, 145, 148, 149, 150, 153, 157, 158, 163, 164, 165, 168, 169, 173, 174, 181, 182, 183, 184, 185, 188, 189, 190, 194, 195, 196, or 197.

[00136] Embodiment 7 is the isolated polynucleotide of embodiment 6, wherein the antigen binding domain is humanized and comprises:

(1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:142, and a light chain variable region having the polypeptide sequence of SEQ ID NO:144;

(2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:142, and a light chain variable region having the polypeptide sequence of SEQ ID NO:145;

(3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:143, and a light chain variable region having the polypeptide sequence of SEQ ID NO:144;

(4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:143, and a light chain variable region having the polypeptide sequence of SEQ ID NO:145;

(5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:148;

(6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:149;

(7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:150;

(8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:148;

(9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:149;

(10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:150;

(11) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:151, and a light chain variable region having the polypeptide sequence of SEQ ID NO:153;

(12) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:152, and a light chain variable region having the polypeptide sequence of SEQ ID NO:153;

(13) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:154, and a light chain variable region having the polypeptide sequence of SEQ ID NO:157;

(14) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:155, and a light chain variable region having the polypeptide sequence of SEQ ID NO:157;

(15) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:156, and a light chain variable region having the polypeptide sequence of SEQ ID NO:158;

(16) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:159, and a light chain variable region having the polypeptide sequence of SEQ ID NO:163;

(17) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:159, and a light chain variable region having the polypeptide sequence of SEQ ID NO:164;

(18) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:160, and a light chain variable region having the polypeptide sequence of SEQ ID NO:163;

(19) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:160, and a light chain variable region having the polypeptide sequence of SEQ ID NO:164;

(20) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:161, and a light chain variable region having the polypeptide sequence of SEQ ID NO:165; or

(21) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:162, and a light chain variable region having the polypeptide sequence of SEQ ID NO:165.

[00137] Embodiment 8 is the isolated polynucleotide of any one of embodiments 1-7, wherein the antigen binding domain is a single chain variable fragment (scFv) that specifically binds human CLDN18.2, preferably human CLDN18.2.

[00138] Embodiment 9 is the isolated polynucleotide of embodiment 8, wherein the single chain variable fragment (scFv) is humanized and comprises a polypeptide sequence at least 95% identical to any one of SEQ ID NOs: 198-215.

[00139] Embodiment 10 is the isolated polynucleotide of any one of embodiments 1-9, wherein the chimeric antigen receptor (CAR) comprise one or more antigen binding domains.

[00140] Embodiment 11 is the isolated polynucleotide of any one of embodiments 1-10, wherein the intracellular signaling domain of the CAR comprises one or more costimulatory domains and one or more activating domains.

[00141] Embodiment 12 is a chimeric antigen receptor (CAR) encoded by the isolated polynucleotide of any one of embodiments 1-11.

[00142] Embodiment 13 is a vector comprising the isolated polynucleotide of any one of embodiments 1-11.

[00143] Embodiment 14 is a host cell comprising the vector of embodiment 13.

[00144] Embodiment 15 is the host cell of embodiment 14, wherein the cell is a CAR-T cell, preferably a human CAR-T cell.

[00145] Embodiment 16 is the host cell of embodiment 14, wherein the cell is a CAR-NK cell, preferably a human CAR-NK cell.

[00146] Embodiment 17 is a method of making a host cell expressing a chimeric antigen receptor (CAR), the method comprising transducing a T cell with the vector of embodiment 13.

[00147] Embodiment 18 is a method of producing a chimeric antigen receptor (CAR)-T cell, the method comprising culturing T cells comprising the isolated polynucleotide comprising a nucleic acid encoding a chimeric antigen receptor (CAR) of any one of embodiments 1-11 under conditions to produce the CAR-T cell and recovering the CAR-T cell.

[00148] Embodiment 19 is a method of making a host cell expressing a chimeric antigen receptor (CAR), the method comprising transducing a NK cell with the vector of embodiment 13.

[00149] Embodiment 20 is a method of producing a chimeric antigen receptor (CAR)-NK cell, the method comprising culturing NK cells comprising the isolated polynucleotide comprising a nucleic acid encoding a chimeric antigen receptor (CAR) of any one of embodiments 1-11 under conditions to produce the CAR-NK cell, and recovering the CAR-NK cell.

[00150] Embodiment 21 is a method of generating a cell comprising a chimeric antigen receptor (CAR), the method comprising contacting a cell with the isolated polynucleotide comprising a nucleic acid encoding a chimeric antigen receptor (CAR) of any one of embodiments 1-11, wherein the isolated polynucleotide is an *in vitro* transcribed RNA or synthetic RNA.

[00151] Embodiment 22 is a method of treating cancer in a subject in need thereof, the method comprising administering to the subject the host cell of any one of embodiments 14-16.

[00152] Embodiment 23 is the method of embodiment 22, wherein the cancer is selected from a lung cancer, a gastric cancer, an esophageal cancer, a bile duct cancer, a cholangiocarcinoma, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma,

a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[00153] Embodiment 24 is a method of treating an inflammatory disease in a subject in need thereof, the method comprising administering to the subject the host cell of any one of embodiments 14-16.

[00154] Embodiment 25 is the method of any one of embodiments 22-24, further comprising administering to the subject in need thereof an agent that increases the efficacy of a cell expressing a CAR molecule.

[00155] Embodiment 26 is the method of any one of embodiments 22-24, further comprising administering to the subject in need thereof an agent that ameliorates one or more side effects associated with administration of a cell expressing a CAR molecule.

[00156] Embodiment 27 is the method of any one of embodiments 22-24, further comprising administering to the subject in need thereof an agent that treats the disease associated with Claudin 18.2.

EXAMPLES

[00157] Example 1: Identification of antigen binding domains that specifically bind CLDN18.2

[00158] The antigen binding domains that specifically bind CLDN18.2 are anti-CLDN18.2 mAbs isolated and sequenced as described in PCT/US19/020872, filed on March 6, 2019, which is incorporated herein by reference in its entirety.

[00159] Sequences of heavy and light chain variable regions for the antigen binding domains that specifically bind CLDN18.2 are provided in Tables 1 and 2, and the CDR regions for the antigen binding domains that specifically bind CLDN18.2 are provided in Tables 3-6.

Table 1: Sequences of heavy chain variable regions for the antigen binding domains that specifically bind CLDN18.2

Name	VH	SEQ ID NO:
2-C3	EVQLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLEWVA TISGGGSYTYYLDSVKGRFTISRDIAKNTLYLQMSSLKSEDTAMYFCARQS RGNAMDYWGQGTSVTVSS	1
2-P8	EVQLQQSGPELVKPGASVKMSCKASGYSFTGYNMHWVKQSHGKSLEWI GYIDPYNGVTNYNQKFKGKATLTVDKSSSTAYVQLNSLTSEDSAVYYCA	3

	RWGGNYVDYWGQGTTLKVSS	
3-E21	EVQLVESGGALVKPGGSLKLSCAASGFTFSKYAMSWVRQTPEKRLEWVA FISNGGSYTYCLDSVKGRFTISRDNAKNTLYLQMSSLRSEDTALYYCARH DKGNALDYWGQGNSVTVSS	5
3-P21	EIQLQQSGAELVKPGASVKISCKASGYSFTGYNMKWVKQSHGKSLEWIG NINPYFGSTNYNQKFKGKATLTVDKSSSTAYMQLNSLTSEDSAVYYCARG AYYGNAMDYWGQGTSVTVSS	7
5-E22	KVQLQQSGPDLVEPGASVKISCKASGYTITDNYMHWVKQKPGQGLEWIG EIYPGSGNTYYNERFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARG FPYYAMDYWGPGTSVTVSS	9
6-J11	DVQLVESGGGLVQPGGSRKLSCAASGFIFSSFGMHWVRQAPEKGLEWVA YISSGRSTMYYADTVKGRFTISRDNPKNTLFLQMTSLRSEDTAMYYCARG GFYGNSLDYWGQGTSVTVSS	11
8-G12	QVQLQQSGPELVKPGASVKISCKASGYAFSDYWMNWVKQRPGKGLEWI GQIYPGYGDTKYNENFKGTATLTADKSSSTAYMQLSSLTSEDSAVYFCAR WGYYGNAMDYWGQGTSVTVSS	13
10-J10	QVQLQQPGAELVKPGASVKLSCKASGYTFTRYRMNWVKQRPGQGLEWI GNIDPSDSETHYNQKFKDKATLTVDKSSSTAYMQLSSLTSEDSAVFYCAR LNYGNCFDYWGQGTTLTVSS	15
10-K2	EVQLQQSGPELVKPGASVKMSCKASGYAFTSYVMHWVKQKPGQGLEWI GYINPYSDGTRYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCTRI YYGNAMDYWGQGTSVTVSS	17
15-D6	QVQLQQPGADLVKPGASVKLSCKASGYTFTSYWINWVKQRPGQGLEWIG NIYPGRSSTNYNEKFKSKATLTVDTSSSTAYMQLSSLASDDSAVYYCSRLS RGNAMDYWGQGTSVTVSS	19

VH: heavy chain variable region

Table 2: Sequences of light chain variable regions for the antigen binding domains that specifically bind CLDN18.2

Name	**VL**	**SEQ ID NO:**
2-C3	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIY WASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFGAGTKLE LK	2
2-P8	DIKMTQSPSSMYASLGERVTITCKASQDINRYLSWFQQKPGKSPKTLIYRANRLVD GVPSRFSGSFSGQDYSLTISSLEYEDMGIYYCLQYDEFPLTFGAGTKLELK	4
3-E21	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIY WASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLSVYYCQNDYFYPLTFGAGTKLE LK	6
3-P21	DIVMTQSPSSLTVTAGGKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIY WASTRESGVPDRFSGSGSGTDFTLTISSVQAEDLAVYYCQNDYFYPLTFGAGTKLE LK	8
5-E22	DIQMNQSPSSLSASLGDTITITCHARQNINVWLSWYQQKSGNIPKLLIYKASNLHTG VPSRFSGSGSGTRFTLTISSLQPEDMATYYCQQGQNYPLTFGGGTKLEIK	10
6-J11	DIVMTQSPSSLTVTAGEKVTMSCKSSLSLLNSGNQKNYLTWYQQKPGQPPKLLIY WASTRESGVPDRFTGSGSGTDFTLTISSMQAEDLAVYSCQNAYSYPLTFGAGTKLE LK	12
8-G12	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIY WASTRESGVPDRFTGSGSGTDFTLTISSVQTEDLAIYYCQNAYIYPLTFGAGTKLEL K	14
10-J10	DIVMTQSPSSLTVTAGEKVTMSCKSSQTLLNSGNQKNYLTWYQQKPGQPPKLLIY WASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVFYCQNDYFYPFTFGSGTKLEI K	16
10-K2	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIY WASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPFTFGSGTKLEI K	18
15-D6	DIVMTQSPSSLTVTAGEKVTMSCRSSQSLLNSGNQKSYLTWYQQKPGQPPKLLIYW	20

	ASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYYYPFTFGSGTKLEIK	

VL: light chain variable region

Table 3: CDR regions 1-3 of heavy chain for the antigen binding domains that specifically bind CLDN18.2

Name	HC CDR1	NO	HC CDR2	NO	HC CDR3	NO
2-C3	GFTFSSYG	21	ISGGGSYT	22	ARQSRGNAMDY	23
2-P8	GYSFTGYN	24	IDPYNGVT	25	ARWGGNYVDY	26
3-E21	GFTFSKYA	27	ISNGGSYT	28	ARHDKGNALDY	29
3-P21	GYSFTGYN	30	INPYFGST	31	ARGAYYGNAMDY	32
5-E22	GYTITDNY	33	IYPGSGNT	34	ARGFPYYAMDY	35
6-J11	GFIFSSFG	36	ISSGRSTM	37	ARGGFYGNSLDY	38
8-G12	GYAFSDYW	39	IYPGYGDT	40	ARWGYYGNAMDY	41
10-J10	GYTFTRYR	42	IDPSDSET	43	ARLNYGNCFDY	44
10-K2	GYAFTSYV	45	INPYSDGT	46	TRIYYGNAMDY	47
15-D6	GYTFTSYW	48	IYPGRSST	49	SRLSRGNAMDY	50

HC: heavy chain; CDR: complementarity determining region; NO: SEQ ID NO

The HC CDRs for the antigen binding domains that specifically bind CLDN18.2 were determined utilizing the IMGT method (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212).

Table 4: CDR regions 1-3 of light chain for the antigen binding domains that specifically bind CLDN18.2

Name	LC CDR1	NO	LC CDR2	NO	LC CDR3	NO
2-C3	QSLLNSGNQKNY	51	WAS	52	QNDYSYPLT	53
2-P8	QDINRY	54	RAN	55	LQYDEFPLT	56
3-E21	QSLLNSGNQKNY	57	WAS	58	QNDYFYPLT	59
3-P21	QSLLNSGNQKNY	60	WAS	61	QNDYFYPLT	62
5-E22	QNINVW	63	KAS	64	QQGQNYPLT	65
6-J11	LSLLNSGNQKNY	66	WAS	67	QNAYSYPLT	68
8-G12	QSLLNSGNQKNY	69	WAS	70	QNAYIYPLT	71
10-J10	QTLLNSGNQKNY	72	WAS	73	QNDYFYPFT	74
10-K2	QSLLNSGNQKNY	75	WAS	76	QNDYSYPFT	77
15-D6	QSLLNSGNQKSY	78	WAS	79	QNDYYYPFT	80

LC: light chain; CDR: complementarity determining region; NO: SEQ ID NO

The LC CDRs for the antigen binding domains that specifically bind CLDN18.2 were determined utilizing the IMGT method (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212).

Table 5: CDR regions 1-3 of heavy chain for the antigen binding domains that specifically bind CLDN18.2

Name	HC CDR1	NO	HC CDR2	NO	HC CDR3	NO
2-C3	GFTFSSYGMS	81	TISGGGSYTYYLDSVKG	82	ARQSRGNAMDY	83

2-P8	GYSFTGYNMH	84	YIDPYNGVTNYNQKFKG	85	ARWGGNYVDY	86
3-E21	GFTFSKYAMS	87	FISNGGSYTYCLDSVKG	88	ARHDKGNALDY	89
3-P21	GYSFTGYNMK	90	NINPYFGSTNYNQKFKG	91	ARGAYYGNAMDY	92
5-E22	GYTITDNYMH	93	EIYPGSGNTYYNERFKG	94	ARGFPYYAMDY	95
6-J11	GFIFSSFGMH	96	YISSGRSTMYYADTVKG	97	ARGGFYGNSLDY	98
8-G12	GYAFSDYWMN	99	QIYPGYGDTKYNENFKG	100	ARWGYYGNAMDY	101
10-J10	GYTFTRYRMN	102	NIDPSDSETHYNQKFKD	103	ARLNYGNCFDY	104
10-K2	GYAFTSYVMH	105	YINPYSDGTRYNEKFKG	106	TRIYYGNAMDY	107
15-D6	GYTFTSYWIN	108	NIYPGRSSTNYNEKFKS	109	SRLSRGNAMDY	110

HC: heavy chain; CDR: complementarity determining region; NO: SEQ ID NO

The HC CDRs for the antigen binding domains that specifically bind CLDN18.2 were determined utilizing a combination of IMGT (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212) and Kabat (Elvin A. Kabat et al, Sequences of Proteins of Immunological Interest 5th ed. (1991)) methods.

Table 6: CDR regions 1-3 of light chain for the antigen binding domains that specifically bind CLDN18.2

Name	**LC CDR1**	**NO**	**LC CDR2**	**NO**	**LC CDR3**	**NO**
2-C3	KSSQSLLNSGNQKNYLT	111	WASTRES	112	QNDYSYPLT	113
2-P8	KASQDINRYLS	114	RANRLVD	115	LQYDEFPLT	116
3-E21	KSSQSLLNSGNQKNYLT	117	WASTRES	118	QNDYFYPLT	119
3-P21	KSSQSLLNSGNQKNYLT	120	WASTRES	121	QNDYFYPLT	122
5-E22	HARQNINVWLS	123	KASNLHT	124	QQGQNYPLT	125
6-J11	KSSLSLLNSGNQKNYLT	126	WASTRES	127	QNAYSYPLT	128
8-G12	KSSQSLLNSGNQKNYLT	129	WASTRES	130	QNAYIYPLT	131
10-J10	KSSQTLLNSGNQKNYLT	132	WASTRES	133	QNDYFYPFT	134
10-K2	KSSQSLLNSGNQKNYLT	135	WASTRES	136	QNDYSYPFT	137
15-D6	RSSQSLLNSGNQKSYLT	138	WASTRES	139	QNDYYYPFT	140

LC: light chain; CDR: complementarity determining region; NO: SEQ ID NO

The LC CDRs for the antigen binding domains that specifically bind CLDN18.2 were determined utilizing a combination of IMGT (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212) and Kabat (Elvin A. Kabat et al, Sequences of Proteins of Immunological Interest 5th ed. (1991)) methods.

[00160] Example 2: Humanization of mouse anti-CLDN18.2 mAbs

[00161] The mouse anti-CLDN18.2 mAbs were humanized to reduce the potential of immunogenicity when used in human patients as described in PCT/US19/020872, filed on March 6, 2019, which is incorporated herein by reference in its entirety. The sequences of the humanized VH and VL regions are shown in Table 7.

Table 7: Sequences of heavy chain and light chain variable regions of humanized antigen binding domains that specifically bind CLDN18.2

VH/VL	SEQUENCE	SEQ ID NO:
2-C3-H1	QVTLRESGPALVKPTQTLTLTCTASGFTFSSYGMSWVRQPPGKALEWVATI SGGGSYTYYNPSLKDRFTISRDISANQLVLKVTNMDPADTATYFCARQSRG NAMDYWGQGTTVTVSS	142
2-C3-H2	QVTLRESGPALVKPTQTLTLTCTFSGFTFSSYGMSWIRQPPGKALEWLATIS GGGSYTYYLDSLKDRFTISRDISKNQVVLTVTNMDPADTATYFCARQSRG NAMDYWGQGTTVTVSS	143
2-C3-L1	DIQMTQSPSTLSASVGDRVTITCKSSQSLLNSGNQKNYLTWYQQKPGKAPK LLIYWASTRESGVPSRFSGSGSGTAFTLTISSLQPDDFATYYCQNDYSYPLTF GGGTKVEIK	144
2-C3-L2	DIQMTQSPSTLSASVGDRVTITCKSSQSLLNSGNQKNYLTWYQQKPGKAPK LLIYWASTRESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQNDYSYPLTF GGGTKVEIK	145
5-E22-H1	QVQLVQSGVEVKKPGASVKVSCKASGYTITDNYMHWVRQAPGQGLEWIG EIYPGSGNTYFNEKFKNRATLTADKSTTTAYMELKSLQFDDTAVYFCARGF PYYAMDYWGQGTTVTVSS	146
5-E22-H3	QVQLVQSGAEVKKPGASVKVSCKASGYTITDNYMHWVRQAPGQGLEWIG EIYPGSGNTYYAEKFKNRATLTADKSISTAYMELSRLRSDDTAVYFCARGF PYYAMDYWGQGTLVTVSS	147
5-E22-L1	EIVMTQSPATLSLSPGERATLSCHARQNINVWLSWYQQKPGQAPRLLIYKA SNLHTGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQGQNYPLTFGGGTK VEIK	148
5-E22-L2	EIVLTQSPATLSLSPGERATLSCHARQNINVWLSWYQQKPGQAPRLLIYKA SNLHTGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQGQNYPLTFGGGTK VEIK	149
5-E22-L3	DIVMTQSPLSLPVTPGEPASISCHARQNINVWLSWYLQKPGQSPQLLIYKAS NLHTGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQGQNYPLTFGQGT KVEIK	150
6-J11-H1	EVQLVESGGGLVQPGGSLRLSCAASGFIFSSFGMHWVRQAPGKGLEWVAY ISSGRSTMYYADSVKGRFTISRDNSKNTLYLQMNSLTAEDTAVYYCARGG FYGNSLDYWGQGTLVTVSS	151
6-J11-H2	EVQLVESGGGLVQPGGSLRLSCAASGFIFSSFGMHWVRQAPGKGLEWVAY ISSGRSTMYYADSVKGRFTISRDNSKNTLYLQMNSLRSEDTAVYYCARGGF YGNSLDYWGQGTLVTVSS	152
6-J11-L1	DIQMTQSPSSLSASVGDRVTITCKSSLSLLNSGNQKNYLTWYQQKPGKAPK LLIYWASTRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYSCQNAYSYPLTF GQGTKVEIK	153
3-E21-H1	QVQLQESGPGLVRPSQTLSLTCTASGFTFSKYAMNWVRQPPGRGLEWVAF ISNGGSYTEYNPSVKGRFTILRDNSKNQLSLRLSSVTAADTAVYYCARHDK GNALDYWGQGSLVTVSS	154
3-E21-H2	QVQLQESGPGLVRPSQTLSLTCTASGFTFSKYAMSWVRQPPGRGLEWVAFI SNGGSYTEYNPSVKGRFTILRDNSKNQLSLKLSSVTAADTAVYYCARHDK GNALDYWGQGSLVTVSS	155
3-E21-H3	EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMSWVRQAPGKGLEWVA AISNGGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARH DKGNALDYWGQGTLVTVSS	156
3-E21-L1	DIQMTQSPSSLSASVGDRVTITCKSSQSLLNSGNQKNYLTWYQQKPGKAPK LLIYWASNLQTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQNDYFYPLTF GQGTKVEIK	157
3-E21-L2	DIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPK LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYFYPLT FGQGTRLEIK	158
3-P21-H1	EIQLVESGGGLVQPGGSLRLSCAASGYSFTGYNIHWVRQAPGKGLEWIGYI NPYFGSTDYADSVKGRATLSVDKSKNTAYLQMNSLRAEDTAVYYCARGA YYGNAMDYWGQGTLVTVSS	159

3-P21-H2	EIQLVESGGGLVQPGGSLRLSCAASGYSFTGYNMKWVRQAPGKGLEWIGN INPYFGSTNYADSVKGRATLSVDKSKNTAYLQMNSLRAEDTAVYYCARG AYYGNAMDYWGQGTLVTVSS	160
3-P21-H3	EIQLVQSGAEVKKPGESLKISCKASGYSFTGYNIGWVRQMPGKGLEWIGIIN PYFGSTRYSPSFQGQATLSVDKSISTAYLQWSSLKASDTAMYYCARGAYY GNAMDYWGQGTLVTVSS	161
3-P21-H4	EIQLVQSGAEVKKPGESLKISCKASGYSFTGYNMKWVRQMPGKGLEWIGII NPYFGSTNYSPSFQGQATLSVDKSISTAYLQWSSLKASDTAMYYCARGAY YGNAMDYWGQGTLVTVSS	162
3-P21-L1	DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKNYVTWYQQKPGKAPK LLIYWASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYFYPLTF GQGTKVEIK	163
3-P21-L2	DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKNYVTWYQQKPGKAPK LLIYWASTRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYFYPLTF GQGTKVEIK	164
3-P21-L3	DIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPK LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYFYPLT FGQGTKVEIK	165
8-G12-H1	EVQLVESGGGLVQPGGSLRLSCAASGYAFSDYWMNWVRQAPGKGLEWIG QIYPGYGDTKHNQRFMDRATLSADKSTSTAYMQMNSLRAEDTAVYFCAR WGYYGNAMDYWGQGTLVTVSS	166
8-G12-H2	QVQLVQSGAEVKKPGASVKVSCKASGYAFSDYWMNWVRQAPGQGLEWI GQIYPGYGDTKYAQKFQGRATLTADKSISTAYMELSRLRSDDTAVYFCAR WGYYGNAMDYWGQGTLVTVSS	167
8-G12-L1	DIQMTQSPSSLSASVGDRVTITCKSSQSLLNSGNQKNYLTWYQQKPGKAPK LLIYWASTRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNAYIYPLTF GQGTKVEIK	168
8-G12-L2	DIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPK LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNAYIYPLT FGGGTKVEIK	169
10-K2-H1	EVQLVESGGGLVQPGGSLRLSCAASGYAFTSYVMHWVRQAPGKGLEWIG YINPYSDGTRHNQRFMDRATLSSDKSTSTAYMQMNSLRAEDTAVYYCTRI YYGNAMDYWGQGTLVTVSS	170
10-K2-H2	QVQLVQSGAEVRKPGASVTVSCKASGYAFTSYVMHWVRQAPGQGLEWIG YINPYSDGTRFAQKFKGRATLTSDKSTSTAFMELSSLRSDDTAIYYCTRIYY GNAMDYWGQGTLVTVSS	171
10-K2-H3	QVQLVQSGAEVKKPGASVKVSCKASGYAFTSYVMHWVRQAPGQGLEWI GYINPYSDGTRFAQKFKGRVTLTSDKSTSTAYMELSSLRSDDTAVYYCTRI YYGNAMDYWGQGTLVTVSS	172
10-K2-L1	DIQMTQSPSSLSASVGDRVTITCKSSQSLLNSGNQKNYLTWYQQKPGKAPK LLIYWASTRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYSYPFTF GQGTKVEIK	173
10-K2-L2	DIVMTQSPLSLPVTPGEAASISCKSSQSLLNSGNQKNYLTWYLQKPGQSPQL LIYWASTRESGVPHRFSGSGSGTEFTLKISRVEAEDVGVYYCQNDYSYPFTF GQGTKVEIK	174
15-D6-H1	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWINWVRQAPGKGLEWIGD IYPGRSSTNYNQNFKDRATLSVDTSKNTAYLQMNSLRAEDTAVYYCSRLS RGNAMDYWGQGTLVTVSS	175
15-D6-H2	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWINWVRQAPGKGLEWIGD IYPGRSSTNYNQNFKGRATLSVDTSKNTAYLQMNSLRAEDTAVYYCSRLS RGNAMDYWGQGTLVTVSS	176
15-D6-H3	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWINWVRQAPGKGLEWIGN IYPGRSSTNYNQNFKGRATLSVDTSKNTAYLQMNSLRAEDTAVYYCSRLS RGNAMDYWGQGTLVTVSS	177
15-D6-H4	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWIHWVRQAPGKGLEWIGY IYPGRSSTNYNEKFKGRATLSVDTSKNTAYLQMNSLRAEDTAVYYCSRLSR GNAMDYWGQGTLVTVSS	178
15-D6-H5	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWINWVRQAPGKGLEWIGY IYPGRSSTNYNEKFKGRATLSVDTSKNTAYLQMNSLRAEDTAVYYCSRLSR GNAMDYWGQGTLVTVSS	179

15-D6-H6	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWINWVRQAPGKGLEWIGN IYPGRSSTNYNEKFKGRATLSVDTSKNTAYLQMNSLRAEDTAVYYCSRLSR GNAMDYWGQGTLVTVSS	180
15-D6-L1	DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKSYMTWYQQKPGKAPK LLIYWASNHASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYYYPFT FGQGTKVEIK	181
15-D6-L2	DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKSYMTWYQQKPGKAPK LLIYWASTRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYYYPFTF GQGTKVEIK	182
15-D6-L3	DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKSYLTWYQQKPGKAPK LLIYWASNHASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYYYPFT FGQGTKVEIK	183
15-D6-L4	DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKSYVTWYQQKPGKAPK LLIYWASHRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYYYPFT FGQGTKVEIK	184
15-D6-L5	DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKSYVTWYQQKPGKAPK LLIYWASHRYTGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQNDYYYPFT FGQGTKVEIK	185
10-J10-H1	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTRYRISWVRQAPGQGLEWIGG IDPSDSETNYAQKFQGRATLTVDKSTSTAYMELSSLRSEDTAVYYCARLNY GNCFDYWGQGTLVTVSS	186
10-J10-H2	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTRYRISWVRQAPGQGLEWIGG IDPSDSETNYAQKFQGRATLTADKSTSTAYMELSSLRSEDTAVYYCARLNY GNCFDYWGQGTLVTVSS	187
10-J10-L1	DIVMTQSPDSLAVSLGERATINCKSSQTLLNSGNQKNYLTWYQQKPGQPPK LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYFYPFT FGQGTRLEIK	188
10-J10-L2	DIVMTQSPDSLAVSLGERATINCKSSQTLLNSGNQKNYLAWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYFYPF TFGQGTKVEIK	189
10-J10-L3	DIVMTQSPDSLAVSLGERATINCKSSQTLLNSGNQKNYLTWYQQKPGQPPK LLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYFYPFT FGQGTKVEIK	190
2-P8-H1	QVQLVQSGAEVKKPGSSVKVSCKASGYSFTGYNLHWVRQAPGQGLEWIG WIDPYNGVTQYNEKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYYCAR WGGNYVDYWGQGTTVTVSS	191
2-P8-H2	QVQLVQSGAEVKKPGASVKVSCKASGYSFTGYNLHWVRQAPGQGLEWIG WIDPYNGVTQYNEKFKGRVTITVDKSTSTAYMELSSLRSEDTAVYYCARW GGNYVDYWGQGTTVTVSS	192
2-P8-H3	QVQLVQSGAEVKKPGSSVKVSCKASGYSFTGYNINWVRQAPGQGLEWIG WIDPYNGVTKYNEKFKGRATLTVDKSTNTAYMELSSLRSEDTAFYYCAR WGGNYVDYWGQGTLVTVSS	193
2-P8-L1	DIQMTQSPSSLSASVGDRVTITCRASQDINRYVSWFQQKPGKAPKTLIYRA NYRYSGVPSRFSGSFSGQDYTLTISSLQPEDFATYYCLQYDEFPLTFGQGTK VEIK	194
2-P8-L2	DIQMTQSPSSLSASVGDRVTITCKASQDINRYVSWFQQKPGKAPKTLIYRA NYRYSGVPSRFSGSFSGQDYTLTISSLQPEDFATYYCLQYDEFPLTFGQGTK VEIK	195
2-P8-L3	DIQMTQSPSSLSASVGDRVTITCKASQDINRYVSWFQQKPGKAPKSLIYRA NYRYSGVPSRFSGSGSGQDYTLTISSLQPEDFATYYCLQYDEFPLTFGQGTK VEIK	196
2-P8-L4	DIQMTQSPSTLSASVGDRVTITCRASQDINRYLSWFQQKPGKAPKTLIYRAN NLASGVPSRFSGSFSGQEYTLTISSLQPDDFATYYCLQYDEFPLTFGQGTKV EIK	197

[00162] The humanized VH and VL regions were fused to the constant regions of human IgG1 heavy chain and kappa light chain, respectively. The humanized mAbs were named as follows:

2-C3-H1L1 refers to the mAb with the 2-C3-H1 heavy chain variable region and the 2-C3-L1 light chain variable region; all the other humanized mAbs adopt the same naming rule.

[00163] Several humanized mAbs were tested for their ability to bind CLDN18.2 and CLDN18.1. Chimeric mAb 15-D6 was also used in the assay. Stable cell lines (HEK293-CLDN18.2 and HEK293-CLDN18.1) expressing human CLDN18.2 and CLDN18.1, respectively, were used in FACS experiments with Alexa Fluor® 488-based detection as described in PCT/US19/020872. The mAbs were tested at 10 μg/mL. The results are shown in FIGs. 1A-1B. "MFI" is "Mean Fluorescence Intensity".

[00164] Additional humanized mAbs were tested for their ability to bind CLDN18.2 using the HEK293-CLDN18.2 stable cell line and the same FACS protocol, with the modification that propidium iodide (PI) was incubated together with the secondary antibody to label dead cells. The results are shown in FIGs. 2A-2D.

[00165] Example 3: Conversion of humanized mAbs to scFvs

[00166] The humanized mAbs were converted to scFvs, each of which consists of one VH and one VL with a $(G_4S)_n$ linker in between (where "n" represents the number of the G_4S repeats). Either the VH or the VL region was placed at the N-terminus of the fusion protein to identify the most effective scFv designs. The sequences of the designed scFvs are shown in Table 8. The scFvs were named as following: 2-C3-H2$(G_4S)_3$L2 refers to the scFv with 2-C3-H2 heavy chain variable region, the $(G_4S)_3$ linker and 2-C3-L2 light chain variable region; all the other scFvs adopted the same naming rule.

Table 8: Sequences of humanized scFvs that specifically bind CLDN18.2

Name	SEQUENCE	SEQ ID NO:
2-C3-H2$(G_4S)_3$L2	QVTLRESGPALVKPTQTLTLTCTFSGFTFSSYGMSWIRQPPGKALEWLATISGGGS YTYYLDSLKDRFTISRDISKNQVVLTVTNMDPADTATYFCARQSRGNAMDYWGQ GTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSTLSASVGDRVTITCKSSQSLLNSG NQKNYLTWYQQKPGKAPKLLIYWASTRESGVPSRFSGSGSGTEFTLTISSLQPDDF ATYYCQNDYSYPLTFGGGTKVEIK	198
2-C3-H2$(G_4S)_4$L2	QVTLRESGPALVKPTQTLTLTCTFSGFTFSSYGMSWIRQPPGKALEWLATISGGGS YTYYLDSLKDRFTISRDISKNQVVLTVTNMDPADTATYFCARQSRGNAMDYWGQ GTTVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSTLSASVGDRVTITCKSSQ SLLNSGNQKNYLTWYQQKPGKAPKLLIYWASTRESGVPSRFSGSGSGTEFTLTISS LQPDDFATYYCQNDYSYPLTFGGGTKVEIK	199
2-C3-L2$(G_4S)_3$H2	DIQMTQSPSTLSASVGDRVTITCKSSQSLLNSGNQKNYLTWYQQKPGKAPKLLIY WASTRESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQNDYSYPLTFGGGTKVEI KGGGGSGGGGSGGGGSQVTLRESGPALVKPTQTLTLTCTFSGFTFSSYGMSWIRQ PPGKALEWLATISGGGSYTYYLDSLKDRFTISRDISKNQVVLTVTNMDPADTATY FCARQSRGNAMDYWGQGTTVTVSS	200

6-J11-H1(G_4S)$_3$L1	EVQLVESGGGLVQPGGSLRLSCAASGFIFSSFGMHWVRQAPGKGLEWVAYISSGRSTMYYADSVKGRFTISRDNSKNTLYLQMNSLTAEDTAVYYCARGGFYGNSLDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCKSSLSLLNSGNQKNYLTWYQQKPGKAPKLLIYWASTRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYSCQNAYSYPLTFGQGTKVEIK	201
6-J11-H1(G_4S)$_4$L1	EVQLVESGGGLVQPGGSLRLSCAASGFIFSSFGMHWVRQAPGKGLEWVAYISSGRSTMYYADSVKGRFTISRDNSKNTLYLQMNSLTAEDTAVYYCARGGFYGNSLDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCKSSLSLLNSGNQKNYLTWYQQKPGKAPKLLIYWASTRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYSCQNAYSYPLTFGQGTKVEIK	202
6-J11-L1(G_4S)$_3$H1	DIQMTQSPSSLSASVGDRVTITCKSSLSLLNSGNQKNYLTWYQQKPGKAPKLLIYWASTRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYSCQNAYSYPLTFGQGTKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFIFSSFGMHWVRQAPGKGLEWVAYISSGRSTMYYADSVKGRFTISRDNSKNTLYLQMNSLTAEDTAVYYCARGGFYGNSLDYWGQGTLVTVSS	203
5-E22-H3(G_4S)$_3$L3	QVQLVQSGAEVKKPGASVKVSCKASGYTITDNYMHWVRQAPGQGLEWIGEIYPGSGNTYYAEKFKNRATLTADKSISTAYMELSRLRSDDTAVYFCARGFPYYAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTPGEPASISCHARQNINVWLSWYLQKPGQSPQLLIYKASNLHTGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQGQNYPLTFGQGTKVEIK	204
5-E22-H3(G_4S)$_4$L3	QVQLVQSGAEVKKPGASVKVSCKASGYTITDNYMHWVRQAPGQGLEWIGEIYPGSGNTYYAEKFKNRATLTADKSISTAYMELSRLRSDDTAVYFCARGFPYYAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTPGEPASISCHARQNINVWLSWYLQKPGQSPQLLIYKASNLHTGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQGQNYPLTFGQGTKVEIK	205
5-E22-L3(G_4S)$_3$H3	DIVMTQSPLSLPVTPGEPASISCHARQNINVWLSWYLQKPGQSPQLLIYKASNLHTGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQGQNYPLTFGQGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTITDNYMHWVRQAPGQGLEWIGEIYPGSGNTYYAEKFKNRATLTADKSISTAYMELSRLRSDDTAVYFCARGFPYYAMDYWGQGTLVTVSS	206
3-E21-H3(G_4S)$_3$L2	EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMSWVRQAPGKGLEWVAAISNGGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHDKGNALDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYFYPLTFGQGTRLEIK	207
3-E21-H3(G_4S)$_4$L2	EVQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMSWVRQAPGKGLEWVAAISNGGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHDKGNALDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYFYPLTFGQGTRLEIK	208
3-E21-L2(G_4S)$_3$H3	DIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYFYPLTFGQGTRLEIKGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSKYAMSWVRQAPGKGLEWVAAISNGGSYTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHDKGNALDYWGQGTLVTVSS	209
3-P21-H2(G_4S)$_3$L1	EIQLVESGGGLVQPGGSLRLSCAASGYSFTGYNMKWVRQAPGKGLEWIGNINPYFGSTNYADSVKGRATLSVDKSKNTAYLQMNSLRAEDTAVYYCARGAYYGNAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKNYVTWYQQKPGKAPKLLIYWASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYFYPLTFGQGTKVEIK	210
3-P21-H2(G_4S)$_4$L1	EIQLVESGGGLVQPGGSLRLSCAASGYSFTGYNMKWVRQAPGKGLEWIGNINPYFGSTNYADSVKGRATLSVDKSKNTAYLQMNSLRAEDTAVYYCARGAYYGNAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKNYVTWYQQKPGKAPKLLIYWASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYFYPLTFGQGTKVEIK	211
3-P21-L1(G_4S)$_3$H2	DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKNYVTWYQQKPGKAPKLLIYWASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYFYPLTFGQGTKVEIKGGGGSGGGGSGGGGSEIQLVESGGGLVQPGGSLRLSCAASGYSFTGYNMKWVRQAPGKGLEWIGNINPYFGSTNYADSVKGRATLSVDKSKNTAYLQMNSLRAEDTAVYYCARGAYYGNAMDYWGQGTLVTVSS	212

15-D6-H5(G_4S)$_3$L4	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWINWVRQAPGKGLEWIGYIYPG RSSTNYNEKFKGRATLSVDTSKNTAYLQMNSLRAEDTAVYYCSRLSRGNAMDY WGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRSSQSL LNSGNQKSYVTWYQQKPGKAPKLLIYWASHRYTGVPSRFSGSGSGTDFTLTISSL QPEDFATYYCQNDYYYPFTFGQGTKVEIK	213
15-D6-H5(G_4S)$_4$L4	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWINWVRQAPGKGLEWIGYIYPG RSSTNYNEKFKGRATLSVDTSKNTAYLQMNSLRAEDTAVYYCSRLSRGNAMDY WGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITC RSSQSLLNSGNQKSYVTWYQQKPGKAPKLLIYWASHRYTGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQNDYYYPFTFGQGTKVEIK	214
15-D6-L4(G_4S)$_3$H5	DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKSYVTWYQQKPGKAPKLLIY WASHRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNDYYYPFTFGQGTKV EIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWINW VRQAPGKGLEWIGYIYPGRSSTNYNEKFKGRATLSVDTSKNTAYLQMNSLRAED TAVYYCSRLSRGNAMDYWGQGTLVTVSS	215

[00167] Fusion proteins of scFvs fused to one (G_4S) linker and human IgG4 Fc (with the order of scFv, G_4S linker and Fc from the N-terminus to the C-terminus) were tested for their ability to bind CLDN18.2. A stable cell line (HEK293-CLDN18.2) expressing human CLDN18.2 was used in FACS experiments with Alexa Fluor® 488-based detection as described in PCT/US19/020872. Propidium iodide was incubated together with the secondary antibody to label dead cells. The binding results are shown in FIGs. 3A-3L.

[00168] Example 4: Construction of chimeric antigen receptor constructs comprising anti-CLDN18.2 antigen binding domains

[00169] To construct a CAR, the mAbs were converted into scFvs using the VH, VL and a $(G_4S)_n$ linker, and the scFv was fused to the N-terminus of the hinge and transmembrane domains derived from human CD8α (aa 114-188, Boursier JP et al., J Biol Chem. 1993;268(3):2013-20). The C-terminal intracellular signaling domain of the CAR was constructed by fusing the intracellular costimulatory domain of CD28 (aa 162-202, Aruffo A and Seed B, Proc Natl Acad Sci USA. 1987;84(23):8573-7) followed by the activation domain from CD3 zeta chain (aa 52-162, Letourneur F and Klausner RD, Proc Natl Acad Sci USA. 1991;88(20):8905-9). The DNA sequence encoding the CAR was assembled and cloned into an expression vector (either retroviral, lentiviral, extrachromosomal or integrated) to generate the CAR construct using standard molecular biology cloning techniques.

[00170] Example 5: Tumor cell killing assay to assess the activity of CAR T cells

[00171] CD4+/CD8+ T cells were isolated using the Pan T isolation kit (Miltenyi biotech, Cat#: 130-096-535), and activated for 3 days by Dynabeads™ Human T-Activator CD3/CD28 (ThermoFisher, Cat#: 11131D) in AIM V medium (ThermoFisher, Cat#: 12055083) containing 10% FBS according to the manufacture instructions. Next, active T cells were continuously

cultured for less than a week in AIM V medium containing 10% FBS and 300 IU/ml IL2 (R&D systems, Cat#: 202-IL-050) and transiently transfected with the 5E22-H3$(G4S)_3$L3 CAR expression plasmid by electroporation to obtain the CAR T cells. Following a 48-hour recovery period, the CAR T cells were used in the assay as the effector cells. Target cells HEK293-CLDN18.2 and HEK293-CLDN18.1 were stained with CFSE (ThermoFisher, Cat#: C34554) and co-cultured with the CAR T cells for 24 hours at the E/T (effector/target) ratio of 2.5:1. Next, the cells were stained with PI (ThermoFisher, Cat#: P3566) and Annexin V (Biolegend, Cat#: 640924) and analyzed by flow cytometry (Attune NxT). Only CFSE positive cells were counted. The tumor cell lysis percentages were calculated as the percentage of PI and/or Annexin V positive cells and are shown in FIG 4.

[00172] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the present description.

CLAIMS

It is claimed:

1. An isolated polynucleotide comprising a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises:

(a) an extracellular domain comprising at least one antigen binding domain that specifically binds claudin 18.2 (CLDN18.2);

(b) a hinge region;

(c) a transmembrane region; and

(d) an intracellular signaling domain.

2. The isolated polynucleotide of claim 1, wherein the antigen binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 21, 22, 23, 51, 52 and 53, respectively;

(2) SEQ ID NOs: 24, 25, 26, 54, 55 and 56, respectively;

(3) SEQ ID NOs: 27, 28, 29, 57, 58 and 59, respectively;

(4) SEQ ID NOs: 30, 31, 32, 60, 61 and 62, respectively;

(5) SEQ ID NOs: 33, 34, 35, 63, 64 and 65, respectively;

(6) SEQ ID NOs: 36, 37, 38, 66, 67 and 68, respectively;

(7) SEQ ID NOs: 39, 40, 41, 69, 70 and 71, respectively;

(8) SEQ ID NOs: 42, 43, 44, 72, 73 and 74, respectively;

(9) SEQ ID NOs: 45, 46, 47, 75, 76 and 77, respectively; or

(10) SEQ ID NOs: 48, 49, 50, 78, 79 and 80, respectively;

wherein the antigen binding domain thereof specifically binds CLDN18.2, preferably human CLDN18.2.

3. The isolated polynucleotide of claim 1, wherein the antigen binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 81, 82, 83, 111, 112 and 113, respectively;

(2) SEQ ID NOs: 84, 85, 86, 114, 115 and 116, respectively;

(3) SEQ ID NOs: 87, 88, 89, 117, 118 and 119, respectively;

(4) SEQ ID NOs: 90, 91, 92, 120, 121 and 122, respectively;

(5) SEQ ID NOs: 93, 94, 95, 123, 124 and 125, respectively;

(6) SEQ ID NOs: 96, 97, 98, 126, 127 and 128, respectively;

(7) SEQ ID NOs: 99, 100, 101, 129, 130 and 131, respectively;

(8) SEQ ID NOs: 102, 103, 104, 132, 133 and 134, respectively;

(9) SEQ ID NOs: 105, 106, 107, 135, 136 and 137, respectively; or

(10) SEQ ID NOs: 108, 109, 110, 138, 139 and 140, respectively;

wherein the antigen binding domain thereof specifically binds CLDN18.2, preferably human CLDN18.2.

4. The isolated polynucleotide of any one of claims 1-3, wherein the antigen binding domain comprises a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, or 19, or a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20.

5. The isolated polynucleotide of any one of claims 1-4, wherein the antigen binding domain comprises:

(1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

(2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;

(3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;

(4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

(5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

(6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;

(7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

(8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

(9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;

(10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20.

6. The isolated polynucleotide of any one of claims 1-3, wherein the antigen binding domain is humanized and comprises a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO: 142, 143, 146, 147, 151, 152, 154, 155, 156, 159, 160, 161, 162, 166, 167, 170, 171, 172, 175, 176, 177, 178, 179, 180, 186, 187, 191, 192, or 193, or a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO: 144, 145, 148, 149, 150, 153, 157, 158, 163, 164, 165, 168, 169, 173, 174, 181, 182, 183, 184, 185, 188, 189, 190, 194, 195, 196, or 197.

7. The isolated polynucleotide of any one of claims 1-3 and 6, wherein the antigen binding domain is humanized and comprises:

(1) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:142, and a light chain variable region having the polypeptide sequence of SEQ ID NO:144;

(2) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:142, and a light chain variable region having the polypeptide sequence of SEQ ID NO:145;

(3) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:143, and a light chain variable region having the polypeptide sequence of SEQ ID NO:144;

(4) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:143, and a light chain variable region having the polypeptide sequence of SEQ ID NO:145;

(5) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:148;

(6) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:149;

(7) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:146, and a light chain variable region having the polypeptide sequence of SEQ ID NO:150;

(8) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:148;

(9) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:149;

(10) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:147, and a light chain variable region having the polypeptide sequence of SEQ ID NO:150;

(11) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:151, and a light chain variable region having the polypeptide sequence of SEQ ID NO:153;

(12) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:152, and a light chain variable region having the polypeptide sequence of SEQ ID NO:153;

(13) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:154, and a light chain variable region having the polypeptide sequence of SEQ ID NO:157;

(14) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:155, and a light chain variable region having the polypeptide sequence of SEQ ID NO:157;

(15) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:156, and a light chain variable region having the polypeptide sequence of SEQ ID NO:158;

(16) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:159, and a light chain variable region having the polypeptide sequence of SEQ ID NO:163;

(17) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:159, and a light chain variable region having the polypeptide sequence of SEQ ID NO:164;

(18) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:160, and a light chain variable region having the polypeptide sequence of SEQ ID NO:163;

(19) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:160, and a light chain variable region having the polypeptide sequence of SEQ ID NO:164;

(20) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:161, and a light chain variable region having the polypeptide sequence of SEQ ID NO:165; or

(21) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:162, and a light chain variable region having the polypeptide sequence of SEQ ID NO:165.

8. The isolated polynucleotide of any one of claims 1-7, wherein the antigen binding domain is a single chain variable fragment (scFv) that specifically binds CLDN18.2, preferably human CLDN18.2.

9. The isolated polynucleotide of claim 8, wherein the single chain variable fragment (scFv) is humanized and comprises a polypeptide sequence at least 95% identical to any one of SEQ ID NOs: 198-215.

10. The isolated polynucleotide of any one of claims 1-9, wherein the chimeric antigen receptor (CAR) comprises one or more antigen binding domains.

11. The isolated polynucleotide of any one of claims 1-10, wherein the intracellular signaling domain comprises one or more costimulatory domains and one or more activating domains.

12. A chimeric antigen receptor (CAR) encoded by the isolated polynucleotide of any one of claims 1-11.

13. A vector comprising the isolated polynucleotide of any one of claims 1-11.

14. A host cell comprising the vector of claim 13.

15. The host cell of claim 14, wherein the host cell is a T cell, preferably a human T cell.

16. The host cell of claim 14, wherein the host cell is a NK cell, preferably a human NK cell.

17. A method of making a host cell expressing a chimeric antigen receptor (CAR), the method comprising transducing a T cell with the vector of claim 13.

18. A method of producing a chimeric antigen receptor (CAR)-T cell, the method comprising culturing T cells comprising the isolated polynucleotide comprising a nucleic acid encoding a chimeric antigen receptor (CAR) of any one of claims 1-11 under conditions to produce the CAR-T cell and recovering the CAR-T cell.

19. A method of making a host cell expressing a chimeric antigen receptor (CAR), the method comprising transducing a NK cell with a vector of claim 13.

20. A method of producing a chimeric antigen receptor (CAR)-NK cell, the method comprising culturing NK cells comprising the isolated polynucleotide comprising a nucleic acid encoding a chimeric antigen receptor (CAR) of any one of claims 1-11 under conditions to produce the CAR-NK cell, and recovering the CAR-NK cell.

21. A method of generating a cell comprising a chimeric antigen receptor (CAR), the method comprising contacting a cell with the isolated polynucleotide comprising a nucleic acid encoding a chimeric antigen receptor (CAR) of any one of claims 1-11, wherein the isolated polynucleotide is an *in vitro* transcribed RNA or synthetic RNA.

22. A method of treating cancer in a subject in need thereof, comprise administering to the subject the host cell of any one of claims 14-16.

23. The method of claim 22, wherein the cancer is selected from a lung cancer, a gastric cancer, an esophageal cancer, a bile duct cancer, a cholangiocarcinoma, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

24. A method of treating an inflammatory disease in a subject in need thereof, comprise administering to the subject the host cell of any one of claims 14-16.

25. The method of any one of claims 22-24, further comprising administering to the subject in need thereof an agent that increases the efficacy of a cell expressing a CAR.

26. The method of any one of claims 22-24, further comprising administering to the subject in need thereof an agent that ameliorates one or more side effects associated with administration of a cell expressing a CAR molecule.

27. The method of any one of claims 22-24, further comprising administering to the subject in need thereof an agent that treats the disease associated with Claudin 18.2.

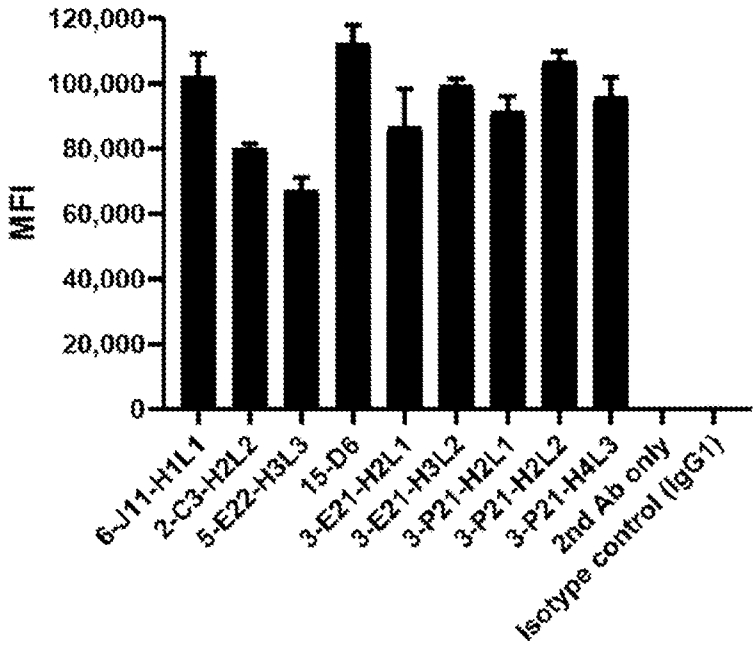
120,000
100,000
80,000
60,000
40,000
20,000
0
MFI
6-J11-H1L1
2-C3-H2L2
5-E22-H3L3
15-D6
3-E21-H2L1
3-E21-H3L2
3-P21-H2L1
3-P21-H2L2
3-P21-H4L3
2nd Ab only
Isotype control (IgG1)

FIG. 1A

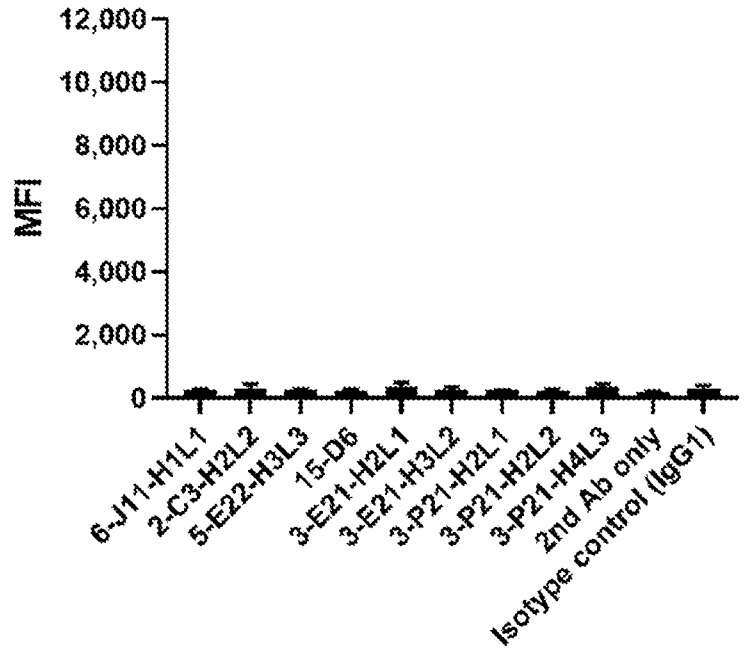
12,000
10,000
8,000
6,000
4,000
2,000
0
MFI
6-J11-H1L1
2-C3-H2L2
5-E22-H3L3
15-D6
3-E21-H2L1
3-E21-H3L2
3-P21-H2L1
3-P21-H2L2
3-P21-H4L3
2nd Ab only
Isotype control (IgG1)

FIG. 1B

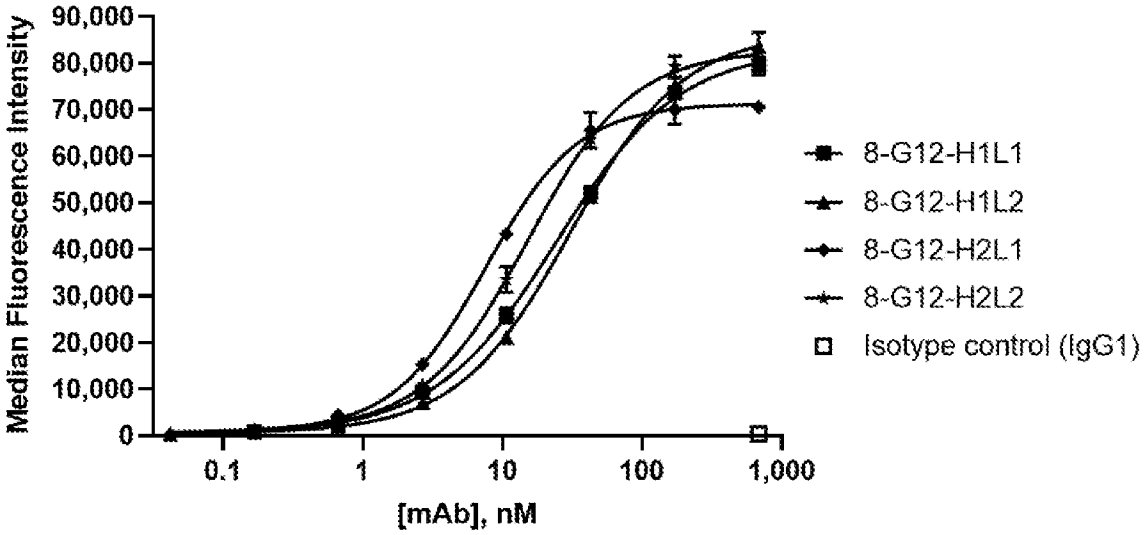
Median Fluorescence Intensity
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
0.1
1
10
100
1,000
[mAb], nM
8-G12-H1L1
8-G12-H1L2
8-G12-H2L1
8-G12-H2L2
Isotype control (IgG1)

FIG. 2A

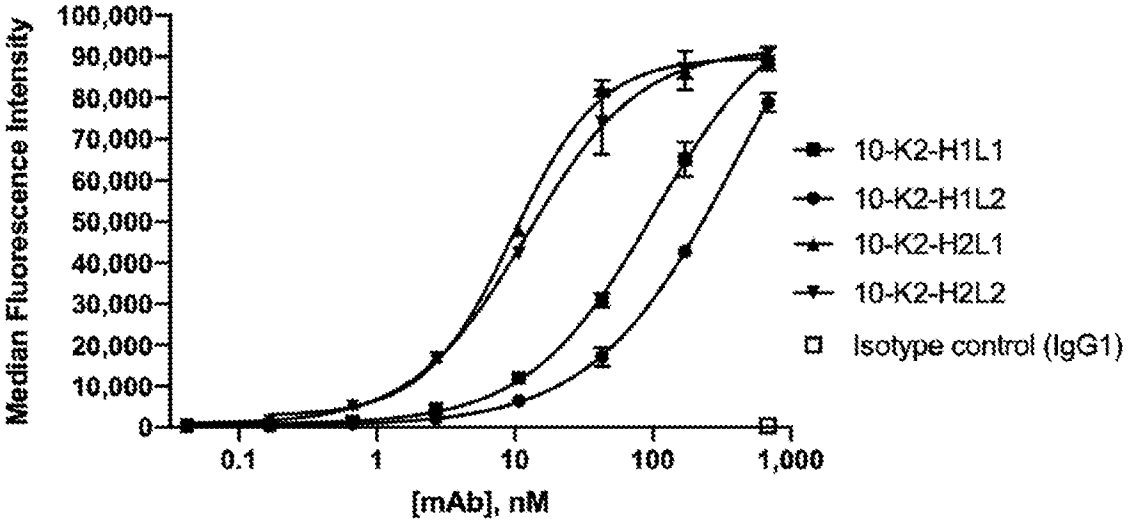
Median Fluorescence Intensity
100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
0.1
1
10
100
1,000
[mAb], nM
10-K2-H1L1
10-K2-H1L2
10-K2-H2L1
10-K2-H2L2
Isotype control (IgG1)

FIG. 2B

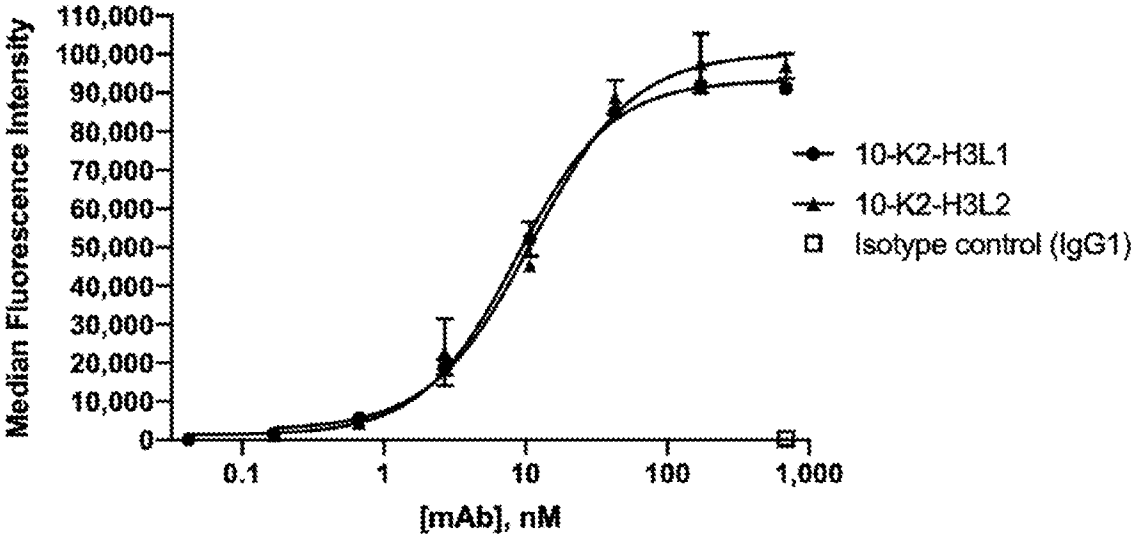
Median Fluorescence Intensity
110,000
100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
0.1
1
10
100
1,000
[mAb], nM
10-K2-H3L1
10-K2-H3L2
Isotype control (IgG1)

FIG. 2C

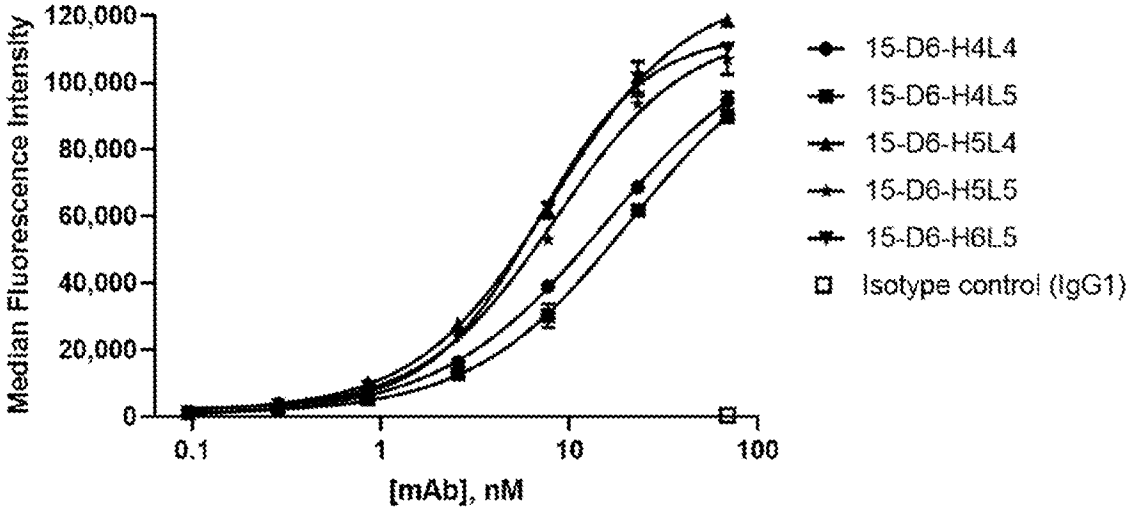
Median Fluorescence Intensity
120,000
100,000
80,000
60,000
40,000
20,000
0
0.1
1
10
100
[mAb], nM
15-D6-H4L4
15-D6-H4L5
15-D6-H5L4
15-D6-H5L5
15-D6-H6L5
Isotype control (IgG1)

FIG. 2D

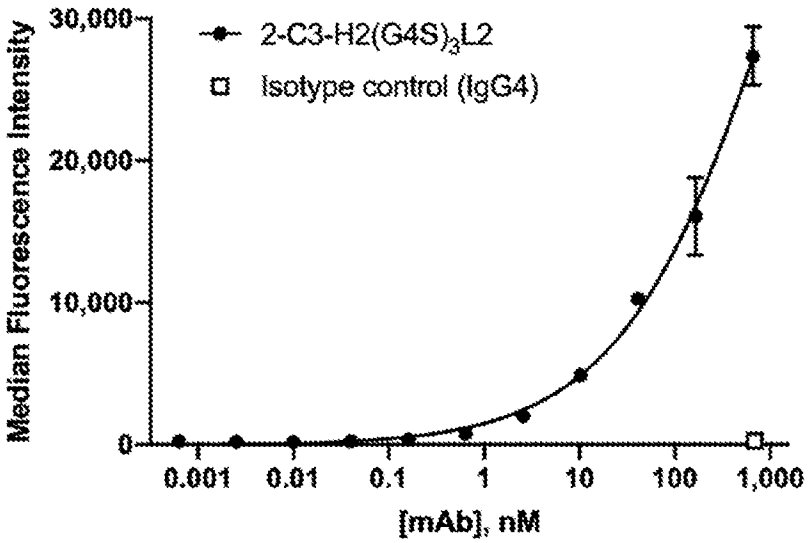
30,000
20,000
10,000
0
Median Fluorescence Intensity
2-C3-H2(G4S)3L2
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3A

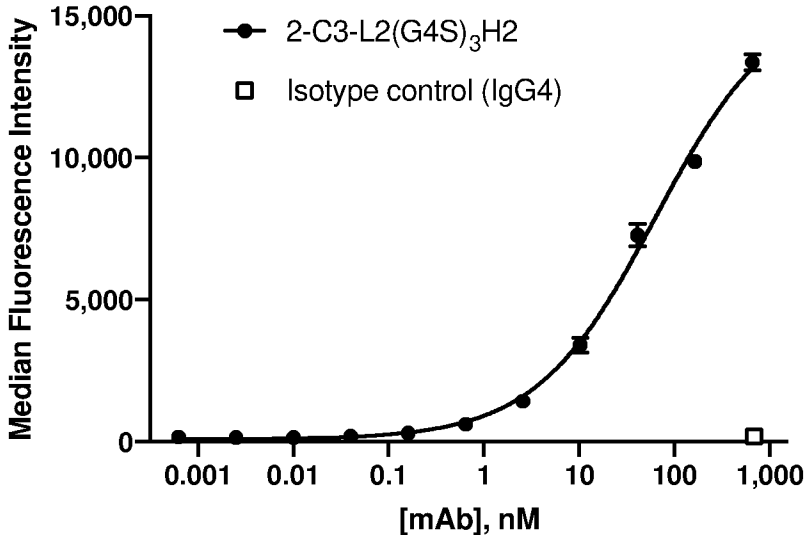
15,000
10,000
5,000
0
Median Fluorescence Intensity
2-C3-L2(G4S)3H2
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3B

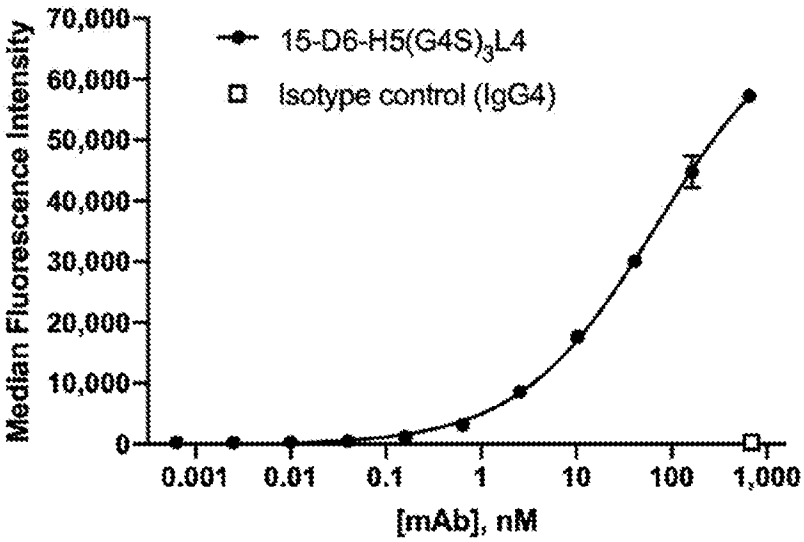
Median Fluorescence Intensity
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
15-D6-H5(G4S)3L4
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3C

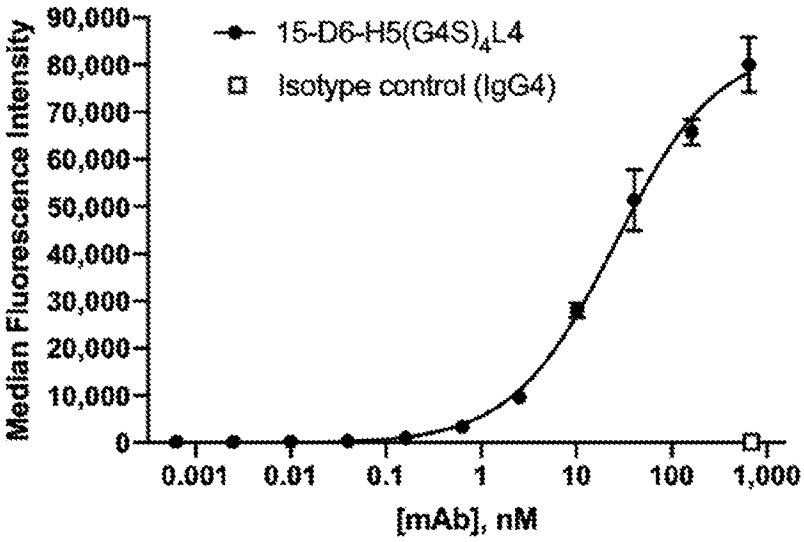
Median Fluorescence Intensity
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
15-D6-H5(G4S)4L4
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3D

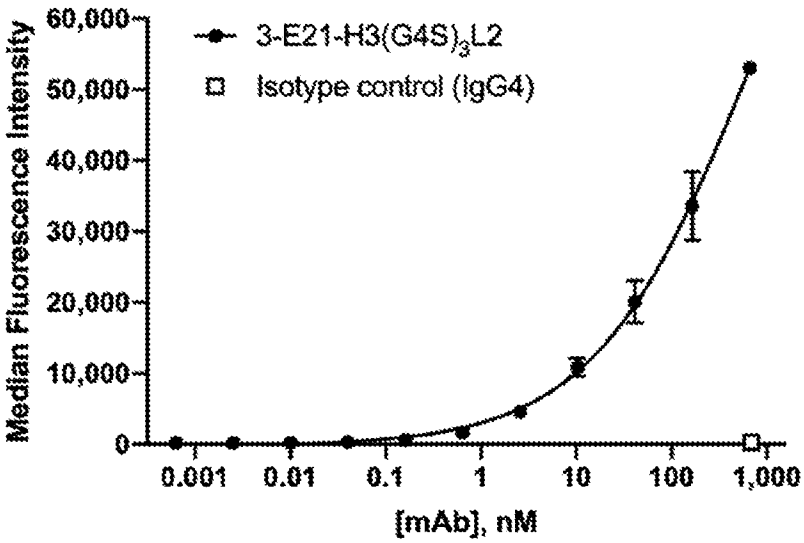
Median Fluorescence Intensity
60,000
50,000
40,000
30,000
20,000
10,000
0
3-E21-H3(G4S)3L2
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3E

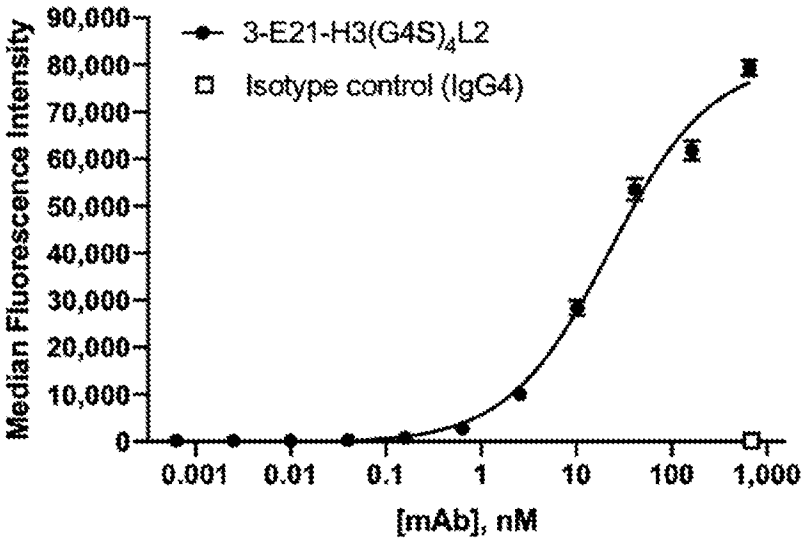
Median Fluorescence Intensity
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
3-E21-H3(G4S)4L2
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3F

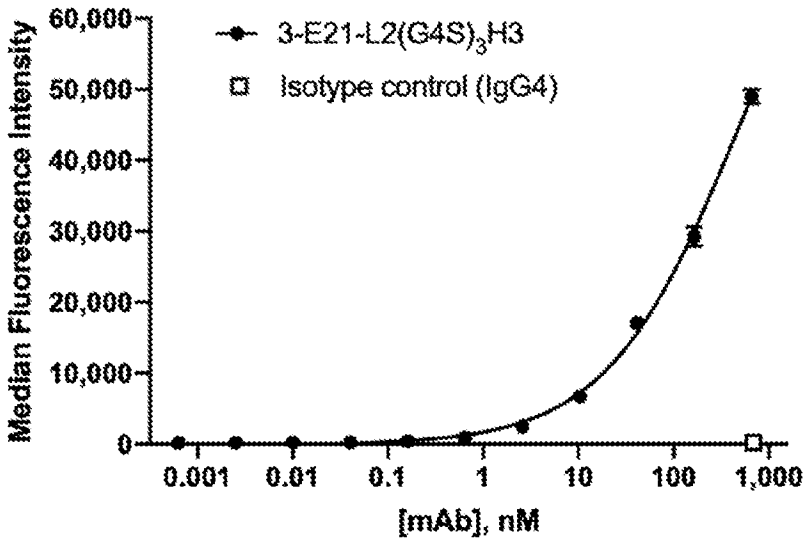
Median Fluorescence Intensity
60,000
50,000
40,000
30,000
20,000
10,000
0
3-E21-L2(G4S)3H3
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3G

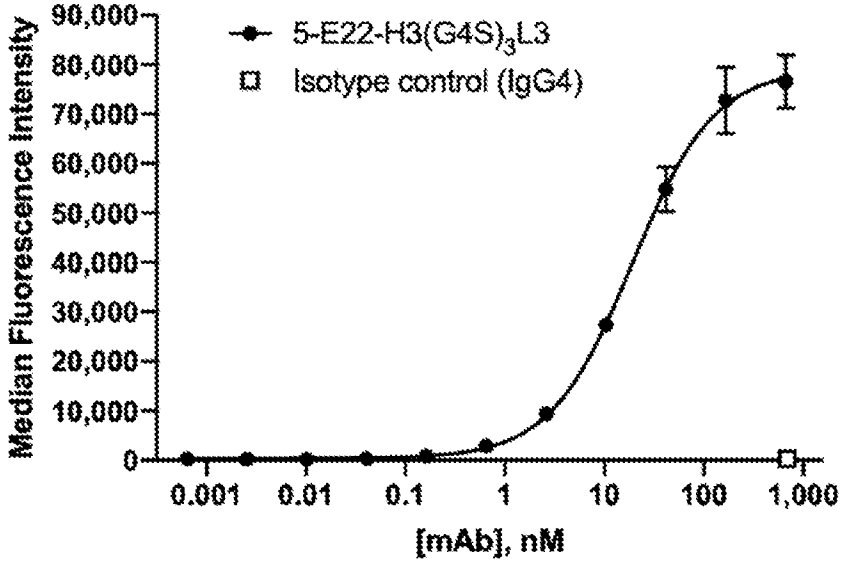
Median Fluorescence Intensity
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
5-E22-H3(G4S)3L3
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3H

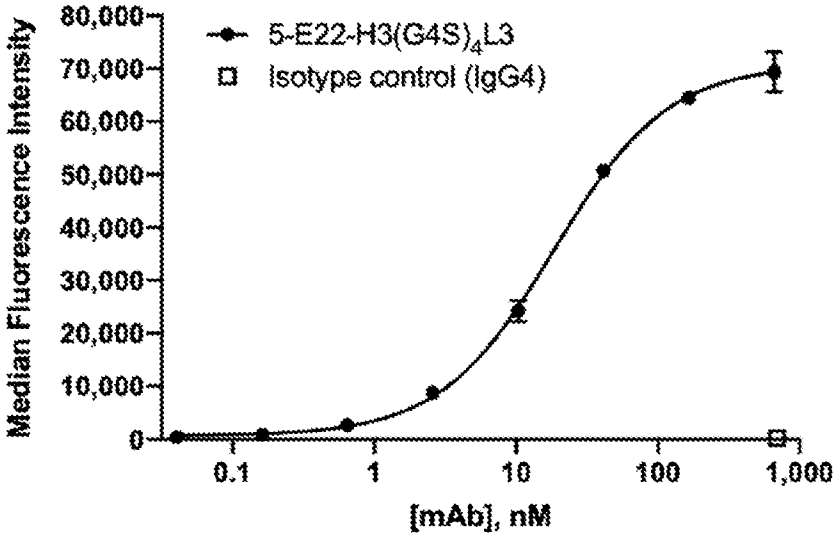
Median Fluorescence Intensity
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
5-E22-H3(G4S)4L3
Isotype control (IgG4)
0.1
1
10
100
1,000
[mAb], nM

FIG. 3I

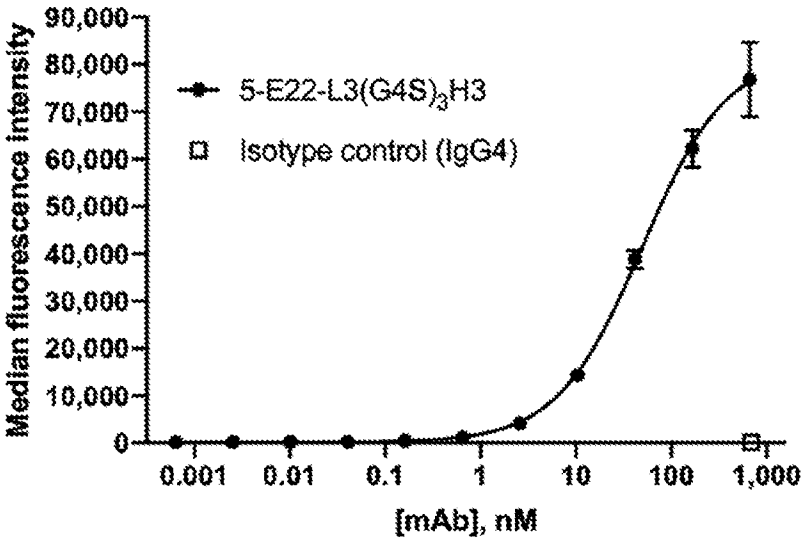
Median fluorescence intensity
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
5-E22-L3(G4S)3H3
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3J

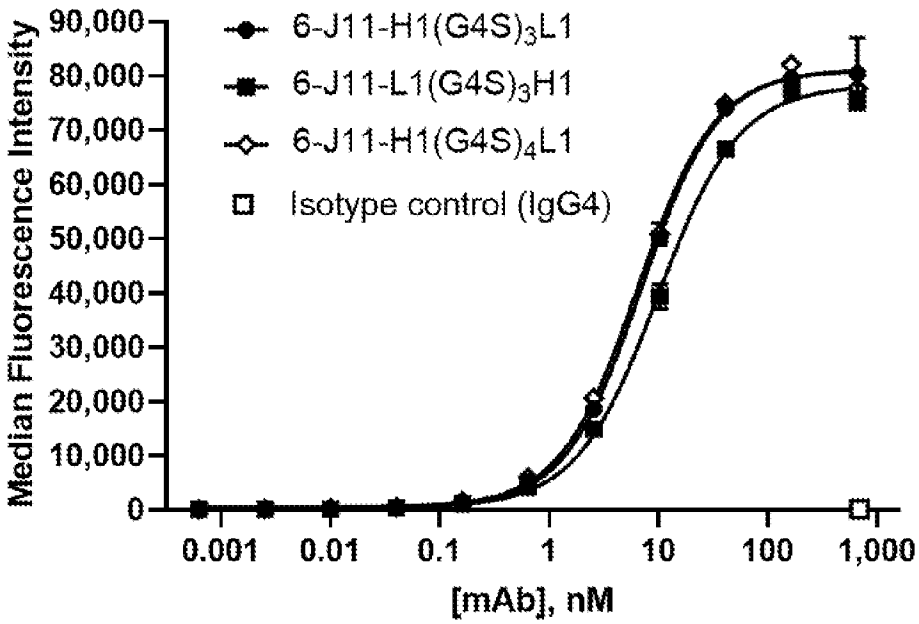
Median Fluorescence Intensity
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
6-J11-H1(G4S)3L1
6-J11-L1(G4S)3H1
6-J11-H1(G4S)4L1
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3K

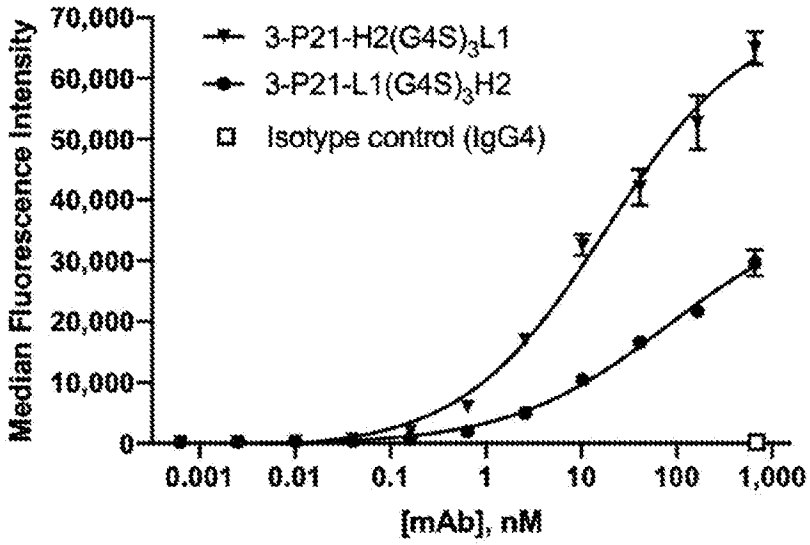
Median Fluorescence Intensity
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
3-P21-H2(G4S)3L1
3-P21-L1(G4S)3H2
Isotype control (IgG4)
0.001
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3L

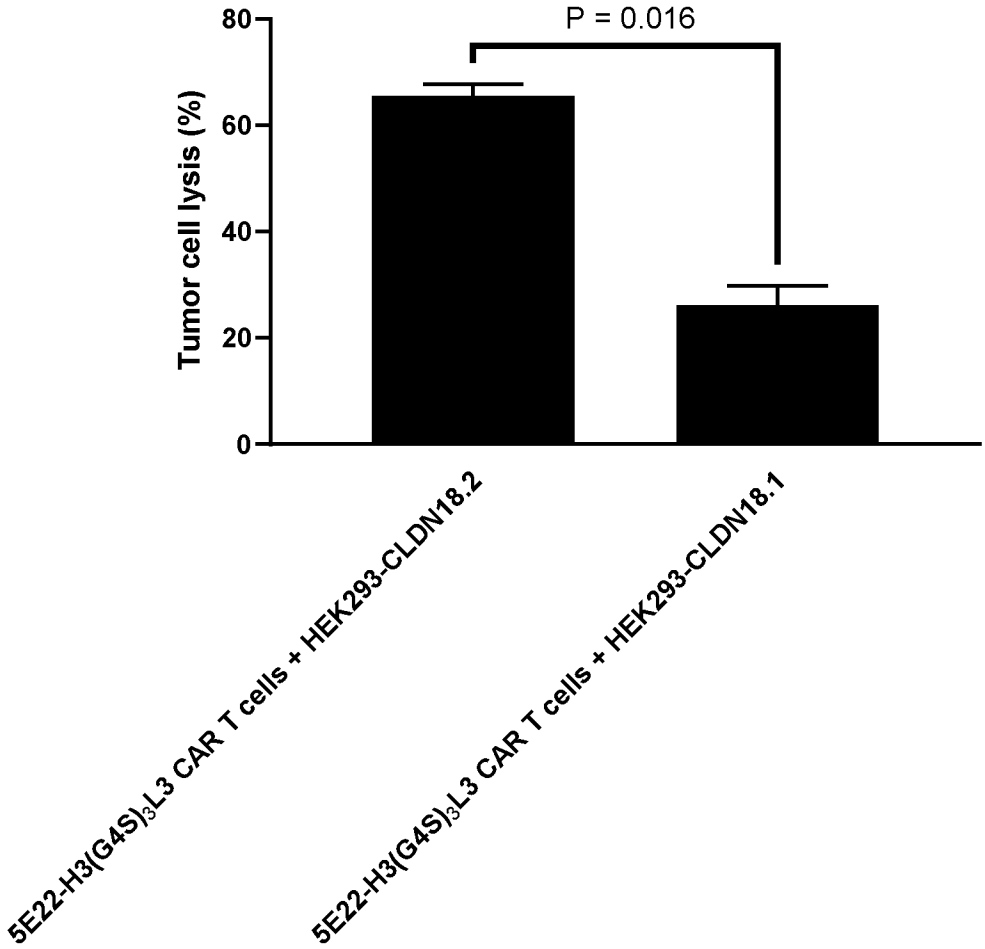
P = 0.016
Tumor cell lysis (%)
80
60
40
20
0
5E22-H3(G4S)3L3 CAR T cells + HEK293-CLDN18.2
5E22-H3(G4S)3L3 CAR T cells + HEK293-CLDN18.1

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/024432

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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

3. Additional comments:

SEQ ID NOs: 1-20 and 142-197 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/024432

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.:
because they relate to subject matter not required to be searched by this Authority, namely:

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.: 5, 7-27
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/024432

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/28; C07K 16/46; C07K 19/00; C12N 15/09; C12N 15/13; C12N 15/62 (2020.01)
CPC - C07K 16/28; C07K 2317/24; C07K 2317/56; C07K 2317/565; C07K 2317/62; C07K 2319/00; C07K 2319/03 (2020.05)

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)
see Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2018/0282389 A1 (BIONTECH CELL & GENE THERAPIES GMBH et al) 04 October 2018 (04.10.2018) entire document	1 ----- 4, 6
Y	US 2018/0326059 A1 (GANYMED PHARMACEUTICALS AG et al) 15 November 2018 (15.11.2018) entire document	4
Y	CA 3030257 A1 (CARSGEN THERAPEUTICS CO LTD et al) 11 January 2018 (11.01.2018) entire document	6
A	US 2016/0272711 A1 (BIONTECH AG et al) 22 September 2016 (22.09.2016) entire document	1-4, 6
P, A	WO 2019/173420 A1 (PHANES THERAPEUTICS, INC.) 12 September 2019 (12.09.2019) entire document	1-4, 6

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

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Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2020	06 AUG 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver Telephone No. PCT Helpdesk: 571-272-4300