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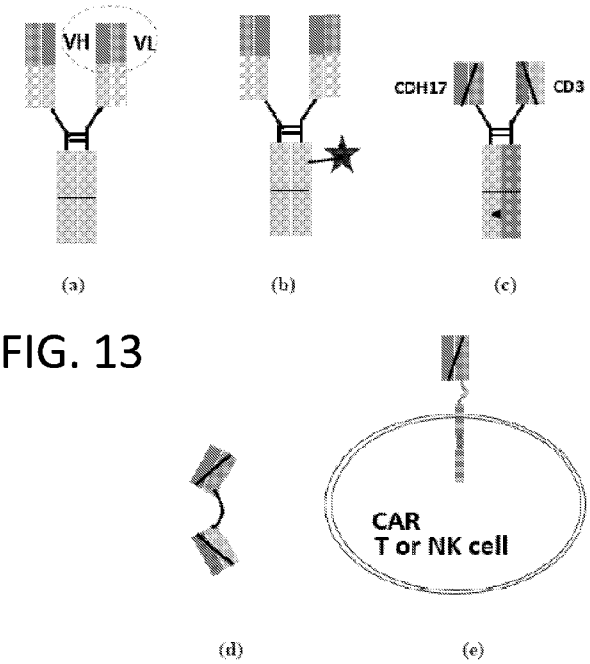

FIG. 13

(57) Abstract: Embodiments of the present disclosure relate to cadherin-17 specific antibodies and cytotoxic cells for cancer treatment. For example, an antibody may have specificity for cadherin-17 and include an amino acid sequence having at least 70% similarity with an amino acid sequence selected from SEQ ID NO: 1-21.

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CADHERIN-17 SPECIFIC ANTIBODIES AND CYTOTOXIC CELLS FOR CANCER TREATMENT

CROSS REFERENCE TO RELATED PATENT APPLICATIONS

This application claims priority over U.S. Provisional Application No. 62,276,855, filed January 9, 2016, titled "Cadherin-17 specific antibodies and cytotoxic cells for cancer treatment," which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

The present disclosure generally relates to the technical field of cancer immunotherapy, and more particularly to cadherin-17 specific antibodies and cytotoxic cells for cancer treatment.

BACKGROUND

Despite the recent advances in drug discovery and clinical imaging, cancer remains one of the deadliest diseases in humans. Our understandings on how tumor initiates, survives under stress, colonizes/metastasizes to distant organs and sites, and becomes resistant to drugs are still limited. The American Cancer Society estimated new cases of cancer in the US in 2014 is 1.6 million, with no approved curative treatment for most of the predominant types of cancer. In China, cancers are among the top killers with increasing incidences and death rates, which are believed attributable to the viral/bacterial endemic (Hepatitis B virus [HBV] and *Helicobacter pylori* infections), environmental pollutions and food contaminations.

Stomach and liver cancers are among the most lethal of malignancies worldwide and over half of the incidences diagnosed in China, causing >1.42 million deaths per year globally, yet there is no effective therapy. New biomarkers and therapeutic targets are thus needed for potential drug development against these aggressive cancers, in particular for those in advanced stages. A proven molecular targeting agent that can eliminate or repress the growth of these two cancers will have important clinical values and significant market impact. These tumors can be resected effectively by surgery if the diseases are diagnosed in early stages. Unfortunately, and very often, most of these cancers are asymptomatic and detected at very advanced

stages when presented in the clinic. Without effective treatment, these patients die shortly after the diagnosis or relapse after salvage therapies.

CART-cell treatments are a kind of adoptive cell therapy that comprises the *ex vivo* modification of T cells to direct an anti-cancer response. CAR-modified T cells can be engineered to target virtually any tumor associated antigen.

Almost all the early-stage CART studies focus on blood cancers including the proof-of-concept study of CART19 (CAR-T cells targeting CD19 antigen) in pediatric acute lymphoblastic leukemia. It remains to be seen whether CAR-T cell therapies and related technologies can repeat the same efficacy in solid tumor indications, such as liver, stomach and lung cancers. Currently, there is a total of 72 clinical trials registered at www.clinicaltrials.gov and 15 studies enrolled in China. Over half of the studies focus on blood cancers with CD19 the most common CAR-T target, followed by GD2 and Her2 specific for a brain tumor and an array of Her2-positive cancers. Thus, there is a huge gap on CAR-T immunotherapy for HCC and GC.

Today, there is only one registered CAR-T phase 1 trial for treating patients with advanced HCC using, KJgpc3-001, a genetically modified T cell which expresses a third-generation GPC3-targeted CAR. GPC3 (glypican-3) is highly expressed in HCC but the limited expression in normal tissues (63.6% vs. 9.2%). Unfortunately, no partial or complete response was observed in the phase I study of anti-GPC3 mAb GC33 (developed by Chugai, a subsidiary of Roche). KJgpc3-001 comprises anti-GPC3 scFv derived from GC33 and an intracellular signaling domain derived from CD28/4-1BB/CD3ζ. KJgpc3-001 could be transduced into patient's T cells with the lentiviral vector to attack the liver cancer. In preclinical studies, all mice treated with KJgpc3-001 survived for longer than 60 days, while the median survival of the saline-treated mice was 33 days. A major concern about KJgpc3-001 is the expression of GPC3 in normal tissues such as gastric glands, kidney tubules, and germ cells. Previously, one patient with breast cancer died after the treatment with HER2-targeted CAR due to the expression of HER2 in lung tissues. The patient experienced respiratory distress within 15 minutes after cell infusion and died 5 days after treatment. Therefore, there is a need for cancer immunotherapy using cadherin-17 specific antibodies and cytotoxic cells for cancer treatment.

SUMMARY

The disclosure provides cadherin-17 specific antibodies and cytotoxic cells.

Embodiments of the present disclosure relate to an antibody having specificity for cadherin-17, comprising an amino acid sequence having at least 70% similarity with an amino acid sequence selected from SEQ ID NO: 1-21.

In some embodiments, the antibody may include an amino acid sequence having at least 80% similarity with an amino acid sequence selected from SEQ ID NO: 1-21.

In some embodiments, the antibody may include an amino acid sequence having at least 95% similarity with an amino acid sequence selected from SEQ ID NO. 1-21.

In some embodiments, the monoclonal antibody is a mouse antibody, a humanized antibody, or a human antibody. In some embodiments, the monoclonal antibody is a human antibody isolated from a phage library screen.

In some embodiments, the antibody may include a variable region of light chain (VL), a variable region of heavy chain (VH), and the VL may include an amino acid sequence having at least 90% similarity with an amino acid sequence selected from SEQ ID NO: 1, 3, 6, 8, 10, 12, 14, and 16. In some embodiments, the VH may include an amino acid sequence having at least 90% similarity with an amino acid sequence selected from SEQ ID NO: 2, 4, 5, 7, 9, 11, 13, and 15.

In some embodiments, the antibody may include a conjugated cytotoxic moiety. In some embodiments, the conjugated cytotoxic moiety may include irinotecan, auristatins, PBDs, maytansines, amantins, spliceosome inhibitors, or a combination thereof. In some embodiments, the conjugated cytotoxic moiety may include a chemotherapeutic agent.

In some embodiments, the antibody is a bispecific antibody.

In some embodiments, the antibody may include specificity for a cell receptor from a cytotoxic T or NK cell. In some embodiments, the antibody is a bispecific antibody having specificity for both cadherin-17 and CD3. In some embodiments, the cell receptor may include 4-1BB, OX40, CD27, CD40, TIM-1, CD28, HVEM, GITR, ICOS, IL12receptor, IL14 receptor, or a derivative or combination thereof.

In some embodiments, the antibody may include a first single-chain variable

fragment (ScFv) having specificity for cadherin-17 and a second sing-chain variable fragment (ScFv) having specificity for CD3, wherein the first ScFv may include a first VH and a first VL, the second ScFv may include a second VH and a second VL. In some embodiments, the first VH may include an amino acid sequence selected from SEQ ID NO: 2, 4, 5, 7, 9, 11, 13, and 15. In some embodiments, the first VL may include an amino acid sequence selected from SEQ ID NO: 1, 3, 6, 8, 10, 12, 14, and 16.

In some embodiments, the second VH may include a corresponding portion of an amino acid sequence of SEQ ID NO: 18.

In some embodiments, the second VL may include a corresponding portion of an amino acid sequence of SEQ ID NO: 18.

In some embodiments, the antibody may include specificity for an immune checkpoint inhibitor. In some embodiments, the checkpoint inhibitor may include PD-1, TIM-3, LAG-3, TIGIT, CTLA-4, PD-L1, BTLA, VISTA, or a combination thereof.

In some embodiments, the antibody may include specificity for an angiogenic factor. In some embodiments, the angiogenic factor may include VEGF.

In some embodiments, the antibody is configured to antagonize the binding of the RGD site in cadherin-17 domain 6 to integrin. In some embodiments, the integrin may include alpha2beta1.

In some embodiments, the antibody is a monoclonal antibody.

Some embodiments of the present disclosure relate to an IgG heavy chain for an antibody, comprising an amino acid sequence having a sequence selected from SEQ ID NO: 2, 4, 5, 7, 9, 11, 13, and 15.

Some embodiments of the present disclosure relate to a light chain for an antibody, comprising an amino acid sequence having a sequence selected from SEQ ID NO: 1, 3, 6, 8, 10, 12, 14, and 16.

Some embodiments of the present disclosure relate to a variable chain for an antibody, comprising an amino acid sequence selected from SEQ ID NO: 1-16.

Some embodiments of the present disclosure relate to a scFv or Fab having specificity for cadherin-17, comprising an amino acid sequence having at least 90% similarity with an amino acid sequence selected from SEQ ID NO: 1-21.

In some embodiments, the scFv or Fab may include specificity for a cell receptor from a cytotoxic T or NK cell. In some embodiments, the scFv or Fab may

include specificity for an immune checkpoint inhibitor. In some embodiments, the scFv or Fab may include specificity for an angiogenic factor.

Some embodiments of the present disclosure relate to a T or NK cell having specificity for cadherin-17, wherein the T or NK cell may include a chimeric antigen receptor, wherein the chimeric antigen receptor may include an amino acid sequence having at least 90% similarity with an amino acid sequence selected from SEQ ID NO: 1-21.

In some embodiments, the chimeric antigen receptor may include an amino acid sequence selected from SEQ ID NO: 1-16.

Some embodiments of the present disclosure relate to an isolated nucleic acid encoding the antibody, the IgG heavy Chain, the light chain, the variable chain, or the ScFv or Fab as described above.

Some embodiments of the present disclosure relate to an expression vector comprising the isolated nucleic acid of those described above. In some embodiments, the vector is expressible in a cell.

Some embodiments of the present disclosure relate to a host cell comprising the nucleic acid as described above.

Some embodiments of the present disclosure relate to a host cell comprising the expression vector as described above.

In some embodiments, the host cell is a prokaryotic cell or a eukaryotic cell.

Some embodiments of the present disclosure relate to a pharmaceutical composition, comprising the antibody of Claim 1-26 and a cytotoxic agent.

In some embodiments, the cytotoxic agent may include cisplatin, gemcitabine, irinotecan, or an anti-tumor antibody.

In some embodiments, the pharmaceutical composition may include the antibody as described above and a pharmaceutically acceptable carrier.

Some embodiments of the present disclosure relate to a method for treating a subject having cancer, comprising administering to the subject an effective amount of the antibody of Claim 1-26 or the T or NK cell of Claims 34-35.

In some embodiments, the cancer is liver cancer, gastric cancer, colon cancer, pancreatic cancer, lung cancer, or a combination thereof.

The objectives and advantages of the disclosure may become apparent from

the following detailed description of preferred embodiments thereof in connection with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments according to the present disclosure may now be described with reference to the FIGs, in which like reference numerals denote like elements.

FIG. 1 shows murine VL and VH sequences of Lic3 and Lic5 (Please note that Lic3 sequences have been updated, i.e. SEQ ID NO. 1 and SEQ ID NO. 2 in prf need to be replaced accordingly).

FIG. 2 shows a homology of Lic3 VL sequence to human VL 2-30/J1 for humanization and humanized variants that are indicated by Hum, where dots indicate the human amino acid and back mutations are indicated by the single amino acid annotation.

FIG. 3 shows a homology of Lic3 VL sequence to human VL 2-24/J1 and Lic3 Vk sequence to human VH Vk2-29 for humanization and humanized variants that are indicated by Hum, where dots indicate the human amino acid and back mutations are indicated by the single amino acid annotation.

FIG. 4 shows a homology of Lic3 VH sequence to human VH 3-11/J4 for humanization and humanized variants that are indicated by Hum, where dots indicate the human amino acid, and back mutations are indicated by the single amino acid annotation.

FIG. 5 shows a homology of Lic3 VH sequence to human VH 3-30/J4 for humanization and humanized variants that are indicated by Hum, where dots indicate the human amino acid, and back mutations are indicated by the single amino acid annotation.

FIG. 6 shows a homology of Lic5 VL sequence to human VL IGKV 2-30 for humanization and humanized variants that are indicated by Hum, where dots indicate the human amino acid, and back mutations are indicated by the single amino acid annotation.

FIG. 7 shows a homology of Lic5 VL sequence to human VL pdb4X0K for humanization and humanized variants that are indicated by Hum, where dots indicate

the human amino acid and back mutations are indicated by the single amino acid annotation.

FIG. 8 shows a homology of Lic5 VH sequence to human VH IGHV3-21 for humanization and humanized variants that are indicated by Hum, where dots indicate the human amino acid, and back mutations are indicated by the single amino acid annotation.

FIG. 9 shows a homology of Lic5 VH sequence to human VH IGHV3-7 for humanization and humanized variants that are indicated by Hum, where dots indicate the human amino acid, and back mutations are indicated by the single amino acid annotation.

FIG. 10 shows a flowchart illustrating the process of isolating human cadherin-17 specific antibodies from an antibody phage library, where human cadherin-17 specific antibodies were isolated from a phage library by multiple rounds of binding to immobilized CDH17 domains 1-3.

FIG. 11 is a graph showing examples of human antibodies isolated from the antibody phage library binding to CDH17.

FIG. 12 shows human CDH17 antibody VL and VH sequences from phage library screen.

FIG. 13 shows a panel of exemplary functional domain configurations for cancer immunotherapeutics generated from CDH17 antibody VH and VL; (a) CDH17 specific antibody; (b) CDH17 antibody drug conjugate; (c) scFv-Fc bispecific antibody (knob in hole); (d) scFv-Fc bispecific antibody (bite); and (e) CAR T or NK cell.

FIG. 14 shows the amino acid sequences of an exemplary CDH17-CD3 bispecific antibody (Knob in hole).

FIG. 15 is a graph showing binding of Lic5 scFv to CDH17 by ELISA.

FIG. 16 is a graph showing binding of Lic3 scFv to CDH17 by ELISA.

FIG. 17 is a graph showing affinity of Lic3 and Lic5 scFv as determined by ELISA, where scFv lic 3 (kd): 11.88 nM, and scFv lic5: 22.06 nM.

FIG. 18 presents photographs showing the internalization of Lic5 into human gastric cancer cells as candidate for antibody drug conjugate; (a) photographs showing the staining of DAPI (blue) and Lic5-FITC (green) at indicated times; (b) photographs showing the merged staining of DAPI (blue) and Lic5-FITC (green) at indicated times;

Lic5-FITC (green) internalization in Oum1 human gastric cancer cells over 30 mins at 37°C was determined by confocal microscopy; nucleus was stained with DAPI.

FIG. 19 presents the binding of humanized Lic3 to CDH17 as determined by ELISA.

FIG. 20 presents examples of CDH17 monoclonal antibodies binding to CDH17 or CDH17 truncates as determined by ELISA.

FIG. 21 presents examples of CDH17 monoclonal antibodies and humanized Lic3 scFvFc (h3scFv) binding to tumor cell lines as determined by FACS. Surprisingly many CDH17 monoclonal antibodies demonstrated different patterns of binding to the different CDH17 expressing tumor cell lines (red boxes).

FIG. 22 presents the design (a) and analysis (b) of second-generation CAR, including an amino acid sequence of hLic3 CAR7a (SEQ ID NO. 25), hLic3 CAR7b (SEQ ID NO. 26), hLic3 CAR8 (SEQ ID NO. 27), and hLic3 CAR9 (SEQ ID NO. 28).

FIG. 23 presents IL2 production as the function of signaling.

FIG. 24 shows an amino acid sequence (SEQ ID NO. 19) of Lic3 2nd generation CAR (Lic3scFv-CD28hinge+TM+endo-CD3zeta endo).

FIG. 25 shows an amino acid sequence (SEQ ID NO. 20) of Lic5 2nd generation CAR (Lic5scFv-CD28hinge+TM+endo-CD3zeta endo).

FIG. 26 shows an amino acid sequence (SEQ ID NO. 21) of HLic26 2nd generation CAR (Lic26scFv-CD28hinge+TM+endo-CD3zeta endo).

FIG. 27 shows an amino acid sequence (SEQ ID NO. 22) of Lic3 3rd generation CAR (Lic3scFv-CD28-4-1BB-CD3zeta endo).

FIG. 28 shows an amino acid sequence (SEQ ID NO. 23) of Lic5 3rd generation CAR (Lic5scFv-CD28hinge+TM+endo-4-1BB endo-CD3zeta endo).

FIG. 29 shows an amino acid sequence (SEQ ID NO. 24) of HuLic26 3rd generation CAR (Lic26scFv-CD28hinge+TM+endo-4-1BB endo-CD3zeta endo).

FIG. 30. shows Lic5 purification and characterization

FIG. 31. shows the chemosensitizing effect of Lic5.

FIG. 32. shows amino acid sequences of the variable regions of the light chain and heavy chain of Lic5.

DETAILED DESCRIPTION

The applications provide antibodies specific for cadherin-17 (CDH17), antibodies targeting tumor cells and anti-tumor immunotherapies using such antibodies. Such immunotherapies include antibodies possessing different modes of cytotoxicity or chimeric antigen receptors that stimulate T or NK cell cytotoxicity.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the present disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, preferred methods and materials are described. For the purposes of the present disclosure, the following terms are defined below.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

By "about" is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

By "coding sequence" is meant any nucleic acid sequence that contributes to the code for the polypeptide product of a gene. By contrast, the term "non-coding sequence" refers to any nucleic acid sequence that does not contribute to the code for the polypeptide product of a gene.

Throughout this specification, unless the context requires otherwise, the words "comprise," "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of." Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory and that no other elements may be present.

By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the

activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but those other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

The terms "complementary" and "complementarity" refer to polynucleotides (i.e., a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands.

By "corresponds to" or "corresponding to" is meant (a) a polynucleotide having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or encoding an amino acid sequence identical to an amino acid sequence in a peptide or protein; or (b) a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein.

As used herein, the terms "function" and "functional" and the like refer to a biological, binding, or therapeutic function.

By "gene" is meant a unit of inheritance that occupies a specific locus on a chromosome and consists of transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (i.e., introns, 5' and 3' untranslated sequences).

"Homology" refers to the percentage number of amino acids that are identical or constitute conservative substitutions. Homology may be determined using sequence comparison programs such as GAP (Deveraux et al., 1984, Nucleic Acids Research 12, 387-395) which is incorporated herein by reference. In this way, sequences of a similar or substantially different length to those cited herein could be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

The term "host cell" includes an individual cell or cell culture which can be or

has been a recipient of any recombinant vector(s) or isolated polynucleotide of the present disclosure. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. A host cell includes cells transfected or infected in vivo or in vitro with a recombinant vector or a polynucleotide of the present disclosure. A host cell which comprises a recombinant vector of the present disclosure is a recombinant host cell.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes.

An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the antibody nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules, therefore, are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The expression "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a

ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The recitation "polynucleotide" or "nucleic acid" as used herein designates mRNA, RNA, cRNA, rRNA, cDNA or DNA. The term typically refers to polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA and RNA.

The terms "polynucleotide variant" and "variant" and the like refer to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridize with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompass polynucleotides that are distinguished from a reference polynucleotide by the addition, deletion or substitution of at least one nucleotide. Accordingly, the terms "polynucleotide variant" and "variant" include polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide, or has increased activity in relation to the reference polynucleotide (i.e., optimized). Polynucleotide variants include, for example, polynucleotides having at least 50% (and at least 51% to at least 99% and all integer percentages in between, e.g., 90%, 95%, or 98%) sequence identity with a reference polynucleotide sequence described herein. The terms "polynucleotide variant" and "variant" also include naturally-occurring allelic variants and orthologs that encode these enzymes.

"Polypeptide," "polypeptide fragment," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues and to variants

and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues are synthetic non-naturally occurring amino acids, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. In certain aspects, polypeptides may include enzymatic polypeptides, or "enzymes," which typically catalyze (i.e., increase the rate of) various chemical reactions.

The recitation polypeptide "variant" refers to polypeptides that are distinguished from a reference polypeptide sequence by the addition, deletion or substitution of at least one amino acid residue. In certain embodiments, a polypeptide variant is distinguished from a reference polypeptide by one or more substitutions, which may be conservative or non-conservative. In certain embodiments, the polypeptide variant comprises conservative substitutions and, in this regard; it is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide. Polypeptide variants also encompass polypeptides in which one or more amino acids have been added or deleted, or replaced with different amino acid residues.

The term "reference sequence" generally refers to a nucleic acid coding sequence, or amino acid sequence, to which another sequence is being compared. All polypeptide and polynucleotide sequences described herein are included as references sequences.

The recitations "sequence identity" or, for example, comprising a "sequence 50% identical to," as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I) or the identical amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Included are nucleotides and polypeptides having at least about 50%, 55%, 60%, 65%,

70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity to any of the reference sequences described herein (see, e.g., Sequence Listing), typically where the polypeptide variant maintains at least one biological activity of the reference polypeptide.

By "statistically significant," it is meant that the result was unlikely to have occurred by chance. Statistical significance can be determined by any method known in the art. Commonly used measures of significance include the p-value, which is the frequency or probability with which the observed event would occur if the null hypothesis were true. If the obtained p-value is smaller than the significance level, then the null hypothesis is rejected. In simple cases, the significance level is defined at a p-value of 0.05 or less.

"Substantially" or "essentially" means nearly totally or completely, for instance, 95%, 96%, 97%, 98%, 99% or greater of some given quantity.

"Treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. For example, for cancer, reduction in the number of cancer cells or absence of the cancer cells; reduction in the tumor size; inhibition (i.e., slow to some extent and preferably stop) of tumor metastasis; inhibition, to some extent, of tumor growth; increase in length of remission, and/or relief to some extent, one or more of the symptoms associated with the specific cancer; reduced morbidity and mortality, and improvement in quality of life issues. Reduction of the signs or symptoms of a disease may also be felt by the patient. Treatment can achieve a complete response, defined as disappearance of all signs of cancer, or a partial response, wherein the size of the tumor is decreased, preferably by more than 50 percent, more preferably by 75%. A patient is also considered treated if the patient experiences stable disease. In a preferred embodiment, the cancer patients are still progression-free in cancer after one year, preferably after 15 months. These parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician of appropriate skill in the art.

The terms "modulating" and "altering" include "increasing" and "enhancing" as well as "decreasing" or "reducing," typically in a statistically significant or a

physiologically significant amount or degree relative to a control. In specific embodiments, immunological rejection associated with transplantation of the blood substitutes is decreased relative to an unmodified or differently modified stem cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, at least 300%, at least 400%, at least 500%, or at least 1000%.

An "increased" or "enhanced" amount is typically a "statistically significant" amount, and may include an increase that is 1.1, 1.2, 1.3, 1.4, 1.5, 1.6 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, or 50 or more times (e.g., 100, 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7. 1.8, etc.) an amount or level described herein.

A "decreased" or "reduced" or "lesser" amount is typically a "statistically significant" amount, and may include a decrease that is about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, or 50 or more times (e.g., 100, 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7. 1.8, etc.) an amount or level described herein.

By "obtained from" is meant that a sample such as, for example, a polynucleotide or polypeptide is isolated from, or derived from, a particular source, such as the desired organism or a specific tissue within the desired organism. "Obtained from" can also refer to the situation in which a polynucleotide or polypeptide sequence is isolated from, or derived from, a particular organism or tissue within an organism. For example, a polynucleotide sequence encoding a reference polypeptide described herein may be isolated from a variety of prokaryotic or eukaryotic organisms, or from particular tissues or cells within a certain eukaryotic organism. A "therapeutically effective amount" refers to an amount of an antibody or a drug effective to "treat" a disease or disorder in a subject. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. See preceding definition of "treating."

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is a treatment that is not consecutively done without interruption, but rather is cyclic in nature.

"Vector" includes shuttle and expression vectors. Typically, the plasmid construct will also include an origin of replication (e.g., the ColE1 origin of replication) and a selectable marker (e.g., ampicillin or tetracycline resistance), for replication and selection, respectively, of the plasmids in bacteria. An "expression vector" refers to a vector that contains the necessary control sequences or regulatory elements for expression of the antibodies including antibody fragment of the present disclosure, in bacterial or eukaryotic cells. Suitable vectors are disclosed below.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full-length monoclonal antibodies), multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity or function.

"Antibody fragments" comprise a portion of a full-length antibody, generally the antigen-binding or variable region of the antibody. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of a Fv comprising only three complementarity determining regions (CDRs) specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual

antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring the production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler et al., Nature 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., Nature 352:624-628 (1991) and Marks et al., J. Mol. Biol. 222:581-597 (1991), for example.

The term "variable" refers to the fact that certain segments of the variable domains (V domains) differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 10-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four frameworks regions (FRs), largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen but exhibit various effector functions, such as participation of the antibody in antibody-

dependent cellular cytotoxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a CDR (e.g. around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the VL, and around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the VH (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the VL, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the VH (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)).

"Chimeric" antibodies (immunoglobulins) have a portion of the heavy and/or light chain identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al. Proc. Natl Acad. Sci. USA 81:6851-6855 (1984)). Humanized antibody as used herein is a subset of chimeric antibodies.

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. In some embodiments, humanized antibodies are human immunoglobulins (recipient or acceptor antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as a mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some embodiments, humanized antibodies are antibodies derived from human cells or from transgenic animals (typically mice) with express human antibody genes.

In one aspect, provided herein are antibodies or antigen-binding fragments thereof having specificity for CDH-17. Tumor-associated antigens may serve as targets for anti-tumor immunotherapies by inhibiting their tumor growth promoting activities and by directing cytotoxic activity to tumor cells. Cadherin-17 is a Class 1 plasma

membrane glycoprotein that belongs to the cadherin superfamily of cell adhesion molecules. It is a non-classical cadherin possessing 7 cadherin or cadherin-like repeats in its ectodomain. Cadherin-17 (CDH-17) is a tumor-associated antigen that participates in tumor growth. CDH-17 expression normally restricted to intestinal epithelial cells of colon, small intestine, and pancreatic ducts are over-expressed in several tumors types including colon adenocarcinoma, gastric adenocarcinoma, hepatocellular carcinoma and pancreatic adenocarcinoma. Tumor growth promoting activity may involve binding between the RGD motif in CDH17 domain 6 and integrins such as $\alpha_2\beta_1$. An abnormal increase in CDH-17 level in blood and in exosomes may serve as prognostic cancer markers.

Using proteomics and oncogenomics approaches, a therapeutic target, liver-intestine cadherin or cadherin-17 (CDH17) is herein disclosed. The target is overexpressed in a majority of gastric carcinoma (GC) and hepatocellular carcinoma as well as in (HCC) pancreas cancer, colon cancer, ovary cancer and lung cancers. RNAi silencing of *CDH17* gene could inhibit tumor growth and metastatic spread in the established HCC mouse models (both xenograft and orthotopic). The underlying antitumor mechanism is based on inactivation of Wnt signaling in concomitance with tumor suppressor pathway reactivation.

The anti-CDH17 antibodies present in this application have shown antitumor effects in multiple *in vitro* and *in vivo* systems of liver cancer and stomach cancers. Such antibodies have i*n vitro* and *in vivo* purification, detection, diagnostic and therapeutic uses. Such antibodies may be developed to support anti-tumor activity by binding selectively to tumor cells and stimulate complement fixation, antibody-dependent cytotoxicity, cytotoxicity mediated by a conjugated drug, lymphocyte mediated cytotoxicity and NK-mediated cytotoxicity. Provided herein are antibodies and humanized antibodies, antigen-binding fragments or chimeric antibody proteins, comprising a heavy chain variable region having an amino acid sequence set forth as a corresponding SEQ ID provided below.

CDH17 antibody sequences may include various type of antibodies such as mouse antibodies (Lic3 and Lic5; FIG. 1) and their humanized variants (FIGS. 2-9), human antibodies isolated from a phage library screen (FIGS. 10-12), monospecific antibodies with isotypes to support ADCC, complement fixation, and drug conjugates

for cytotoxic anti-cancer activity (preferred conjugate moieties being irinotecan, auristatins, PBDs, maytansines, amantins, spliceosome inhibitors and other chemotherapeutic agents) (FIG. 13), bispecific antibodies, including various engineered antibody fragments (Fab, scFv, diabodies etc). Preferred forms include knobs-into holes and "Bite" (Example sequence in FIG. 14).

In some embodiments, mouse CDH17 antibodies, Lic5 scFv and Lic3 scFv, display their ability to bind CDH17 in an ELISA assay, respectively (FIGS. 15-16). The binding affinity of Lic3 and Lic5 scFv can also be determined by ELISA, where the Kd of scFv lic 3 and scFv lic5 were 11.88 nM and 22.06 nM, respectively (FIG. 17).

In another aspect, the internalization of Lic5 into human gastric cancer cells is indicative of a candidate for antibody drug conjugate as shown in FIG. 18; (a) photographs showing the staining of DAPI (blue) and Lic5-FITC (green) at indicated times; (b) photographs showing the merged staining of DAPI (blue) and Lic5-FITC (green) at indicated times; Lic5-FITC (green) internalization in Oum1 human gastric cancer cells over 30 mins at 37°C was determined by confocal microscopy; nucleus was stained with DAPI.

In some embodiments, over 200 CDH17 monoclonal antibodies were generated by standard hybridoma technology. Humanized Lic3 scFvFc was expressed from CHO cells transfected with four cDNA expression construct clones (hL3.1 – 3.4). Culture media from transfected or mock transfected CHO was added to a 96-well plate coated with CDH17Fc. Binding was determined using anti-mouse Fc HRP conjugate. Binding was compared to that of a mouse Lic3 scFvFc (mLic3 scFv) also produced in CHO cells (FIG. 19).

In one aspect, mice were immunized with recombinant CDH17 possessing the entire ectodomain of 7 cadherin repeats (D1-7) fused to a modified human IgG4 Fc (CDH17Fc). Hybridoma culture media were a screen for binding to CDH17Fc and lack of binding to IgG4 Fc. CDH17Fc truncates possessing D1, D1-2, D1-3, D3-4, D6 and D5-7 were generated by standard PCR methodology. CDH17 antibodies were identified that bound specifically to each truncate and examples are presented in FIG. 20. A panel of 13 novel CDH17 monoclonal antibodies was analyzed by ELISA for binding to CDH17 possessing all 7 ectodomains or truncates possessing domains1-2, domains 3-4, domains 5-6 or domain 6. Antibody epitopes were localized to different domains.

Surprisingly the binding of certain antibodies was found to be restricted to CDH17 produced in CHO cells whereas the binding of other antibodies was found to be restricted to CDH17 produced in 293F cells.

In another aspect, antibodies were identified that bound to D1, D6 and D1 and D6 domains that have been implicated in CDH17 dependent homotypic and heterotypic interactions. The binding of CDH17 antibodies to CDH17 positive tumor cell lines, by flow cytometry, yielded unanticipated results (FIG. 21). Many different binding profiles were observed suggesting that there are different forms of CDH17 expressed in a cell type-specific manner. This notion is further supported by differential binding of CDH17 antibodies to recombinant CDH17 produced in CHO cells versus 293F cells. Therefore, these novel CDH17 antibodies may be used to selectively target CDH17 expressed in one tissue and not another.

In some embodiments, a second-generation CAR was designed and analyzed, in which CAR7, 7b, 8 and 9. CARs were constructed with humanized Lic3 scFv, CD8 or IgG1 hinges, CD8 or CD28 transmembrane domains, CD137 or CD28 endodomains and CD3zeta (isoforms 1/2 or 3/4) endodomains (FIG. 22A). Jurkat cells at 4 days post-lentivirus transduction were analyzed by FACS analysis, which shows the GFP expression and CAR expression by soluble recombinant CDH17Fc binding (FIG. 22B). The CDH17 binding to CARs was determined by using an anti-Fc fluorochrome conjugate. Furthermore, Jurkat cells expressing different CARs were seeded in 96 well plates coated with CDH17Fc, EGFRFc (control), or no protein (NA). Immunocult (CD3-CD28 complex) was added to some uncoated wells containing Jurkat cells. Il-2 levels in the culture media was measured by ELISA after 48 hours of stimulation (FIG. 23), indicating that IL2 production can be assessed as the function of signaling.

In some embodiments, CDH17 antibodies may antagonize the binding of the RGD site in CDH17 domain 6 to integrins including alpha2beta1.

In another aspect, bispecific antibodies having specificity for CDH17 are disclosed. The bispecific antibodies may have at least one of the following features including, without limitation, killing tumor cells by linking tumor cell (CDH17) with cytotoxic T or NK cell receptors (e.g. CDH17/CD3) in a manner to activate cellular cytotoxicity, increasing anti-cancer activity by binding a lymphoid/NK/monocytic cell activator (e.g. 4-1BB, OX40, CD27, CD40, TIM-1, CD28, HVEM, GITR, ICOS, IL12receptor,

IL14 receptor) stimulating cytotoxicity or chemokine production and immune cell recruitment, blocking an immune checkpoint inhibitor (PD-1, TIM-3, LAG-3, TIGIT, CTLA-4, PD-L1, BTLA, VISTA), block an angiogenic factor (e.g. VEGF). CDH17 may be used in combination treatment with cytotoxic drugs (e.g. cisplatin, gemcitabine, irinotecan) or other anti-tumor antibodies.

In some embodiments, CDH17 specific scFv is engineered as second or third generation chimeric antigen receptors (CAR) to support T and NK cell-mediated tumor cell cytotoxicity (sequences and designs in FIG. 24-26). CDH17 specific CAR T and CAR NK cells may kill different types of solid tumors including but not limited to those of liver, gastric, colon, pancreatic, and lung. Hence, CDH17 may be used for various conditions related to, for example, liver and stomach cancers.

CDH17 is a prominent cancer biomarker overexpressed in both liver and stomach cancers but not in the normal healthy adult tissues. CDH17 is highly expressed in the metastatic phenotype of cancers, and blockage of CDH17 reduces markedly lung metastasis of HCC. Monoclonal antibodies targeting CDH17 marker are able to inhibit the growth of liver and stomach tumors. CDH17 humanized antibody can be used clinically to treat cancer patients with indication of CDH17 biomarker in the tumor tissues and/or in serum samples. Anti-CDH17 scFv molecules are selected to generate CAR T vector for proof-of-concept in vivo model studies.

Tumor antigens derived from clinical samples were selected for the antibodies with specificity directed against the tumor-restricted CDH17 epitopes. As such, both Lic5 and Lic3 mAbs were shown to bind tumor cells of HCC and GC only. By lowering and/or varying the antibody affinity (through computational modeling), CDH17-CAR-T cells will have enhanced differential binding to the tumor cell surface.

The most solid tumor is surrounded by vasculature and immune cells, which is characterized by an immune-suppressive environment. The presence of a large number of regulatory T cells and blockade of immune checkpoint factors (PD1 and TIM-3) in cancers will favor the immune tolerance conditions that might impact the effectiveness of CAR-T-cells. To address this issue, an anti-PD1/anti-Tim3 bispecific component in the CAR-T vehicle was created leading to the circumventing of the immune checkpoint barrier. In addition, once this anti-PD1/Tim3 component is engaged in the tumor microenvironment, it will switch to the anti-tumor cytotoxic

activity of the CAR-T cells to enhance the efficacy and improve the safety.

Chimeric antigen receptor (CAR), also known as artificial T cell receptor, is engineered immunoreceptor, which is grafted the specificity of a monoclonal antibody onto a T cell facilitated by retroviral vectors. The CAR-engineered T cells can recognize and kill the cancer cells and demonstrate significant clinical benefits.

Chimeric IgG Fc-fusion protein for clinical applications is disclosed herein. Example chimeric IgG-fusion protein includes the soluble form of ICAM-1 and ICAM-2 for therapeutic intervention of autoimmune diseases and viral infections. The CAR-T vector is constructed with a scFv domain derived from a mAb, linker, and cytoplasmic domain, in which different generations of CAR-T vector have evolved from the simple form of CD3-zeta which contains 3 ITAMs (for activation and co-stimulatory signals) to the recent 3rd generation containing CD28 and OX40 to provide proliferative / survival signals. From the CDH17-specific clones (Lic3 and Lic5) disclosed herein, the scFv segments were designed for cloning into the 3rd generation of lentiviral based CAR-T vector.

Examples

The present disclosure is further described with reference to the following examples. These examples are provided for purposes of illustration only and are not intended to be limiting unless otherwise specified. Thus, the present disclosure should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Example 1. Study Design and Methodology

The effect of Lic5 alone or in combination with cisplatin or epirubicin on tumor growth inhibition and animal survival were tested using an orthotopic tumor xenograft model. Human HCC cell lines MHCC97-L (97L) and PLC/PRF/5 (PLC) were used. Single Lic5, cisplatin and epirubicin treatment inhibited the growth of cultured HCC cells using cell proliferation assay, while more significant reductions were observed when cisplatin or epirubicin was used together with Lic5. Similar trends of growth inhibition were observed when the same experimental grouping was applied to treat orthotopic tumor-bearing nude mice.

Treatment of Lic5 enhanced survivals of orthotopic tumor-bearing nude mice

when compared to the control group. Among all experimental groups, combined Lic5 and epirubicin group yielded the best survival. For next phase antibody humanization, the complementarity determining regions (CDRs) on variable regions on the light and heavy chain of Lic5 were identified. Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies), 100 units/ml penicillin and streptomycin (Life Technologies). Cultured cells were maintained in a humidified atmosphere at 37°C with 5% carbon dioxide.

Example 2. Production and Characterization of Lic5, an in-house Monoclonal Antibody against CDH17

An in-house hybridoma cell line secreting Lic5 was generated as described [5]. Hybridoma cells were maintained in DMEM supplemented with 10% FBS and 100 units/ml penicillin and streptomycin. To collect Lic5, hybridoma cells were cultured in serum-free hybridoma-SFM (Life Technologies) for 6 days. The cultured supernatant was collected and purified using Protein G column (Life Technologies). The concentration of the purified Lic5 was estimated using RC DC Protein Assay (Bio-Rad, Hercules, CA, USA). The purity of Lic5 was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing condition, followed by Coomassie Blue staining. Immunoreactivity and specificity of Lic5 were confirmed by western blot using 97L cell lysate and by immunohistochemistry using paraffin-embedded human HCC tissue. The use of clinical specimen for research was approved by the Institutional Review Board of The University of Hong Kong/Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB). The procedures for SDS-PAGE, Coomassie Blue staining and western blot were routinely performed, while the method for immunohistochemistry was described below.

Example 3. *In vitro* Cell Proliferation Assay

A pre-determined number of 97L and PLC cells was seeded onto 96-well culture plates for 1 day. 150 ug/ml mouse IgG (Sigma-Aldrich, St Louis, MO, USA) as a control for Lic5. After treatment, the proliferation of treated cells was measured using Cell Proliferation Kit I (Roche, Indianapolis, IN, USA) and as described. In brief, MTT reagent was incubated with the treated cells for 4 hours. Colorimetric signals were then measured after addition of 10% SDS. Independent cell proliferation MTT assays were

performed at least twice for result confirmation.

Example 4. Orthotopic Tumor Xenograft Model

Male nude mice were obtained from Laboratory Animal Unit of The University of Hong Kong, Hong Kong. Animals were housed in individually ventilated cages under a 12-hour light/12-hour dark cycle and with free access to autoclaved water and chow. Animal experiments performed in this study were approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR) of our institute. To allow *in vivo* viewing of the growing orthotopic liver tumors, previously established luciferase-labelled 97L cell line was used. 5 x 106 luciferase- labeled 97L cells were injected subcutaneously into nude mice to allow the growth of the subcutaneous tumors. These subcutaneous tumors were harvested when their sizes reached 200-300 mm3 and used as tumor seeds to establish orthotopic liver tumors in a separate group of nude mice following the standard procedure. Five days after orthotopic tumor implantation, mice received different treatments as following via intraperitoneal administration 3 times/week for 3 weeks (10-11 mice in each group): (1) 6 mg/kg Lic5; (2) 1 mg/kg cisplatin; (3) 1 mg/kg epirubicin; (4) combined treatment of Lic5 and cisplatin; (5) combined treatment of Lic5 and epirubicin; (6) 6 mg/kg mouse IgG as Lic5 control. Every week after treatment, orthotopic tumor growth and metastasis were monitored by live imaging in an IVIS 100 *In Vivo* Imaging System (Perkin Elmer, Waltham, MA, USA) after intraperitoneal injection of 150 mg/kg potassium luciferin (Gold Biotechnology, St Louis, MO, USA). For each measurement, a net photon count was obtained for each tumor. At the end of the experiment, orthotopic tumors were collected and subjected to formalin fixation and paraffin embedding.

Example 5. Animal Survival Study

Orthotopic liver tumors were established as above using 97L cells. Orthotopic tumor-bearing mice received the following treatments intraperitoneally 3 times/week for 3 weeks (11-15 mice in each group): (1) 8 mg/kg Lic5; (2) 1 mg/kg cisplatin; (3) 1 mg/kg epirubicin; (4) combined treatment of Lic5 and cisplatin; (5) combined treatment of Lic5 and epirubicin; (6) 8 mg/kg mouse IgG as Lic5 control. Animal survival was continuously monitored till 6 weeks after treatment.

Example 6. Immunohistochemistry

Immunohistochemistry was performed on paraffin-embedded orthotopic

tumor xenografts and human HCC specimen as described. In brief, 6 um sections were prepared for deparaffinization and rehydration. After antigen retrieval and hydrogen peroxide treatment, sections were blocked with 3% bovine serum albumin before staining with mouse monoclonal antibody against alpha-catenin (1:200; BD Biosciences, San Jose, CA, USA) and Lic5 (0.0625 ug/ml) overnight at 4°C. Signals were detected using EnVision+ System-HRP Labelled Polymer Anti-mouse (Dako, Carpinteria, CA, USA) and visualized using Liquid DAB+ Substrate Chromogen System (Dako). Counterstaining was performed using hematoxylin. Images were captured using DXM1200F digital camera (Nikon, Melville, NY, USA).

Example 7. Cloning of Variable Regions of Lic5

Total RNA was extracted from Lic5-secreting hybridoma cells using TRIzol reagent (Life Technologies) and converted to cDNA using SuperScript III First-Strand Synthesis System (Life Technologies). Variable region cDNA fragments from heavy chain and light chain of Lic5 were amplified using Ig-Primer Set (Novagen, Germany) and Platinum Taq DNA Polymerase High Fidelity (Life Technologies). Variable region of light chain was amplified by the 5' variable region primer (5'-ATGGAGACAGACACACTCCTGCTAT-3') and 3' primer on the constant region, while variable region of heavy chain was obtained by the 5' variable region primer (5'-ATGAACTTYGGGYTSAGMTTGRTTT-3', in which Y=C/T, S=C/G, M=A/C, and R=A/G) and 3' primer on the constant region. PCR products after resolving on a 1.5% agarose gel were purified and sequenced (BGI, Hong Kong).

Example 8. Identification of CDR of Lic5

The nucleotide sequences of variable region of heavy chain and light chain of Lic5 were used to derive the amino acid sequences, which were then numbered according to Kabat numbering scheme. CDR was identified according to Kabat definition (www.bioinf.org.uk).

Example 9. Statistical Analyses

Statistical analyses were performed using SPSS version 19 (SPSS Inc., Chicago, IL, USA). Data presented in bar chart are expressed as mean ± SD/SEM. Student's *t*-test was used to calculate the significance of the difference between treatment groups and control group in both cell proliferation MTT assay and animal tumor xenograft experiments. Kaplan-Meier method was employed for survival analyses, while the

differences in survival were calculated using log- rank test. A *p*-value of less than 0.05 was considered statistically significant.

Example 10. Establishment and Purifying Large Quantity of Lic5 from Hybridoma Cell Culture Supernatant

To obtain Lic5 with high purity and quantity for *in vitro* and *in vivo* experiments, Lic5- secreting hybridoma cells were cultured in serum-free medium. Purifying Lic5 has thus far achieved a good antibody purity and yield for establishing a pipeline. Coomassie Blue staining result revealed two prominent bands of about 55 and 27 kDa corresponding to the heavy and light chain of antibody on Day 6 culture supernatant, while these bands were not observed on Day 1 culture supernatant. With the presence of a detectable level of antibody, culture supernatant collected on Day 6 was subjected to Protein G affinity chromatography for antibody purification. Successful binding of antibody onto the Protein G column was confirmed when not detecting the presence of antibody in the flow- through after loading Day 6 culture supernatant onto the column. Antibody elution performed on different fractions (eluent E1 to E4) revealed the second and third elution fraction contained most abundant antibody. The two stained bands corresponding to the heavy and light chain of the antibody are the predominant bands detected on the protein gel, implicating our antibody purity with more than 90% (FIG. 30(A)). Using our antibody purification pipeline, a favorable antibody was collected amount of 23.88 ± 6.96 mg Lic5 from each liter of culture supernatant collected on Day 6.

The purified Lic5 demonstrated high immunoreactivity against CDH17. A single band of about 120 kDa corresponding to the apparent molecular weight of CDH17 was detected in CDH17-expressing 97L cells when Lic5 was used as a primary antibody for western blot (FIG. 30(B)). When Lic5 was used for immunohistochemistry to detect CDH17 in human HCC tissue, discrete stains appeared at the cell membrane and cytoplasm (FIG. 30(C)). The immunohistochemical stains generated by using Lic5 in this study was similar to the previous report with the use of a commercial antibody against CDH17 for immunohistochemistry.

Example 11. Survival Benefit of Lic5

The chemosensitizing effect of Lic5 towards another chemotherapeutic drug epirubicin WAS examined. The drug testing with or without Lic5 in an orthotopic

tumor xenograft model was performed.

Before treating orthotopic tumor-bearing mice with the purified Lic5, the anti-tumor effect of Lic5 alone or combined with cisplatin or epirubicin in cultured HCC cell lines *in vitro* was examined using cell proliferation MTT assay (FIG. 31(A)). When cell proliferation assay was performed on cultured 97L cells, treatment with cisplatin and epirubicin for 2 days resulted in 31.6% and 21.9% growth inhibition, respectively. The combination with Lic5 further enhanced the growth inhibition effect by 1.74-fold for cisplatin and 1.98-fold for epirubicin, which led to a growth inhibition of 55.1% and 43.3%, respectively (FIG. 31(A), left panel). Similar growth inhibition was observed in cultured PLC cells, in which treatment with cisplatin resulted in 37.6% growth inhibition and combined treatment with Lic5 further increased the growth inhibition by 1.73- fold (65.2%). Unlike the significant growth inhibition of epirubicin on cultured 97L cells, this drug only inhibited the growth of cultured PLC cells by 8.1%. Likewise, its combination with Lic5 enhanced the growth inhibition by 4.62-fold and led to a significant inhibition of 37.4% (FIG. 31(B), right panel).

Lic5 was purified using Protein G affinity chromatography from culture supernatant of Lic5-secreting hybridoma cell line. FIG. 31(A) shows that, on the Coomassie Blue-stained gel, culture supernatant (culture S/N) from Day 6 (D6) contained higher antibody amount than Day 1 (D1). Two stained bands with sizes of about 55 kDa and 27 kDa corresponding to the heavy chain and light chain of antibody were visualized on the gel. No antibody was detected in the flow-through (FT) after passing the Day 6 culture supernatant on the Protein G column. Antibody elution was performed on 4 fractions (E1 to E4). The most abundant antibodies were eluted in fractions E2 and E3, which was collected and pooled. In addition, FIG. 31(A) shows that single treatment of Lic5, cisplatin or epirubicin for 2 days inhibited the growth of cultured 97L and PLC cells when compared to the mouse IgG-treated cells in cell proliferation MTT assay. Further growth inhibition was observed when the cultured cells were treated under combined treatment of Lic5 with cisplatin or epirubicin. Bars represent mean ± SD.

FIG. 31(B) shows that, using purified Lic5 for the western blot on 97L cell lysate, a single band corresponding to the apparent molecule weight of CDH17 was revealed. In FIG. 31(B), Luciferase-labelled 97L cells were used to establish orthotopic tumor

xenografts in nude mice. Single and combined treatments of Lic5 with or without chemotherapeutic drugs cisplatin and epirubicin were applied to these animals for 3 weeks. The data shown are net photon count relative to the control IgG group obtained 3 weeks after treatment. Single treatment yielded tumor growth inhibition, while the further reduction in tumor growth was observed when Lic5 was combined with cisplatin or epirubicin. Bars represent mean + SEM.

FIG. 31(C) presents that, using purified Lic5 for immunohistochemistry on human HCC tissue, discrete stains corresponding to the membrane and cytoplasmic localizations of CDH17 were detected. In FIG. 31(C), Orthotopic tumor xenografts were established in nude mice using 97L cells. These animals were treated with single or combined treatment of Lic5 and epirubicin. Combined treatment of Lic5 and epirubicin led to most favorable survival as revealed in the Kaplan-Meier curve. A statistically significant difference in survivals was reached when the combined treatment group was compared with mouse IgG control group ($p = 0.017$). Abbreviations used: C, cisplatin; E, epirubicin. **, $p < 0.05$; ns, statistically not significant

After the chemosensitizing effect of Lic5 towards cisplatin and epirubicin treatment on cultured HCC cells was confirmed by using cell proliferation MTT assay, the next step was to examine whether similar growth inhibition can be observed in an *in vivo* setting by animal tumor xenograft experiments. Luciferase-labelled 97L cells were used for developing orthotopic tumor xenografts for treatments as this enables live monitoring of tumor growth. When compared to IgG control group, cisplatin treatment inhibited the xenograft growth by 48.6%. Such treatment when combined with Lic5 further augmented the xenograft growth inhibition by 1.64-fold to 79.6%. Despite such inhibition, the combined treatment data did not yield a statistically significant value when compared to control group ($p = 0.08$). For epirubicin, its combined treatment with Lic5 manifested a further reduction of xenograft growth from 37.9% for single epirubicin treatment to 86.4% (i.e. 2.28-fold). However, such drop in xenograft growth still did not reach a statistically significant value ($p = 0.07$) (FIG. 31(B)). At the end of the experiment, the orthotopic tumors were collected for analyzing the expression and localization of alpha-catenin, which is known as a downstream molecule of CDH17 and is a key member of the Wnt signaling pathway. Both combined treatment of Lic5 with cisplatin or epirubicin reduced the overall

expression and cytoplasmic localization of alpha-catenin when compared to the single treatment of chemotherapeutic drugs. Such immunohistochemical results further strengthen the tumor-suppressing effect mediated by Lic5 via its action on altering the expression and localization of alpha-catenin.

As shown in FIG. 31(B), a trend of tumor growth reduction was observed for every treatment group when compared to control group. The survival rate of orthotopic tumor- bearing mice for each treatment group *versus* control group was compared. The animal survival rate of Lic5 group reached 63.6%, which was much higher than that of the control group (26.6%). Although the survival rate between the control group and epirubicin group was similar (26.6% *versus* 25%), combined treatment of Lic5 and epirubicin led to the most significant increase in survival rate to 81% among all experimental groups when compared to control group ($p = 0.017$) (FIG. 31(C)). However, such significant difference in survival for cisplatin group and cisplatin with Lic5 group did not reach any statistically significant values when compared to control group. Taken together, this set of animal experiments has demonstrated the chemosensitizing effect of Lic5 in particular for epirubicin on tumor growth inhibition and survival rate induction.

Example 13. Identification of CDR on Variable Regions of Lic5

Each monoclonal antibody has its unique variable regions on heavy chain and light chain, for which each of them is composed of a sequence of amino acids that determines the antigen binding property of this antibody. To obtain the variable region sequences from heavy chain and light chain of Lic5, each variable region was amplified using discrete primer pairs (FIG. 32, left panel). After Sanger sequencing of the amplified products, the nucleotide sequences were translated into amino acid sequences by *in silico* method. Kabat numbering scheme and CDR definition were employed to identify the three CDRs on the variable regions of heavy chain and light chain of Lic5 using the deduced amino acid sequences (FIG. 32, right panel). PCR followed by Sanger sequencing were used to obtain the variable region sequences of light chain and heavy chain of Lic5 from cDNA prepared from Lic5-secreting hybridoma cells (FIG. 32, left panel). Based on the Kabat numbering scheme and CDR definition, the six CDRs on the two variable regions (VL: SEQ ID NO. 29 and VH: SEQ ID NO. 30) were identified and highlighted in different colors (FIG. 32, right panel)

The results from the examples have demonstrated the anti-tumor and chemosensitizing effects of Lic5 in treating HCC xenografts, showing that the monoclonal antibodies disclosed herein may be useful to treat other cancers in addition to HCC. Lic5 was a monoclonal antibody produced in parallel with Lic3 using recombinant ectodomain 1-2 of CDH17 as an immunogen. Lic5 targets the extracellular region of CDH17; it can bind to intact form or carboxyl- terminal truncated form of CDH17 when used for therapeutic purpose. Therefore, Lic5 can be used to target other cancers with CDH17 overexpression. In addition to HCC, a number of cancers are found overexpressing CDH17, which include gastric adenocarcinoma, esophageal adenocarcinoma, and colorectal cancers. Among these cancer types, CDH17 overexpression was present in the majority of colorectal and esophageal adenocarcinoma cancers. As such, these CDH17- overexpressing cancers may be susceptible to the action of Lic5. Several studies performing CDH17 suppression counteracted tumorigenesis as manifested by reduced growth of cultured cells or tumor xenografts after treatment. Thus, targeting CDH17 to counteract tumorigenesis works well in other cancers overexpressing CDH17 in addition to HCC.

The results from the examples show that CDH17 seems to be linked with α -catenin-associated network. In one embodiment, targeting CDH17 using Lic5 inactivates CDH17/α-catenin axis by interfering with the expression and localization of the axis components such as α -catenin. In another cancer type, CDH17 was bridged to integrin- related pathway during colorectal tumorigenesis because of the intrinsic integrin-binding affinity of CDH17. CDH17 also affects two other tumorigenic pathways related to Ras/Raf/MEK/ERK and NFγB, such that its suppression inactivated these pathways in gastric cancer. Given these observations, it remains to be determined whether Lic5 could also block these pathways as a mean to suppress tumorigenesis.

In addition to testing the anti-tumor effects of Lic5, three CDRs on each variable region on heavy and light chains of Lic5 were identified. These CDRs are responsible for determining antibody binding affinity and specificity.

Pharmaceutical Compositions

The term "effective amount" refers to an amount of a drug effective to achieve a desired effect, e.g., to ameliorate disease in a subject. Where the disease is cancer, the effective amount of the drug may inhibit (for example, slow to some extent, inhibit

or stop) one or more of the following example characteristics including, without limitation, cancer cell growth, cancer cell proliferation, cancer cell motility, cancer cell infiltration into peripheral organs, tumor metastasis, and tumor growth. Wherein the disease is cancer, the effective amount of the drug may alternatively do one or more of the following when administered to a subject: slow or stop tumor growth, reduce tumor size (for example, volume or mass), relieve to some extent one or more of the symptoms associated with the cancer, extend progression-free survival, result in an objective response (including, for example, a partial response or a complete response), and increase overall survival time. To the extent the drug may prevent growth and/or kill existing cancer cells, it is cytostatic and/or cytotoxic.

With respect to the formulation of suitable compositions for administration to a subject such as a human patient in need of treatment, the antibodies disclosed herein may be mixed or combined with pharmaceutically acceptable carriers known in the art dependent upon the chosen route of administration. There are no particular limitations to the modes of application of the antibodies disclosed herein, and the choice of suitable administration routes and suitable compositions are known in the art without undue experimentation.

Although many forms of administration are possible, an example administration form would be a solution for injection, in particular for intravenous or intra-arterial injection. Usually, a suitable pharmaceutical composition for injection may include pharmaceutically suitable carriers or excipients such as, without limitation, a buffer, a surfactant, or a stabilizer agent. Example buffers may include, without limitation, acetate, phosphate or citrate buffer. Example surfactants may include, without limitation, polysorbate. Example stabilizer may include, without limitation, human albumin.

Similarly, persons skilled in the art have the ability to determine the effective amount or concentration of the antibodies disclosed therein to effective treat a condition such as cancer. Other parameters such as the proportions of the various components of the pharmaceutical composition, the administration does and frequency may be obtained by a person skilled in the art without undue experimentation. For example, a suitable solution for injection may contain, without limitation, from about 1 to about 20, from about 1 to about 10 mg antibodies per ml.

The example dose may be, without limitation, from about 0.1 to about 20, from about 1 to about 5 mg/Kg body weight. The example administration frequency could be, without limitation, once per day or three times per week.

While the present disclosure has been described with reference to particular embodiments or examples, it may be understood that the embodiments are illustrative and that the disclosure scope is not so limited. Alternative embodiments of the present disclosure may become apparent to those having ordinary skill in the art to which the present disclosure pertains. Such alternate embodiments are considered to be encompassed within the scope of the present disclosure. Accordingly, the scope of the present disclosure is defined by the appended claims and is supported by the foregoing description.

In summary, the novel panel of CDH17 antibodies, whose variable domains are derived from CDH17 mAb, will enable the generation of unique CARs for cancer treatment. The cell type specificity of CDH17 antibodies may enable more specific targeting of CDH17 expressing tumor cells relative to CDH17 expressed in normal cells or tissues. T cells or NK cells expressing two CARs may also be generated in which one CAR is more specific for CDH17 on tumor cells and the second CAR is moderately specific for a tumor associated antigen (TAA) on the same tumor cells. The combinatorial specificity is enabling a more selective targeting of a tumor cell signature. Alternatively, a CDH17 CAR may support more specific targeting of a moderately specific tumor specific TAA CAR through a different mechanism. For example, a moderately specific colon or gastric cancer TAA CAR may be fused to a CD3zeta endodomain. This first generation CAR may not support a robust killing response and has a short in vivo half-life of 1 to several weeks. A CDH17 CAR may be coexpressed that is fused to CD28 or CD28 and CD137 endodomains which will provide a strong costimulatory signal to the 1st generation CAR. The CDH17 CAR will bind and activate a robust tumor-killing response in colon and stomach where CDH17 is normally expressed. The co-expressed 1st generation CAR would be selected for binding a target that is not normally expressed in colon or stomach (or other tissue where CDH17 is normally expressed) and hence would be selectively activated to kill colon or stomach cancer cells.

While the disclosure has been described with reference to particular

embodiments, it will be understood that the embodiments are illustrative and that the disclosure scope is not so limited. Alternative embodiments of the present disclosure will become apparent to those having ordinary skill in the art to which the present disclosure pertains. Such alternate embodiments are considered to be encompassed within the scope of the present disclosure. Accordingly, the scope of the present disclosure is defined by the appended claims and is supported by the foregoing description.

The embodiments are merely for illustrating the present disclosure and are not intended to limit the scope of the present disclosure. It should be understood for persons in the technical field that certain modifications and improvements may be made and should be considered under the protection of the present disclosure without departing from the principles of the present disclosure.

CLAIMS

What is claimed is:

1. An antibody having specificity for cadherin-17, comprising an amino acid sequence having at least 70% similarity with an amino acid sequence selected from SEQ ID NO: 1-21.

2. The antibody of Claim 1, comprising an amino acid sequence having at least 80% similarity with an amino acid sequence selected from SEQ ID NO: 1-21.

3. The antibody of Claim 1, comprising an amino acid sequence having at least 95% similarity with an amino acid sequence selected from SEQ ID NO. 1-21.

4. The antibody of Claim 1, wherein the monoclonal antibody is a mouse antibody, a humanized antibody, or a human antibody.

5. The antibody of Claim 1, wherein the monoclonal antibody is a human antibody isolated from a phage library screen.

6. The antibody of Claim 1, comprising a variable region of light chain (VL), a variable region of heavy chain (VH), wherein the VL comprises an amino acid sequence having at least 90% similarity with an amino acid sequence selected from SEQ ID NO: 1, 3, 6, 8, 10, 12, 14, and 16.

7. The antibody of Claim 6, wherein the VH comprises an amino acid sequence having at least 90% similarity with an amino acid sequence selected from SEQ ID NO: 2, 4, 5, 7, 9, 11, 13, and 15.

8. The antibody of Claim 1, further comprising a conjugated cytotoxic moiety.

9. The antibody of Claim 8, wherein the conjugated cytotoxic moiety comprises irinotecan, auristatins, PBDs, maytansines, amantins, spliceosome inhibitors, or a combination thereof.

10. The antibody of Claim 8, wherein the conjugated cytotoxic moiety comprises a chemotherapeutic agent.

11. The antibody of Claim 1, wherein the antibody is a bispecific antibody.

12. The antibody of Claim 11, having specificity for a cell receptor from a cytotoxic T or NK cell.

13. The antibody of Claim 12, wherein the antibody is a bispecific antibody having specificity for both cadherin-17 and CD3.

14. The antibody of Claim 12, wherein the cell receptor comprises 4-1BB, OX40, CD27, CD40, TIM-1, CD28, HVEM, GITR, ICOS, IL12receptor, IL14 receptor, or a derivative or combination thereof.

15. The antibody of Claim 12, comprising a first single-chain variable fragment (ScFv) having specificity for cadherin-17 and a second sing-chain variable fragment (ScFv) having specificity for CD3, wherein the first ScFv comprises a first VH and a first VL, the second ScFv comprises a second VH and a second VL.

16. The antibody of Claim 15, wherein the first VH comprises an amino acid sequence selected from SEQ ID NO: 2, 4, 5, 7, 9, 11, 13, and 15.

17. The antibody of Claim 15, wherein the first VL comprises an amino acid sequence selected from SEQ ID NO: 1, 3, 6, 8, 10, 12, 14, and 16.

18. The antibody of Claim 15, wherein the second VH comprises a corresponding portion of an amino acid sequence of SEQ ID NO: 18.

19. The antibody of Claim 15, wherein the second VL comprises a corresponding portion of an amino acid sequence of SEQ ID NO: 18.

20. The antibody of Claim 11, having specificity for an immune checkpoint inhibitor.

21. The antibody of Claim 20, wherein the checkpoint inhibitor comprises PD-1, TIM-3, LAG-3, TIGIT, CTLA-4, PD-L1, BTLA, VISTA, or a combination thereof.

22. The antibody of Claim 11, having specificity for an angiogenic factor.

23. The antibody of Claim 22, wherein the angiogenic factor comprises VEGF.

24. The antibody of Claim 1, wherein the antibody is configured to antagonize the binding of the RGD site in cadherin-17 domain 6 to integrin.

25. The antibody of Claim 24, wherein the integrin comprises alpha2beta1.

26. The antibody of Claim 1, wherein the antibody is a monoclonal antibody.

27. An IgG heavy chain for an antibody, comprising an amino acid sequence having a sequence selected from SEQ ID NO: 2, 4, 5, 7, 9, 11, 13, and 15.

28. A light chain for an antibody, comprising an amino acid sequence having a sequence selected from SEQ ID NO: 1, 3, 6, 8, 10, 12, 14, and 16.

29. A variable chain for an antibody, comprising an amino acid sequence selected from SEQ ID NO: 1-16.

30. A scFv or Fab having specificity for cadherin-17, comprising an amino acid sequence having at least 90% similarity with an amino acid sequence selected from SEQ ID NO: 1-21.

31. The scFv or Fab of Claim 30, having specificity for a cell receptor from a cytotoxic T or NK cell.

32. The scFv or Fab of Claim 30, having specificity for an immune checkpoint inhibitor.

33. The scFv or Fab of Claim 30, having specificity for an angiogenic factor.

34. A T or NK cell having specificity for cadherin-17, wherein the T or NK cell comprises a chimeric antigen receptor, wherein the chimeric antigen receptor comprises an amino acid sequence having at least 90% similarity with an amino acid sequence selected from SEQ ID NO: 1-21.

35. The T or NK cell of Claim 34, wherein the chimeric antigen receptor comprises an amino acid sequence selected from SEQ ID NO: 1-16.

36. An isolated nucleic acid encoding the antibody of Claim 1-26, the IgG heavy Chain of Claim 27, the light chain of Claim 28, the variable chain of Claim 29, or the ScFv or Fab of Claims 30-33.

37. An expression vector comprising the isolated nucleic acid of Claim 36.

38. The expression vector of Claim 37, wherein the vector is expressible in a cell.

39. A host cell comprising the nucleic acid of Claim 36.

40. A host cell comprising the expression vector of Claim 37.

41. The host cell of Claim 40, wherein the host cell is a prokaryotic cell or a eukaryotic cell.

42. A pharmaceutical composition, comprising the antibody of Claim 1-26 and a cytotoxic agent.

43. The pharmaceutical composition of Claim 42, wherein the cytotoxic agent comprises cisplatin, gemcitabine, irinotecan, or an anti-tumor antibody.

44. The pharmaceutical composition, comprising the antibody of Claim 1-26 and a pharmaceutically acceptable carrier.

45. A method for treating a subject having cancer, comprising administering to the subject an effective amount of the antibody of Claim 1-26 or the T or NK cell of Claims 34-35.

46. The method of Claim 45, wherein the cancer is liver cancer, gastric cancer, colon cancer, pancreatic cancer, lung cancer, or a combination thereof.

Lic3 VL	DVLMTQIPLSLTVSLGDQASISC**RSSQSIVHSNGNTYLE**WYLQRPGQSPKLLI **YKVSNRFS**GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC**FQGSHVPLI**FGA GTKLELK (SEQ ID NO. 1)
Lic3 VH	EVQLVESGGGLVKPGGSLKLSCAASGFSF**SDYYMY**WVRQAPEKRLEWVA **SISFDGIYTYYTDRVKG**RFTISRDNAKNNLYLQMSSLKSEDTAMYYCAR **DRPAWFPY**WGQGTLVTVSA (SEQ ID NO. 2)
Lic5 VL	DIVLTQTTLSLNVSLGDQASISC**RSSQSIVHSNGNTYLE**WYLQRPGQSPKLLI **YKVSNRFS**GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC**FQGSHVPLI**FGA GTKLELKRAD (SEQ ID NO. 3)
Lic5 VH	EVQLEESGGGLVKPGGSLKLPCAASGSSF**SDFYMY**WVRQTPEKRLEWVA **SISFDGIYTYYTDRVKG**RFTISRDNAKNNLYLQMSSLKSEDTAMYYCAR **DRPAWFPY**WGQGTLVTVSA (SEQ ID NO. 4)

FIG. 1

```
Lic3VL    DIVLTQTPLSLTVSLGDQASISCRSSQSIVHSNGNTYLGWYLQRPGQSPKLLIYKVSNR
2-30/J1   DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTYLGWFQQRPGQSPRRLIYKVSNR
          *:*:**:**** *:**: **********************: *******: ********

Hum1      .......................RSSQSIVHSNG....................KVSNR
Hum2      ...........T...........RSSQSIVHSNG....................KVSNR
Hum3      .................Q.....RSSQSIVHSNG....................KVSNR
Hum4      .......................RSSQSIVHSNG.......L............KVSNR
Hum5      .......................RSSQSIVHSNG................L...KVSNR
Hum6      .......................RSSQSIVHSNG....................KVSNR

Lic3VL    FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLTFGAGTKLELK
2-30/J1   FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGQGTKVEIK
          ****************************:**************** ***:*:*

Hum1      FS................................FQGSHVPLT..........
Hum2      FS................................FQGSHVPLT..........
Hum3      FS................................FQGSHVPLT..........
Hum4      FS................................FQGSHVPLT..........
Hum5      FS................................FQGSHVPLT..........
Hum6      FS................................FQGSHVPLT..A.......
```

FIG. 2

```
Lic3VL          DIVLTQTPLSLTVSLGDQASISCRSSQSIVESNGNTYLEWYLQRPGQSPKLLIYKVSNRF
2-24/J1         DIVMTQTPLSSPVTLGQPASISCRSSQSIVESNGNTYLGWLQQRPGQPPRLLIYKVSNRF
                ***:****** *:**: ********************** ***** *:**********

Hum1            .......................RSSQSIVESNG.....................KVSNRF
Hum2            ..........L............RSSQSIVESNG.....................KVSNRF
Hum3            ..........T............RSSQSIVESNG.....................KVSNRF
Hum4            ................Q.....RSSQSIVESNG.....................KVSNRF
Hum5            .......................RSSQSIVESNG.......Y.............KVSNRF
Hum6            .......................RSSQSIVESNG..............S.....KVSNRF
Hum7            .......................RSSQSIVESNG.....................KVSNRF


Lic3VL          SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLTFGAGTKLELK
2-24/J1         SGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGQGTKVEIK
                ***********:**************:**************** ***:*:*

Hum1            S..............................FQGSHVPLT..........
Hum2            S..............................FQGSHVPLT..........
Hum3            S..............................FQGSHVPLT..........
Hum4            S..............................FQGSHVPLT..........
Hum5            S..............................FQGSHVPLT..........
Hum6            S..............................FQGSHVPLT..........
Hum7            S..............................FQGSHVPLT..A.......


Lic3Vk          DVLMTQIPLSLTVSLGDQASISCRSSQSIVHSNGNTYLEWYLQRPGQSPKLLIYKVSNRF
Vk2-29          DIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRF
                *:*** ****:*: *: ***************************:*****:**********

Hum1            ......I.............RSSQSIVHSNG.....................KVSNRF
Hum2            ............L.......RSSQSIVHSNG.....................KVSNRF
Hum3            ..............Q.....RSSQSIVHSNG.....................KVSNRF
Hum4            .......................RSSQSIVHSNG.............K....KVSNRF
Hum5            .......................RSSQSIVHSNG.....................KVSNRF


Lic3Vk          SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLTFGAGTKLELK
Vk2-29          SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGAGTKLELK
                ****************************:************************

Hum1            S..............................FQGSHVPLT..........
Hum2            S..............................FQGSHVPLT..........
Hum3            S..............................FQGSHVPLT..........
Hum4            S..............................FQGSHVPLT..........
Hum5            S.........................L.....FQGSHVPLT..........
```

FIG. 3

```
Lic3VH    QVQLQESGGGLVKPGGSLKLSCAASGFSFSDYYMYWVRQAPEKRLEWVASISFDGTYTYY
3-11/J4   QVQLVESGGGLVKPGGSLRLSCAASGFSFSDYYMYWIRQAPGKGLEWVSSISFDGTYTYY
          **** *************:*****************:**** * ****:***********

Hum1      ..............................DYYMY..............SISFDGTYTYY
Hum2      ....Q.........................DYYMY..............SISFDGTYTYY
Hum3      ..............................DYYMY......E.......SISFDGTYTYY
Hum4      ..............................DYYMY.........R....SISFDGTYTYY
Hum5      ..............................DYYMY.............ASISFDGTYTYY
Hum6      ..............................DYYMY..............SISFDGTYTYY
Hum7      ..............................DYYMY..............SISFDGTYTYY

Lic3VH    TDRVKGRFTISRDNAKNNLYLQMSSLKSEDTAMYYCARDRPAWFPYWGQGTLVTVSA
3-11/J4   TDRVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRPAWFPYWGQGTLVTVS-
          *****************.*****.**::****:***********************

Hum1      TDRVKG................................DRPAWFPY..........
Hum2      TDRVKG................................DRPAWFPY..........
Hum3      TDRVKG................................DRPAWFPY..........
Hum4      TDRVKG................................DRPAWFPY..........
Hum5      TDRVKG................................DRPAWFPY..........
Hum6      TDRVKG...........N....................DRPAWFPY..........
Hum7      TDRVKG.................S..............DRPAWFPY..........
```

FIG. 4

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Lic3VH   QVQLQESGGGLVKPGGSLKLSCAASGFSFSDYYMYWVRQAPEKRLEWVASISFDGTYTYY
3-30/J4  QVQLVESGGGVVQPGRSLRLSCAASGFSFSDYYMYWVRQAPGKGLEWVASISFDGTYTYY
         **** *****:*:** **:********************** * ****************

Hum1     ..............................DYYMY..............SISFDGTYTYY
Hum2     ....Q.........................DYYMY..............SISFDGTYTYY
Hum3     ...............G..............DYYMY..............SISFDGTYTYY
Hum4     ..............................DYYMY......E.......SISFDGTYTYY
Hum5     ..............................DYYMY........R.....SISFDGTYTYY
Hum6     ..............................DYYMY..............SISFDGTYTYY
Hum7     ..............................DYYMY..............SISFDGTYTYY
Hum8     ...........................T..DYYMY..............SISFDGTYTYY

Lic3VH   TDRVKGRFTISRDNAKNNLYLQMSSLKSEDTAMYYCARDRPAWFPYWGQGTLVTVSA
3-30/J4  TDRVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRPAWFPYWGQGTLVTVSA
         **************:**.*****.**::****:************************

Hum1     TDRVKG................................DRPAWFPY...........
Hum2     TDRVKG................................DRPAWFPY...........
Hum3     TDRVKG................................DRPAWFPY...........
Hum4     TDRVKG................................DRPAWFPY...........
Hum5     TDRVKG................................DRPAWFPY...........
Hum6     TDRVKG...........N....................DRPAWFPY...........
Hum7     TDRVKG.................S..............DRPAWFPY...........
Hum8     TDRVKG................................DRPAWFPY...........
```

FIG. 5

```
Lic5VL    DIVLTQTTLSLNVSLGDQASISCRSSQSIVHSNGNTYLEWYLQRPGQSPKLLIYKVSNRF
IGKV2-30  DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGNTYLNWFQQRPGQSPRRLIYKVSNRD
          *:*:**: *** *:**: ***********:*:*:*****:*: *******: ********

Hum1      .......................RSSQSIVHSNGNTYLE...............KVSNRF
Hum2      .......T...............RSSQSIVHSNGNTYLE...............KVSNRF
Hum3      ............N..........RSSQSIVHSNGNTYLE...............KVSNRF
Hum4      .................Q.....RSSQSIVHSNGNTYLE...............KVSNRF
Hum5      .......................RSSQSIVHSNGNTYLE..L............KVSNRF
Hum6      .......................RSSQSIVHSNGNTYLE...........L...KVSNRF

Lic5VL    SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLTFGAGTKLELKRAD
IGKV2-30  SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQGTHWPVVFGGGTKLTVL---
          ***************************:*****:**:* *:.**.**** :

Hum1      .................................FQGSHVPLT..........
Hum2      .................................FQGSHVPLT..........
Hum3      .................................FQGSHVPLT..........
Hum4      .................................FQGSHVPLT..........
Hum5      .................................FQGSHVPLT..........
Hum6      .................................FQGSHVPLT..........
```

FIG. 6

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Lic5VL   -DIVLTQTTLSLNVSLGDQASISCRSSQSIVHSNGNTYLEWYLQRPGQSPKLLIYKVSNR
4X0K     KDIVMTQTPSSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNR
          ***:***   **  ***************************:***************

Hum1      .......................RSSQSIVHSNGNTYLE...............KVSNR
Hum2      ...L...................RSSQSIVHSNGNTYLE...............KVSNR
Hum3      .......T...............RSSQSIVHSNGNTYLE...............KVSNR
Hum4      ...........N...........RSSQSIVHSNGNTYLE...............KVSNR
Hum5      ...L...T...............RSSQSIVHSNGNTYLE...............KVSNR
Hum6      .......T...N...........RSSQSIVHSNGNTYLE...............KVSNR

Lic5VL   FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLTFGAGTKLELKRAD
4X0K     FSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSLVPPTFGAGTKLELKRGA
         ******************************:*******  **  ***********.

Hum1     FS................................FQGSHVPLT.............
Hum2     FS................................FQGSHVPLT.............
Hum3     FS................................FQGSHVPLT.............
Hum4     FS................................FQGSHVPLT.............
Hum5     FS................................FQGSHVPLT.............
Hum6     FS................................FQGSHVPLT.............
```

FIG. 7

```
Lic5VH     EVQLEESGGGLVKPGGSLKLPCAASGSSFSDFYMYWVRQTPEKRLEWVASISFDGTYTYY
IGHV3-21   EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYIYY
           **** *************:* ***** :**.: * ****:* * ****:*** ..:* **

Hum1       ..............................DFYMY..............SISFDGTYTYY
Hum2       ....E.........................DFYMY..............SISFDGTYTYY
Hum3       ....................P.........DFYMY..............SISFDGTYTYY
Hum4       ..........................S...DFYMY..............SISFDGTYTYY
Hum5       ..............................DFYMY......E.......SISFDGTYTYY
Hum6       ..............................DFYMY........R.....SISFDGTYTYY
Hum7       ....E...............P.........DFYMY..............SISFDGTYTYY
Hum8       ..............................DFYMY......E.R.....SISFDGTYTYY

Lic5VH     TDRVKGRFTISRDNAKNNLYLQMSSLKSEDTAMYYCARDRPAWFPYWGQGTLVTVSA
IGHV3-21   ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRPAWFPYWGQGTLVTVSA
           :* **************.*****.**::****:************************

Hum1       TDRVKG................................DRPAWFPY...........
Hum2       TDRVKG................................DRPAWFPY...........
Hum3       TDRVKG................................DRPAWFPY...........
Hum4       TDRVKG................................DRPAWFPY...........
Hum5       TDRVKG................................DRPAWFPY...........
Hum6       TDRVKG................................DRPAWFPY...........
Hum7       TDRVKG................................DRPAWFPY...........
Hum8       TDRVKG................................DRPAWFPY...........
```

FIG. 8

```
Lic5VH    EVQLEESGGGLVKPGGSLKLPCAASGSSFSDFYMYWVRQTPEKRLEWVASISFDGTYTYY
IGHV3-7   EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYY
          **** *******:*****:* ***** :**.::* ****:* * *****.*. **: .**
Hum1      ..............................DFYMY..............SISFDGTYTYY
Hum2      ....E.........................DFYMY..............SISFDGTYTYY
Hum3      ....................P.........DFYMY..............SISFDGTYTYY
Hum4      ..........................S...DFYMY..............SISFDGTYTYY
Hum5      ..............................DFYMY......E.......SISFDGTYTYY
Hum6      ..............................DFYMY........R.....SISFDGTYTYY
Hum7      ..............................DFYMY..............SISFDGTYTYY
Hum8      ..............................DFYMY..............SISFDGTYTYY

Lic5VH    TDRVKGRFTISRDNAKNNLYLQMSSLKSEDTAMYYCARDRPAWFPYWGQGTLVTVSA
IGHV3-7   VDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDRPAWFPYWGQGTLVTVSA
          .* **************.*****.**::****:************************
Hum1      TDRVKG................................DRPAWFPY...........
Hum2      TDRVKG................................DRPAWFPY...........
Hum3      TDRVKG................................DRPAWFPY...........
Hum4      TDRVKG................................DRPAWFPY...........
Hum5      TDRVKG................................DRPAWFPY...........
Hum6      TDRVKG................................DRPAWFPY...........
Hum7      TDRVKG...........N....................DRPAWFPY...........
Hum8      TDRVKG..................S.............DRPAWFPY...........
```

FIG. 9

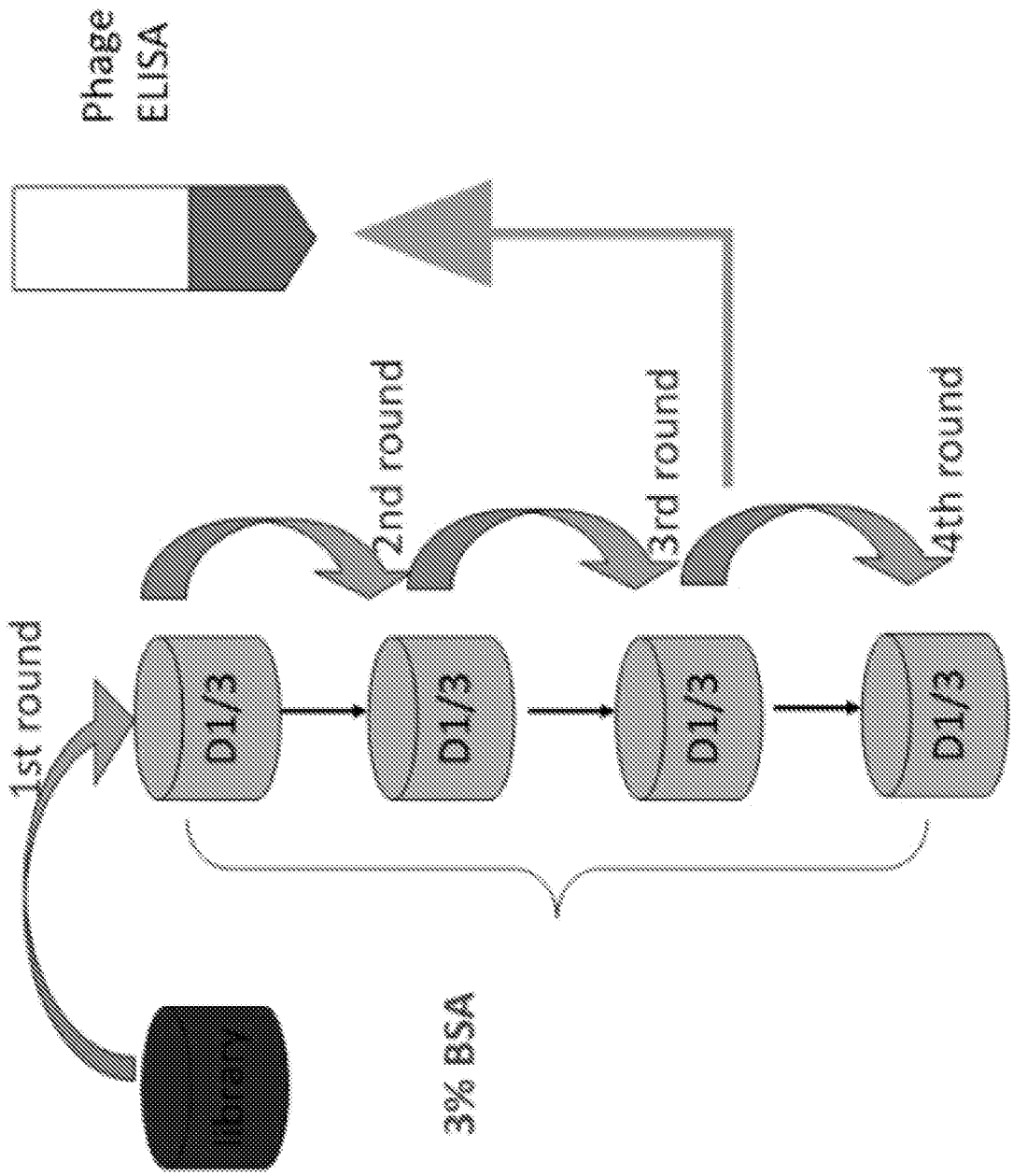

Phage
ELISA
2nd round
3rd round
4th round
1st round
D1/3
D1/3
D1/3
D1/3
3% BSA

FIG. 10

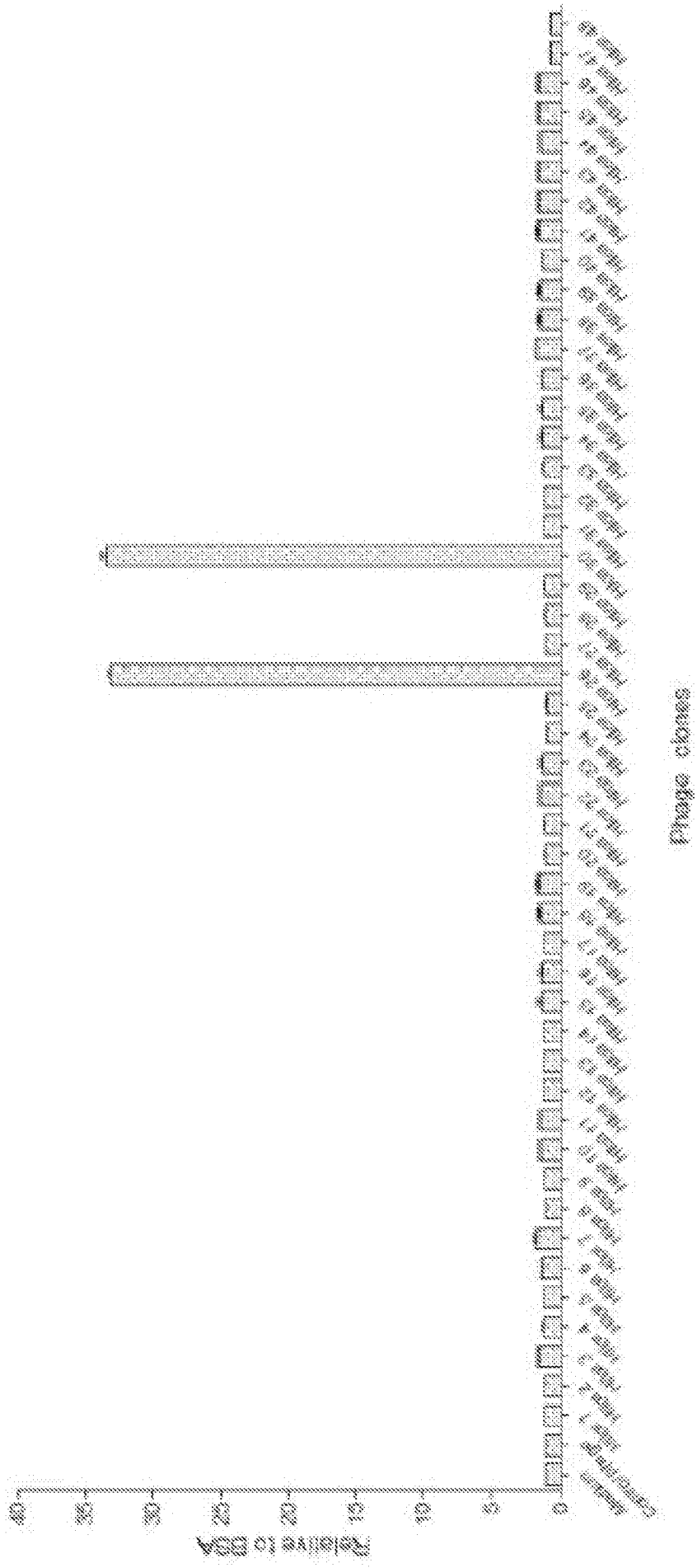


FIG. 11

HuLica26	VH (SEQ ID NO. 5): AEVQLVETGGGLVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVA VISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCASRF GMDVWGQGT
	VL (SEQ ID NO. 6): MFVMSQSPLSLPVTLGQPASISCRSSQSLVHSDGNTYLSWFQQRPGQSPRRL IYNVFNRDSGVPDRFSGSGSGTDFTLEISRVEAEDVGVYYCMQGTHWPFTF GQGTKLE
HuLica 30	VH (SEQ ID NO. 7): AEVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVA VISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGR WFDPWGQGT
	VL (SEQ ID NO. 8): SVVTQPPSVSGAPGQRVTISCTGSSSNIGAGYGVQWYQQFPGTAPKLLIYG NNNRPSGVPDRFSGSKSDTSASLAITGLRAEDEADYYCQSYDSSLSGWVFGGG
HuLica 66	VH (SEQ ID NO. 9): AEVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC AQGSGWYYWGQGT
	VL (SEQ ID NO. 10): DVVMTQSPLSLPVTLGQPASISCRSSRGLVHSDGNTYLTWFQQRPGQSPRR LIYKVSSRDSGVPDRFSGSGSGTDFTLKINRVEAEDVGVYYCMQGTHWPW TFGQGTKVE

FIG. 12

HuLica 78	VH (SEQ ID NO. 11): AEVQLVESGSELKKPGASVKVSCKASGYTFNRYAMNWVRQAPGQGL EWMGWINTNTGNPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYY CARGRRGAFDIWGQGT
	VL (SEQ ID NO. 12): IVLTQSPLSLPVTPGEPASISCRSSQSLLHRNGYNYLDWYLQKPGQSPQLLIY LGSNRASGVPDRFSGSGSGTDFTLQISRVEAEDVGVYYCMQALLTPRTFGQ GTKVEI
HuLica 85	VH (SEQ ID NO. 13): AEVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWV AVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR SYSNFDYWGQGT
	VL (SEQ ID NO. 14): DVVMTQSPLSLPVTLGQPASISCRSSRSLVYSDGSTYLNWYQQRPGQSPRRL IYKVSNRDAGVPDRFSGSGSATYFTLKISRVEAEDVGVYFCMQGTHWPWTF GQGTKVE
HuLica 94	VH (SEQ ID NO. 15): AEVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVA VISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAQGS GWYYWGQGT
	VL (SEQ ID NO. 16): DVVMTQSPLSLPVTLGQPASISCRSSRGLVHSDGNTYLTWFQQRPGQSPRRL IYKVSSRDSGVPDRFSGSGSGTDFTLKINRVEAEDVGVYYCMQGTHWPWT FGQGTKVE

FIG. 12 (CONTINUED)

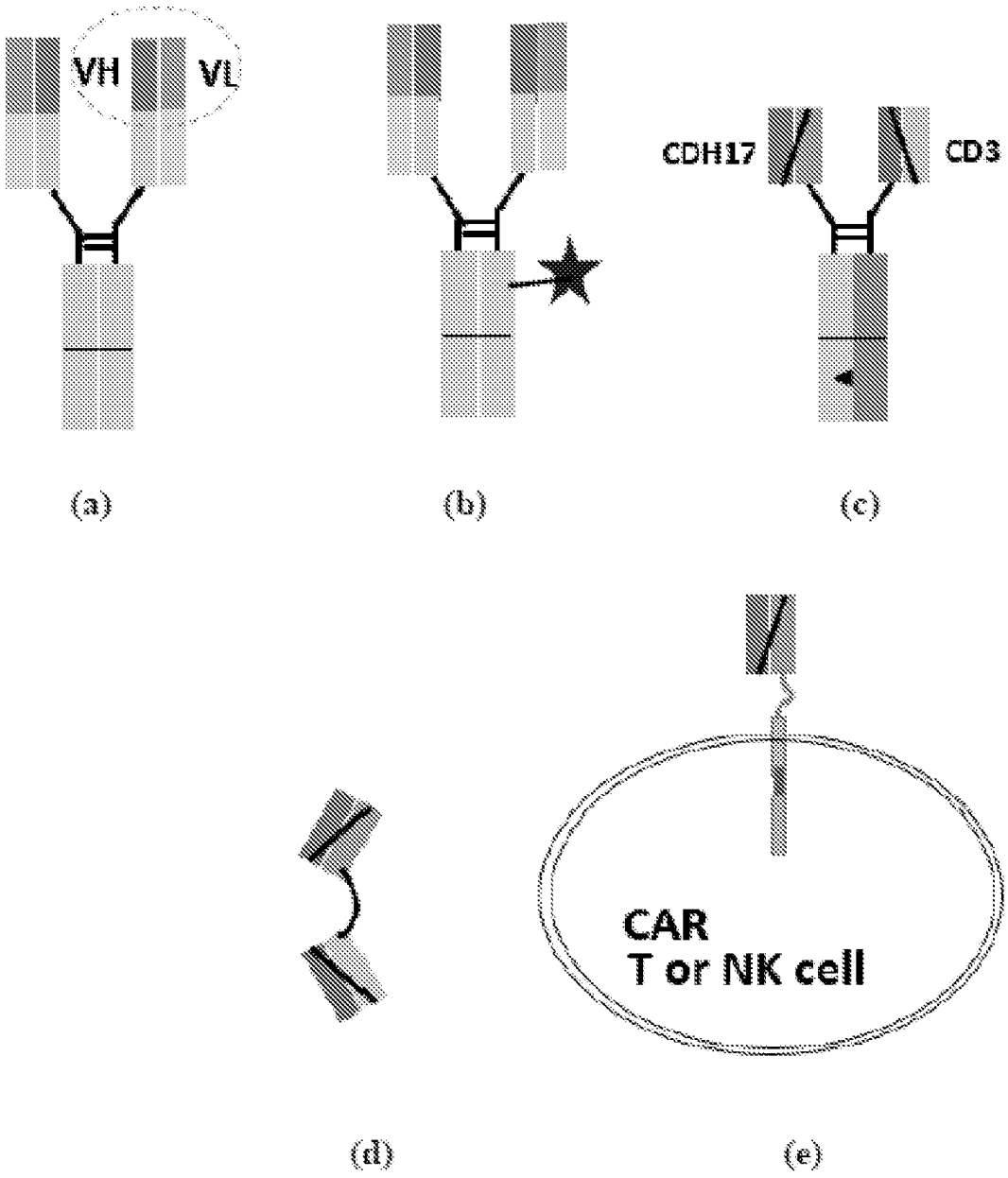


FIG. 13

CDH17 arm:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYYMYWVRQAPGKGLEWVASISFDGT
YTYYTDRVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRPAWFPYWGQGTLV
TVSAGGGGSGGGGSGGGGSGDIVMTQTPLSLSVTPGQPASISCRSSQSIVHSNGNTY
LEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQG
SHVPLTFGAGTKLELKGAPGGGSGEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSPEEMTKNQVSLYC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK** (SEQ ID NO. 17)

CD3 arm:

EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPGKGLEWVALI
NPYKGVTTYADSVKGRFTISVDKSKNTAYLQMNSLRAEDTAVYYCARSGYYG
DSDWYFDVWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRV
TITCRASQDIRNYLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTL
TISSLQPEDFATYYCQQGNTLPWTFGQGTKVEIKRTGAPGGGSGEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSPEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSRL
TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK** (SEQ ID NO 18)

FIG. 14

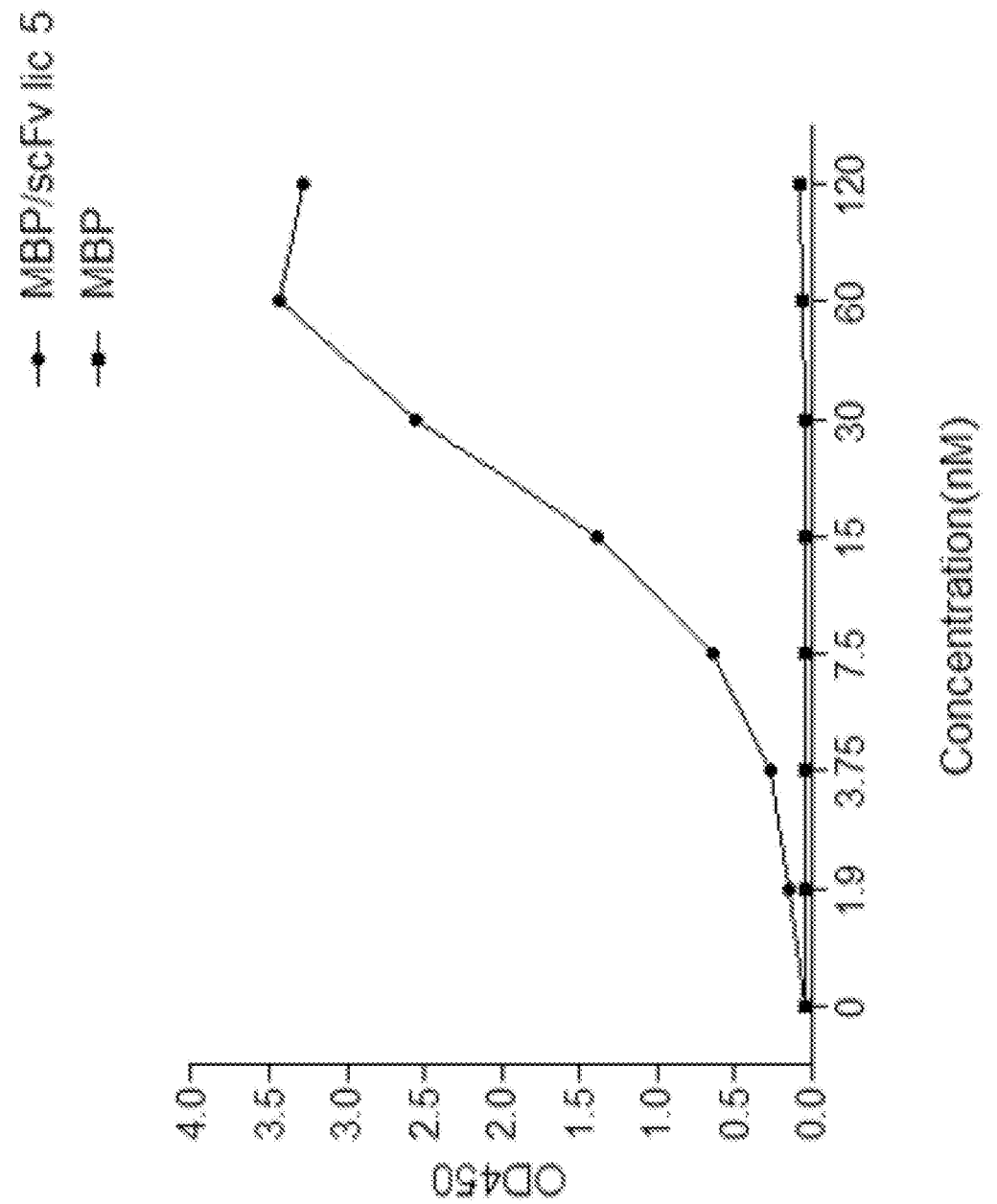

MBP/scFv IIc 5
MBP
OD450
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1.9
3.75
7.5
15
30
60
120
Concentration(nM)

FIG. 15

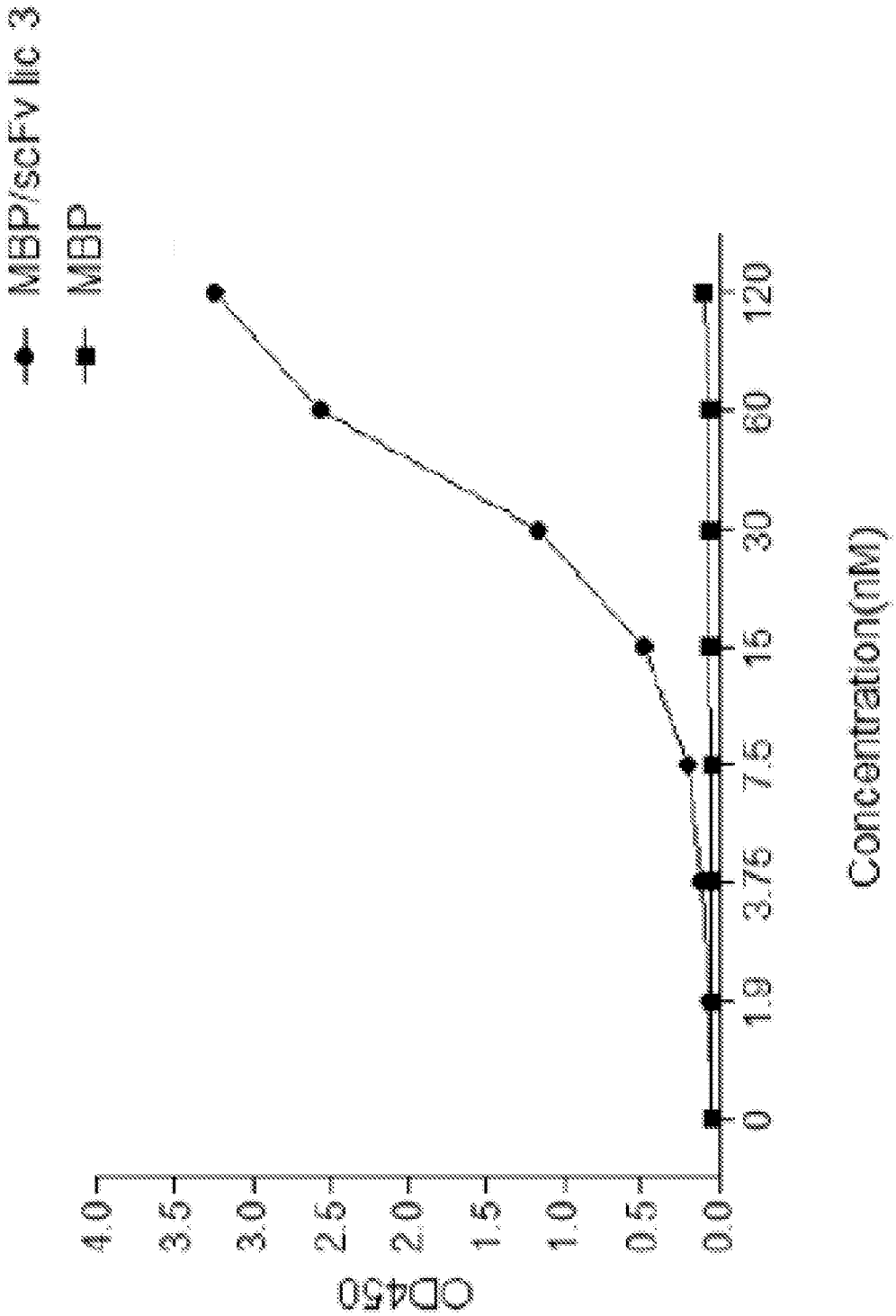

MBP/scFv IIc 3
MBP
OD450
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1.9
3.75
7.5
15
30
60
120
Concentration(nM)

FIG. 16

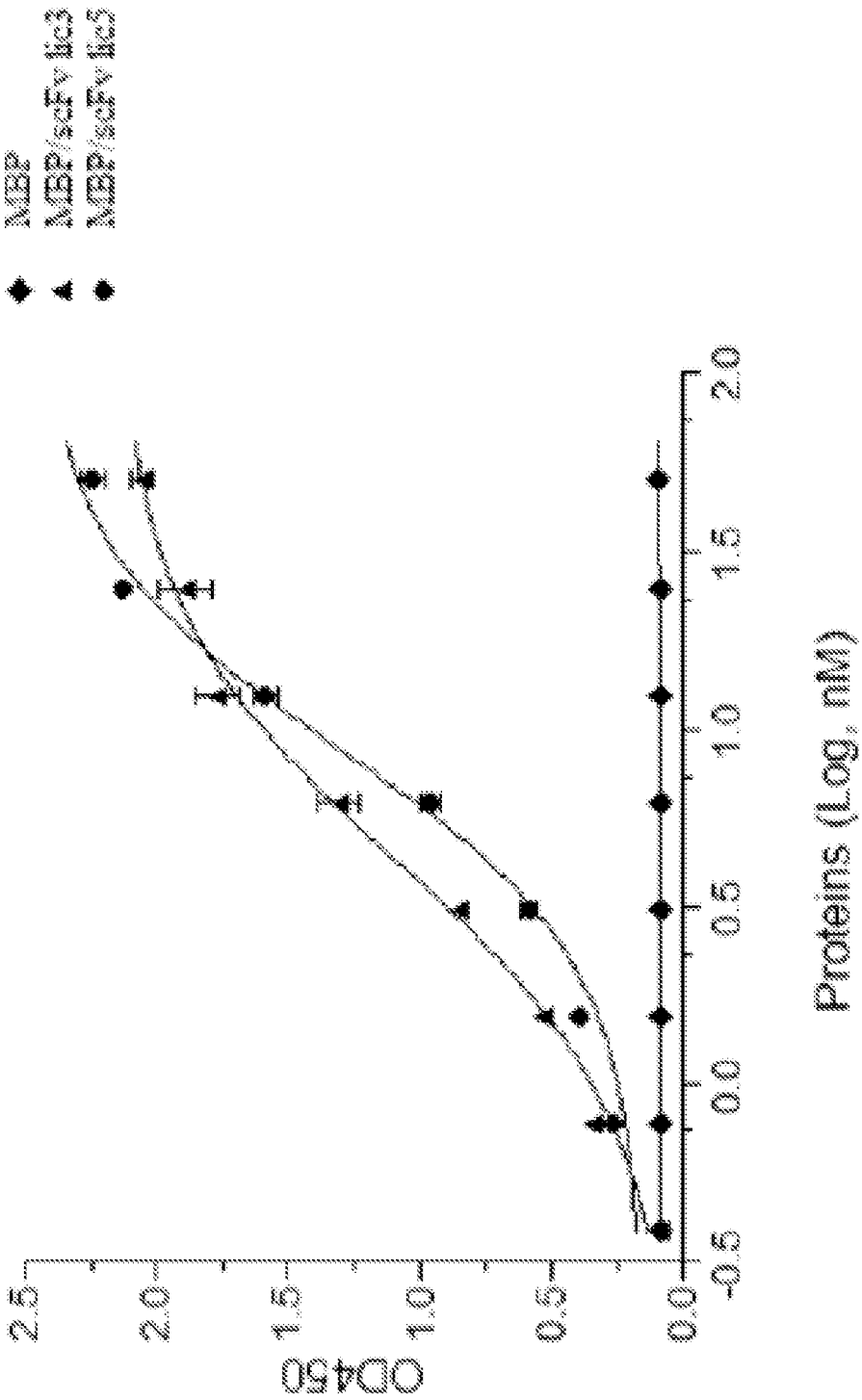

MBP
MBP/scFv lic3
MBP/scFv lic3
OD450
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0.0
0.5
1.0
1.5
2.0
Proteins (Log, nM)

FIG. 17

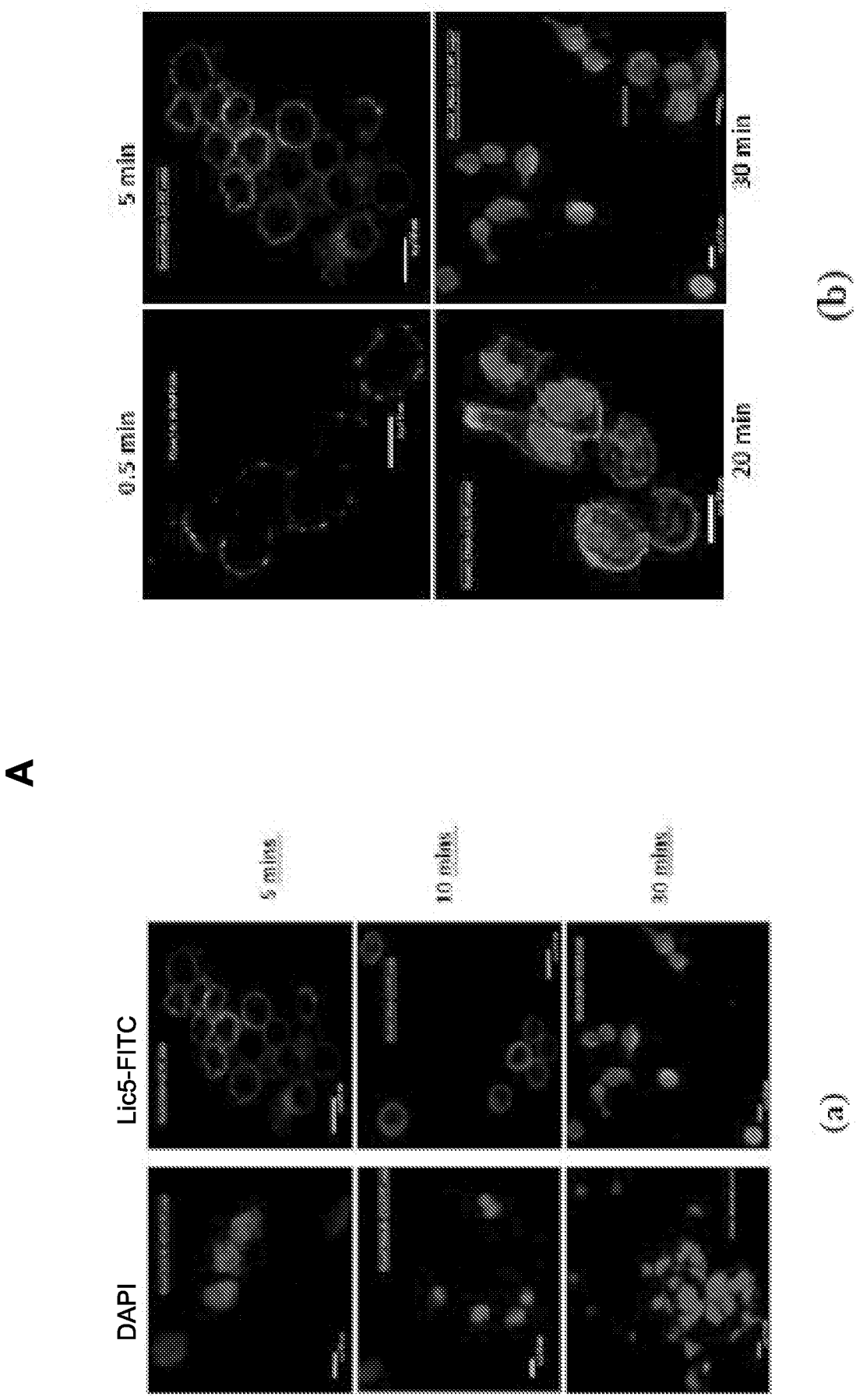

A
DAPI
Lic5-FITC
5 mins
10 mins
30 mins
(a)
0.5 min
5 min
20 min
30 min
(b)

FIG. 18

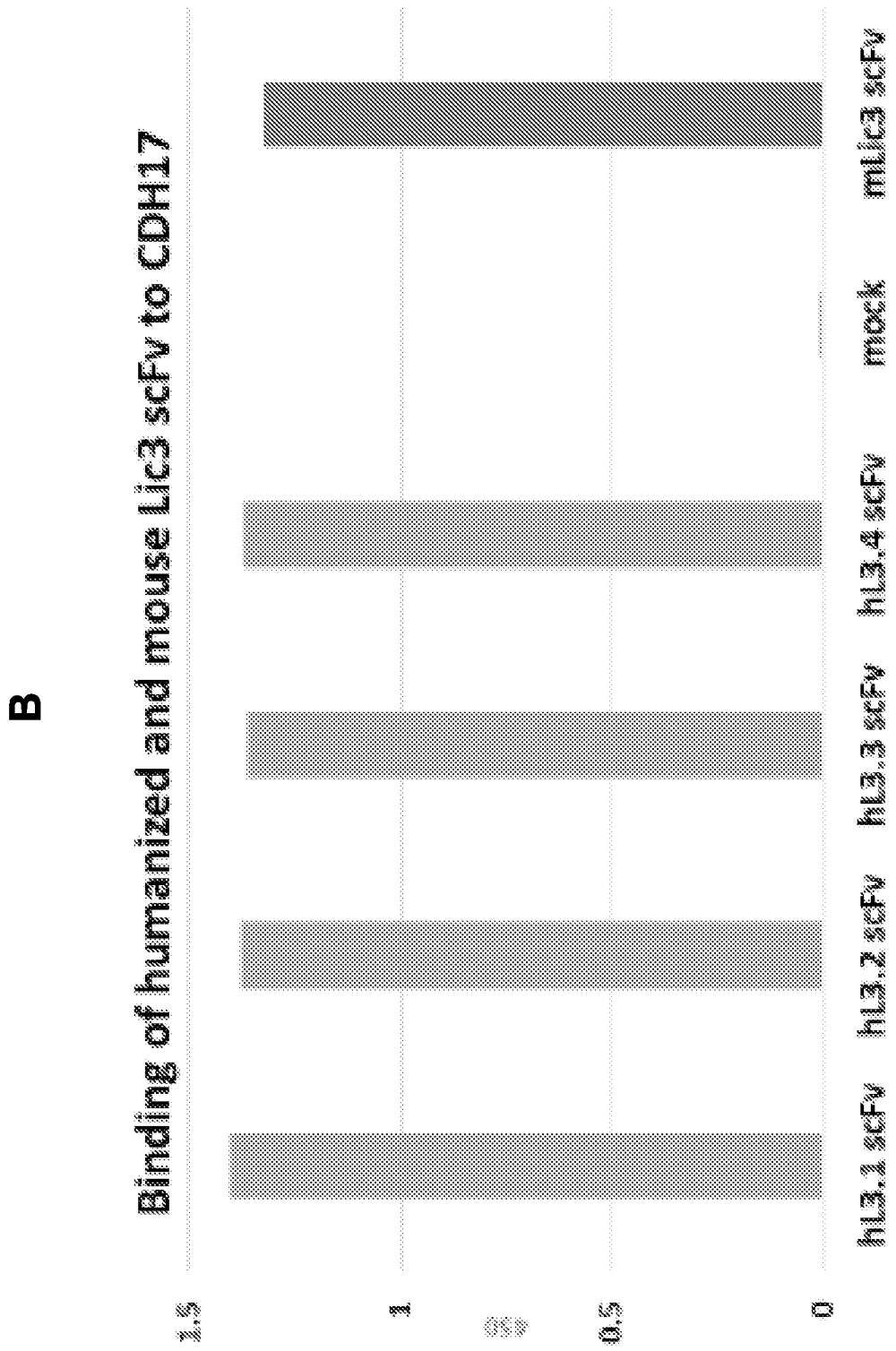


FIG. 18 (CONTINUED)

Examples of CDH17 monoclonal antibodies binding to CDH17 and CDH17 truncates													
	1E12	5F6	6C2	8E8	9A6	2H11	8G3	9C6	9F10	10C12	7C5-C10	8G5-A4	9B5-G2
BSA	0.093	0.069	0.081	0.130	0.063	0.061	0.063	0.078	0.062	0.071	0.065	0.058	0.057
CDH17 D1-2	0.062	0.059	0.057	0.059	0.056	0.531	0.057	0.053	0.064	0.309	0.056	0.080	0.069
CDH17 D3-4	0.061	0.063	0.063	0.058	1.151	0.057	0.061	0.054	1.333	0.055	0.054	0.054	0.058
CDH17 D5-7	0.061	1.445	0.059	0.060	0.065	0.058	0.056	0.056	0.056	0.060	0.059	0.054	0.059
CDH17 D6	0.062	1.403	0.070	0.061	0.058	0.058	0.056	0.058	0.057	0.055	0.057	0.059	0.061
CHO CDH17F c	0.158	1.334	0.619	0.152	1.095	1.365	0.613	0.434	1.306	0.428	1.003	0.717	0.602
293F CDH17F c	1.029	1.556	0.679	0.972	1.081	1.511	0.655	0.556	1.097	0.357	0.927	0.744	0.053
IgG control	0.071	0.068	0.063	0.061	0.066	0.061	0.062	0.061	0.064	0.060	0.068	0.065	0.055

FIG. 19

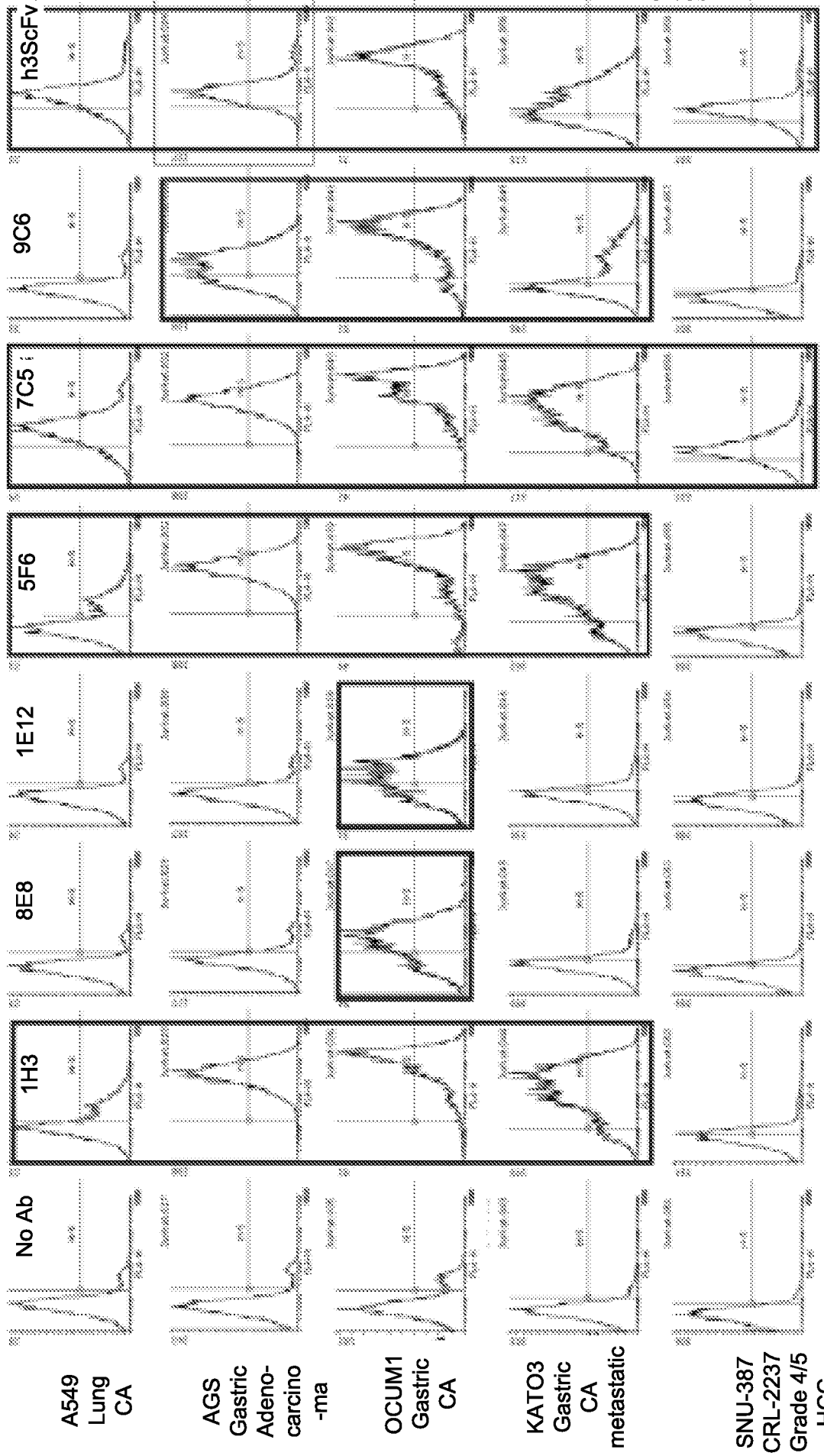

No Ab
1H3
8E8
1E12
5F6
7C5
9C6
h3ScFv
A549 Lung CA
AGS Gastric Adeno-carcino -ma
OCUM1 Gastric CA
KATO3 Gastric CA metastatic
SNU-387 CRL-2237 Grade 4/5 HCC

FIG. 20

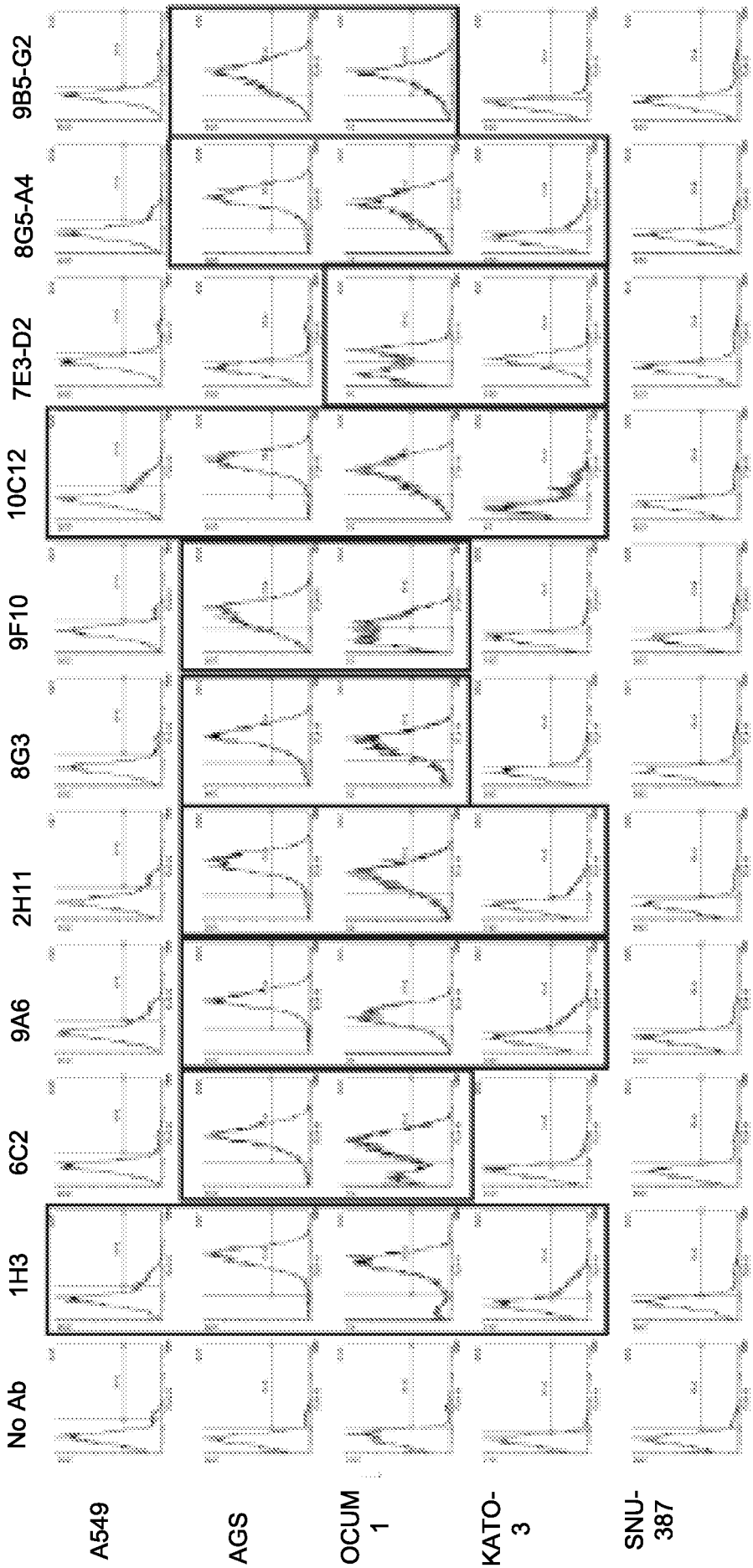
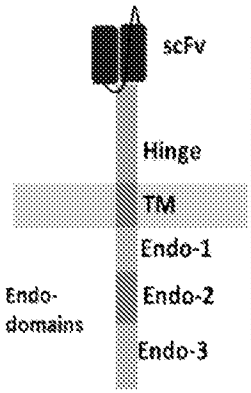

No Ab
1H3
6C2
9A6
2H11
8G3
9F10
10C12
7E3-D2
8G5-A4
9B5-G2
A549
AGS
OCUM 1
KATO-3
SNU-387

FIG. 21



CAR:	scFv	Hinge	TM	Endo-1	Endo-2	Endo-3
CAR7	hLic3	8	8	CD137	3z- iso1/2	--
CAR7b	hLic3	8	8	CD137	3z-iso3/4 +K	--
CAR8	hLic3	8	8	CD28	3z –iso1/2	--
CAR9	hLic3	IgG CPPC	28	CD137	3z –iso1/2	-

SEQ ID NO. 25: hLic3 CAR7a

QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYYMYWVRQAPGKGLEWVASISFDGTYTYYTDRVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARDRPAWFPYWGQGTLVTVSAGGGGSGGGGSGGGGSGDIVMTQTPLSLSVTPGQPASI
SCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPL
TFGAGTKLELKGAPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY
CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD
KRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
PR

SEQ ID NO. 26: hLic3 CAR7b

QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYYMYWVRQAPGKGLEWVASISFDGTYTYYTDRVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARDRPAWFPYWGQGTLVTVSAGGGGSGGGGSGGGGSGDIVMTQTPLSLSVTPGQPASI
SCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPL
TFGAGTKLELKGAPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY
CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLD
KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP
R

SEQ ID NO. 27: hLic3 CAR8

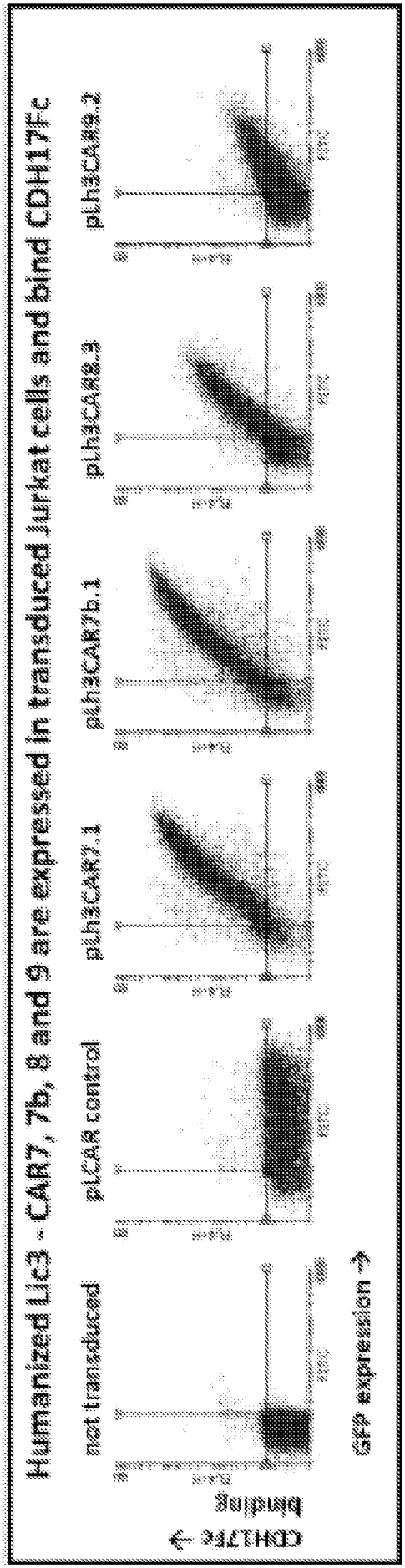
QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYYMYWVRQAPGKGLEWVASISFDGTYTYYTDRVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARDRPAWFPYWGQGTLVTVSAGGGGSGGGGSGGGGSGDIVMTQTPLSLSVTPGQPASI
SCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPL
TFGAGTKLELKGAPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY
CRSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
LDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
LPPR

SEQ ID NO. 28: hLic3 CAR9

QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYYMYWVRQAPGKGLEWVASISFDGTYTYYTDRVKGRFTISRDNSKNT
LYLQMNSLRAEDTAVYYCARDRPAWFPYWGQGTLVTVSAGGGGSGGGGSGGGGSGDIVMTQTPLSLSVTPGQPASI
SCRSSQSIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPL
TFGAGTKLELKGAPGGGSGEPKSSDKTHTCPPCPAPELLGGPDFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIF
KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

FIG. 22

a



b

FIG. 22 (CONTINUED)

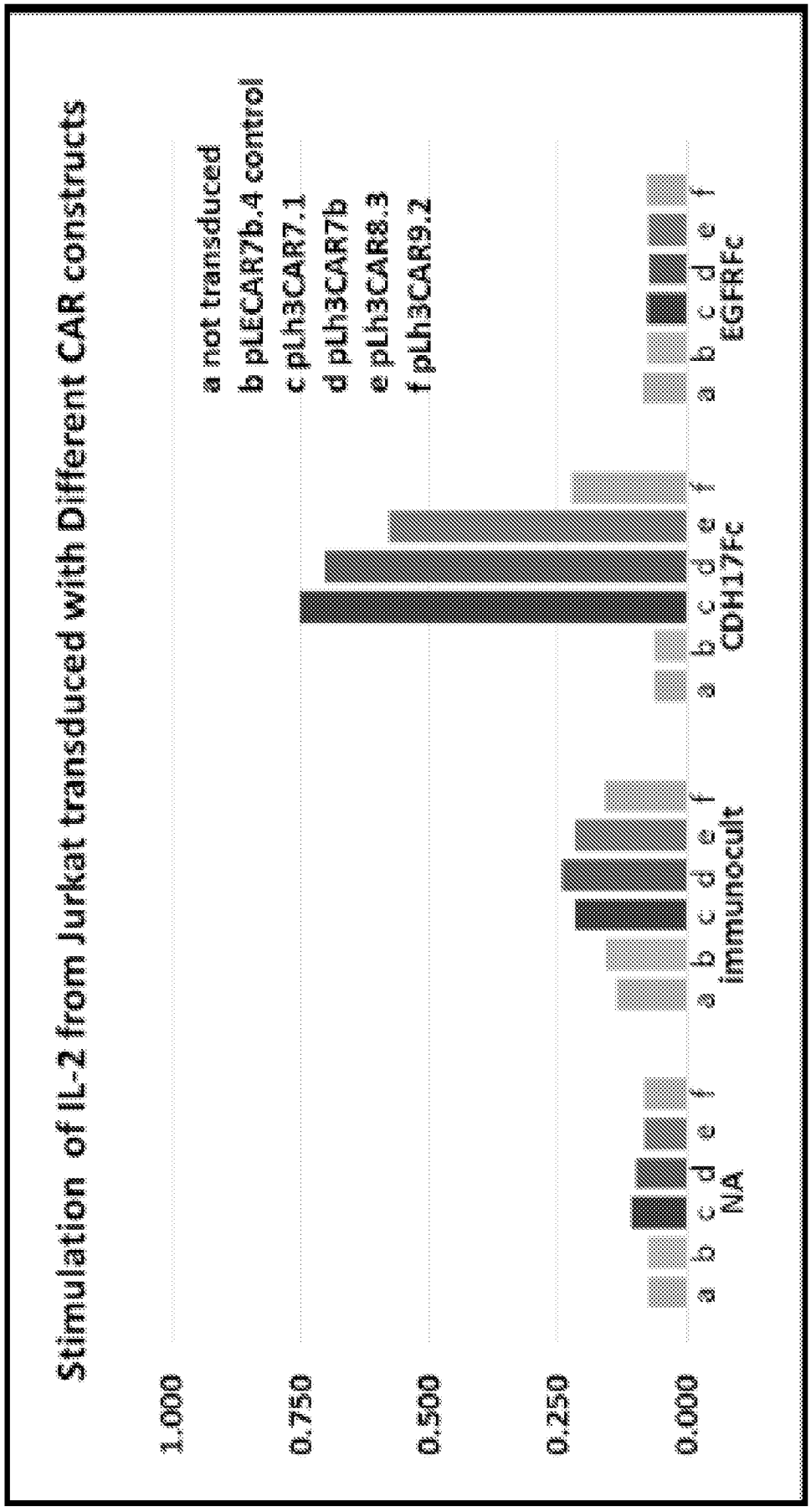

Stimulation of IL-2 from Jurkat transduced with Different CAR constructs
a not transduced
b pLECAR7b.4 control
c pLh3CAR7.1
d pLh3CAR7b
e pLh3CAR8.3
f pLh3CAR9.2
1.000
0.750
0.500
0.250
0.000
a b c d e f
NA
a b c d e f
immunocult
a b c d e f
CDH17Fc
a b c d e f
EGFRFc

FIG. 23

SEQ ID NO. 19: Lic3 2nd generation CAR (Lic3scFv-CD28hinge+TM+endo-CD3zeta endo)

QVQLQESGGGLVKPGGSLKLSCAASGFSFSDYYMYWVRQAPEKRLEWVASISFDGTYTYYTDRVKGRFTISRDNAKN
NLYLQMSSLKSEDTAMYYCARDRPAWFPYWGQGTLVTVSAGGGGSGGGGSGGGGSDIVLTQTPLSLTVSLGDQASIS
CRSSQSIVHSNGMTYLGWYLQRPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLT
FGAGTKLELKRADLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK
HYQPYAPPRDFAAYRSGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

FIG. 24

SEQ ID NO. 20: Lic5 2nd generation CAR (Lic5scFv-CD28hinge+TM+endo-CD3zeta endo)

EVQLEESGGGLVKPGGSLKLPCAASGSSFSDFYMYWVRQTPEKRLEWVASISFDGTYTYYTDRVKGRFTISRDNAKNN
LYLQMSSLKSEDTAMYYCARDRPAWFPYWGQGTLVTVSAGGGGSGGGGSGGGGSDIVLTQTTLSLNVSLGDQASIC
RSSQSIVHSNGNTYLEWYLQRPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLTF
GAGTKLELKRADLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKH
YQPYAPPRDFAAYRSGGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

FIG. 25

SEQ ID NO. 21: HLic26 2nd generation CAR (Lic26scFv-CD28hinge+TM+endo-CD3zeta endo)

AEVQLVETGGGLVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSK
NTLYLQMNSLRAEDTAVYYCASRFGMDVWGQGTGGGSGGGGSGGGGSGGGGSMFVMSQSPLSLPVTLGQPASISCRSSQ
SLVHSDGNTYLSWFQQRPGQSPRRLIYNVFNRDSGVPDRFSGSGSGTDFTLEISRVEAEDVGVYYCMQGTHWPFTFG
QGTKLELCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSGGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYN
ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

FIG. 26

SEQ ID NO. 22: Lic3 3rd generation CAR (Lic3scFv-CD28-4-1BB-CD3zeta endo)

QVQLQESGGGLVKPGGSLKLSCAASGFSFSDYYMYWVRQAPEKRLEWVASISFDGTYTYYTDRVKGRFTISRDNAKN
NLYLQMSSLKSEDTAMYYCARDRPAWFPYWGQGTLVTVSAGGGGSGGGGSGGGGSDIVLTQTPLSLTVSLGDQASI
SCRSSQSIVHSNGNTYLGWYLQRPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVP
LTFGAGTKLELKRADLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPT
RKHYQPYAPPRDFAAYRSGGGRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADAP
AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPR

FIG. 27

SEQ ID NO. 23: Lic5 3rd generation CAR (Lic5scFv-CD28hinge+TM+endo-4-1BB endo-CD3zeta endo)

EVQLEESGGGLVKPGGSLKLPCAASGSSFSDFYMYWVRQTPEKRLEWVASISFDGTYTYYTDRVKGRFTISRDNAKNN
LYLQMSSLKSEDTAMYYCARDRPAWFPYWGQGTLVTVSAGGGGSGGGGSGGGGSDIVLTQTTLSLNVSLGDQASIS
CRSSQSIVHSNGNTYLEWYLQRPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPL
TFGAGTKLELKRADLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPT
RKHYQPYAPPRDFAAYRSGGGRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADAP
AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPR

FIG. 28

SEQ ID NO. 24: HuLic26 3rd generation CAR (Lic26scFv-CD28hinge+TM+endo-4-1BB endo-CD3zeta endo)

AEVQLVETGGGLVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSK
NTLYLQMNSLRAEDTAVYYCASRFGMDVWGQGTGGGSGGGGSGGGGSGGGGSMFVMSQSPLSLPVTLGQPASISCRSSQ
SLVHSDGNTYLSWFQQRPGQSPRRLIYNVFNRDSGVPDRFSGSGSGTDFTLEISRVEAEDVGVYYCMQGTHWPFTFG
QGTKLELCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAYRSGGGRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADAPAYQQGQN
QLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY
QGLSTATKDTYDALHMQALPPR

FIG. 29

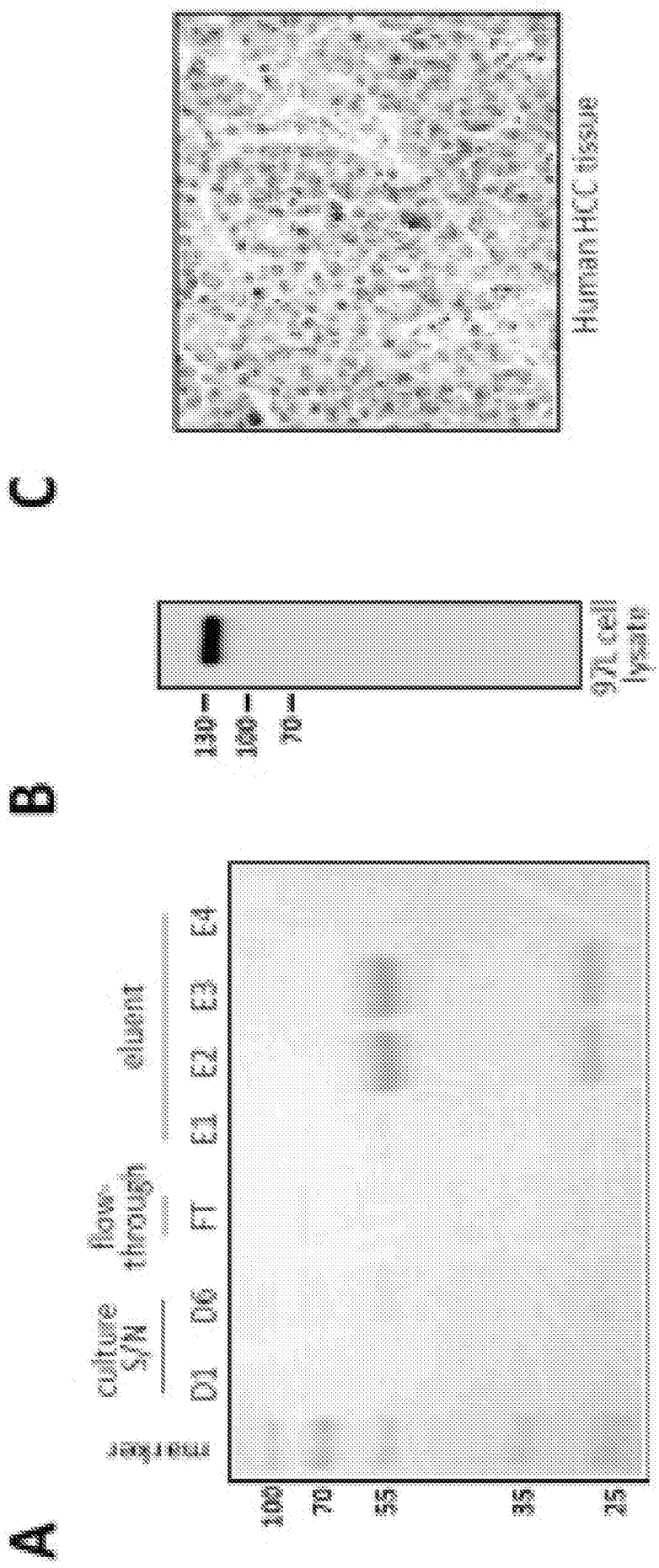

A
B
C
culture syn
flow-through
elumt
D1
D6
FT
E1
E2
E3
E4
100
70
55
35
25
130
100
70
97L cell lysate
Human HCC tissue

FIG. 30

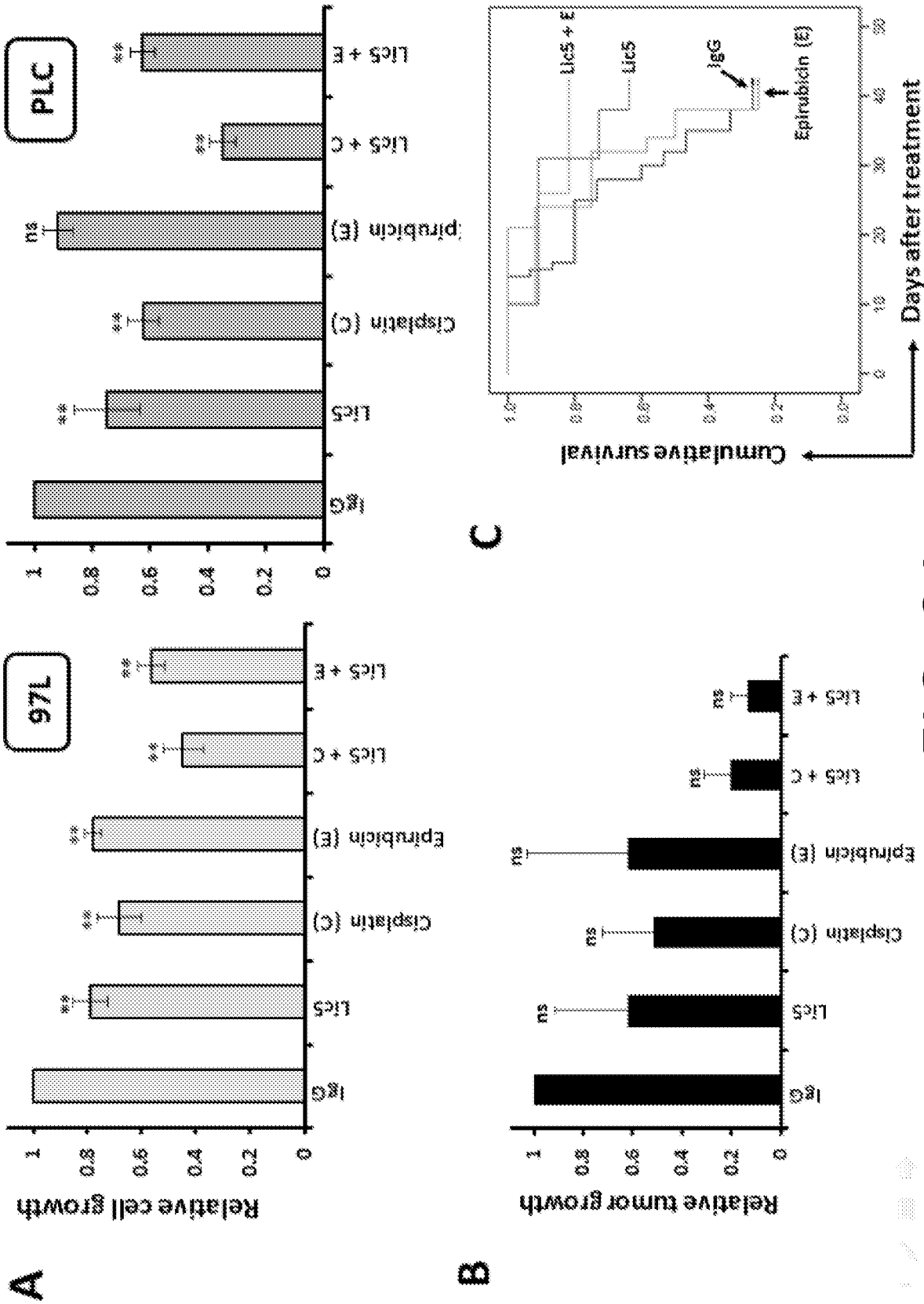

A
97L
PLC
Relative cell growth
IgG
Lic5
Cisplatin (C)
Epirubicin (E)
Lic5 + C
Lic5 + E
**
ns
B
Relative tumor growth
C
Cumulative survival
Days after treatment
Lic5 + E
Lic5
IgG
Epirubicin (E)

FIG. 31

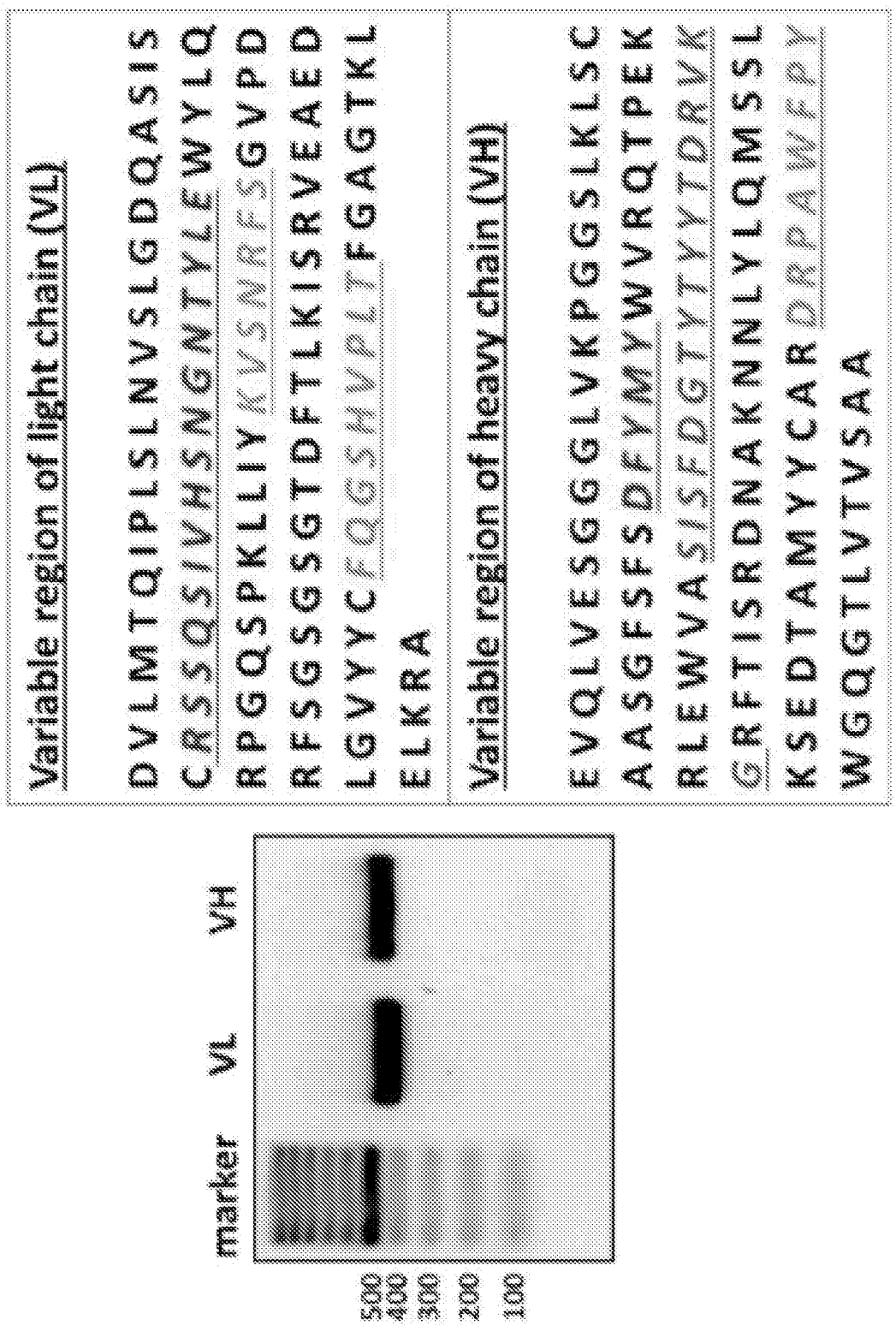

Variable region of light chain (VL)
DVLMTQIPLSLNVSLGDQASIS
CRSSQSIVHSNGNTYLEWYLQ
RPGQSPKLLIYKVSNRFSGVPD
RFSGSGSGTDFTLKISRVEAED
LGVYYCFQGSHVPLTFGAGTKL
ELKRA
Variable region of heavy chain (VH)
EVQLVESGGGLVKPGGSLKLSC
AASGFSFSDFYMYWVRQTPEK
RLEWVASISFDGTYTYYTDRVK
GRFTISRDNAKNNLYLQMSSL
KSEDTAMYYCARDRPAWFPY
WGQGTLVTVSAA
marker
VL
VH
500
400
300
200
100

FIG. 32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/012648

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. [X] forming part of the international application as filed:

      [X] 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: 1-30 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/012648

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395; C07K 16/18; C12N 15/12 (2017.01)
CPC - A61K 2039/505; C07K 2317/56; C12N 15/11 (2017.02)

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
USPC - 424/130.1; 530/387.3; 536/23.5 (keyword delimited)

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.
A	US 2015/0322151 A1 (AMGEN INC) 12 November 2015 (12.11.2015) entire document	1-46
A	US 2014/0322218 A1 (AMGEN RESEARCH (MUNICH)) 30 October 2014 (30.10.2014) entire document	1-46
A	US 2010/0092978 A1 (LUK et al) 15 April 2010 (15.04.2010) entire document	1-46
A	US 2010/0028357 A1 (MATSUBARA et al) 04 February 2010 (04.02.2010) entire document	1-46
A	WO 2010/123874 A1 (OXFORD BIOTHERAPEUTICS LTD.) 28 October 2010 (28.10.2010) entire document	1-46

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2017	06 APR 2017
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 PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774