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(54) **Title**: ANTIBODIES AGAINST IMMUNOGENIC GLYCOPEPTIDES, COMPOSITION COMPRISING THE SAME AND USE THEREOF

(57) **Abstract**: Disclosed herein are antibodies which specifically bind to at least one epitope defined by the immunogenic glycopeptide. Other aspects of the present disclosure are pharmaceutical composition comprising the antibody; and method for preventing and/or treating Globo-H- positive cancer.

ANTIBODIES AGAINST IMMUNOGENIC GLYCOPEPTIDES, COMPOSITION COMPRISING THE SAME AND USE THEREOF

Field of the Invention

[0001] The present invention relates to the field of immunotherapy of cancer. More particularly, the disclosed invention relates to an antibody against an immunogenic glycopeptide, a pharmaceutical composition comp comprising the antibody and to the use thereof in cancer therapy.

Background of the Invention

[0002] Globo H is a hexasaccharide and belongs to a large number of tumor-associated carbohydrate antigens that are overexpressed on the surface of various epithelial cancer cells, including breast, colon, ovarian, pancreatic, lung, and prostate cancer cells. The aberrant expression of Globo H renders it an attractive candidate for immunotherapy and the development of cancer vaccines for Globo H-positive cancers.

[0003] However, like most carbohydrate antigens, Globo H is often tolerated by the immune system, and consequently, the immunogenicity induced by Globo H is limited. Further, the production of antibody against a specific immunogen typically involves the cooperative interaction of two types of lymphocytes, B-cells and helper T-cells. Yet, Globo H alone cannot activate helper T-cells, which also attributes to the poor immunogenicity of Globo H. Accordingly, the immunization with Globo H is often typified by low titer of immunoglobulin M (IgM) and failure to class switch to immunoglobulin G (IgG), as well as ineffective antibody affinity maturation.

[0004] Various approaches have been developed to address the above-mentioned deficiencies. In certain researches, foreign carrier proteins or peptides having T-epitopes (such as keyhole limpet hemocyanin (KLH) or detoxified tetanus toxoid (TT)) have been conjugated with carbohydrate antigens hoping to enhance the immunogenicity of the carbohydrate antigens. US 20010048929 provided a multivalent immunogenic molecule, comprising a carrier molecule containing at least one functional T-cell epitope, and multiple different carbohydrate fragments each linked to the carrier molecule and each containing at least one functional B-cell epitope, wherein said carrier molecule imparts enhanced immunogenicity to said multiple carbohydrate fragments and wherein the carbohydrate fragment is Globo H, LeY or STn. US 20120328646 provides a carbohydrate based vaccine containing Globo H (B cell epitope) chemically conjugated to the immunogenic carrier diphtheria toxin cross-reacting material 197 (DT-CRM 197) (Th epitope) via a p-nitrophenyl

linker, which provides immunogenicity in breast cancer models, showing delayed tumorigenesis in xenograft studies. US 20120263749 relates to a polyvalent vaccine for treating cancer comprising at least two conjugated antigens selected from a group containing glycolipid antigen such as Globo H, a Lewis antigen and a ganglioside, polysaccharide antigen, mucin antigen, glycosylated mucin antigen and an appropriate adjuvant.

[0005] Nonetheless, conjugation of carbohydrates to a carrier protein poses several new problems. According to Ingale et al., the foreign carrier protein and the linker for attaching the carrier protein and the carbohydrate may elicit strong B-cell responses, thereby leading to the suppression of an antibody response against the carbohydrate epitope (*Ingale S. et al. Robust immune responses elicited by a fully synthetic three-component vaccine. Nat Chem Biol. 2007 Oct;3(10):663-7. Epub 2007 Sep 2*). Furthermore, Ingale *et al.* also indicated that the conjugation chemistry is difficult to control, resulting in conjugates with ambiguities in composition and structure, which may affect the reproducibility of an immune response. Considering the above-mentioned factors, Ingale et al. concluded that it is not surprising that preclinical and clinical studies using carbohydrate-protein conjugates have led to mixed results. For example, Kuduk *et al.* taught that the immunization with a trimeric cluster of Tn-antigens conjugated to KLH in the presence of the adjuvant QS-21 elicited modest titers of IgG antibodies in mice (*Kuduk SD, et al. Synthetic and immunological studies on clustered modes of mucin-related Tn and TF O-linked antigens: the preparation of a glycopeptide-based vaccine for clinical trials against prostate cancer. J Am Chem Soc. 1998;120:12474–12485*); while Slovin *et al.* taught that the same vaccine gave low median IgG and IgM antibody titers in a clinical trial of relapsed prostate cancer patients (*Slovin SF, et al. Fully synthetic carbohydrate-based vaccines in biochemically relapsed prostate cancer: clinical trial results with alpha-N-acetylgalactosamine-O-serine/threonine conjugate vaccine. J Clin Oncol. 2003;21:4292–4298*).

[0006] Moreover, for cancer patients with hypoimmune status; particular in patients receiving chemotherapy or radiation therapy, as well as late-stage cancer patients, the efficacy of active immune intervention is often limited, for these patients may not be able to produce sufficient antibodies to elicit the anti-tumor effect.

[0007] In view of the foregoing, there exists a need in the art for developing alternative strategies for improving the immunization and/or therapeutic efficacy of carbohydrate-based vaccines.

Summary of the Invention

[0008] The following presents a simplified summary of the disclosure in order to provide a basic understanding to the reader. This summary is not an extensive overview of the disclosure and it does not identify key/critical elements of the present invention or delineate the scope of the present invention. Its sole purpose is to present some concepts disclosed herein in a simplified form as a prelude to the more detailed description that is presented later.

[0009] The present disclosure is directed to an antibody which specifically binds to Globo H according to any of the above-mentioned aspect/embodiments of the present disclosure.

[0010] According to certain embodiments, the antibody is a monoclonal antibody.

[0011] According to optional embodiments, the antibody is a chimeric or humanized antibody.

[0012] In still another aspect, the present disclosure is directed to a pharmaceutical composition for treating a cancer in a subject in need thereof.

[0013] According to one embodiment of the present disclosure, the pharmaceutical composition comprises (1) a therapeutically effective amount of the antibody according to any of the above-mentioned aspects/embodiments of the present disclosure, and optionally (2) a pharmaceutically acceptable carrier.

[0014] In still yet another aspect, the present disclosure is directed to a method of treating a cancer in a subject in need thereof.

[0015] According to embodiments of the present disclosure, the method includes administering to the subject the pharmaceutical composition the antibody or pharmaceutical composition according to any of the above-mentioned aspects/embodiments of the present disclosure.

Brief Description of the Drawing

[0016] Figures 1A to E illustrate results of cell binding assay (A: Isotype; B: VK9; C: MZ-2; D: Control serum; E: α-Globo H serum).

[0017] Figures 2 illustrate the binding affinity of anti-Globo H IgG antibody to primary ovarian cancer cells according to a few working examples of the present disclosure.

[0018] Figure 3 is a FACS graph illustrating the result of a serial glycan-bead binding analysis, according to one working example of the present disclosure.

[0019] Figures 4A and B shows the simulation of protein folding of anti-Globo H IgG antibody, according to one working example of the present disclosure (A: Mouse MZ-2 monoclonal antibody; B: Humanized MZ-2 monoclonal antibody).

[0020] Figures 5A to E show the binding affinity of anti-Globo H IgG antibody, representing the association and Dissociation curve fitting and dissociation constant, according to one working example of the present disclosure (A: mMZ-2; B: cMZ-2; C:hMZ-2L; D: MK-1; E: hMZ-2Lw).

[0021] Figure 6 shows that the human serum containing more than 1.6 μg/ml of hMZ-2Lw antibody elicited complement-dependent cytotoxicity in breast cancer, MCF-7 cells.

[0022] Figure 7 shows that hMZ-2Lw antibody in a concentration higher than about 10 and 20μg/ml resulted in a dose-dependent cytotoxicity in human ovarian cancer cell line TOV21G.

[0023] Figure 8 shows that hMZ-2Lw antibody in a concentration higher than about 10 and 20μg/ml resulted in a dose-dependent cytotoxicity in human pancreatic cancer cell line, HPAC.

[0024] Figure 9A and B show antigen-dependent cell-mediated cytotoxicity (ADCC) activity of the present hMZ-2Lw and MK1 antibodies on MCF-7 cells (breast cancer cell line).

[0025] Figures.10A and B show antigen-dependent cell-mediated cytotoxicity (ADCC) activity of the present hMZ-2Lw and MK1 antibodies on TOV21G cells (ovarian cancer cell line).

[0026] Figure 11 shows administration of both hMZ-2Lw and MK1 antibodies significantly inhibit the tumor growth, while control IgG antibody did not substantially affect the tumor growth.

[0027] Figure 12 shows results of hMZ-2 antibody in breast cancer subcutaneous model MCF-7 cell.

[0028] Figures 13A and B shows the results of hMZ-2 antibody in pancreatic cancer subcutaneous model HPAC cell. (A: Photographs of tumors; B: Reduction of tumor size)

[0029] Figure 14 shows that MZ-2 antibody inhibits migration of Globo H-expressing TOV21G cells.

[0030] Figure 15 shows that MZ-2 antibody inhibits migration of Globo H-expressing TOV21G cells.

Detailed Description of the Invention

[0031] The present invention is based, at least, on the finding that recombinant antibodies specifically bind to Globo H for treating cancer expression tumor-associated carbohydrate antigens.

Definition

[0032] Unless otherwise defined herein, scientific and technical terminologies employed in the present disclosure shall have the meanings that are commonly understood and used by one of ordinary skill in the art. Unless otherwise required by context, it will be understood that singular terms shall include plural forms of the same and plural terms shall include the singular. Specifically, as used herein and in the claims, the singular forms "a" and "an" include the plural reference unless the context clearly indicates otherwise. Also, as used herein and in the claims, the terms "at least one" and "one or more" have the same meaning and include one, two, three, or more.

[0033] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in the respective testing measurements. Also, as used herein, the term "about" generally means within 10%, 5%, 1%, or 0.5% of a given value or range. Alternatively, the term "about" means within an acceptable standard error of the mean when considered by one of ordinary skill in the art. Other than in the operating/working examples, or unless otherwise expressly specified, all of the numerical ranges, amounts, values and percentages such as those for quantities of materials, durations of times, temperatures, operating conditions, ratios of amounts, and the likes thereof disclosed herein should be understood as modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the present disclosure and attached claims are approximations that can vary as desired. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0034] The term "antigen" as used herein is defined as a substance capable of eliciting an immune response. Said immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. As used herein, the term "immunogen" refers to an antigen capable of inducing the production of an antibody. Also,

the term "immunogenicity" generally refers to the ability of an immunogen or antigen to stimulate an immune response.

[0035] The term "epitope" refers to a unit of structure conventionally bound by an immunoglobulin VH/VL pair. An epitope defines the minimum binding site for an antibody, and thus represent the target of specificity of an antibody.

[0036] The term "antibody" as used herein refers to a whole antibody molecule or a fragment, variant or derivative thereof, which is capable of recognizing or binding to an antigen. Most natural antibodies have two heavy chains and two light chains linked to each other by disulfide bonds. The light chain includes a variable domain (VL) and a constant domain (CL); while the heavy chain includes a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is, composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR).

[0037] As used herein, "antibody variable domain" refers to the portions of the light and heavy chains of antibody molecules that include amino acid sequences of Complementarity Determining Regions (CDRs; i.e., CDR1, CDR2, and CDR3), and Framework Regions (FRs). According to the methods used herein, the amino acid positions assigned to CDRs and FRs can be defined according to Kabat (Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md., 1987 and 1991)). Amino acid numbering of antibodies or antigen binding fragments is also according to that of Kabat.

[0038] As used herein, the term "Complementarity Determining Regions" (CDRs), i.e., CDR1, CDR2, and CDR3) refers to the amino acid residues of an antibody variable domain the presence of which are necessary for antigen binding. Each variable domain typically has three CDR regions identified as CDR1, CDR2 and CDR3. H-CDR refers to the CDR of the heavy chain and L-CDR refers to the CDR of the light chain.

[0039] As used herein, the term "monoclonal antibody" refers to an antibody molecule obtained from a single type of antibody-producing cells.

[0040] The term "chimeric antibody" refers to an antibody comprising a variable region from one source or species and at least a portion of a constant region derived from a different source or species, usually prepared by recombinant DNA techniques. It is preferred that the CDRs of a chimeric antibody have one origin, while the remainder of the antibody has a different origin. In particular, in the present invention the chimeric antibody may be a humanized antibody in which the antigen binding sequences/variable domains of a non-human antibody have been grafted onto human antibody framework regions.

[0041] As used herein, the term "humanized antibody" refers to forms of antibodies that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The humanized forms of rodent antibodies will essentially comprise the same CDR sequences of the parental rodent antibodies, although certain amino acid substitutions may be included to increase affinity, increase stability of the humanized antibody, or for other reasons. However, as CDR loop exchanges do not uniformly result in an antibody with the same binding properties as the antibody of origin, changes in framework residues (FR), residues involved in CDR loop support, might also be introduced in humanized antibodies to preserve antigen binding affinity.

[0042] Unless specified otherwise, in the peptide notation used herein, the left-hand direction is the amino-terminal (N-terminal) direction and the right-hand direction is the carboxy-terminal (C-terminal) direction, in accordance with standard usage and convention.

[0043] "Percentage (%) amino acid sequence identity" with respect to the amino acid sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percentage sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring

alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, sequence comparison between two amino acid sequences was carried out by computer program Blastp (protein-protein BLAST) provided online by Nation Center for Biotechnology Information (NCBI). Specifically, the percentage amino acid sequence identity of a given amino acid sequence A to a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has a certain % amino acid sequence identity to a given amino acid sequence B) is calculated by the formula as follows:

$$\frac{X}{Y} \times 100\%$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program BLAST in that program's alignment of A and B, and where Y is the total number of amino acid residues in A or B, whichever is shorter.

[0044] As discussed herein, minor variations in the amino acid sequences of proteins/polypeptides are contemplated as being encompassed by the presently disclosed and claimed inventive concept(s), providing that the variations in the amino acid sequence maintain at least 80% such as at least, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic aspartate, glutamate; (2) basic lysine, arginine, histidine; (3) nonpolar alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: serine and threonine are aliphatic-hydroxy family; asparagine and glutamine are an amide-containing family; alanine, valine, leucine and isoleucine are an aliphatic family; and phenylalanine, tryptophan, and tyrosine are an aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Fragments or analogs of proteins/polypeptides can be readily prepared by those of ordinary skill in the art.

Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains.

[0045] As used herein, "antibody mutant" or "antibody variant" refers to an amino acid sequence variant of the species-dependent antibody wherein one or more of the amino acid residues of the species-dependent antibody have been modified. Such mutants necessarily have less than 100% sequence identity or similarity with the species-dependent antibody. In one embodiment, the antibody mutant will have an amino acid sequence having at least 75% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the species-dependent antibody, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%. Identity or similarity with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical (i.e., same residue) or similar (i.e., amino acid residue from the same group based on common side-chain properties, see below) with the species-dependent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence outside of the variable domain shall be construed as affecting sequence identity or similarity.

[0046] As used herein, the term "antigen-binding portion" of an antibody (or simply "antigen portion"), refers to full length or one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH1 domains; a $F(ab)_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the V_H and CH1 domains; a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward et al., 1989 Nature 341:544-546), which consists of a V_H domain; and an isolated complementarity determining region (CDR), or any fusion proteins comprising such antigen-binding portion.

[0047] Unless contrary to the context, the term "treatment" are used herein broadly to include a preventative (e.g., prophylactic), curative, or palliative measure that results in a desired pharmaceutical and/or physiological effect. Preferably, the effect is therapeutic in terms of partially or completely curing or preventing cancer. Also, the terms “treatment” and

"treating" as used herein refer to application or administration of the present immunogenic glycopeptide, antibody, or pharmaceutical composition comprising any of the above to a subject, who has cancer, a symptom of cancer, a disease or disorder secondary to cancer, or a predisposition toward cancer, with the purpose to partially or completely alleviate, ameliorate, relieve, delay onset of, inhibit progression of, reduce severity of, and/or reduce incidence of one or more symptoms or features of cancer. Generally, a "treatment" includes not just the improvement of symptoms or decrease of markers of the disease, but also a cessation or slowing of progress or worsening of a symptom that would be expected in absence of treatment. The term "treating" can also be used herein in a narrower sense which refers only to curative or palliative measures intended to ameliorate and/or cure an already present disease state or condition in a patient or subject.

[0048] The term "preventing" as used herein refers to a preventative or prophylactic measure that stops a disease state or condition from occurring in a patient or subject. Prevention can also include reducing the likelihood of a disease state or condition from occurring in a patient or subject and impeding or arresting the onset of said disease state or condition.

[0049] The term "effective amount" as used herein refers to the quantity of a component which is sufficient to yield a desired response. Effective amount may be expressed, for example, in grams, milligrams or micrograms or as milligrams per kilogram of body weight (mg/kg). The term also refers to an amount of a pharmaceutical composition containing an active component or combination of components. The specific effective or sufficient amount will vary with such factors as the particular condition being treated, the physical condition of the patient (e.g., the patient's body mass, age, or gender), the type of mammal or animal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compounds or its derivatives.

[0050] As used herein, the term "therapeutically effective amount" refers to the quantity of an active component which is sufficient to yield a desired therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound or composition are outweighed by the therapeutically beneficial effects.

[0051] As used herein, a "pharmaceutically acceptable carrier" is one that is suitable for use with the subjects without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio. Also, each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the pharmaceutical composition. The carrier can be in the form of a solid, semi-solid, or liquid

diluent, cream or a capsule. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, and is selected to minimize any degradation of the active agent and to minimize any adverse side effects in the subject.

[0052] The term "subject" refers to a mammal including the human species that is treatable with antibody. The term “subject” is intended to refer to both the male and female gender unless one gender is specifically indicated.

[0053] All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[0054] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as those commonly understood to one of ordinary skill in the art to which this invention pertains. Although any known methods, devices, and materials may be used in the practice or testing of the invention, the methods, devices, and materials in this regard are described herein.

Antibodies for Prevention and/or Treatment Cancer

[0055] Provided herein are novel recombinant anti-Globo H antibodies and anti-Globo H-binding peptides specifically binding to Globo H or its derivatives, and methods of their use in anti-tumor immunotherapies, such as the treatment of cancer. Once bound to a cancer antigen, antibodies can induce antibody-dependent cell-mediated cytotoxicity, activate the complement system, and prevent a receptor interacting with its ligand (such as Globo H). In one embodiment, the compositions comprising the anti-Globo H-binding peptides or anti-Globo H antibodies described herein are useful in anti-cancer therapies. Furthermore, the compositions comprising the anti-Globo H-binding peptides or anti-Globo H antibodies can combine with an additional anti-tumor agent. In particular, the present embodiments provide the complementarity determining region (CDR) sequences of specific anti-Globo H antibodies, which can be used in a variety of anti-Globo H-binding peptides. In particular, the present invention provides a humanized or chimeric antibody or an antigen-binding fragment thereof capable of binding to Globo H or its derivatives.

[0056] In one aspect, the present invention provides an isolated anti-Globo H antibody or an antigen-binding portion thereof, comprising at least one of a heavy chain complementarity determining region 1 (H-CDR1) consisting of the amino acid residues of GFSLSTFDMGVG (SEQ ID NO: 1), GSSLSTFDVGVG (SEQ ID NO: 2), GFSLGTFDLGIG (SEQ ID NO: 3), GFSLSTFDLGIG (SEQ ID NO: 4) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 1 to 4; a heavy chain CDR2 (H-CDR2) consisting of the amino acid residues of HIWWDDDKYYNPA (SEQ ID NO:5), HIWGDDDKYYNPA (SEQ ID NO: 6) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 5 and 6; and a heavy chain CDR3 (H-CDR3) consisting of the amino acid residues of LYGNYLTSFYCDY (SEQ ID NO: 7), or LSGNYLTSFYCDY (SEQ ID NO: 8), LYGNYLRSYYCDY (SEQ ID NO: 9) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 7 to 9; and at least one of a light chain CDR1 (L-CDR1) consisting of the amino acid residues of SASSSVSYMH (SEQ ID NO: 10), SASSRVSYMH (SEQ ID NO:11), SARSSVSYMH (SEQ ID NO:12), RASSSVSYMH (SEQ ID NO:13) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 10 to 13; a light chain CDR2 (L-CDR2) consisting of the amino acid residues of ATSNLAS (SEQ ID NO:14), WTSDRYS (SEQ ID NO:15), DTSKLAS (SEQ ID NO:16) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 14 to 16; and a light chain CDR3 (L-CDR3) consisting of the amino acid residues QQWSSNPFT (SEQ ID NO: 17), QQWSSNPLT (SEQ ID NO: 18), QQHLHIPYT (SEQ ID NO: 19) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 17 to 19; such that said isolated antibody or antigen-binding portion thereof binds to Globo H. Preferably, the sequence identity as mentioned above is at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[0057] In a further embodiment, the present invention provides an isolated anti-Globo H antibody or an antigen-binding portion thereof, comprising (i) a heavy chain variable region comprising a heavy chain variable region comprising H-CDR1 selected from the group consisting of SEQ ID NOs: 1 to 4, H-CDR2 selected from the group consisting of SEQ ID NOs: 5 and 6 and H-CDR3 selected from the group consisting of SEQ ID NOs: 7 to 9, and (ii) light chain variable regions comprising L-CDR1 selected from the group consisting of SEQ ID NOs: 10 to 13, L-CDR2 selected from the group consisting of SEQ ID NOs: 14 to 16 and L-CDR3 selected from the group consisting of SEQ ID NOs: 17 to 19. Preferably, H-CDR1

is SEQ ID NO:3; H-CRD2 is SEQ ID NO:5; H-CDR3 is SEQ ID NO:8; L-CDR1 is SEQ ID NO:10; L-CDR2 is SEQ ID NO:16; and L-CDR3 is SEQ ID NO:18.

[0058] In further embodiments, H-CDR1 has the amino acid sequence of SEQ ID NO:3 or SEQ ID NO:4; H-CDR2 has the amino acid sequence of SEQ ID NO:5; H-CDR3 has the amino acid sequence of SEQ ID NO: 8; L-CDR1 has the amino acid sequence of SEQ ID NO:12; L-CDR2 has the amino acid sequence of SEQ ID NO:16 and L-CDR3 has the amino acid sequence of SEQ ID NO:18.

[0059] In one aspect, the present invention provides a heavy chain variable region comprising a heavy chain variable region comprising H-CDR1 selected from the group consisting of SEQ ID NOs: 1 to 4, H-CDR2 selected from the group consisting of SEQ ID NOs: 5 and 6 and H-CDR3 selected from the group consisting of SEQ ID NOs: 7 to 9. In a further embodiment, the present invention provides a heavy chain variable region comprising SEQ ID NO: 3 as H-CDR1, SEQ ID NO: 5 H-CDR2 and SEQ ID NO: 8 as H-CDR3.

[0060] In one aspect, the present invention provides a light chain variable region comprising L-CDR1 selected from the group consisting of SEQ ID NOs: 10 to 13, L-CDR2 selected from the group consisting of SEQ ID NOs: 14 to 16 and L-CDR3 selected from the group consisting of SEQ ID NOs: 17 to 19. In a further embodiment, the present invention provides a light chain variable region comprising SEQ ID NO: 12 as L-CDR1, SEQ ID NO: 16 as L-CDR2 and SEQ ID NO: 18 as L-CDR3.

[0061] In one embodiment, the isolated anti-Globo H antibody is a monocloncal antibody. Monoclonal antibodies to Globo H can be made according to knowledge and skill in the art. For example, it can be made by injecting test subjects with Globo H conjugate of the invention and then isolating hybridomas expressing antibodies having the desired sequence or functional characteristics.

[0062] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant production of antibodies will be described in more detail below.

[0063] In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the conventional techniques known in the art; for example, the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling, as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries. Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0064] In one embodiment, the present invention provides an isolated anti-Globo H antibody, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to any of the amino acid sequences of SEQ ID NOs: 140 to 163, and (ii) a light chain variable region comprising an amino acid sequence having at least 80% identical to any of the amino acid sequence of SEQ ID NO: 164 to 199. Preferably, the sequence identity as mentioned above is at least 90%, 91%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[0065] In one embodiment, the present invention provides an isolated anti-Globo H antibody, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to the amino acid sequences of SEQ ID NO: 147, and (ii) a light chain variable region comprising an amino acid sequence having at least 80% identical to the amino acid sequence of SEQ ID NO: 195. In a further embodiment, the present invention provides an isolated anti-Globo H antibody, comprising (i) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 147, and (ii) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 195 (MZ-2 antibody). The nucleotides encoding the heavy chian variable region having the amino acid sequence of SEQ ID NO: 147 are as follows:

CAGGTTACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGACCTCCCAGACCCTCAGTCTGACTTGT
TCTTTCTCTGGGTTTTCACTGGGCACTTTTGATTTGGGTATAGGCTGGATTCGTCAGCCTTCAGGGA
AGGGTCTGGAGTGGCTGGCGCACATCTGGTGGGATGATGATAAGTACTATAATCCAGCCCTGAAG
AGTCGGCTCACAATCTCCAAGGATACCTCCAAAAACCAGGTATTCCTCAAGATCGCCAATGTGGAC
ACTGCAGACTCTGCCACATATTACTGTGCTCGGCTCTCTGGAAACTACCTCACGTCGTTCTACTGTG
ACTACTGGGGCCAAGGCACCACTCTCACAGTGTCCTCA (SEQ ID NO: 236)

The nucleotides encoding the light chian variable region having the amino acid sequence of SEQ ID NO: 195 are as follows:

CAAATTGTTCTCACCCAGTCTCCAGCAATCGTGTCTGCATCTCCAGGGGAGAAGGTCACCATGACC
TGCAGTGCCAGATCAAGTGTAAGTTATATGCACTGGTACCAGCAGAAGTCAGGCACCTCCCCCAA

AAGATGGATTTATGACACATCCAAACTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTC TGGGACCTCTTATTCTCTCACAATCAGCAGCATGGAGGCTGAAGACGCTGCCACTTATTACTGCCA GCAGTGGAGTAGTAATCCACTCACGTTCGGTGCTGGGACCAAGCTGGAACTGAAACGG (SEQ ID NO: 237)

Heavy chain variable region of MZ-2 series

QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDMGVGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSR LTISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:140)

QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDMGVGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSR LTISKDTSKNQVFLKIANVDTADSATYYCARLSGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:141)

QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDMGVGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSR LTISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLRSYYCDYWGQGTTLTVSS (SEQ ID NO:142)

QVTLKESGPGILQTSQTLSLTCSFSGSSLSTFDVGVGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:143)

QVTLKESGPGILQTSQTLSLTCSFSGSSLSTFDVGVGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLSGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:144)

QVTLKESGPGILQTSQTLSLTCSFSGSSLSTFDVGVGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:145)

QVTLKESGPGILQTSQTLSLTCSFSGFSLGTFDLGIGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCAR LYGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:146)

QVTLKESGPGILQTSQTLSLTCSFSGFSLGTFDLGIGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLSGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:147)

QVTLKESGPGILQTSQTLSLTCSFSGFSLGTFDLGIGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLRSYYCDYWGQGTTLTVSS (SEQ ID NO:148)

QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDLGIGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCAR LYGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:149)

QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDLGIGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCAR LSGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:150)

QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDLGIGWIRQPSGKGLEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLRSYYCDYWGQGTTLTVSS (SEQ ID NO:151)

QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDMGVGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:152)

QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDMGVGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLSGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:153)

QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDMGVGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLRSYYCDYWGQGTTLTVSS (SEQ ID NO:154)

QVTLKESGPGILQTSQTLSLTCSFSGSSLSTFDVGVGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:155)

QVTLKESGPGILQTSQTLSLTCSFSGSSLSTFDVGVGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVFLKIANVDTADSATYYCARLSGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:156)

QVTLKESGPGILQTSQTLSLTCSFSGSSLSTFDVGVGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRL
TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLRSYYCDYWGQGTTLTVSS (SEQ ID NO:157)
QVTLKESGPGILQTSQTLSLTCSFSGFSLGTFDLGIGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRL
TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:158)
QVTLKESGPGILQTSQTLSLTCSFSGFSLGTFDLGIGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRL
TISKDTSKNQVFLKIANVDTADSATYYCARLSGNYLTSFYCDY WGQGTTLTVSS (SEQ ID NO:159)
QVTLKESGPGILQTSQTLSLTCSFSGFSLGTFDLGIGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRL
TISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLRSYYCDYWGQGTTLTVSS (SEQ ID NO:160)
QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDLGIGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRLT
ISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLTSFYCDY WGQGTTLTVSS (SEQ ID NO:161)
QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDLGIGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRLT
ISKDTSKNQVFLKIANVDTADSATYYCARLSGNYLTSFYCDYWGQGTTLTVSS (SEQ ID NO:162)
QVTLKESGPGILQTSQTLSLTCSFSGFSLSTFDLGIGWIRQPSGKGLEWLAHIWGDDDKYYNPALKSRLT
ISKDTSKNQVFLKIANVDTADSATYYCARLYGNYLRSYYCDYWGQGTTLTVSS (SEQ ID NO:163)

Light chain variable region of MZ-2 series

QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYATSNLAS
GVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:164)
QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:165)
QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:166)
QIVLTQSPAIVSASPGEKVTMTCSASSRVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:167)
QIVLTQSPAIVSASPGEKVTMTCSASSRVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:168)
QIVLTQSPAIVSASPGEKVTMTCSASSRVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:169)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:170)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYATSNLAS
GVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:171)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:172)
QIVLTQSPAIVSASPGEKVTMTCRASSSVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:173)
QIVLTQSPAIVSASPGEKVTMTCRASSSVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:174)
QIVLTQSPAIVSASPGEKVTMTCRASSSVSYMHWYQQKSGTSPKRWIYATSNLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:175)

QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYWTSDRYSGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:176)
QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYWTSDRYSGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:177)
QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYWTSDRYSGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:178)
QIVLTQSPAIVSASPGEKVTMTCSASSRVSYMHWYQQKSGTSPKRWIYWTSDRYS
GVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:179)
QIVLTQSPAIVSASPGEKVTMTCSASSRVSYMHWYQQKSGTSPKRWIY
WTSDRYSGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:180)
QIVLTQSPAIVSASPGEKVTMTCSASSRVSYMHWYQQKSGTSPKRWIYWTSDRYSGVPARFSGSGSGT
SYSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:181)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYWTSDRYSGVPARFSGSGSGT
SYSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:182)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYWTSDRYSGVPARFSGSGSGT
SYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:183)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYWTSDRYS
GVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:184)
QIVLTQSPAIVSASPGEKVTMTCRASSSVSYMHWYQQKSGTSPKRWIYWTSDRYSGVPARFSGSGSGT
SYSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:185)
QIVLTQSPAIVSASPGEKVTMTCRASSSVSYMHWYQQKSGTSPKRWIYWTSDRYSGVPARFSGSGSGT
SYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:186)
QIVLTQSPAIVSASPGEKVTMTCRASSSVSYMHWYQQKSGTSPKRWIYWTSDRYSGVPARFSGSGSGT
SYSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:187)
QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:188)
QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:189)
QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:190)
QIVLTQSPAIVSASPGEKVTMTCSASSRVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:191)
QIVLTQSPAIVSASPGEKVTMTCSASSRVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:192)
QIVLTQSPAIVSASPGEKVTMTCSASSRVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:193)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:194)

QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:195)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:196)
QIVLTQSPAIVSASPGEKVTMTCRASSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPFTFGAGTKLELKR (SEQ ID NO:197)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELKR (SEQ ID NO:198)
QIVLTQSPAIVSASPGEKVTMTCSARSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFSGSGSGTS
YSLTISSMEAEDAATYYCQQHLHIPYTFGAGTKLELKR (SEQ ID NO:199)

[0066] In another embodimetn, the isolated anti-Globo H antibody of the presetn invention is a humanized or chimeric antibody or fragment thereof capable of specifically binding to Globo H.

[0067] In a further embodiment, the present invention provides a humanized anti-Globo H antibody or an antigen-binding portion thereof, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to any of the amino acid sequences of SEQ ID NOs: 20 to 43, and (ii) a light chain variable region comprising an amino acid sequence having at least 80% identical to any of the amino acid sequence of SEQ ID NO: 44 to 79 and 200 to 235. Preferably, the sequence identity as mentioned above is at least 90%, 91%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[0068] In another further embodiment, the present invention provides a humanized anti-Globo H antibody or an antigen-binding portion thereof, comprising (i) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 20 to 43, and (ii) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 44 to 79 and 200 to 235. In another embodiment, the humanized anti-Globo H antibody or an antigen-binding portion thereof comprises a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 27, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 75 (hMZ-2Lw antibody). In another embodiment, the humanized anti-Globo H antibody or an antigen-binding portion thereof comprises a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 27, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 231.

Heavy chain variable region of hMZ-2 series

(QITLKESGPTLVKPTQTLTLTCTFS**********WIRQPPGKALEWLA**********LKSRLTITKDTS KNQVVLTMTNMDPVDTATYYCAR**********WGQGTLVTVSS)

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWWDDDKYYNPA LKSRLTITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 20)

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWWDDDKYYNPA LKSRLTITKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 21)

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWWDDDKYYNPA LKSRLTITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 22)

QITLKESGPTLVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 23)

QITLKESGPTLVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 24)

QITLKESGPTLVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 25)

QITLKESGPTLVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 26)

QITLKESGPTLVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 27)

QITLKESGPTLVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 28)

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 29)

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 30)

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 31)

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 32)

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 33)

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 34)

QITLKESGPTLVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL
TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 35)
QITLKESGPTLVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL
TITKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 36)
QITLKESGPTLVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL
TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 37)
QITLKESGPTLVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL
TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 38)
QITLKESGPTLVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL
TITKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 39)
QITLKESGPTLVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL
TITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 40)
QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRLT
ITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 41)
QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRLT
ITKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 42)
QITLKESGPTLVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRLT
ITKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 43)

Light chain variable region of hMZ-2 series

(EIVLTQSPSSLSASVGDRVTITC**********WYQQKPGKAPKLLIY*******GVPSRFSGSGSGTDFTFTI
SSLQPEDIATYYC*********FGGGTKLEIKR)

EIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 44)
EIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 45)
EIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 46)
EIVLTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 47)
EIVLTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 48)
EIVLTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 49)
EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 50)

EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYC QQWSSNPLT FGGGTKLEIKR (SEQ ID NO: 51)
EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 52)
EIVLTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 53)
EIVLTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 54)
EIVLTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYATSNLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 55)
EIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 56)
EIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 57)
EIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 58)
EIVLTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYC QQWSSNPFT FGGGTKLEIKR (SEQ ID NO: 59)
EIVLTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 60)
EIVLTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 61)
EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 62)
EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 63)
EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 64)
EIVLTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 65)
EIVLTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 66)
EIVLTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYWTSDRYSGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 67)
EIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 68)

EIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 69)
EIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 70)
EIVLTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 71)
EIVLTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 72)
EIVLTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 73)
EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 74)
EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 75)
EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 76)
EIVLTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 77)
EIVLTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 78)
EIVLTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTDF
TFTISSLQPEDIATYYCQQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 79)
EIQMTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 200)
EIQMTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 201)
EIQMTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 202)
EIQMTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 203)
EIQMTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 204)
EIQMTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 205)
EIQMTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 206)
EIQMTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLT FGGGTKLEIKR (SEQ ID NO: 207)

EIQMTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 208)
EIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 209)
EIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 210)
EIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYATSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 211)
EIQMTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 212)
EIQMTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 213)
EIQMTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 214)
EIQMTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFT FGGGTKLEIKR (SEQ ID NO: 215)
EIQMTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 216)
EIQMTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 217)
EIQMTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 218)
EIQMTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 219)
EIQMTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 220)
EIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 221)
EIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 222)
EIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYWTSDRYS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 223)
EIQMTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 224)
EIQMTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 225)
EIQMTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 226)
EIQMTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 227)

EIQMTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 228)
EIQMTQSPSSLSASVGDRVTITCSASSRVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 229)
EIQMTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 230)
EIQMTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 231)
EIQMTQSPSSLSASVGDRVTITCSARSSVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 232)
EIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPFTFGGGTKLEIKR (SEQ ID NO: 233)
EIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQWSSNPLTFGGGTKLEIKR (SEQ ID NO: 234)
EIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKLLIYDTSKLAS
GVPSRFSGSGSGTDFTLTISSLQPEDIATYYC QQHLHIPYTFGGGTKLEIKR (SEQ ID NO: 235)

[0069] In a further embodiment, the present invention provides a humanized anti-Globo H antibody or an antigen-binding portion thereof, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to any of the amino acid sequences of SEQ ID NOs: 80 to 103, and (ii) a light chain variable region comprising an amino acid sequence having at least 80% identical to any of the amino acid sequence of SEQ ID NOs: 104 to 139. Preferably, the sequence identity as mentioned above is at least 90%, 91%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[0070] In another further embodiment, the present invention provides a humanized anti-Globo H antibody or an antigen-binding portion thereof, comprising (i) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 80 to 103, and (ii) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 104 to 139.

[0071] In another embodiment, the humanized anti-Globo H antibody or an antigen-binding portion thereof comprises a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 90, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 135 (MK1 antibody).

Heavy chain variable region of MK1 series

(QVTLKESGPALVKPTQTLTLTCTFS**********WIRQPPGKALEWLA**********LKSRLTISKDT
SKNQVVLTMTNMDPVDTATYYCAR**********WGQGTLVTVSS)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWWDDDKYYNPALKS RLTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 80)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWWDDDKYYNPALKS RLTISKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 81)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWWDDDKYYNPALKS RLTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 82)

QVTLKESGPALVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWWDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 83)

QVTLKESGPALVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWWDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 84)

QVTLKESGPALVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWWDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 85)

QVTLKESGPALVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 86)

QVTLKESGPALVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 87)

QVTLKESGPALVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 88)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 89)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 90)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWWDDDKYYNPALKSRL TISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 91)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 92)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 93)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDMGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 94)

QVTLKESGPALVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 95)

QVTLKESGPALVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 96)

QVTLKESGPALVKPTQTLTLTCTFSGSSLSTFDVGVGWIRQPPGKALEWLAHIWGDDDKYYNPALKSR LTISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 97)

QVTLKESGPALVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 98)

QVTLKESGPALVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 99)

QVTLKESGPALVKPTQTLTLTCTFSGFSLGTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVVLTMTNMDPVDTATYYCAR LYGNYLRSYYCDY WGQGTLVTVSS (SEQ ID NO: 100)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 101)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVVLTMTNMDPVDTATYYCARLSGNYLTSFYCDYWGQGTLVTVSS (SEQ ID NO: 102)

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTFDLGIGWIRQPPGKALEWLAHIWGDDDKYYNPALKSRL TISKDTSKNQVVLTMTNMDPVDTATYYCARLYGNYLRSYYCDYWGQGTLVTVSS (SEQ ID NO: 103)

Light chain variable region of MK1 series

(DVVMTQSPAFLSVTPGEKVTITC**********WYQQKPDQAPKLLIK*******GVPSRFSGSGSGTDFTFT ISSLEAEDAATYYC*********FGQGTKLEIKR)

DVVMTQSPAFLSVTPGEKVTITCSASSSVSYMHWYQQKPDQAPKLLIKATSNLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 104)
DVVMTQSPAFLSVTPGEKVTITCSASSSVSYMHWYQQKPDQAPKLLIKATSNLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 105)
DVVMTQSPAFLSVTPGEKVTITCSASSSVSYMHWYQQKPDQAPKLLIKATSNLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 106)
DVVMTQSPAFLSVTPGEKVTITCSASSRVSYMHWYQQKPDQAPKLLIKATSNLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 107)
DVVMTQSPAFLSVTPGEKVTITCSASSRVSYMHWYQQKPDQAPKLLIKATSNLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 108)
DVVMTQSPAFLSVTPGEKVTITCSASSRVSYMHWYQQKPDQAPKLLIKATSNLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 109)
DVVMTQSPAFLSVTPGEKVTITCSARSSVSYMHWYQQKPDQAPKLLIKATSNLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 110)
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FTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 111)
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FTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 112)
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FTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 113)
DVVMTQSPAFLSVTPGEKVTITCRASSSVSYMHWYQQKPDQAPKLLIKATSNLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 114)
DVVMTQSPAFLSVTPGEKVTITCRASSSVSYMHWYQQKPDQAPKLLIKATSNLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 115)
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DFTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 116)
DVVMTQSPAFLSVTPGEKVTITCSASSSVSYMHWYQQKPDQAPKLLIKWTSDRYSGVPSRFSGSGSGT
DFTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 117)
DVVMTQSPAFLSVTPGEKVTITCSASSSVSYMHWYQQKPDQAPKLLIKWTSDRYSGVPSRFSGSGSGT
DFTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 118)
DVVMTQSPAFLSVTPGEKVTITCSASSRVSYMHWYQQKPDQAPKLLIKWTSDRYSGVPSRFSGSGSGT
DFTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 119)
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DFTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 120)
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DFTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 121)

DVVMTQSPAFLSVTPGEKVTITCSARSSVSYMHWYQQKPDQAPKLLIKWTSDRYSGVPSRFSGSGSGT
DFTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 122)
DVVMTQSPAFLSVTPGEKVTITCSARSSVSYMHWYQQKPDQAPKLLIKWTSDRYSGVPSRFSGSGSGT
DFTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 123)
DVVMTQSPAFLSVTPGEKVTITCSARSSVSYMHWYQQKPDQAPKLLIKWTSDRYSGVPSRFSGSGSGT
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DVVMTQSPAFLSVTPGEKVTITCRASSSVSYMHWYQQKPDQAPKLLIKWTSDRYSGVPSRFSGSGSGT
DFTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 127)
DVVMTQSPAFLSVTPGEKVTITCSASSSVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 128)
DVVMTQSPAFLSVTPGEKVTITCSASSSVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 129)
DVVMTQSPAFLSVTPGEKVTITCSASSSVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 130)
DVVMTQSPAFLSVTPGEKVTITCSASSRVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 131)
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FTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 132)
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FTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 133)
DVVMTQSPAFLSVTPGEKVTITCSARSSVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 134)
DVVMTQSPAFLSVTPGEKVTITCSARSSVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 135)
DVVMTQSPAFLSVTPGEKVTITCSARSSVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
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DVVMTQSPAFLSVTPGEKVTITCRASSSVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPFTFGQGTKLEIKR (SEQ ID NO: 137)
DVVMTQSPAFLSVTPGEKVTITCRASSSVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQWSSNPLTFGQGTKLEIKR (SEQ ID NO: 138)
DVVMTQSPAFLSVTPGEKVTITCRASSSVSYMHWYQQKPDQAPKLLIKDTSKLASGVPSRFSGSGSGTD
FTFTISSLEAEDAATYYCQQHLHIPYTFGQGTKLEIKR (SEQ ID NO: 139)

[0072] A humanized antibody has one or more amino acid residues from a source that is non-human. The non-human amino acid residues are often referred to as "import" residues, and are typically taken from an "import" variable domain. Humanization can be performed generally following conventional method known in the art, by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in non-human, for example, rodent antibodies.

[0073] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. The sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies.

[0074] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[0075] In one embodiment, the present invention provides a chimeric anti-Globo H antibody or an antigen-binding portion thereof, comprising (i) a heavy chain variable region

comprising an amino acid sequence having at least 85% identical to any of the amino acid sequences of SEQ ID NOs: 140-163 wherein the last third sequence V of the amino acid sequences is changed as I, and (ii) a light chain variable region comprising an amino acid sequence having at least 80% identical to any of the amino acid sequence of SEQ ID NO:164-199. Preferably, the sequence identity as mentioned above is at least 90%, 91%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

[0076] In one embodiment, the present invention provides a chimeric anti-Globo H antibody, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to the amino acid sequences of SEQ ID NO: 147 wherein the last third sequence V of the amino acid sequence is changed as I, and (ii) a light chain variable region comprising an amino acid sequence having at least 85% identical to the amino acid sequence of SEQ ID NO: 195. In a further embodiment, the present invention provides an isolated anti-Globo H antibody (cMZ-2), comprising (i) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 147 wherein the last third sequence V of the amino acid sequences is changed as I, and (ii) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 195.

[0077] The production of the chimeric antibody can be produced according to conventional methods known in the art. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397. In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454 which are incorporated herein by reference in their entireties) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. In some embodiments, the constant domains of the light and heavy chains of, for example, a mouse monoclonal antibody can be substituted 1) for those regions of, for example, a human antibody to generate a chimeric antibody or 2) for a non-immunoglobulin polypeptide to generate a fusion antibody. In other embodiments, the constant regions are truncated or removed to generate the desired antibody fragment of a monoclonal antibody. Furthermore, site-directed or high-density mutagenesis of the variable region can be used to optimize specificity, affinity, etc. of a monoclonal antibody.

Compositions of Antibodies of the Invention

[0078] In another aspect, the present invention provides a pharmaceutical composition comprising an anti-Globo H antibody or an antigen-binding portion thereof. The present antibody can also be formulated into a pharmaceutical composition. In addition to the antibody or an antigen-binding portion thereof, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

[0079] Administration of the anti-Globo H antibody or an antigen-binding portion thereof described herein, can include formulation into pharmaceutical compositions or pharmaceutical formulations for parenteral administration, e.g., intravenous; mucosal, e.g., intranasal; ocular, or other mode of administration. In some embodiments, the anti-Globo H antibody or an antigen-binding portion thereof described herein can be administered along with any pharmaceutically acceptable carrier compound, material, or composition which results in an effective treatment in the subject. Thus, a pharmaceutical formulation/composition for use in the methods described herein can contain an anti-Globo H antibody or an antigen-binding portion thereof as described herein in combination with one or more pharmaceutically acceptable carriers.

[0080] The phrase "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent, media, encapsulating material, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in maintaining the stability, solubility, or activity of, an anti-Globo H antibody or an antigen-binding portion thereof as described herein. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. The terms "excipient", "carrier", "pharmaceutically acceptable carrier", or the like are used interchangeably herein.

[0081] The anti-Globo H antibody or an antigen-binding portion thereof as described herein can be specially formulated for administration of the compound to a subject in solid, liquid or gel form, including those adapted for the following: (1) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (2) topical

application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (3) intravaginally or intrarectally, for example, as a pessary, cream or foam; (4) ocularly; (5) transdermally; (6) transmucosally; or (79) nasally. Additionally, a anti-Globo H antibody or an antigen-binding portion thereof as described herein can be implanted into a patient or injected using a drug delivery system.

[0082] According to various working examples presented below, adult C57BL/6 mice (weight 20-25 grams) administered with the present antibody twice a week achieved reduced tumor size on 3 to 21 days after inoculation. Hence, in certain embodiments of the present disclosure, the therapeutically effective amount of the antibody for mice could be expressed as 0.8-100 mg/kg body weight. HED of the above-mentioned murine effective amount is about 0.65-81.5 mg/kg body weight. According to various embodiments of the present disclosure, when the subject is human, the therapeutically effective amount of the antibody can be at least 1 mg/kg. Depending on the type and severity of the disease, about 1 mg/kg to 150 mg/kg (e.g., 0.1-20 mg/kg) of an anti-Globo H antibody or an antigen-binding portion thereof as described herein is an initial candidate dosage for administration to a subject, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 mg/kg to about 100 mg/kg or more, depending on the factors mentioned above. Typical dosages include, for example, 5 mg/kg, 10 mg/kg, 20 mg/kg, and 30 mg/kg. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until, for example, the cancer is treated, as measured by the methods described above or known in the art.

Modes of Administration

[0083] In another aspect, the invention provides a method of treating and/or preventing a cancer, comprising administering to a subject in need thereof a therapeutically effective amount of an anti-Globo H antibody or an antigen-binding portion thereof as described herein or a pharmaceutical composition comprising an anti-Globo H antibody or an antigen-binding portion thereof as described herein.

[0084] The administration of the antibody or the pharmaceutical composition comprising the same to a subject confers passive protection to the subject and thereby provides the intended therapeutic effect to a cancer, such as tumor-associated carbohydrate-expressing cancer; preferably, breast cancer, ovarian cancer, pancreatic cancer, prostate cancer, colorectal cancer and lung cancer.

[0085] In some embodiments, the anti-Globo H antibody or an antigen-binding portion thereof as described herein or a pharmaceutical composition comprising an anti-Globo H

antibody or an antigen-binding portion thereof as described herein is administered to a subject having a cancer to be inhibited by any mode of administration that delivers the agent systemically or to a desired surface or target, and can include, but is not limited to, injection, infusion, instillation, and inhalation administration. To the extent that anti-Globo H antibody or an antigen-binding portion thereof as described herein or a pharmaceutical composition comprising an anti-Globo H antibody or an antigen-binding portion thereof as described herein can be protected from inactivation in the gut, oral administration forms are also contemplated. "Injection" includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection and infusion. In preferred embodiments, the anti-Globo H antibody or an antigen-binding portion thereof as described herein or a pharmaceutical composition comprising an anti-Globo H antibody or an antigen-binding portion thereof as described herein for use in the methods described herein are administered by intravenous infusion or injection.

[0086] The phrases "parenteral administration" and "administered parenterally" as used herein, refer to modes of administration other than enteral and topical administration, usually by injection. The phrases "systemic administration," "administered systemically", "peripheral administration" and "administered peripherally" as used herein refer to the administration of the bispecific or multispecific polypeptide agent other than directly into a target site, tissue, or organ, such as a tumor site, such that it enters the subject's circulatory system and, thus, is subject to metabolism and other like processes.

[0087] The methods provided herein for inhibiting or treating cancer in subject having or at risk for cancer by administering to the subject a therapuetically effective amount of an anti-Globo H antibody or an antigen-binding portion thereof as described herein or a pharmaceutical composition comprising an anti-Globo H antibody or an antigen-binding portion thereof as described herein, can, in some embodiments, further comprise administration one or more additional treatments such as angiogenic inhibitors, chemotherapy, radiation, surgery, or other treatments known to those of skill in the art to prevent and/or treat cancer.

[0088] The following Examples are provided to elucidate certain aspects of the present invention and to aid those of skilled in the art in practicing this invention. These Examples are in no way to be considered to limit the scope of the invention in any manner. Without further elaboration, it is believed that one skilled in the art can, based on the description

herein, utilize the present invention to its fullest extent. All publications cited herein are hereby incorporated by reference in their entirety.

Example

Example 1 Production of Monoclonal Antibody, Chimeric Antibody and Humanized Antibody against Globo H

[0089] Adult female C57BL/6 mice (n=3 each group; 5 weeks old; average weight 16-20 grams; purchased from Biolasco, Taiwan) were immunized by subcutaneous injection with 6 μg of Globo H-PADRE glycopeptide wherein PARDE represents a polypeptide of AKXVAAWTLKAAA (SEQ ID NO:238), and 50 μl of complete Freund's adjuvant (CFA; from Sigma). Four immunizations were given at a 2-week interval. Three days after the fourth immunization, immunized splenocytes were harvest and washed with serum-free medium. Subsequently, $1x10^8$ of single cell suspended splenocytes were mixed with $2x10^7$ of FO cells, and cell fusion was performed in 1 ml of 50% PEG 1500 solution (Roche) at 37°C followed by drop-wise addition of 13 ml of warmed RPMI medium (Gibco). Fused cells were centrifuged and washed twice with complete medium. Cells were then re-suspended in complete medium with 1x BM-Conditioned H1 Hybridoma cloning supplement (Roche) and seeded into 96-well plates. For target specific B cell-myeloma cells fusion, immunized splenocytes were incubated with Globo H-biotin (10 μg/ml) in serum-free RPMI medium for 3 hours at 4°C. After being washed three times with the same medium, Globo H-binotin-bearing cells were resuspended at a concentration of $1x10^8$ cells/ml and incubated with streptavidin (50 μg/ml) for 30 minutes at 4°C. Meanwhile, FO cells were incubated with 50 μg/ml of NHS-biotin for 1 hour at 4°C. Both treated cells were then washed three times with serum-free RPMI medium. Then, $1x10^8$ splenocytes and $2x10^7$ FO cells were mixed together, and chemical cell fusion was performed as describe above. After cell fusion, cells were cultured in RPMI 1640 medium containing 1xHAT medium (Gibco) for further selection.

[0090] Mmonoclonal antibody-producing hybridoma cell lines were screened through limited dilution by ELISA assay on plate coated with Globo H-biotin antigen. Five clones (named MZ-1 to MZ-5, respectively) capable of secreting high-titers of anti-Globo H IgG or IgM antibodies were obtained.

[0091] Supernatants from these hybridoma lines were also subjected to cell binding assay. Briefly, 100 μl of the supernatant from the hybridoma culture was incubated with 2x105 of MCF-7 cells and then analyzed by flow cytometry with appropriate fluorescent secondary antibody mentioned below. The cells were washed once with 2 ml of 1x PBS.

After centrifugation, the wash buffer was discarded and cells were resuspended in 100 μl of 1:100 diluted PE anti-mouse IgG-Fc (Jackson immunoresearch) or 100 μl of 1:100 diluted PE anti-mouse IgM (eBioscience) and incubated again at room temperature for 20 minutes. The cells were washed with PBS and resuspended in 200 μl of 1x PBS after centrifugation. The binding of antibodies with cells were detected by flow cytometry. The results provided in Figures 1A to E reveal that the monoclonal antibody produced by MZ-2 hybridoma (see Fig. 1C) (hereinafter, the MZ-2 antibody) had good binding affinity to MCF-7 cells. For comparison purpose, a commercially available anti-Globo H IgG3 antibody (see Fig. E), VK9 antibody (see Fig. B) (eBioscience), was also analyzed.

[0092] Variable regions of heavy and light chains of MZ-2 antibody were first cloned to human IgG1 and kappa chain conserved region expression vectors to form mouse-human chimeric MZ-2 (cMZ-2). The cMZ-2 antibody has been gone through humanization by CDR-grafting based on structure analysis and sequence homology. The humanized MZ-2 antibody (hMZ-2) could be readily expressed as a recombinant IgG in mammalian cells and retains the antigen-binding affinity and specificity of the parent mouse antibody. We further improved its binding affinity with selective mutations of CDR and framework sequences that flank the CDR. The humanized construct was further transfected into FO cells by electroporation and selected by associated antibiotics to generate stable expression clones. Antibodies produced from the FO cells were checked by flow cytometry analysis. To obtain large-scale hMZ-2 antibodies for anti-tumor assay in vivo, 1x106 of hMZ-2 expression FO cells were first i.p. injected into NOD/SCID mice. Ascites from these mice were harvest after 2 weeks and passed through protein G agarose column to purify the generated antibody. The thus-obtained hMZ-2 antibodies were sequenced. The hMZ-2 antibody, hMz-2Lw (variable region of heavy chain: SEQ ID No. 27; variable region of light chain: SEQ ID No. 75 and MK1 (variable region of heavy chain: SEQ ID No. 290; variable region of light chain: SEQ ID No.135), were used in the following examples.

Example 2 Binding Affinity of MZ-2 Monoclonal Antibody to Primary Ovarian Cancer Cells

[0093] Primary human ovarian cancer tissues were obtained under the approval of IRB and patient consent. Ovarian cancer tissues were digested by MASC Tumor Dissociation kit (MASC, 130-095-929). The single cell suspension was stained with MZ-2 monoclonal antibody (10 μg/ml ; 100μl) for 30 minutes at 4°C., and stained with secondary antibody anti-mouse IgG1 PE(1:50; eBioscience, 2-405-82), then fixed with 4% paraformaldehyde for 1 min at RT. The cell were then stained with FITC-conjugated mouse anti-human EpCAM

(1:50; biolegend, 324204), and mouse anti-human SSEA4 (1:50;biolegend, 330408) for 30 min at 4°C. Flow cytometric analysis was conducted on a Becton Dickinson FACScan (BD FACSCalibur). Figures 2 demonstrate that the present MZ-2 monoclonal antibody exhibited good binding to primary ovarian cancer cells. Most epithelial primary cancer cells were stained with either MZ-2 or SSEA4 antibody showing by representative flow cytometry. EpCAM staining here represents a marker of epithelial cell.

Example 3 Specificity Assay of MZ-2 Antibody

[0094] Biotinylated-carbohydrates were conjugated on BD Cytometric Beads via Avidin by Functional Bead Conjugation Buffer Set (BD). First, 75 μL of the functional beads were sonicated and incubated with 1.9 μL of 1M DTT at room temperature for 1 hr. At the same time, 20 uL of each biotin-carbohydrate (0.2mg/mL) was mixed with 90 μg of maleimide activated neutrAvidin (1mg/mL in coupling buffer; Pierce) and incubated at room temperature for 1 hr. The beads were washed 3 times with 1 mL of coupling buffer and resuspended in 20 μL of coupling buffer. The carbohydrate were then mixed with designed beads and incubated with further one hour. 2 μL of N-Ethylmaleimide (2mg/mL on DMSO, Pierce) was then added into the conjugated beads and incubated for further 15 min. After 3 time wash, the beads were suspended in 500 μL of storage buffer and ready for use.

[0095] For detecting the specificity and relative titer of anti-Globo H antibody, each set of carbohydrate-conjugated beads were mixed and diluted to 1:50 of each. 50 μL of bead mixture was then transfer to V buttom 96-well plate, and mixed with 50 μL of 1:1000 diluted serum or 1 μg/mL of MZ-2 antibody. After incubation for 30 min, the beads were washed with 150 μL of wash buffer and further stained with 100 μL of PE-conjugated 2Ab (Jackson Immunoresearch). The binding of anti-Globo H antibody were detected by FACS. Figure 3 show the specificity of MZ-2 antibody is indicated by the fact that MZ-2 and chimeric MZ-2 antibodies only bind to Globo H-conjugated beads, but not SSEA3- or SSEA4-conjugated beads.

Example 4 Simulation of Protein Folding in Mouse and Humanized MZ-2 Monoclonal Antibody

[0096] The 3-D structure of variable regions in original and humanized MZ-2 clones were simulated by Prediction of Immunoglobulin Structure online program. Figures 4A and B show the simulation of protein folding of mouse MZ-2 monoclonal antibody (Fig. 4A) and humanized MZ-2 monoclonal antibodies (Fig. 4B).

Example 5 Association and Dissociation Curve Fitting and KD Calculation of Antibodies of the Invention

[0097] The binding affinity of anti-Globo H antibody was detected by Biolayer interferometry using FortrBio OcTet system. Briefly, the sensors were first soaked in 20 μM of biotinylated Globo H to coat Globo H on their surface. The MZ-2 clone (mMZ-2), mouse-human chimerical MZ-2 clone (cMZ-2), and humanized MZ-2 clones (hMZ-2L, MK-1or hMZ-2Lw) were serially diluted into 1333, 444.4, 148.1, 49.4 and 16.5 nM, and incubated with Globo H coated sensors separately. 10mM of Glycine (pH 1.5) was used as Regeneration buffer. Figures 5A and B show biacore full binding kinetic analysis of MZ-2 antibodies were carried out. Detailed binding kinetic parameters (association rate, ka, dissociation rate, kd, and affinity constant, KD) were determined by full kinetic analysis. Sensograms for each antibody are shown. The KD values of three humanized MZ-2 antibodies (hMZ-2L (see Fig. 5C), MK-1 (see Fig. 5D) and hMZ-2Lw (see Fig. 5E)) and chimeric MZ-2 (cMZ-2) antibody (see Fig. 5B) are similar.

Example 6 CDC assay of Chimeric MZ-2 (IgG1 kappa) on Breast MCF-7 cells

[0098] MCF-7, TOV21G Globo H(+), and HPAC cells were adjusted to 2 x 106 cells/ mL in RPMI 1640 medium and aliquot 50 μL of diluted cells into flow tubes. The chimeric MZ-2 antibody was diluted to 2x designed concentration in RPMI 1640 medium and aliquot 50 μL to each flow tube with cancer cells. Human IgG (Fitzgerald, 31-AI06) was diluted into the same concentration as a control. After 15 min, 100 μL of normal human serum from healthy human donor was added into each tube and incubated at 37°C for 2hr. The cells were washed once with complete medium and resuspended in 200 μL of complete medium with 0.5 μg/mL propidium iodide. The percentage of dead cells was analyzed using FACS.

[0099] Results of complement-dependent cytotoxicity (CDC) assay, as reported in Figure 6, indicate that the human serum containing more than 1.6 μg/ml of hMZ-2Lw antibody elicited complement-dependent cytotoxicity in breast cancer, MCF-7 cells. The CDC increased in a dose-dependent manner. Similar trends were also observed in ovarian and pancreatic cancer cells in which hMZ-2Lw antibody in a concentration higher than about 10 and 20μg/ml resulted in a dose-dependent cytotoxicity in human ovarian cancer cell line TOV21G (Figure 7), or the pancreatic cancer cell line, HPAC (see Figure 8).

Example 7 ADCC Assay of hMZ-2Lw and MK1 Antibody on MCF-7 cells

[00100] Briefly, 7.5 x 10^3 (100 uL) of each cells were pre-seeded into the designed wells of 96-well assay plate (Corning Cat.# 3917) and incubated overnight in a CO2 incubator at 37°C. The humanized anti-Globo H antibody clone hMZ-2Lw or MK1 was diluted into 3x

highest concentration of assay and 4x serial dilution by 8 times. Noraml human IgG in the same concentration was used as control. ADCC assay was performed by ADCC Reporter Bioassay kit (Promega Cat.# G7010) at a E/T ratio of 10:1 and detected by chemoluminescent reader (EnSpire 2300, PerkinElmer). The folds of induced ADCC response were calculated as equation below:

Calculate Fold of Induction = RLU (induced–background) /RLU (no antibody control–background)

[00101] Figures 9A and B and Figures.10A and B respectively summarizes the antigen-dependent cell-mediated cytotoxicity (ADCC) activity of the present hMZ-2Lw and MK1 antibodies on MCF-7 cells (breast cancer cell line) and TOV21G cells (ovarian cancer cell line). In general, these results demonstrate that the present humanized MZ-2 antibody may elicit efficient and dose-dependent ADCC in MCF-7 and TOV21G cells.

Example 8 Anti-tumorEffect of Humanized MZ-2 Antibody, hMZ-2Lw and MK1

[00102] For establishing an intra-peritoneal ovarian tumor model, $1x10^6$ of TOV21G cells were intraperitoneally (i.p.) injected into 5-week-old female NU/NU mice (BioLASCO Taiwan). Two days later, mice were divided into 4 groups and administered with either 100μg (at a therapeutic dose of 5 mg/kg) of Human IgG, anti-GloboH antibodies hMZ-2Lw or MK1 twice a week through tail vein (i.v.) route. Untreated mice were set as control. To monitor the tumor growth, tumor bearing mice were i.p. injected 200μL of luciferin (3.9mg/ml) and the chemoluminance intensity of each mouse was detected by a non-invasive IVIS system (Xenogen) with fixed exposure condition in each different batch of experiment. Representative photograph in Figure 11 illustrates administration of both hMZ-2Lw and MK1 antibodies significantly inhibit the tumor growth, while control IgG antibody did not substantially affect the tumor growth. However, the present hMZ-2 antibody significantly reduced the tumor size. By 24 days after treatment, tumor was nearly eliminated in mice given hMZ-2Lw antibody.

[00103] For establishing a subcutaneous breast tumor model, 17b-estradiol (Innovative Research of America, SE-121) was first subcutaneously (s.c.) implanted into NU/NU mice (BioLASCO Taiwan). Three days later, $3x10^6$ MCF-7 cells mixed with matrigel were s.c. implanted (xenograft) into mice. Eighteen days after tumor challenge, mice were divided into 3 groups (n = 7), and treated by a therapeutic dose (5 mg/kg) of human IgG (100 μg/mouse) or 100 μg/mouse of hMZ-2Lw antibody i.v. (through tail vein) twice a week. Mice without treatment were set as control. Tumor size was measured weekly by calipers. The results of hMZ-2 antibody in breast cancer subcutaneous model MCF-7 cell are shown in

Figure 12. Figures 13A and B shows the results of hMZ-2 antibody in pancreatic cancer subcutaneous model HPAC cell.

Example 9 MZ-2 Antibody Inhibits Migration of Globo H-expressing TOV21G cells (I)

[00104] Briefly, 2×10^5 of Globo H-positive TOV21G cells were mixed with 5 μg of mouse IgG1 isotype or MZ-2 antibody (hMZ-2Lw) in 1 mL of culture medium in and incubated at 37°C for 15 min. These cells were then washed three times with 1x PBS and resuspended to 2×10^5 cells/mL in culture medium. One hundred micro liters of cells were transferred into the inserts of transwell (Corning) and incubated in CO_2 incubator for 24 hr. Cells without antibody treatment, or Globo H-negative TOV21G cells were used as controls. Those cells on the top side of transwell membrane were removed by cotton rod. Migrated cells through the membrane (bottom side) were fixed with 4% formaldehyde and stained with crystal violate. The numbers of migrated cells were counted by tissue scanner (TissueGnostics). Figure 14 shows that MZ-2 antibody inhibits migration of Globo H-expressing TOV21G cells.

Example 10 MZ-2 Antibody Inhibits migration of Globo H-expressing TOV21G cells (II)

[00105] Briefly, 2×10^5 of Globo H-expressing TOV21G cells were mixed with 5 μg of mouse IgG1 isotype (eBioscience) or MZ-2 antibody (hMZ-2Lw) in 0.5 mL of culture medium and incubated at 37°C for 15 min. Those cells were then washed three times with 1x PBS, and resuspended to 4×10^5 cells/mL in culture medium. One hundred micro liters of cells were transferred into a well of wound healing culture-inserts (Ibid Cat# 80241) in a 24-well plate and incubated for 8 hours in CO_2 incubator. Cells without antibody treatment, or Globo H-negative TOV21G cells were used as controls. The insert were then removed and the cells were kept cultured for another 18 hours. Cell images were took by CCD camera from 0 hr to 18 hr after insert removal. Note that the gap of cells treated by MZ-2 antibody is larger than that treated by IgG1 isotype control. Figure 15 shows that MZ-2 antibody inhibits migration of Globo H-expressing TOV21G cells.

Example 11 Assays of Other Antibodies of the Invention

[00106] The antibodies of the invention having the heavy chain variable regions and the light chain variable regions as described herein were subjected to the binding affinity assay of Example 2, association and dissociation assay of Example 5, CDC assay of Example 6, ADCC assay of Example 7 and anti-tumor assay of Example 8 and show similar results to cMZ-2 antobody, hMZ-2Lw antibody and MK-1 antibody. For example, the KD value of the

antibody having a heavy chain variable region of SEQ ID NO: 27, and a light chain variable region of SEQ ID NO: 231 is in the range of $1x\ 10^{-7}$ to $1x10^{-10}$ nM.

Claims

What is claimed is:

1. An isolated anti-Globo H antibody or an antigen-binding portion thereof, comprising a humanized anti-Globo H antibody or an antigen-binding portion thereof, comprising at least one of a heavy chain complementarity determining region 1 (H-CDR1) consisting of the amino acid residues of GFSLSTFDMGVG (SEQ ID NO: 1), GSSLSTFDVGVG (SEQ ID NO: 2), GFSLGTFDLGIG (SEQ ID NO: 3), GFSLSTFDLGIG (SEQ ID NO: 4) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 1 to 4; a heavy chain CDR2 (H-CDR2) consisting of the amino acid residues of HIWWDDDKYYNPA (SEQ ID NO:5), HIWGDDDKYYNPA (SEQ ID NO: 6) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 5 and 6; and a heavy chain CDR3 (H-CDR3) consisting of the amino acid residues of LYGNYLTSFYCDY (SEQ ID NO: 7), or LSGNYLTSFYCDY (SEQ ID NO: 8), LYGNYLRSYYCDY (SEQ ID NO: 9) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 7 to 9; and

at least one of a light chain CDR1 (L-CDR1) consisting of the amino acid residues of SASSSVSYMH (SEQ ID NO: 10), SASSRVSYMH (SEQ ID NO:11), SARSSVSYMH (SEQ ID NO:12), RASSSVSYMH (SEQ ID NO:13) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 10 to 13; a light chain CDR2 (L-CDR2) consisting of the amino acid residues of ATSNLAS (SEQ ID NO:14), WTSDRYS (SEQ ID NO:15), DTSKLAS (SEQ ID NO:16) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 14 to 16; and a light chain CDR3 (L-CDR3) consisting of the amino acid residues QQWSSNPFT (SEQ ID NO: 17), QQWSSNPLT (SEQ ID NO: 18), QQHLHIPYT (SEQ ID NO: 19) or a variant having amino acid sequence with at least 80% identity to any of SEQ ID NOs: 17 to 19; such that said isolated recombinant antibody or antigen-binding portion thereof binds to Globo H.

2. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 1, wherein the sequence identity is at least 90%.

3. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 1, which is a monoclonal antibody, chimeric antibody or humanized antibody.

4. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 1, comprising (i) a heavy chain variable region comprising a heavy chain variable region comprising H-CDR1 selected from the group consisting of SEQ ID NOs: 1 to 4, H-CDR2 selected from the group consisting of SEQ ID NOs: 5 and 6 and H-CDR3 selected from the

group consisting of SEQ ID NOs: 7 to 9, and (ii) light chain variable regions comprising L-CDR1 selected from the group consisting of SEQ ID NOs: 10 to 13, L-CDR2 selected from the group consisting of SEQ ID NOs: 14 to 16 and L-CDR3 selected from the group consisting of SEQ ID NOs: 17 to 19.

5. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 1, wherein H-CDR1 is SEQ ID NO:3; H-CRD2 is SEQ ID NO:5; H-CDR3 is SEQ ID NO:8; L-CDR1 is SEQ ID NO:10; L-CDR2 is SEQ ID NO:16; and L-CDR3 is SEQ ID NO:18.

6. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 1, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to any of the amino acid sequences of SEQ ID NOs: 140 to 163, and (ii) a light chain variable region comprising an amino acid sequence having at least 80% identical to any of the amino acid sequence of SEQ ID NO: 164 to 199.

7. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 6, wherein the sequence identity as mentioned above is at least 90%.

8. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 6, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to the amino acid sequences of SEQ ID NO: 147, and (ii) a light chain variable region comprising an amino acid sequence having at least 80% identical to the amino acid sequence of SEQ ID NO: 195.

9. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 6, comprising (i) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 147, and (ii) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 195

10. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 1, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to any of the amino acid sequences of SEQ ID NOs: 20 to 43, and (ii) a light chain variable region comprising an amino acid sequence having at least 80% identical to any of the amino acid sequence of SEQ ID NO: 44 to 79 and 200 to 235.

11. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 9, wherein the sequence identity as mentioned above is at least 90%.

12. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 9, comprising (i) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 20 to 43, and (ii) a light chain variable region

comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 44 to 79 and 200 to 235.

13. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 9, comprises (i) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 27, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 75; or (ii) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 27, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 231.

14. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 1, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to any of the amino acid sequences of SEQ ID NOs: 80 to 103, and (ii) a light chain variable region comprising an amino acid sequence having at least 85% identical to any of the amino acid sequence of SEQ ID NOs: 104 to 139.

15. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 9, comprising (i) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 80 to 103, and (ii) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 104 to 139.

16. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 14, comprises a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 90, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 135..

17. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 1,, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to any of the amino acid sequences of SEQ ID NOs: 140-163 wherein the last third sequence "V" of the amino acid sequences is changed to "I", and (ii) a light chain variable region comprising an amino acid sequence having at least 80% identical to any of the amino acid sequence of SEQ ID NO:164-199

18. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 17, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 85% identical to the amino acid sequences of SEQ ID NO: 147 wherein the last third sequence "V" of the amino acid sequence is changed to "I", and (ii) a light chain variable region comprising an amino acid sequence having at least 85% identical to the amino acid sequence of SEQ ID NO: 195.

19. The isolated anti-Globo H antibody or an antigen-binding portion thereof of Claim 17, comprising (i) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 147 wherein the last third sequence "V" of the amino acid sequence is changed to "I", and (ii) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO: 195.

20. A pharmaceutical composition, comprising any of the isolated anti-Globo H antibody or an antigen-binding portion thereof of Claims 1 to 19 and a pharmaceutically acceptable carrier.

21. The pharmaceutical composition of Claim 20, which further comprises an anti-tumor drug.

22. A method of treating and/or preventing a cancer, comprising administering to a subject in need thereof a therapeutically effective amount of any of the isolated anti-Globo H antibody or an antigen-binding portion thereof of Claims 1 to 20.

23. The method of Claim 22, wherein the cancer is a tumor-associated carbohydrate-expressing cancer.

24. The method of Claim 22, wherein the cancer is a breast cancer, ovarian cancer, pancreatic cancer, prostate cancer, colorectal cancer or lung cancer.

25. The method of Claim 22, wherein the cancer is a migration cancer.

26. The method of Claim 22, which further comprises administration one or more additional treatments selected from such as angiogenic inhibitors, chemotherapy, radiation or surgery.

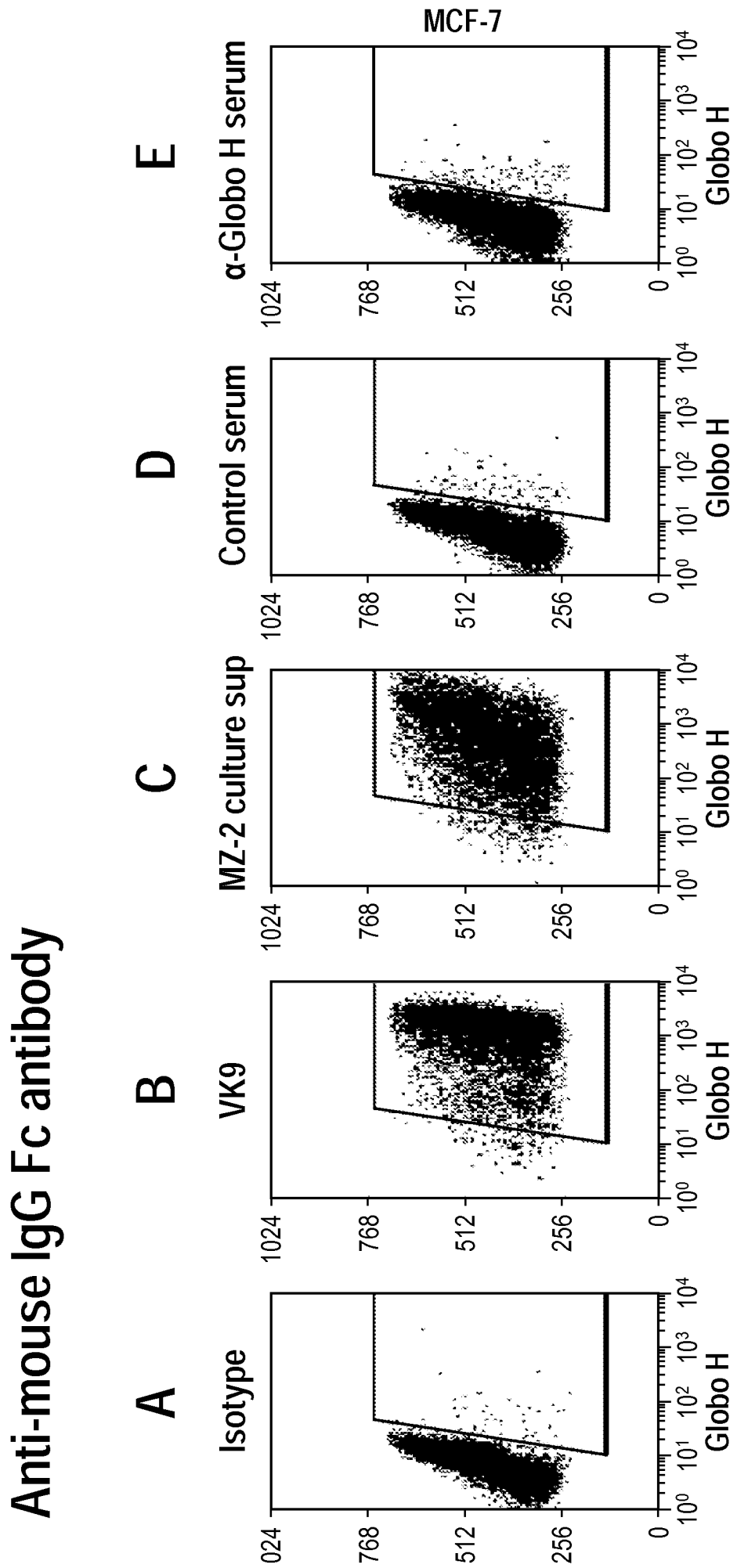

MCF-7
A
Isotype
B
VK9
C
MZ-2 culture sup
D
Control serum
E
α-Globo H serum
Anti-mouse IgG Fc antibody
Globo H
1024
768
512
256
0
10^0
10^1
10^2
10^3
10^4

Fig. 1

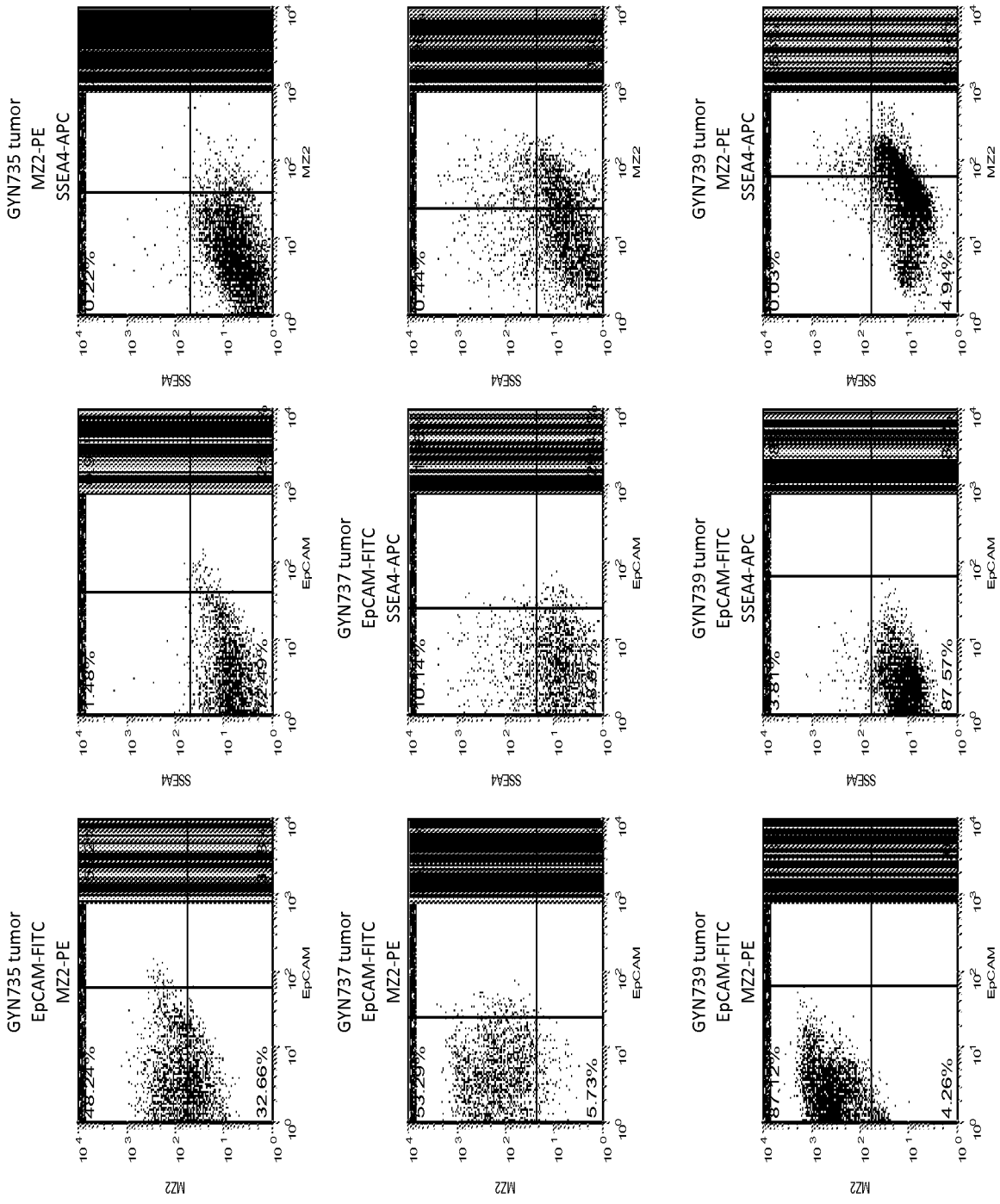

GYN735 tumor
EpCAM-FITC
MZ2-PE
48.24%
32.66%
GYN737 tumor
EpCAM-FITC
MZ2-PE
53.29%
5.73%
GYN739 tumor
EpCAM-FITC
MZ2-PE
87.12%
4.26%
GYN735 tumor
EpCAM-FITC
SSEA4-APC
1.48%
72.49%
GYN737 tumor
EpCAM-FITC
SSEA4-APC
10.14%
GYN739 tumor
EpCAM-FITC
SSEA4-APC
3.81%
87.57%
GYN735 tumor
MZ2-PE
SSEA4-APC
0.22%
GYN737 tumor
MZ2-PE
SSEA4-APC
0.44%
GYN739 tumor
MZ2-PE
SSEA4-APC
0.03%
4.94%
MZ2
EpCAM
SSEA4

Fig. 2

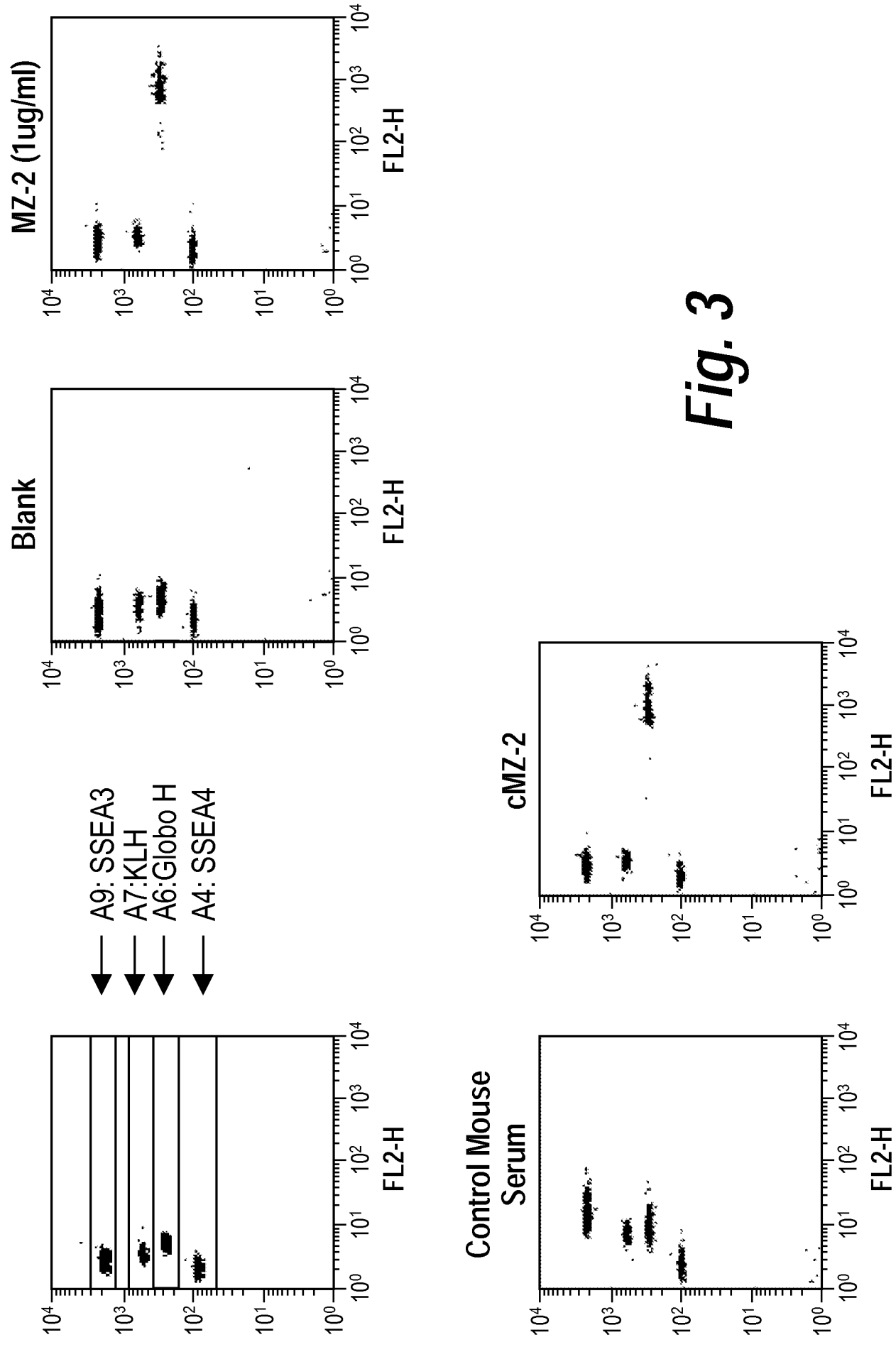

MZ-2 (1ug/ml)
Blank
cMZ-2
Control Mouse Serum
A9: SSEA3
A7:KLH
A6:Globo H
A4: SSEA4
FL2-H

Fig. 3

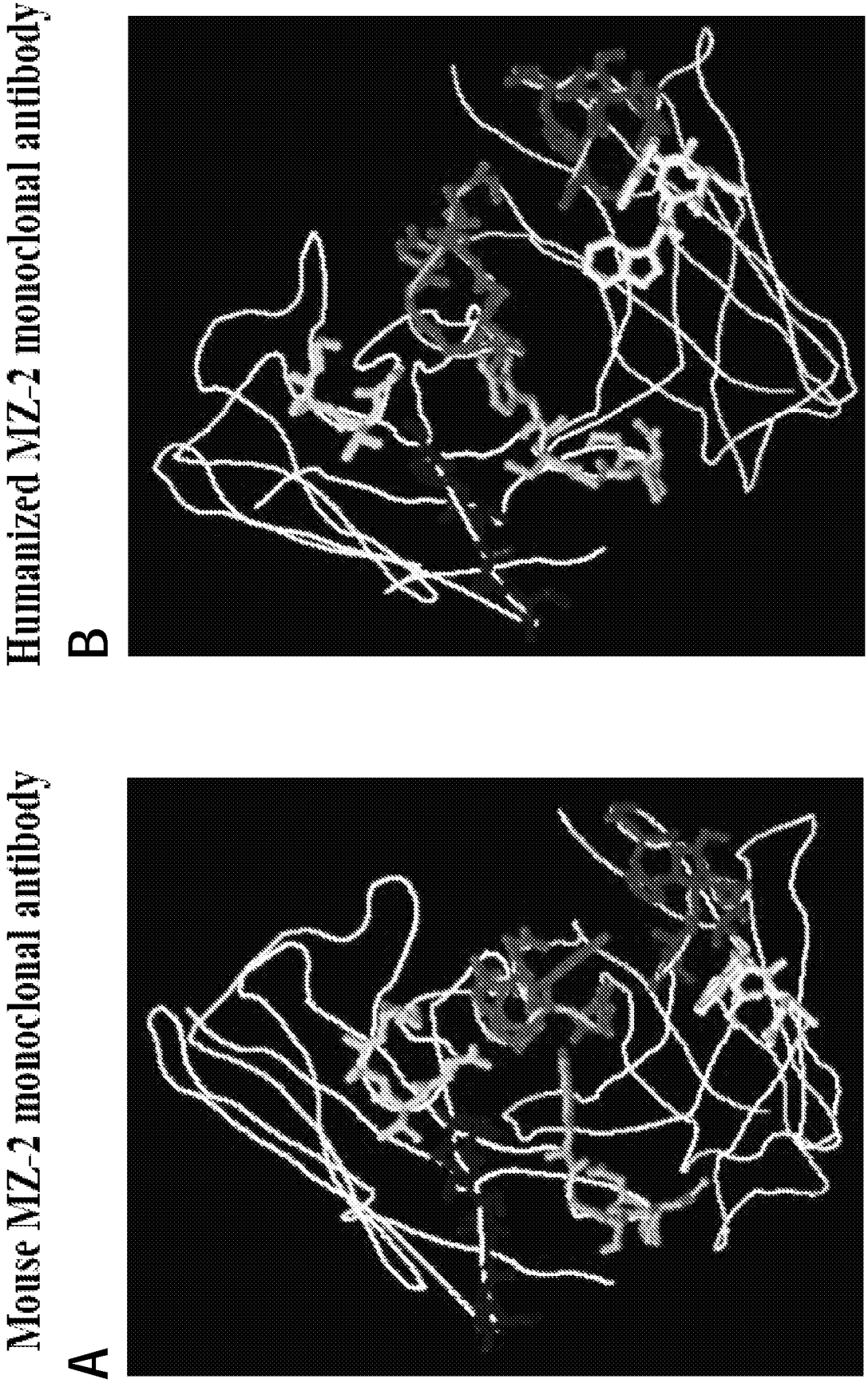
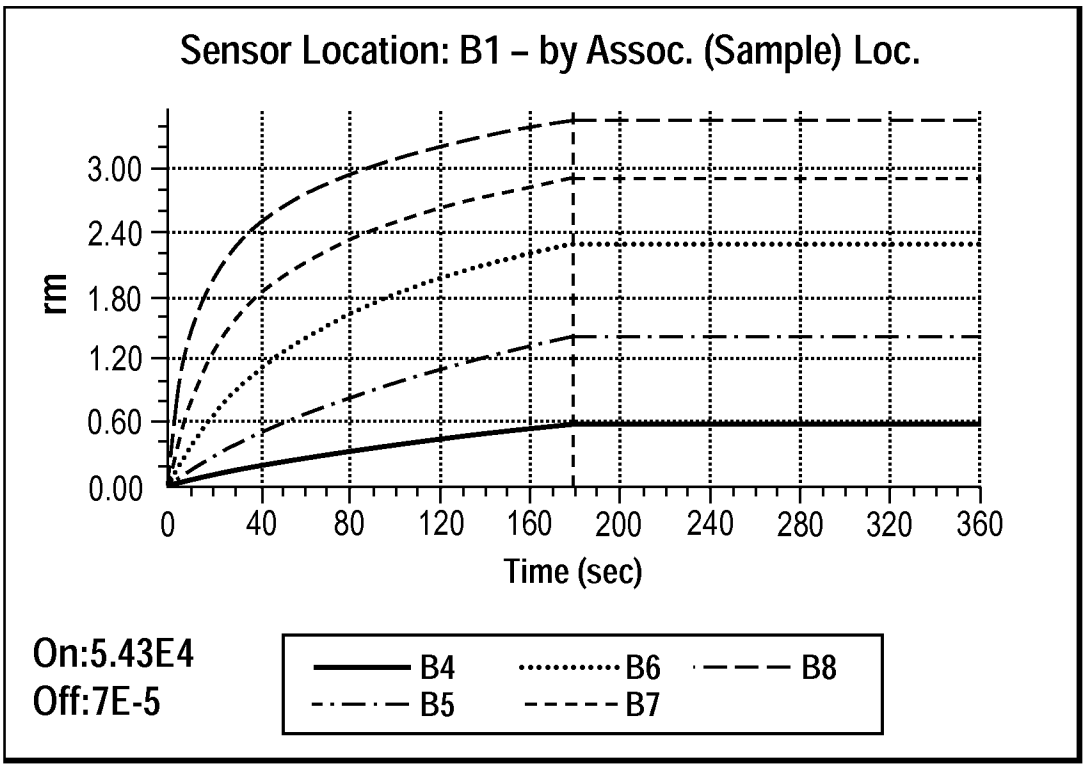

A
Mouse MZ-2 monoclonal antibody
B
Humanized MZ-2 monoclonal antibody

Fig. 4

A

mMZ-2 (KD=1.29x10^{-9})



B

cMZ-2 (KD=1.27x10^{-8})

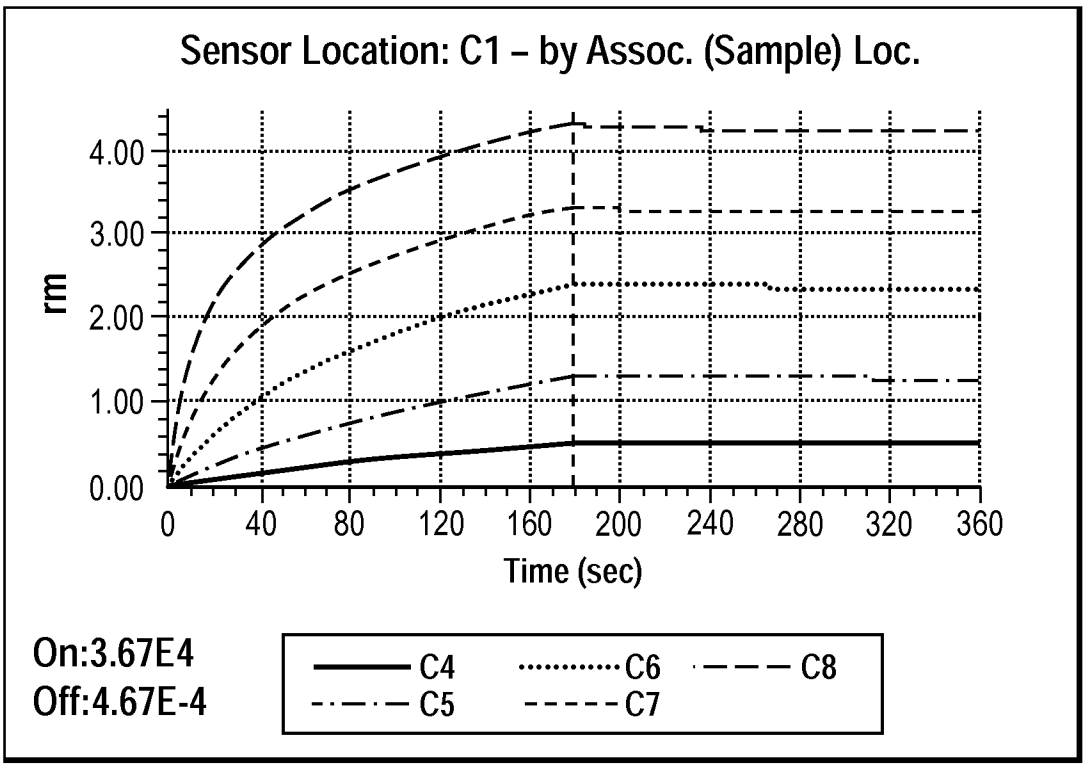
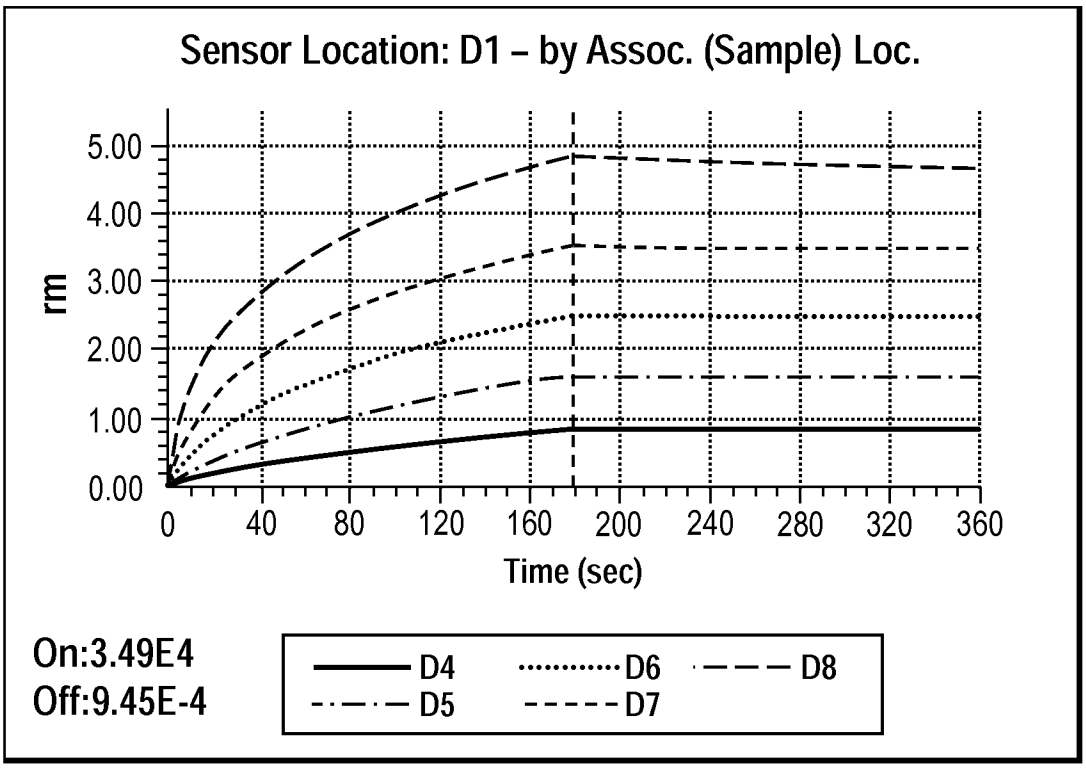


Fig. 5

C

hMZ-2L (KD=2.71x10^{-8})



D

MK-1 (KD=9.7x10^{-9})

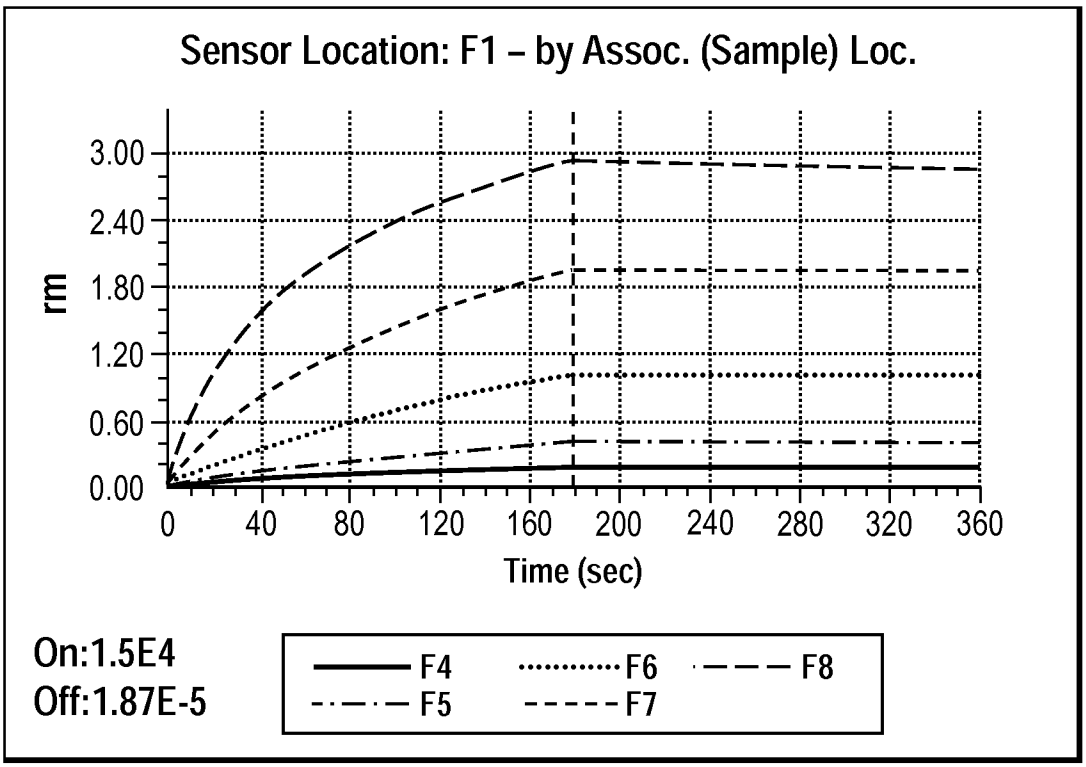


Fig. 5

E

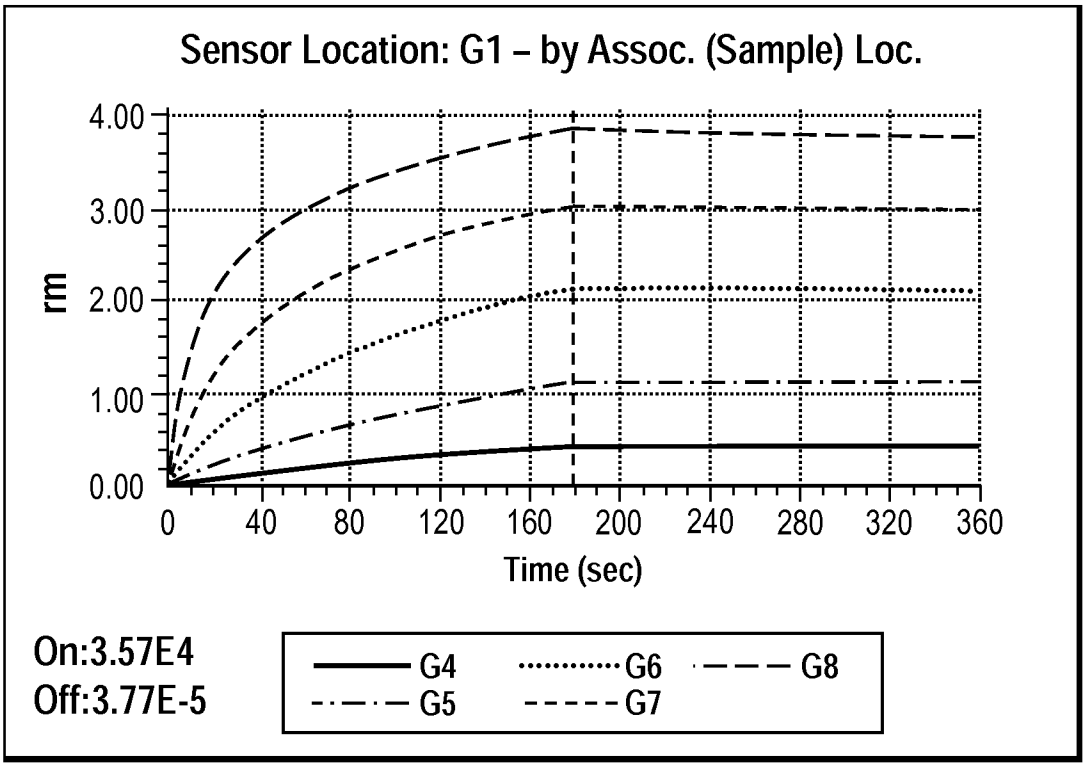

hMZ-2Lw (KD=1.48x10-8)
Sensor Location: G1 – by Assoc. (Sample) Loc.
4.00
3.00
2.00
1.00
0.00
rm
0
40
80
120
160
200
240
280
320
360
Time (sec)
On:3.57E4
Off:3.77E-5
G4
G5
G6
G7
G8

Fig. 5

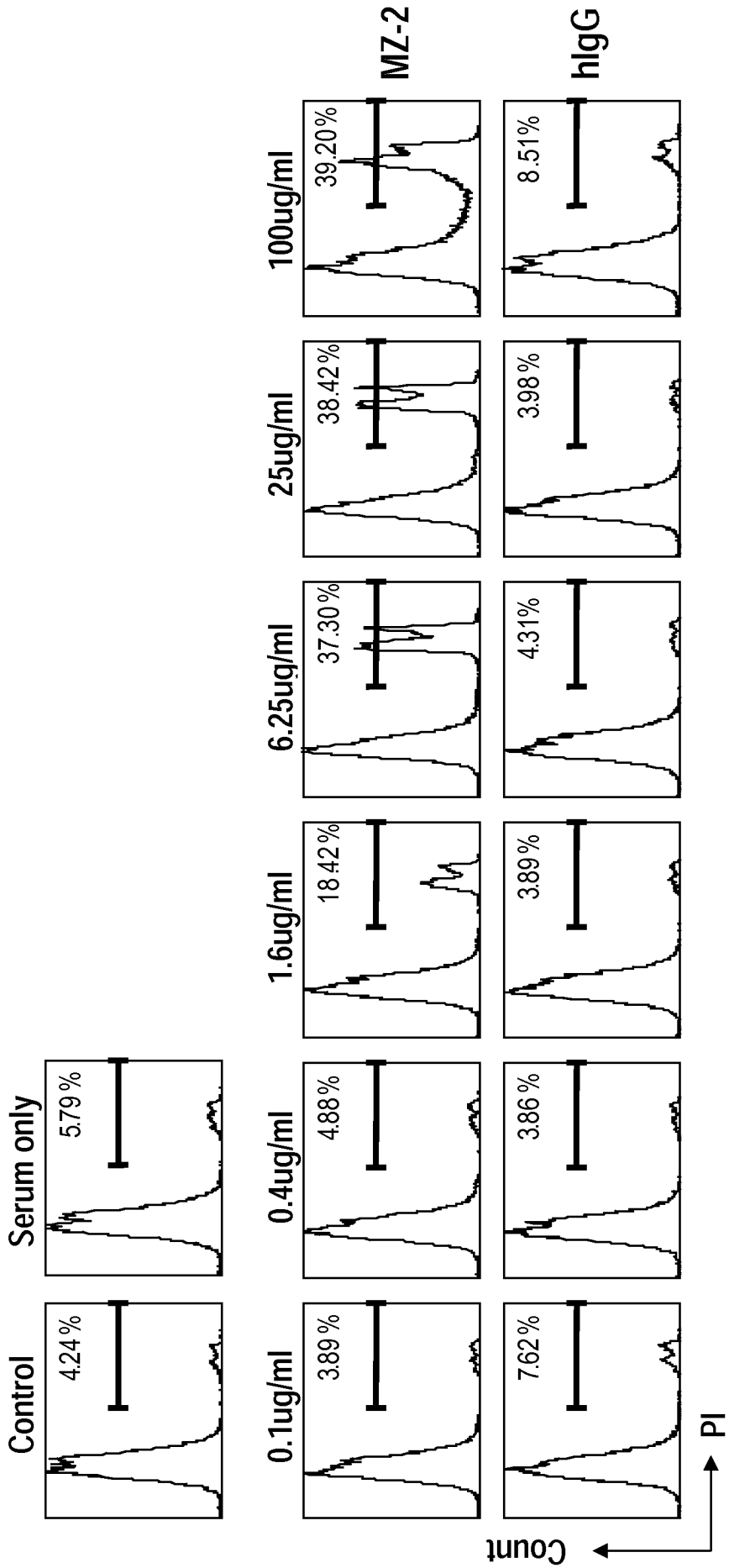

MZ-2
hIgG
100ug/ml
25ug/ml
6.25ug/ml
1.6ug/ml
0.4ug/ml
0.1ug/ml
Serum only
Control
39.20%
8.51%
38.42%
3.98%
37.30%
4.31%
18.42%
3.89%
4.88%
3.86%
3.89%
7.62%
5.79%
4.24%
Count
PI

Fig. 6

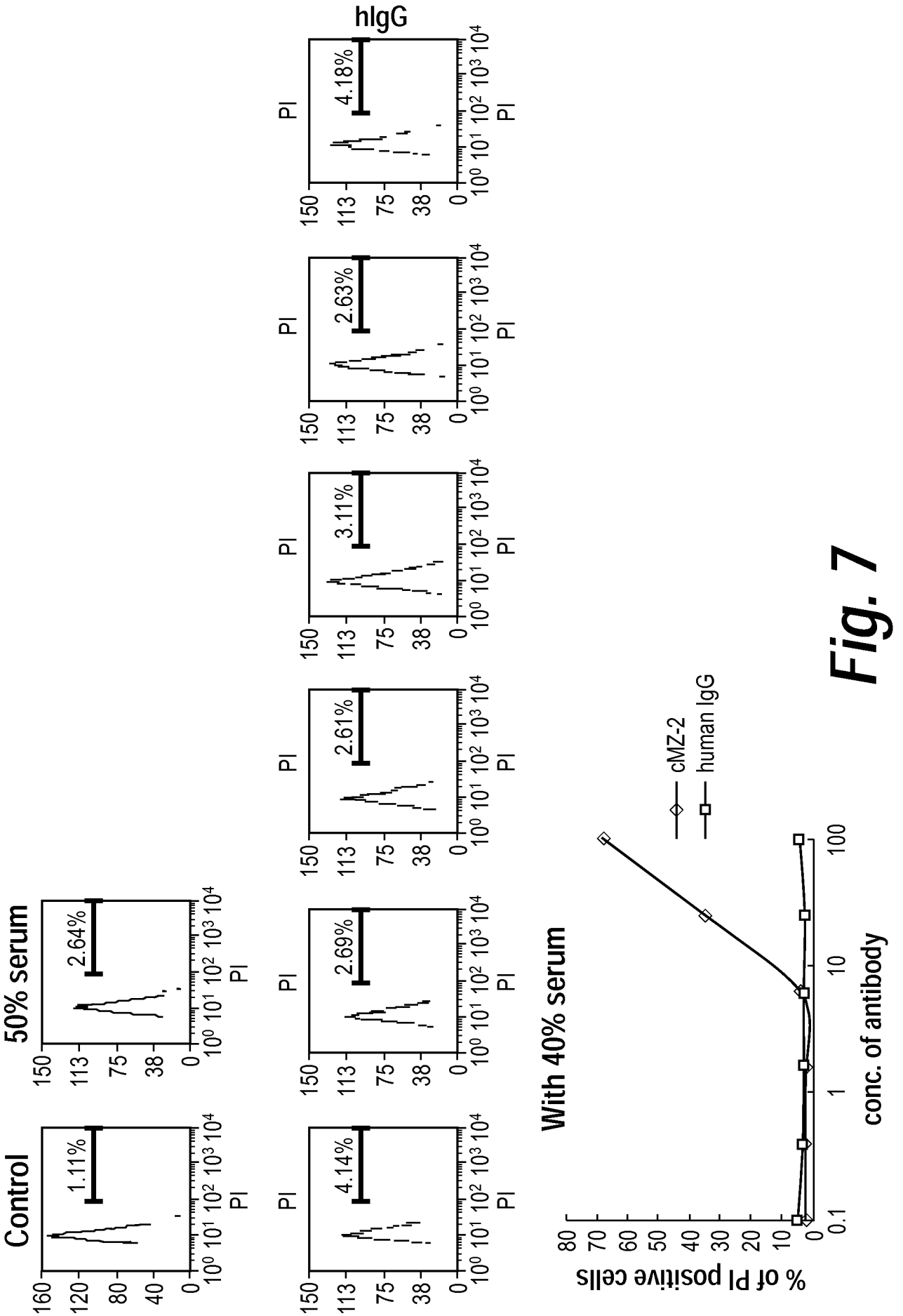

Control
50% serum
hIgG
1.11%
2.64%
2.69%
4.14%
2.61%
3.11%
2.63%
4.18%
PI
With 40% serum
cMZ-2
human IgG
% of PI positive cells
conc. of antibody

Fig. 7

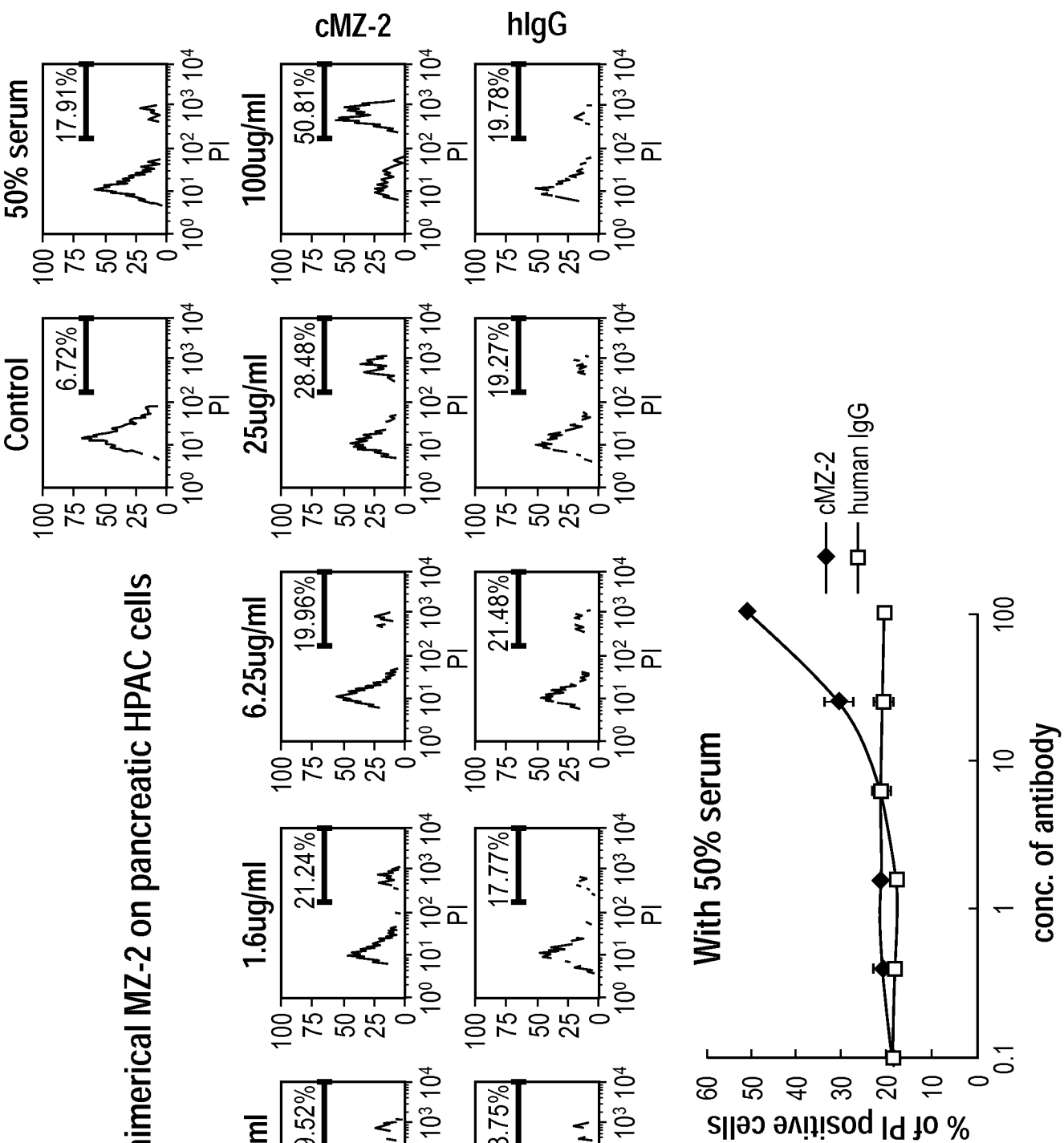

CDC assay of chimerical MZ-2 on pancreatic HPAC cells
With 50% serum
cMZ-2
hIgG
Control
50% serum
0.1ug/ml
0.4ug/ml
1.6ug/ml
6.25ug/ml
25ug/ml
100ug/ml
6.72%
17.91%
18.71%
19.52%
21.24%
19.96%
28.48%
50.81%
18.69%
18.75%
17.77%
21.48%
19.27%
19.78%
PI
With 50% serum
% of PI positive cells
conc. of antibody
cMZ-2
human IgG

Fig. 8

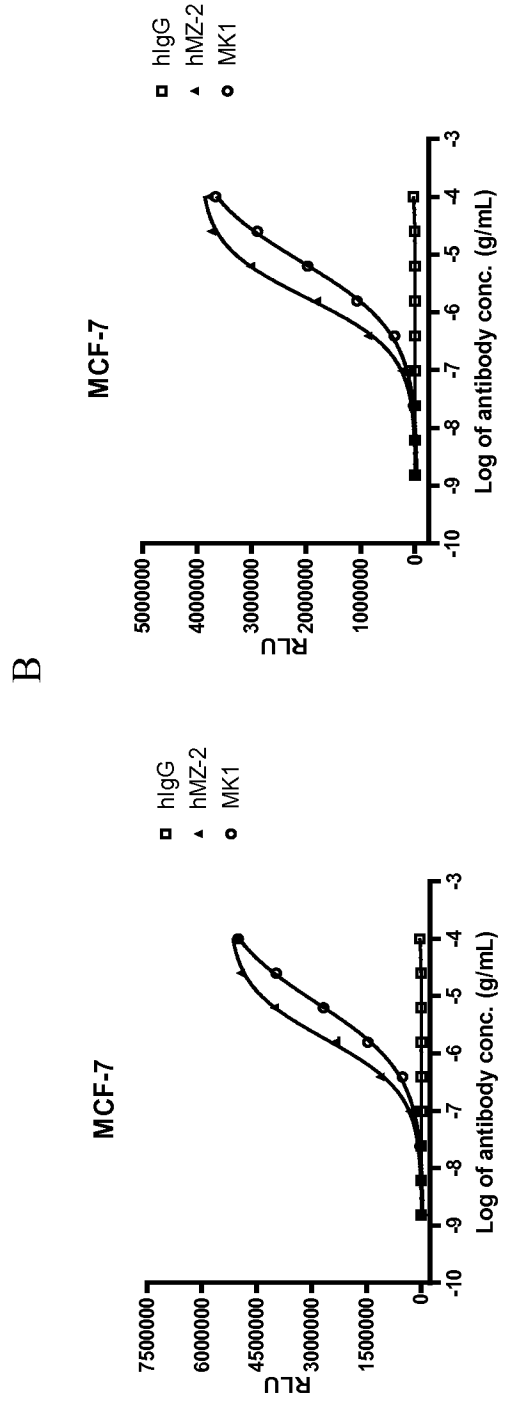

A
MCF-7
hIgG
hMZ-2
MK1
RLU
7500000
6000000
4500000
3000000
1500000
0
-10
-9
-8
-7
-6
-5
-4
-3
Log of antibody conc. (g/mL)
B
MCF-7
hIgG
hMZ-2
MK1
RLU
5000000
4000000
3000000
2000000
1000000
0
-10
-9
-8
-7
-6
-5
-4
-3
Log of antibody conc. (g/mL)

Fig 9.

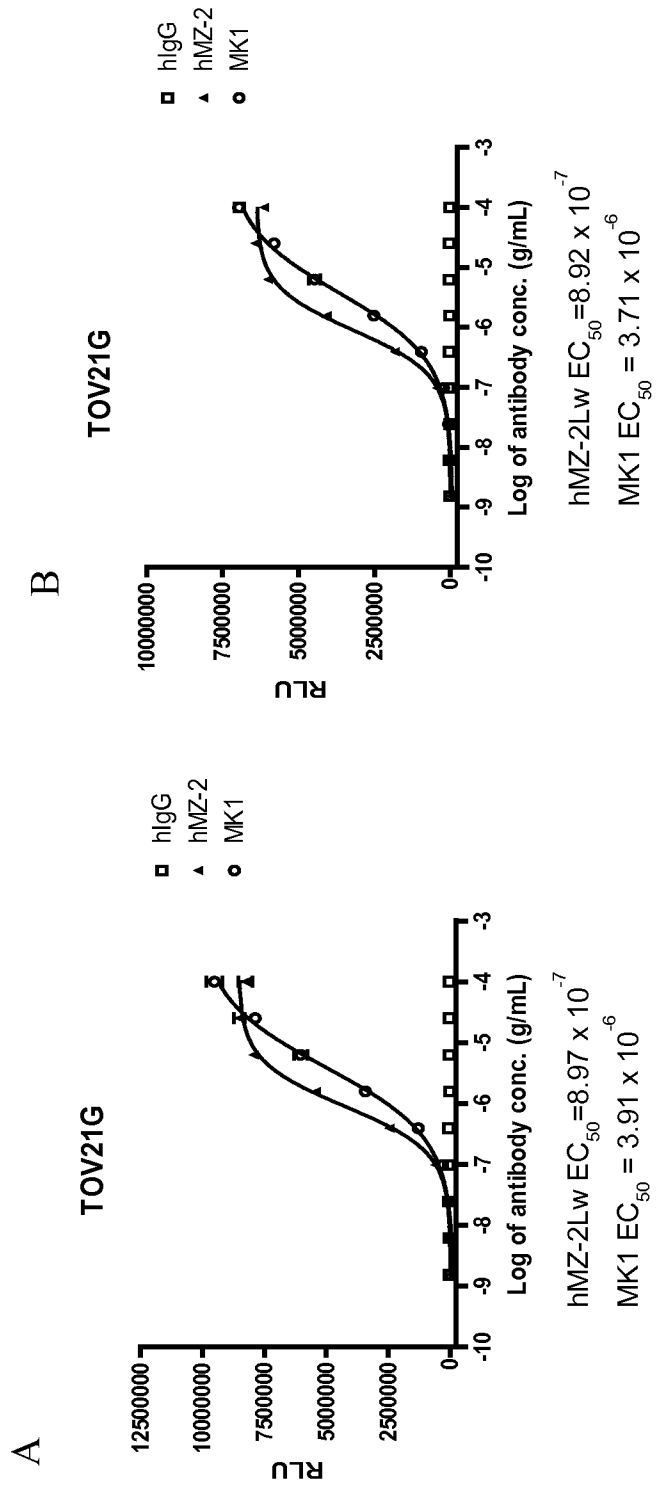

A
TOV21G
hIgG
hMZ-2
MK1
RLU
Log of antibody conc. (g/mL)
hMZ-2Lw EC50=8.97 x 10-7
MK1 EC50 = 3.91 x 10-6
B
TOV21G
hIgG
hMZ-2
MK1
RLU
Log of antibody conc. (g/mL)
hMZ-2Lw EC50=8.92 x 10-7
MK1 EC50 = 3.71 x 10-6

Fig 10

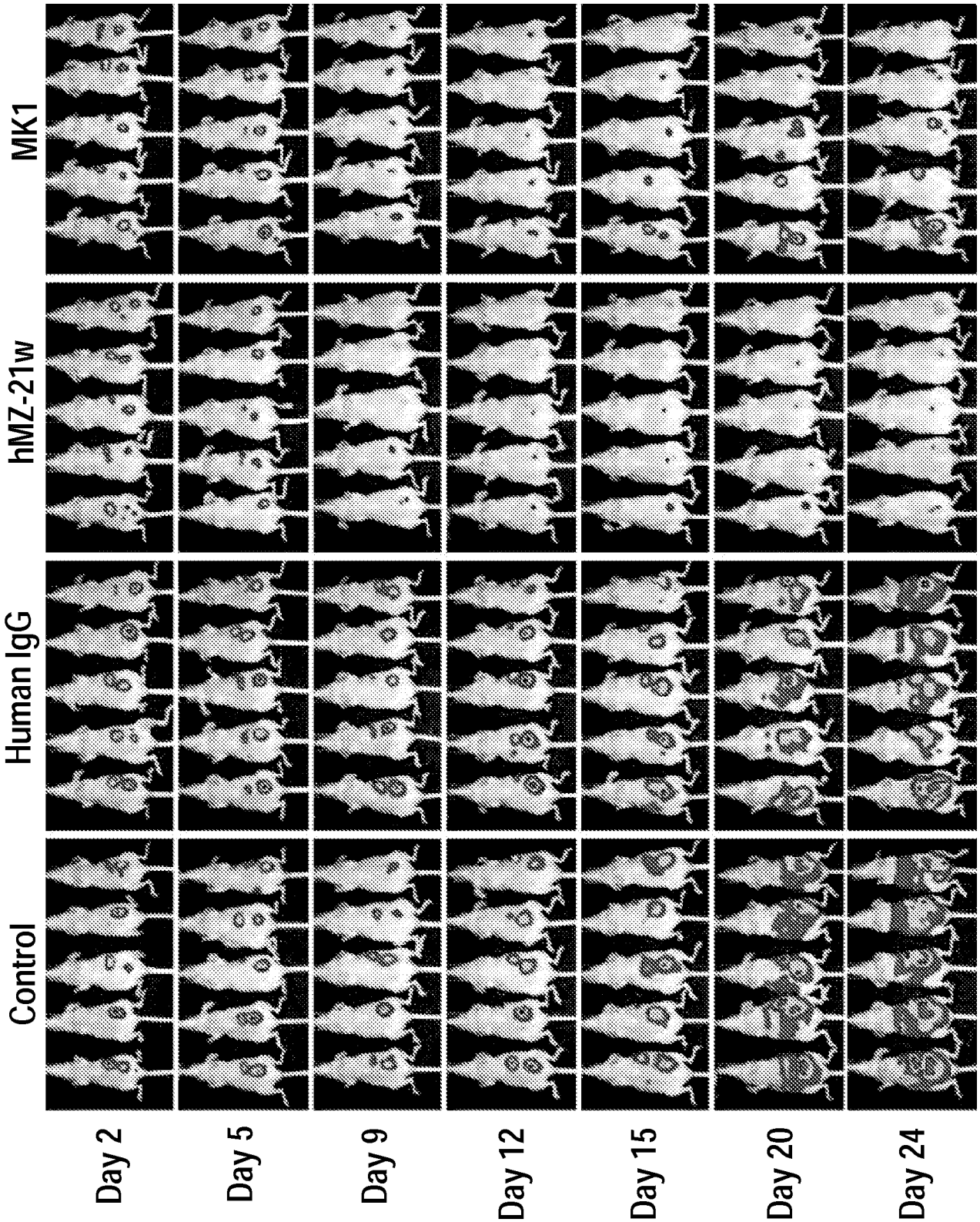

Control
Human IgG
hMZ-21w
MK1
Day 2
Day 5
Day 9
Day 12
Day 15
Day 20
Day 24

Fig. 11

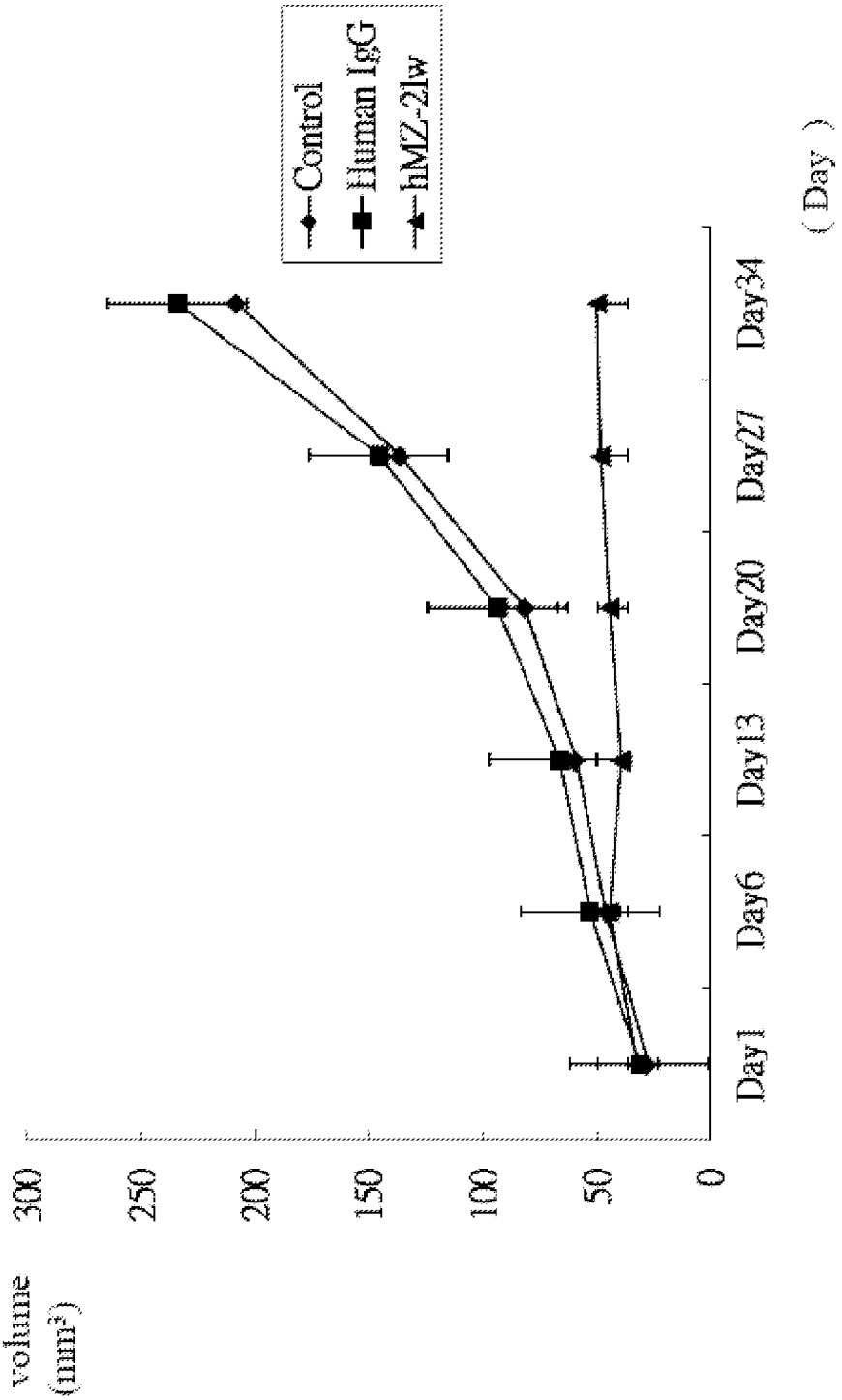
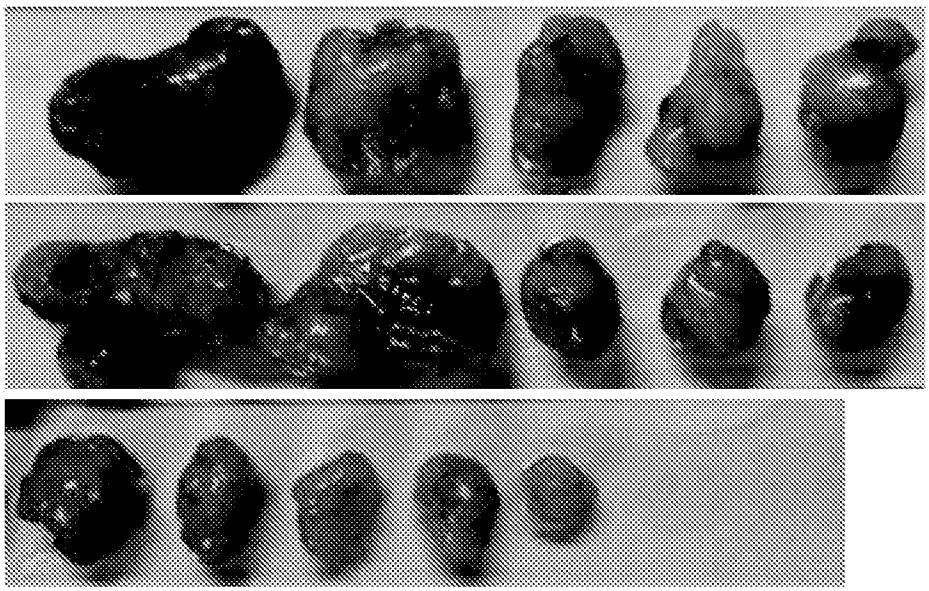

Control
Human IgG
hMZ-2lw
volume (mm³)
300
250
200
150
100
50
0
Day1
Day6
Day13
Day20
Day27
Day34
(Day)

Fig. 12

A.



B.

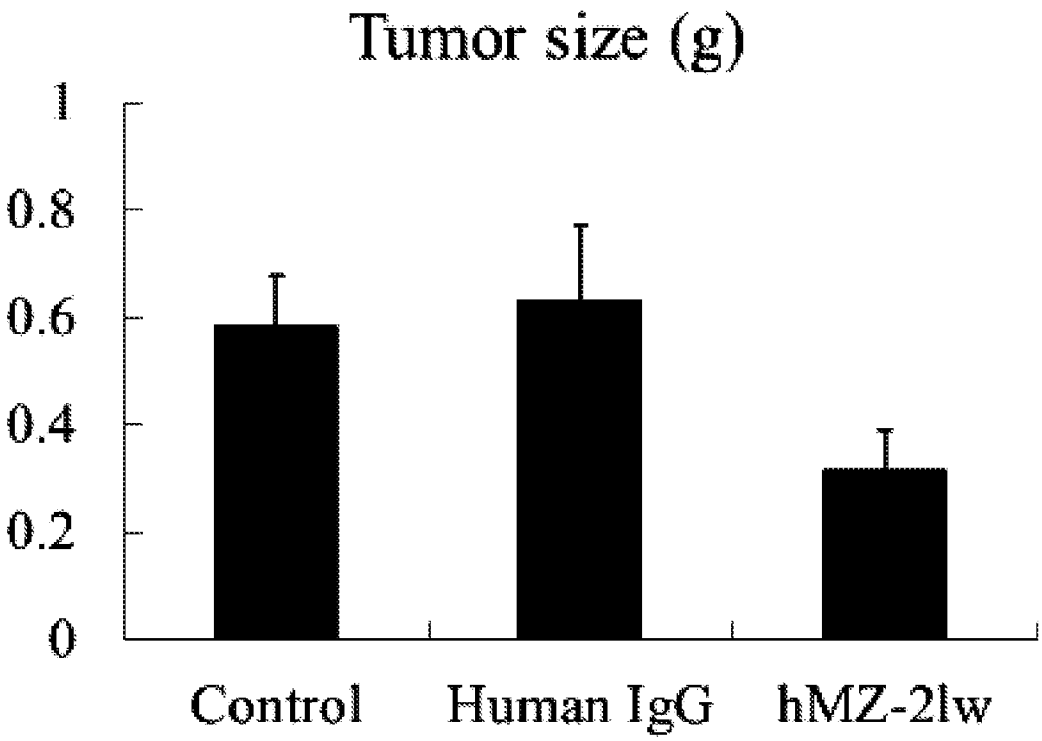


Fig. 13

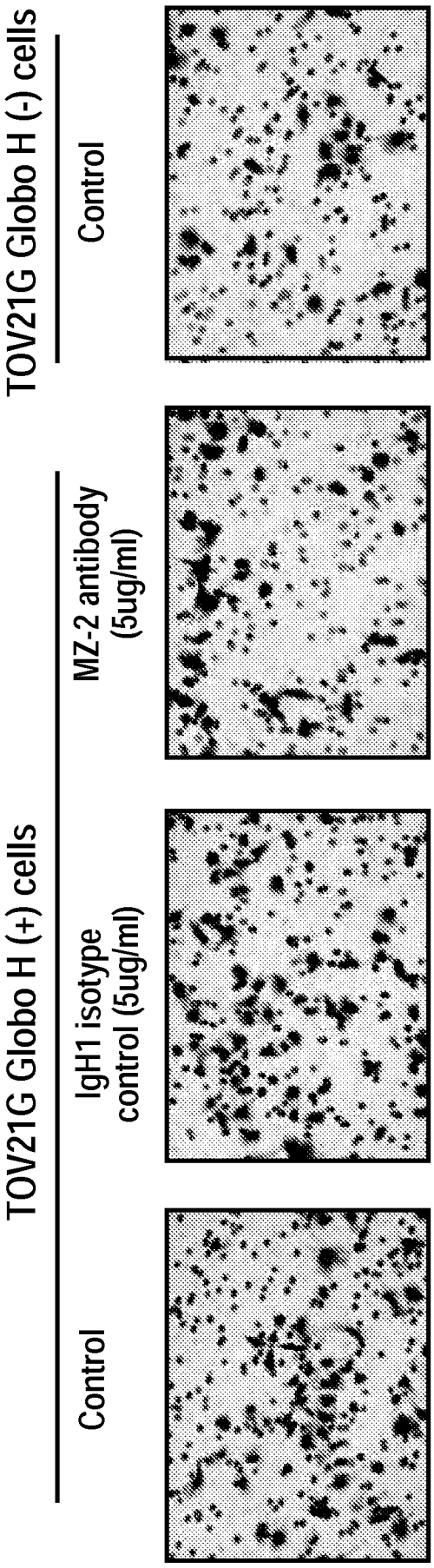

TOV21G Globo H (+) cells
TOV21G Globo H (-) cells
Control
IgH1 isotype control (5ug/ml)
MZ-2 antibody (5ug/ml)
Control

Fig. 14

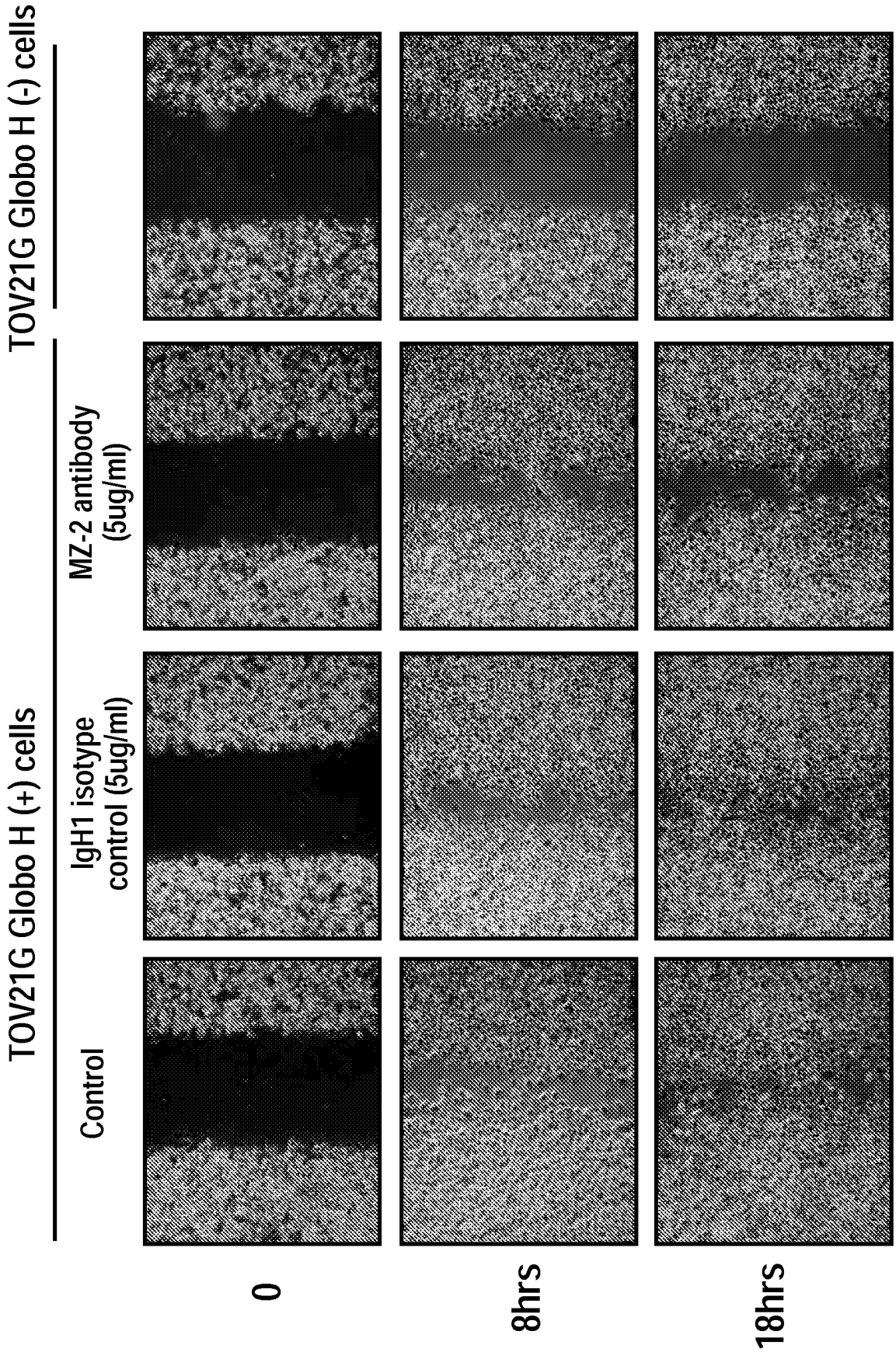

TOV21G Globo H (+) cells
TOV21G Globo H (-) cells
Control
IgH1 isotype control (5ug/ml)
MZ-2 antibody (5ug/ml)
0
8hrs
18hrs

Fig. 15