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(54) Title: MONOCLONAL AND HUMANIZED ANTIBODIES TO A CANCER GLYCOPEPTIDE

(57) Abstract: The present invention discloses a mouse-human chimeric antibody preferably recognizes the MUC1 glycopeptide epitope RPAPGS (GalNAc) TAPPAHG on the surface of cancer cells, and the encoding sequences, wherein the monoclonal antibody having a light chain and a heavy chain. Moreover, the present invention provides humanized light and heavy chains, and the encoding sequences. The results of paired expression show that humanized antibodies also recognize the MUC1 glycopeptide epitope RPAPGS (GalNAc) TAPPAHG on the surface of cancer cells, and show the same specificity as the parental antibody.

Monoclonal and humanized antibodies to a cancer glycopeptide

FIELD OF THE INVENTION

The present invention relates to the field of biotechnology, particularly to the monoclonal and humanized antibodies or a functional fragment thereof against a cancer glycopeptide, and use of the same.

BACKGROUND OF THE INVENTION

Cancer cells expresss abnormal glycoconjugates which are immune-suppressive biomarcromolecules to subvert immune surveillance (Figure 1). Abnormal glycosylated tumor mucins, such as MUC1, bind to BAX and activate anti-apoptotic pathway [1-2]. Furthermore, MUC1 glycans bind to signaling molecule of lymphocytes, Galectin-9, and inhibit the cytotoxic function of natural killer cells [3-4]. MUC1 is the highest expressed mucin in lung cancer, which has been widely studied in the treatment of lung cancer (small cell lung cancer and NSCLC). Our previous studies, including analysis of mRNA expression profiles of glycoproteins in 212 cases of lung cancer, confirmed that MUC1 is the preferred target for immunotherapy [5]. Previous studies by others also found that MUC1 protein bearing Tn and sialyl Tn sugar residues are expressed by breast, gastric, colon, pancreatic, and other cancer types [6-9].

It has been a challenge to generate highly specific antibodies against MUC1 glycopeptides. Glycans are poorly immunogenic due to their high hydrophilicity and lack of charges. The immunogenicity of the peptide part of a glycopeptide is far higher then their glycan part. Therefore antibodies which can recognize both the peptide and glycan parts are rare. Lakshminarayanan et al. found that 90% of antibodies from mice immunized by a glycopeptide can be inhibited by synthetic peptide part of the same glycopeptide. In other words, 90% of binding activities of antibodies induced by a glycopeptide vaccine are directed at peptide part [10].

MUC1 has been as a target for immunotherapy for decades. Most antibodies generated against MUC1 are induced by synthetic peptides containing the tandem repeat domain of MUC1, without glycan modification. These anitbodies were found to be safe in phase I and II clinical trials [11]. However, no significant clinical benefits have been observed. It is believed that the antibody-dependent cell-mediated cytotoxicity is not sufficient to eliminate tumor in patients. Antibody-drug conjugates, chimeric antigen receptor (CAR) transduced T cells, have been reported to target several forms of MUC1 peptides or glycopeptides. huDS6-DM4 developed by Sanofi, recognizes a tumor-associated sialoglyco-epitope on MUC1, although the exact epitope sequence remains unclear (12). 5E5, a monoclonal antibody which binds to GalNAc (Tn) modified 60 mer tandem repeat sequence (13, 14), has shown great promise in the treatment of solid tumor

(pancreatic cancer) when its VL and VH regions were used in the design of chimeric antigen receptor for T cell therapy (15).

For anti-glycopeptides antibodies with therapeutic value, they must have the high specificity to recognize tumor but not healthy tissues. While tumor tissues are known to express unique glycan structures such as Tn antigen (GalNAc), Sialyl Tn antigen (NeuAc alpha2,6 GalNAc), glycan structures are poorly immunogenic, and can not induce antibodies with high affinity. In order to obtain glycopeptide-specific antibodies which can recognize both glycans and polypeptides, we have screened mice immunized by tumor cells, and selected those few mice with higher serum antibody response to glycopeptide compared to non-glycosylated control peptide, and generated monoclonal antibodies specific to glycopeptides.

Murine antibodies must be humanized for therapeutic utilizations such as antibody-drugs, CAR (chimeric antigen receptor) T-cell therapy, in vivo antibody-based diagnostic reagents, etc. CDR (complimentary determination region) graft is the graft of mouse CDR of variable region, which recognizes antigen and determines the specificity of antibody. By grafting CDR of a mouse monoclonal antibody into variable region of a human antibody, and replacing human antibody's CDR, will gain the human antibody's binding to specific antigen, and reduces its immunogenicity in human.

Therefore, the inventors designed humanized light chains hVL1 and hVL2 sequence as well as humanzied heavy chains hVH1, hVH2, hVH3, hVH4, hVH5 sequences for cVL gene and cVH gene of murine 16A. Humanized antibodies were designed by creating multiple hybrid sequences that fuse select parts of the parental antibody sequence with the human framework sequences. Using the 3D model, these humanized sequences were methodically analyzed by eye and computer modeling to isolate the sequences that would most likely retain antigen binding [16]. The goal was to maximize the amount of human sequence in the final humanized antibodies while retaining the original antibody specificity.

SUMMARY OF THE INVENTION

Objects of the present invention include providing the humanized and monoclonal antibodies or a functional fragment thereof against a cancer glycopeptide, and use of the same.

In a first aspect of the present invention, there is provided a humanized antibody or a functional fragment thereof, wherein the humanized antibody recognizes the MUC1 glycopeptide epitope, RPAPGS(GalNAc)TAPPAHG on the surface of cancer cells.

The humanized antibodies are preferably monoclonal.

In a preferred embodiment, the humanized antibody functional or a fragment thereof comprises: a heavy chain sequence contains a variable region having CDRH1, CDRH2, and CDRH3, and the CDRH1 comprises an amino acid sequence set forth in SEQ ID NO: 28, the CDRH2 comprises the amino acid sequences set forth in SEQ ID NOS: 29, and the CDRH3 comprises an amino acid sequence set forth in SEQ ID NO: 30; and

a light chain sequence contains a variable region having CDRL1, CDRL2, and CDRL3, and the CDRL1 comprises the amino acid sequences set forth in SEQ ID NO: 31, the CDRL2 comprises an amino acid sequence set forth in SEQ ID NO: 32, and the CDRL3 comprises an amino acid sequence set forth in SEQ ID NO: 33.

In another preferred embodiment, the humanized antibody or a functional fragment thereof comprises the variable region of the heavy chain sequence, the variable region comprises an amino acid sequence set forth in any one of SEQ ID NOs: 21-25.

In another preferred embodiment, the humanized antibody or a functional fragment thereof comprises the variable region of the light chain sequences, the variable region comprises an amino acid sequences set forth in SEQ ID NO: 26 or SEQ ID NO: 27.

In another preferred embodiment, the humanized antibody or a functional fragment thereof comprises humanized heavy chain sequences hVH1, hVH2, hVH3, hVH4, and hVH5 comprising an amino acid sequence set forth in SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11 and SEQ ID NO: 13, respectively.

In a yet preferred embodiment, the present invention provides the humanized antibody or a functional fragment thereof comprises humanized light chain sequences hVL1 and hVL2 comprising an amino acid sequences set forth in SEQ ID NO: 15 and SEQ ID NO: 17, respectively.

In a still preferred embodiment, there is provided a nucleotide sequence encoding the heavy chain hVH1, hVH2, hVH3, hVH4, and hVH5 of the humanized antibody or a functional fragment thereof as above mentioned, wherein the nucleotide sequence is depicted in SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12 and SEQ ID NO: 14, respectively. In a yet preferred embodiment, the present invention provides a nucleotide sequence encoding the light chain hVL1 and hVL2 of the humanized antibody or a functional fragment thereof as above mentioned, wherein the nucleotide sequence is depicted in SEQ ID NO: 16, and SEQ ID NO: 18.

In a second aspect of the present invention, there is provided a mouse-human chimeric antibody 16A or a functional fragment thereof, wherein the mouse-human chimeric antibody recognizes the MUC1 glycopeptide epitope, RPAPGS(GalNAc)TAPPAHG on the surface of cancer cells.

In a preferred embodiment, the mouse-human chimeric antibody 16A or a functional fragment thereof comprises a heavy chain sequence cVH having an amino acid sequence depicted in SEQ ID NO: 1, and a light chain sequence cVL having an amino acid sequence depicted in SEQ ID NO: 2.

In another preferred embodiment, the present invention provides a nucleotide sequence encoding the heavy chain cVH of the mouse-human chimeric antibody 16A or a functional fragment thereof having the nucleotide sequence depicted in SEQ ID NO: 3. In a yet preferred embodiment, the present invention provides a nucleotide sequence encoding the light chain cVL of the mouse-human chimeric antibody 16A or a functional fragment thereof, wherein the gene has the nucleotide sequence depicted in SEQ ID NO: 4.

All the sequences are listed in Table 1 below.

In a third aspect of the present invention, there is provided an expression vector, wherein said expression vector comprises the nucleotide sequences the encoding the heavy chain hVH1, hVH2, hVH3, hVH4, and hVH5 of the humanized antibody as above mentioned, and/or the nucleotide sequences encoding the light chain hVL1 and hVL2 of the humanized antibody as above mentioned.

In a fourth aspect of the present invention, there is provided a host cell, wherein the cell comprises the expression vector as above mentioned, or has the nucleotide sequences as above mentioned integrated into its genome.

In a fifth aspect of the present invention, there is provided a pharmaceutical composition, comprises the mouse-human chimeric antibody 16A, which contains the VL and VH regions of mouse monoclonal antibody 16A and the constant region of human IgG1, or a functional fragment thereof and a pharmaceutically acceptable carrier.

In a sixth aspect of the present invention, there is provided a pharmaceutical composition, comprises the humanized antibody or a functional fragment thereof as above mentioned and a

pharmaceutically acceptable carrier.

The present still provides the use of the humanized antibody or a functional fragment thereof as above mentioned in the prevention or treatment of the diseases such as cancers.

The present also provides the use of the mouse-human chimeric antibody 16A or a functional fragment thereof in the prevention or treatment of the diseases such as cancers.

The present still provides a method for preventing or treating cancers, wherein said method comprises administering to a subject in need an effective amount of the humanized antibody or a functional fragment thereof, or the mouse-human chimeric antibody 16A or a functional fragment thereof as above mentioned.

Other aspects of the present invention will be apparent to one skilled in the art in view of the present disclosure.

Table 1. Sequences of the present invention

Chain	name	Abbreviation	SEQ ID NO.
Amino acid sequence of chimeric 16A, heavy chain 159-16A-1-hIgG1 HC	H2267.ami	cVH.ami	SEQ ID NO.:1
Amino acid sequence of chimeric 16A, light chain 159-16A-1-hLambda 2 LC	L2267.ami	cVL.ami	SEQ ID NO.:2
Nucleotide sequence of chimeric 16A 159-16A-1-hIgG1 HC	H2267.nt	cVH.nt	SEQ ID NO.:3
Nucleotide sequence of chimeric 16A 159-16A-1-hLambda 2 LC	L2267.nt	cVL.nt	SEQ ID NO.:4
Amino acid sequence of humanized heavy chain H2987	Humanized HC 1.ami	hVH1.ami	SEQ ID NO.:5
Nucleotide sequence of humanized heavy chain H2987	Humanized HC 1.nt	hVH1.nt	SEQ ID NO.:6
Amino acid sequence of	Humanized HC	hVH2.ami	SEQ ID NO.:7

humanized heavy chain H2988	2.ami		
Nucleotide sequence of humanized heavy chain H2988	Humanized HC 2.nt	hVH2.nt	SEQ ID NO.:8
Amino acid sequence of humanized heavy chain H2989	Humanized HC 3.ami	hVH3.ami	SEQ ID NO.:9
Nucleotide sequence of humanized heavy chain H2989	Humanized HC 3.nt	hVH3.nt	SEQ ID NO.:10
Amino acid sequence of humanized heavy chain H2990	Humanized HC 4.ami	hVH4.ami	SEQ ID NO.:11
Nucleotide sequence of humanized heavy chain H2990	Humanized HC 4.nt	hVH4.nt	SEQ ID NO.:12
Amino acid sequence of humanized heavy chain H2991	Humanized HC 5.ami	hVH5.ami	SEQ ID NO.:13
Nucleotide sequence of humanized heavy chain H2991	Humanized HC 5.nt	hVH5.nt	SEQ ID NO.:14
Amino acid sequence of humanized light chain L2987	Humanized LC 1.ami	hVL1.ami	SEQ ID NO.:15
Nucleotide sequence of humanized light chain L2987	Humanized LC 1.nt	hVL1.nt	SEQ ID NO.:16
Amino acid sequence of humanized light chain L2988	Humanized LC2.ami	hVL2.ami	SEQ ID NO.:17
Nucleotide sequence of humanized light chain L2988	Humanized LC2.nt	hVL2.nt	SEQ ID NO.:18
The amino acid sequence of variable region of heavy chain of chimeric 16A 159-16A-1-hIgG1 HC [VH2267]	VH2267.ami	[VH2267.ami]	SEQ ID NO.:19
The amino acid sequence of variable region of light chain of	VL2267.ami	[VL2267.ami]	SEQ ID NO.:20

chimeric 16A 159-16A-1-hLambda 2 LC [VL2267]			
Amino acid sequence of variable region of humanized heavy chain VH2987 (Humanized HC 1)	VH2987.ami	VH2987.ami	SEQ ID NO.:21
Amino acid sequence of variable region of humanized heavy chain VH2988 (Humanized HC 2)	VH2988.ami	VH2988.ami	SEQ ID NO.22
Amino acid sequence of variable region of humanized heavy chain VH2989 (Humanized HC 3)	VH2989.ami	VH2989.ami	SEQ ID NO.:23
Amino acid sequence of variable region of humanized heavy chain VH2990 (Humanized HC 4)	VH2990.ami	VH2990.ami	SEQ ID NO.:24
Amino acid sequence of variable region of humanized heavy chain VH2991 (Humanized HC 5)	VH2991.ami	VH2991.ami	SEQ ID NO.:25
Amino acid sequence of variable region of humanized light chain VL2987 (Humanized LC 1)	VL2987.ami	VL2987.ami	SEQ ID NO.:26
Amino acid sequence of variable region of humanized light chain VL2988 (Humanized LC2)	VL2988.ami	VL2988.ami	SEQ ID NO.:27
Amino acid sequence of the Complimentary Determination Region of humanized heavy chain	VH-CDR1.ami	VH-CDR1.ami	SEQ ID NO.:28

VH-CDR1			
Amino acid sequence of the Complimentary Determination Region of humanized heavy chains VH-CDR2	VH-CDR2.ami	VH-CDR2.ami	SEQ ID NO.:29
Amino acid sequences of the Complimentary Determination Region of humanized heavy chain VH-CDR3	VH-CDR3.ami	VH-CDR3.ami	SEQ ID NO.:30
Amino acid sequence of the Complimentary Determination Region of humanized heavy chain VL-CDR1	VL-CDR1.ami	VL-CDR1.ami	SEQ ID NO.:31
Amino acid sequence of the Complimentary Determination Region of humanized heavy chains VL-CDR2	VL-CDR2.ami	VL-CDR2.ami	SEQ ID NO.:32
Amino acid sequence of the Complimentary Determination Region of humanized heavy chain VL-CDR3	VL-CDR3.ami	VL-CDR3.ami	SEQ ID NO.:33

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates tumor glycoconjugates promote tumor growth and subvert immune surveillance. Mucin MUC1 directly binds to BAX molecule and blocks apoptotic pathways of tumor cells. Mucin glycoproteins bind to galectins of NK cells and T cells, and induce the apoptosis of immune cells.

Fig. 2 illustrates generation of monoclonal antibodies by immunizing mice with xenogenic tumor cell lines lacking core-1 β3-galactosyltransferase activity. (A) C57B6 strain of mice were intravenously immunized by Jurkat cell line transfected by MUC1 gene; (B) MUC1 epitopes

expressed on tumor cell surface stimulate B cells to produce antibodies. Tumor cell antigens provide CD4 T cell help to B cells. (C) Antibody responses toward glycopeptide can be detected by ELISA experiments. Monoclonal antibodies can be selected by using specific glycopeptides.

Fig. 3 depicts amino acid and DNA sequences of 16A chimeric antibody. Each variable region is showed by dark area.

Fig. 4 shows humanization degree of CDR grafted antibody.

Fig. 5 depicts amino acid and DNA sequence of humanized antibody. Each variable region is showed by dark area.

Fig. 6 illustrates specificity of chimeric and humanized antibodies as measured by ELISA. Pep1 is glycopeptide RPAPGS(GalNAc)TAPPAHG, Pep 2 is control polypeptide without glycosylation. Y-axis is the value of OD, X-axis is the concentrations of antibodies (ng/ml).

Fig. 7 illustrates binding of chimeric and humanized antibodies to lung cancer cell line H838. Lung cancer cell line H838 were stained with chimeric parental and humanized antibodies (hVH1hVL2, hVH2hVL2, hVH3hVL2, hVH4hVL2, and hVH5hVL2) with different concentrations. Solid line is the staining first by humanized antibodies and then fluorescence-labeled secondary antibody; dashed line is the staining by secondary antibody alone. The overlap of solid line and dashed line indicates the lowest staining concentration.

Fig. 8 illustrates the anti-tumor efficacy of 16A antibody. Left panel: 16A antibody drug group; Right panel: control IgG group. Each group contained 5 mice. The tumor growth curve of every mouse was presented. Data are representative of 3 independent experiments (The 16 A antibody inhibited the growth of tumor cell line).

Fig. 9 illustrates the specific binding of 16A antibody to tissue section of a representative lung adenocarcinoma patient. Only tumor tissue is stained as positive, but not the peritumoral lung tissue.

DETAILED DESCRIPTION OF THE INVENTION

The following non-limiting examples are provided to further illustrate embodiments of the invention disclosed herein. It will be appreciated by those of skill in the art that the techniques disclosed in the examples that follow representative approaches that have been found to function well in the practice of the invention and thus can be considered to constitute examples of modes for its practice. However, those of skill in the art will, in light of the present disclosure,

appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1. Cloning of cVL and cVH genes of 16A monoclonal antibody

Total RNA was extracted from 16A murine hybridoma (from the University of Texas MD Anderson Cancer Center; reference: Int J Oncol 41(6):1977-84, 12/2012) by QIAGEN RNeasy Mini reagent (QIAGEN). cDNA was synthesized by by SMARTer RACE reagent (CLONTECH). The primer used for reverse transcription was Oligo-dT. cDNA was used as PCR template to clone cVH gene and cVL gene. Universal primer A mix (CLONTECH) and 5'-GGGRCCARKG GATAGACHGATGG-3' (designed according to the C segment of mouse IgG antibody heavy chain sequence) were used as cloning primers of cVH gene. Universal primer A mix (CLONTECH) and 5'-5′-CTTCAGAGGA AGGGTGGAAACAGG-3' (designed according to the C segment of mouse IgG antibody light chain sequence) were used as cloning primers of cVL gene. cVH and cVL PCR fragments were sequenced by 3130XL ABI DNA sequencer.

Example 2. Design and expression of murine 16A chimeric antibody

The encoding gene of Chimeric 16A antibody is a hybrid structure wherein the murine 16A VH and VL gene fragments jointed to C region fragments of human IgG1. Amino acid and cDNA sequences of 16A chimeric antibody are as shown in Figure 3. The VL and VH genes were synthesized by Sydlabs, MA. The synthesized genes were verified by DNA sequencing using 3130XL ABI DNA sequencer. Chimeric VL and chimeric VH genes were built into pcDNA3.1 expression vector (Invitrogen), as pcDNA3.1- chimeric VL and pcDNA3.1-chimeric-VH, respectively.

Example 3. Expression and purification of chimeric antibody

HEK293 cells were cultured in serum-free media (DMEM, Life Technologies). pcDNA3.1-chimeric VL and pcDNA3.1-chimeric-VH were transiently transfected simultaneously by electroporation (Maxcyte). HEK293 cells were cultured for 5 additional days after electroporation, and culture supernatant was used in subsequent testing of antibody titer. Then culture supernatant was combined, and antibody was purified by Protein A affinity chromatography column (GE Healthcare).

Example 4. Design and expression of murine 16A humanized (CDR grafted) antibody

CDR of 16A antibody variable region directly determines the specificity of antibody. By grafting CDR of mouse monoclonal antibody into variable region of human antibody, we designed light

chain hVL1 and hVL2 sequences, and heavy chain hVH1, hVH2, hVH3, hVH4 and hVH5 sequences. We hereby used hVL2 sequence in subsequent testing of antibody function.

Selection of the human antibody framework was based on BLAST search by mouse cVH and cVL amino acid sequences of 16A against the human hVH and hVL databases (IMGT®, the international ImMunoGeneTics information system®) respectively.

Humanized antibody was generated by grafting 16A CDR region to human antibody frame work. Furthermore, several amino acid sites were optimized by using computer 3D modeling. The aim was to obtain humanized sequences with the highest humanness score, while the specificity of 16A antibody is remained. The calculation method of humanized extent was according to Reference 16. Predicted humanness score was as shown in Figure 4.

Amino acid and cDNA sequences of humanized 16 antibody were as shown in Figure 5.

Example 5. Measurement of the binding activity of monoclonal antibodies to glycopeptides

ELISA plates were coated with streptavidin (1.5 μg/ml, Millipore) for overnight at 4 degree, and blocked by 1% BSA for 1 hour at room temperature. 2 μg/ml biotinylated glycopeptides (RPAPGS(GalNAc)TAPPAHG) were attached to streptavidin coated plates. Serially diluted chimeric or humanized 16A antibodies (antibody concentrations as shown in Figure 6) were incubated with glycopeptides. After washing three times with PBS 0.05% Tween-20, the plates were then incubated with HRP-conjugated goat-anti-mouse secondary antibody. After three washes, the plates were incubated with DAB reagent. Non-glycosylated control peptide was used at the same concentrations to measure its binding to chimeric or humanized 16A antibodies.

Affinity of 16A chimeric antibody and humanized antibody for glycopeptides was higher than control polypeptides, particularly the hVH5hVL2. As shown in Figure 6, strong binding to glycopeptide was found even at concentration of 10 ng/ml for the antibodies (OD=2.0). Whereas the antibody binding to control peptide (Peptide 2) was very low at 10ng/ml antibody concentration (OD=ELISA background). The only difference between Peptide 1 and Peptide 2 is Peptide 2 had no sugar (GalNAc) modification.

Minimal concentration of chimeric and humanized antibodies to bind antigen RPAPGS(GalNAc)TAPPAHG, as determined by ELISA.

Antibody	Minimum concentration of binding to glycopeptide（ng/ml）
Chimeric	0.15
hVH1hVL2	20

hVH2hVL2	156
hVH3hVL2	78
hVH4hVL2	78
hVH5hVL2	0.15

Example 6. Measurement of antibody binding to tumor cells by flow cytometry staining

Lung cancer cell line H838 was obtained from the University of Texas M.D. Anderson Cancer Center. Cells were cultured in 10% RPMI 1640 medium. Different concentrations of chimeric antibody or humanized antibodies were used as primary antibody for staining, washed three times with PBS, then incubated with PE-conjugated mouse-anti-human IgG (BioLegend). The stained cells were analyzed by FACS Caliber flow cytometer (BD Biosciences, San Jose, CA). Staining results were as shown in Figure 7.

Minimal concentration of chimeric and humanized antibodies to bind antigen, determined by cell surface staining of lung cancer cell line H838.

Antibody	Minimum concentration of binding to lung cancer cell line H838（μg / ml）
Chimeric	0.0125
hVH1hVL2	0.025
hVH2hVL2	0.025
hVH3hVL2	0.025
hVH4hVL2	0.05
hVH5hVL2	0.025

Example 7. Antitumor efficacy of 16A monoclonal antibody

C3H mice (Jackson Laboratory, ME) were inoculated with Ag104-MUC1 cell line, a mouse fibrosarcoma cell line stably transfected by MUC1 gene (9). 6-week old C3H mice were inoculated with 2 million tumor cells subcutaneously. 100 micrograms of 16A antibody were administered by intraperotoneal injection at the same day of tumor inoculation. 16A antibody drug was given at 100 microgram per mouse every 3 days. Control mouse IgG antibody (from Southern Biotech, AL) was used to treat the tumor-bearing mice in the control group. The perpendicular diameters of tumor were measured and the tumor area was used to represent tumor burden. In mice treated by 16A monoclonal antibody, the tumor growth is significantly inhibited.

Example 8. Specific binding of 16A antibody to cancer but not peritumoral tissue

Immunohistochemistry was performed as previously described (9). Briefly, 5-μm paraffin-fixed tissue sections were deparaffinized in xylene and rehydrated through using a gradient of alcohol

(100, 95 to 80%, Sigma, St. Louis, MO). Antigen retrieval was carried out for 30 min using PT Module (Lab Vision Corp., USA) in Tris-EDTA buffer (pH 9.0). After cooling down, the slides were thoroughly washed in distilled water and washed three times in 1X phosphate-buffered saline (PBS), 2 min each. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (Sigma), then in methanol for 10 min at room temperature followed by rinsing for 2 min in 1X PBS three times. Nonspecific binding of the primary antibody was blocked by incubating the sections with 10% normal horse serum for 30 min at room temperature. Sections were then incubated with primary anti-16A monoclonal antibody at 4°C overnight, at 1 μg/ml concentration.

The second day, after washing three times in 1X PBS (2 min each), the slides were incubated with secondary anti-mouse IgG-biotin antibody (1:200, Vectastain Elite ABC kit; Vector laboratories, CA, USA) at room temperature for 1 h and rinsed in 1X PBS three times (2 min each). After another 1-h incubation with the avidin-biotin peroxidase complex (1:100, Vectastain Elite ABC Kit; Vector Laboratories, CA, USA) and repeated washing steps with 1X PBS, visualization was performed with the chromagen 3,3′ -diaminobenzidine (DAB, Dako, Carpinteria, CA, USA). The slides were counterstained with hematoxylin and coverslipped with PerMount. Sections of Jurkat-pcDNA-IRES-eGFP-MUC1 and Jurkat-pcDNA-IRES-eGFP were used as positive and negative controls, respectively. Isotype IgG and omission of the primary antibody were used as negative controls for staining.

All references cited in the present disclosure are hereby incorporated herein by reference as if each was individually incorporated herein by reference. In addition, it is understood that those skilled in the art will, in light of the teaching described hereinabove, make various changes and modifications to the present invention without departing from the spirit of the present invention, and these equivalents are deemed to fall within the scope of the present invention as defined in the appended claims.

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Feldman MD, Young R, Keith B, Schreiber H, Clausen H, Johnson LA, June CH. Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma. Immunity. 2016 Jun 21;44(6):1444-54.

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Claims

What is claimed is:

1. A humanized antibody or a functional fragment thereof, characterized in that the humanized antibody comprises: a heavy chain sequence contains a variable region having CDRH1, CDRH2, and CDRH3, and the CDRH1 comprises an amino acid sequence set forth in SEQ ID NO: 28, the CDRH2 comprises the amino acid sequences set forth in SEQ ID NOS: 29, and the CDRH3 comprises an amino acid sequence set forth in SEQ ID NO: 30; and

a light chain sequence contains a variable region having CDRL1, CDRL2, and CDRL3, and the CDRL1 comprises the amino acid sequences set forth in SEQ ID NO: 31, the CDRL2 comprises an amino acid sequence set forth in SEQ ID NO: 32, and the CDRL3 comprises an amino acid sequence set forth in SEQ ID NO: 33.

2. The humanized antibody or a functional fragment thereof according to Claim 1, wherein the humanized antibody comprises the variable region of the heavy chain sequence, the variable region comprises an amino acid sequence set forth in any one of SEQ ID NOs: 21-25.

3. The humanized antibody or a functional fragment thereof according to Claim 1, wherein the humanized antibody comprises the variable region of the light chain sequences, the variable region comprises an amino acid sequences set forth in SEQ ID NO: 26 or SEQ ID NO: 27.

4. The humanized antibody or a functional fragment thereof according to any one of Claims 1-3, wherein the humanized antibody comprises the heavy chain sequence comprises an amino acid sequence set forth in any one of SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11 and SEQ ID NO: 13.

5. The humanized antibody or a functional fragment thereof according to any one of Claims 1-4, wherein the humanized antibody comprises the lignt chain sequence comprises an amino acid sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 17.

6. A mouse-human chimeric antibody 16A or a functional fragment thereof, characterized in that the mouse-human chimeric antibody comprises a variable region of heavy chain having an amino acid sequence set forth in SEQ ID NO: 19, and a variable region of light chain having an amino acid sequence set forth in SEQ ID NO: 20, and the constant region of human IgG1.

7. The mouse-human chimeric antibody or a functional fragment thereof according to claim 6, wherein the mouse-human chimeric antibody comprises a heavy chain sequence having an amino acid sequence set forth in SEQ ID NO: 1, and a light chain sequence having an amino acid sequence set forth in SEQ ID NO: 2.

8. A nucleotide sequence encoding the heavy chain of the humanized antibody or a functional fragment thereof according to any one of claims 1 to 5, wherein the nucleotide sequence is depicted in any one of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12 and SEQ

ID NO: 14.

9. A nucleotide sequence encoding the light chain of the humanized antibody or a functional fragment thereof according to any one of claims 1 to 5, wherein the nucleotide sequence is depicted in SEQ ID NO: 16, or SEQ ID NO: 18.

10. An expression vector, wherein said expression vector comprises the sequence according to claim 8 or claim 9.

11. A host cell, wherein the cell comprises the expression vector according to claim 10 or the sequence according to claim 8 or claim 9 integrated into its genome.

12. A pharmaceutical composition comprises the humanized antibody or a functional fragment thereof according to any one of claims 1 to 5, or the mouse-human chimeric antibody or a functional fragment thereof according to claim 6 or claim 7, and a pharmaceutically acceptable carrier.

13. Use of the humanized antibody or a functional fragment thereof according to any one of claims 1 to 5, or the mouse-human chimeric antibody or a functional fragment thereof according to claim 6 or claim 7 in the prevention or treatment of cancers.

14. A method for preventing or treating cancers, wherein said method comprises administering to a subject in need an effective amount of the humanized antibody or a functional fragment thereof according to any one of claims 1 to 5, or the mouse-human chimeric antibody or a functional fragment thereof according to claim 6 or claim 7.

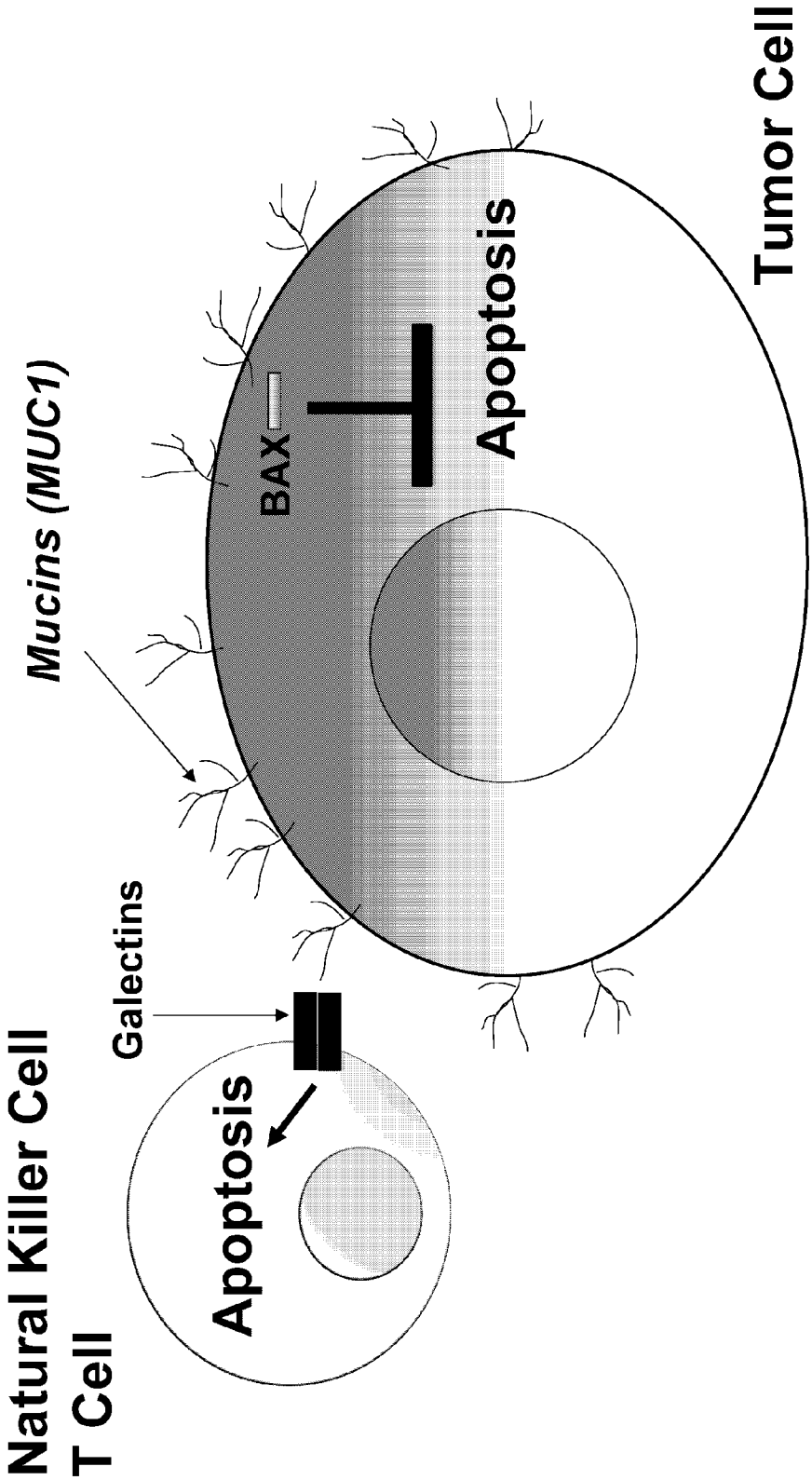
Natural Killer Cell
T Cell
Galectins
Apoptosis
Mucins (MUC1)
BAX
Apoptosis
Tumor Cell

Fig. 1

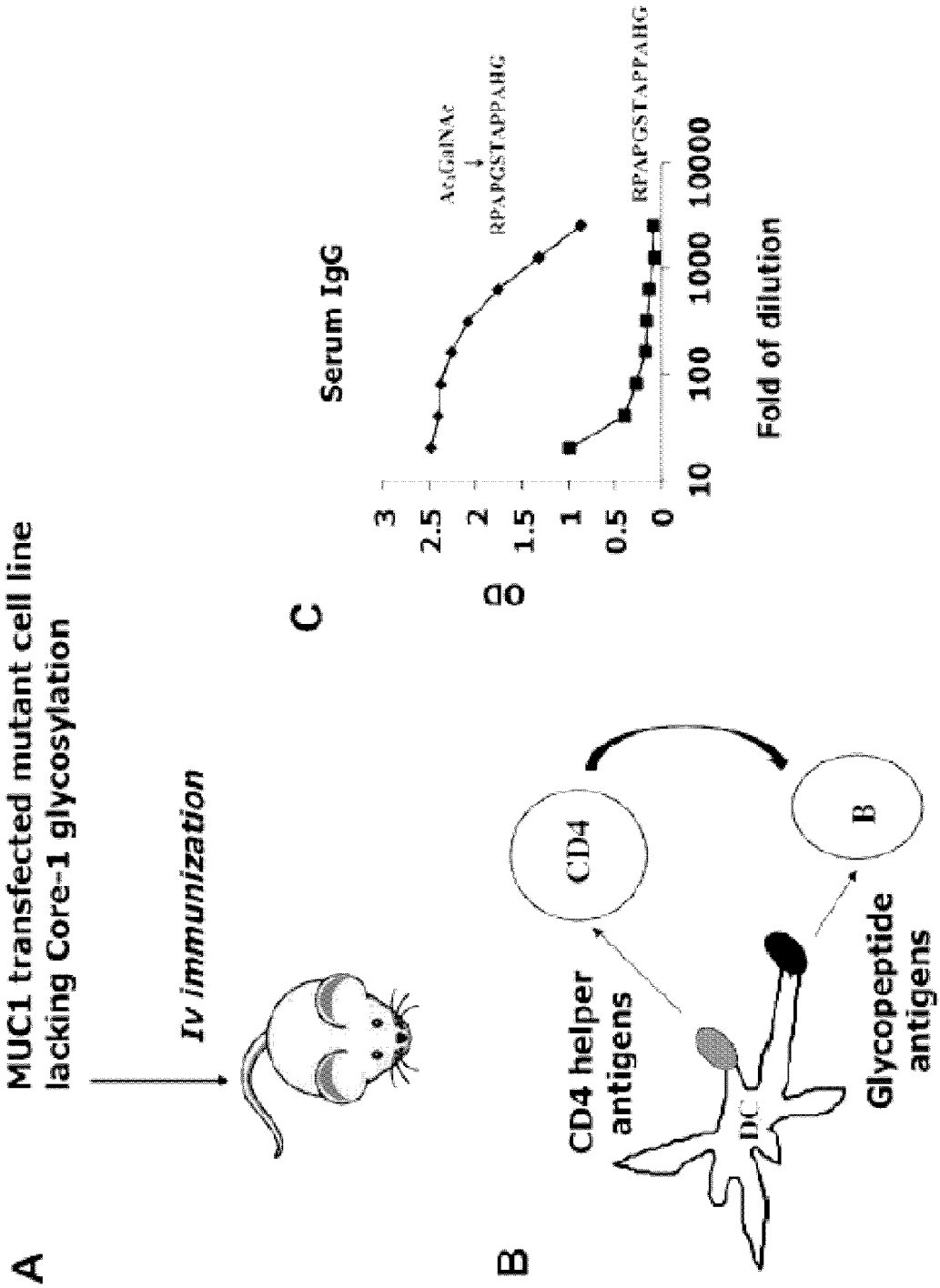
A
MUC1 transfected mutant cell line lacking Core-1 glycosylation
Iv immunization
B
CD4
B
CD4 helper antigens
Glycopeptide antigens
DC
C
Serum IgG
OD
3
2.5
2
1.5
1
0.5
0
10
100
1000
10000
Fold of dilution
AcGalNAc
RPAPGSTAPPAHG
RPAPGSTAPPAHG

Fig. 2

The amino acid sequence of heavy chain of chimeric 16A

159-16A-1-hIgG1 HC [H2267]:
MDPKGSLSWRILLFLSLAFELSYGEVKLHQSGGGLVQPGGFLKISCVVSGIDFSRYWMSWVRRAPGKGLEWIGEI
TPDSNTINYVPSLKDNFGISRDNAKNTLFLQMTKVPSEDTALYFCASYYEGFAYWGQGTLVTVSAASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPG*

The amino acid sequence of light chain of chimeric 16A

159-16A-1-hLambda 2 LC [L2267]:
MSVPTQVLGLLLLWLTDARCQAVVTQESALTTSPGETVTLTCRSSTGAVITSNYANWVQEKPDHLFTGLIGRTYN
RVPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNHFVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA
NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTV
EKTVAPTECS*

Variable region

The amino acid sequence of variable region of heavy chain of chimeric 16A

159-16A-1-hIgG1 HC [VH2267]:
EVKLHQSGGGLVQPGGFLKISCVVSGIDFSRYWMSWVRRAPGKGLEWIGEITPDSNTINYVPSLKDNFGISRDNA
KNTLFLQMTKVRSEDTALYFCASYYEGFAYWGQGTLVTVSA

The amino acid sequence of variable region of light chain of chimeric 16A

159-16A-1-hLambda 2 LC [VL2267]:
QAVVTQESALTTSPGETVTLTCRSSTGAVITSNYANWVQEKPDHLFTGLIGRTYNRVPGVPARFSGSLIGDKAAL
TITGAQTEDEAIYFCALWYSNHFVFGGGTKLTVL

Fig. 3

The DNA sequence of heavy chain of chimeric 16A

159-16A-1-hIgG1 HC [H2267]:

ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCCTGAGCCTGGCCTTCGAGCTGAGCTACGGCGAG
GTGAAGCTTCACCAGTCTGGAGGTGGCCTGGTGCAGCCTGGAGGATTCCTGAAAATCTCCTGTGTAGTCTCAGGA
ATCGATTTTAGTAGATACTGGATGAGTTGGGTTCGGCGGGCTCCAGGGAAAGGACTAGAATGGATTGGAGAAATT
ACTCCAGATAGCAATACAATAAACTATGTACCATCTCTAAAGGATAATTTCGGCATCTCCAGAGACAACGCCAAA
AATACGCTGTTCCTGCAAATGACCAAAGTGAGATCTGAGGACACAGCCCTTTATTTCTGTGCATCCTACTACGAG
GGATTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTAGCACCAAGGGCCCCAGCGTGTTCCCT
CTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGAACCGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAG
CCCGTGACCGTGTCCTGGAACAGCGGCGCTCTGACCAGCGGAGTGCACACCTTCCCTGCCGTGCTGCAGAGCAGC
GGCCTGTACTCCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTG
AACCACAAGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCTGCGACAAGACCCACACCTGCCCT
CCCTGCCCCGCCCCCGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCTCCCAAGCCCAAGGACACCCTGATG
ATCAGCCGCACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGG
TACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGGGAGGAGCAGTACAACTCCACCTACCGCGTG
GTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCC
CTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGGGAGCCTCAGGTGTACACCCTGCCC
CCCAGCCGCGACGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATC
GCCGTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACAGCGACGGC
AGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCGGATAG

The DNA sequence of light chain of chimeric 16A

159-16A-1-hLambda 2 LC [L2267]:

ATGTCCGTGCCTACCCAGGTGCTGGGACTGCTGCTGCTGTGGCTGACCGACGCCAGATGTCAGGCTGTTGTGACT
CAGGAATCTGCACTCACCACATCACCTGGTGAAACAGTCACACTCACTTGTCGCTCAAGTACTGGGGCTGTTATA
ACTAGTAACTATGCCAACTGGGTCCAAGAAAAACCAGATCATTTATTCACTGGTCTAATAGGTCGTACCTACAAC
CGAGTTCCAGGTGTTCCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTCACCATCACAGGGGCA
CAGACTGAGGATGAGGCAATATATTTCTGTGCTCTATGGTACAGCAACCATTTCGTGTTCGGTGGAGGAACCAAA
CTGACTGTCCTAGGACAGCCTAAGGCCGCTCCTTCCGTGACCCTGTTCCCTCCATCCTCCGAGGAACTGCAGGCC
AACAAGGCCACCCTCGTGTGCCTGATCTCCGACTTCTACCCTGGCGCCGTGACCGTGGCCTGGAAGGCTGATAGC
TCTCCTGTGAAGGCCGGCGTGGAAACCACCACCCCTTCCAAGCAGTCCAACAACAAATACGCCGCCTCCTCCTAC
CTGTCCCTGACCCCTGAGCAGTGGAAGTCCCACCGGTCCTACAGCTGCCAAGTGACCCACGAGGGCTCCACCGTG
GAAAAGACCGTGGCTCCTACCGAGTGCTCCTGA

Fig. 3 (continued)

The humanness scores of humanized light chains

Chain Name	Note	Full-length (Framework+CDR) Cutoff = 84	Framework Only Cutoff = 88
L2267 (Chimeric Parental)	Light chain	62.34	70.95
L2987 (VL1)	Regular humanized	82.98	96.33
L2988 (VL2)	Regular humanized	78.63	90.57

The humanness scores of humanized light chains

Chain Name	Note	Full-length (Framework+CDR) Cutoff = 79	Framework Only Cutoff = 84
H2267 (Chimeric Parental)	Parental	63.62	69.31
H2987 (VH1)	Regular humanized; 6 highlighted residues changed	82.72	91.67
H2988 (VH2)	Regular humanized:; 6 highlighted residues changed	81.68	90.80
H2989 (VH3)	Regular humanized; 5 highlighted residues changed	80.22	88.62
H2990 (VH4)	No change in highlighted residues	76.59	84.25
H2991 (VH5)	Balanced change; 3 highlighted residues changed	79.40	87.53

Fig. 4

Amino acid and nucleotide sequences of humanized heavy chains

> H2987 (Humanized HC 1)
MDPKGSLSWRILLFLSLAFELSYGEVQLVESGGGLVQPGGSLRLSCAVSGIDFSRYWMSWVRQA
PGKGLEWVAEITPDSNTINYVPSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCASYYEGFAY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPG**

ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCCTGAGCCTGGCCTTCGAGC
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GCGACAGGCTCCTGGCAAGGGCCTGGAATGGGTGGCCGAGATCACCCCCGACTCCAACAC
CATCAACTACGTGCCCTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAACGCCAAGAAC
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CCTACTACGAGGGCTTCGCCTATTGGGGCCAGGGCACCCTCGTGACCGTGTCCTCTGCTAG
CACCAAGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGAAC
CGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAA
CAGCGGCGCTCTGACCAGCGGAGTGCACACCTTCCCTGCCGTGCTGCAGAGCAGCGGCCT
GTACTCCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
TGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCT
GCGACAAGACCCACACCTGCCCTCCCTGCCCCGCCCCCGAGCTGCTGGGCGGACCCAGCG
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CTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGA
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CCGCGTGGTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAA
GTGCAAGGTGAGCAACAAGGCCCTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAG
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AACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATCGCCGTGGAGT
GGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACAGCG
ACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAA
CGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTG
AGCCTGAGCCCCGGATAGTAA

Variable region

Fig. 5

> H2988 (Humanized HC 2)
MDPKGSLSWRILLFLSLAFELSYGEVQLVESGGGLVQPGGSLRLSCAVSGIDFSRYWMSWVRQA
PGKGLEWVGEITPDSNTINYVPSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCASYYEGFAY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPG**

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CATCAACTACGTGCCCTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAACGCCAAGAAC
TCCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGATACCGCCGTGTACTACTGCGCCT
CCTACTACGAGGGCTTCGCCTATTGGGGCCAGGGCACCCTCGTGACCGTGTCCTCTGCTAG
CACCAAGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGAAC
CGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAA
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AACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATCGCCGTGGAGT
GGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACAGCG
ACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAA
CGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTG
AGCCTGAGCCCCGGATAGTAA

Fig. 5 (continued)

> H2989 (Humanized HC 3)
MDPKGSLSWRILLFLSLAFELSYGEVKLVESGGGLVQPGGSLRLSCAVSGIDFSRYWMSWVRQA
PGKGLEWVGEITPDSNTINYVPSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYFCASYYEGFAY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPG**

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CTCTGAGACTGTCCTGCGCCGTGTCCGGCATCGACTTCTCCCGGTACTGGATGTCCTGGGT
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CATCAACTACGTGCCCTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAACGCCAAGAAC
TCCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGATACCGCCGTGTACTTCTGCGCCT
CCTACTACGAGGGCTTCGCCTATTGGGGCCAGGGCACCCTCGTGACCGTGTCCTCTGCTAG
CACCAAGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGAAC
CGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAA
CAGCGGCGCTCTGACCAGCGGAGTGCACACCTTCCCTGCCGTGCTGCAGAGCAGCGGCCT
GTACTCCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
TGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCT
GCGACAAGACCCACACCTGCCCTCCCTGCCCCGCCCCCGAGCTGCTGGGCGGACCCAGCG
TGTTCCTGTTCCCTCCCAAGCCCAAGGACACCCTGATGATCAGCCGCACCCCCGAGGTGAC
CTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGA
CGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGGGAGGAGCAGTACAACTCCACCTA
CCGCGTGGTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAA
GTGCAAGGTGAGCAACAAGGCCCTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAG
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GGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACAGCG
ACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAA
CGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTG
AGCCTGAGCCCCGGATAGTAA

Fig. 5 (continued)

> H2990 (Humanized HC 4)
MDPKGSLSWRILLFLSLAFELSYGEVQLVESGGGLVQPGGFLRLSCVVSGIDFSRYWMSWVRQA
PGKGLEWVGEITPDSNTINYVPSVKGNFGISRDNAKNSLFLQMNSLRAEDTAVYFCASYYEGFAY
WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPG**

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TGAGCTACGGCGAAGTGCAGCTGGTGGAATCTGGCGGCGGACTGGTGCAGCCTGGCGGCT
TTCTGAGACTGTCCTGCGTGGTGTCCGGCATCGACTTCTCCCGGTACTGGATGTCCTGGGT
GCGACAGGCTCCTGGCAAGGGCCTGGAATGGGTGGGAGAGATCACCCCCGACTCCAACAC
CATCAACTACGTGCCCTCCGTGAAGGGCAACTTCGGCATCTCCAGAGACAACGCCAAGAACT
CCCTGTTCCTGCAGATGAACAGCCTGCGGGCCGAGGATACCGCCGTGTACTTCTGCGCCTC
CTACTACGAGGGCTTCGCCTATTGGGGCCAGGGCACCCTCGTGACCGTGTCCTCTGCTAGC
ACCAAGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGAACC
GCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAAC
AGCGGCGCTCTGACCAGCGGAGTGCACACCTTCCCTGCCGTGCTGCAGAGCAGCGGCCTG
TACTCCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCT
GCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCTG
CGACAAGACCCACACCTGCCCTCCCTGCCCCGCCCCCGAGCTGCTGGGCGGACCCAGCGT
GTTCCTGTTCCCTCCCAAGCCCAAGGACACCCTGATGATCAGCCGCACCCCCGAGGTGACC
TGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGAC
GGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGGGAGGAGCAGTACAACTCCACCTAC
CGCGTGGTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG
TGCAAGGTGAGCAACAAGGCCCTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAGG
GCCAGCCCCGGGAGCCTCAGGTGTACACCCTGCCCCCCAGCCGCGACGAGCTGACCAAGA
ACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATCGCCGTGGAGTG
GGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACAGCGA
CGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAA
CGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTG
AGCCTGAGCCCCGGATAGTAA

Fig. 5 (continued)

> H2991 (Humanized HC 5)
MDPKGSLSWRILLFLSLAFELSYGEVQLVESGGGLVQPGGSLRLSCVVSGIDFSRYWMSWVRQ
APGKGLEWVGEITPDSNTINYVPSVKGRFGISRDNAKNSLYLQMNSLRAEDTAVYFCASYYEGFA
YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPG**

ATGGACCCCAAGGGCAGCCTGAGCTGGAGAATCCTGCTGTTCCTGAGCCTGGCCTTCGAGC
TGAGCTACGGCGAAGTGCAGCTGGTGGAATCTGGCGGCGGACTGGTGCAGCCTGGCGGAT
CTCTGAGACTGTCCTGCGTGGTGTCCGGCATCGACTTCTCCCGGTACTGGATGTCCTGGGT
GCGACAGGCTCCTGGCAAGGGCCTGGAATGGGTGGGAGAGATCACCCCCGACTCCAACAC
CATCAACTACGTGCCCTCCGTGAAGGGCCGGTTCGGCATCTCCAGAGACAACGCCAAGAAC
TCCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGATACCGCCGTGTACTTCTGCGCCT
CCTACTACGAGGGCTTCGCCTATTGGGGCCAGGGCACCCTCGTGACCGTGTCCTCTGCTAG
CACCAAGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGAAC
CGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAA
CAGCGGCGCTCTGACCAGCGGAGTGCACACCTTCCCTGCCGTGCTGCAGAGCAGCGGCCT
GTACTCCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATC
TGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAGCCTAAGAGCT
GCGACAAGACCCACACCTGCCCTCCCTGCCCCGCCCCCGAGCTGCTGGGCGGACCCAGCG
TGTTCCTGTTCCCTCCCAAGCCCAAGGACACCCTGATGATCAGCCGCACCCCCGAGGTGAC
CTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGA
CGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTCGGGAGGAGCAGTACAACTCCACCTA
CCGCGTGGTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAA
GTGCAAGGTGAGCAACAAGGCCCTGCCCGCTCCCATCGAGAAGACCATCAGCAAGGCCAAG
GGCCAGCCCCGGGAGCCTCAGGTGTACACCCTGCCCCCCAGCCGCGACGAGCTGACCAAG
AACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCCTCCGACATCGCCGTGGAGT
GGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCTCCCGTGCTGGACAGCG
ACGGCAGCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAA
CGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTG
AGCCTGAGCCCCGGATAGTAA

Fig.5 (continued)

Amino acid and nucleotide sequences of humanized light chains

> L2987 (Humanized LC 1)
MSVPTQVLGLLLLWLTDARCQAVVTQEPSLTVSPGGTVTLTCGSSTGAVITSNYANWFQQKPGQ
APRTLIGRTYNKVPWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNHFVFGGGTKLTV
LGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN
KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS**

ATGTCCGTGCCTACCCAGGTGCTGGGACTGCTGCTGCTGTGGCTGACCGACGCCAGATGTC
AGGCTGTCGTGACCCAGGAACCTTCCCTGACCGTGTCTCCTGGCGGCACCGTGACCCTGAC
CTGTGGATCTTCTACCGGCGCTGTGATCACCTCCAACTACGCCAACTGGTTCCAGCAGAAGC
CAGGCCAGGCTCCTAGAACCCTGATCGGCAGAACCTACAACAAGGTGCCATGGACCCCTGC
CCGGTTCTCCGGATCTCTGCTGGGAGGAAAGGCCGCTCTGACCCTGTCTGGTGCCCAGCCT
GAGGATGAGGCCGAGTACTACTGCGCCCTGTGGTACTCCAACCACTTCGTGTTCGGCGGAG
GCACCAAGCTGACCGTGCTGGGACAGCCTAAGGCCGCTCCTTCCGTGACCCTGTTCCCTCC
ATCCTCCGAGGAACTGCAGGCCAACAAGGCCACCCTCGTGTGCCTGATCTCCGACTTCTAC
CCTGGCGCCGTGACCGTGGCCTGGAAGGCTGATAGCTCTCCTGTGAAGGCCGGCGTGGAA
ACCACCACCCCTTCCAAGCAGTCCAACAACAAATACGCCGCCTCCTCCTACCTGTCCCTGAC
CCCTGAGCAGTGGAAGTCCCACCGGTCCTACAGCTGCCAAGTGACCCACGAGGGCTCCAC
CGTGGAAAAGACCGTGGCTCCTACCGAGTGCTCCTGATAA

> L2988 (Humanized LC2)
MSVPTQVLGLLLLWLTDARCQAVVTQEPSLTVSPGGTVTLTCGSSTGAVITSNYANWVQQKPGQ
APTGLIGRTYNKVPWTPARFSGSLLGDKAALTLSGAQPEDEAEYFCALWYSNHFVFGGGTKLTV
LGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN
KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS**

ATGTCCGTGCCTACCCAGGTGCTGGGACTGCTGCTGCTGTGGCTGACCGACGCCAGATGTC
AGGCTGTCGTGACCCAGGAACCTTCCCTGACCGTGTCTCCTGGCGGCACCGTGACCCTGAC
CTGTGGATCTTCTACCGGCGCTGTGATCACCTCCAACTACGCCAACTGGGTGCAGCAGAAG
CCAGGCCAGGCTCCTACCGGCCTGATCGGCAGAACCTACAACAAGGTGCCATGGACCCCTG
CCCGGTTCTCCGGATCTCTGCTGGGCGATAAGGCCGCTCTGACCCTGTCTGGTGCCCAGCC
TGAGGATGAGGCCGAGTACTTCTGCGCCCTGTGGTACTCCAACCACTTCGTGTTCGGCGGA
GGCACCAAGCTGACCGTGCTGGGACAGCCTAAGGCCGCTCCTTCCGTGACCCTGTTCCCTC
CATCCTCCGAGGAACTGCAGGCCAACAAGGCCACCCTCGTGTGCCTGATCTCCGACTTCTA
CCCTGGCGCCGTGACCGTGGCCTGGAAGGCTGATAGCTCTCCTGTGAAGGCCGGCGTGGA
AACCACCACCCCTTCCAAGCAGTCCAACAACAAATACGCCGCCTCCTCCTACCTGTCCCTGA
CCCCTGAGCAGTGGAAGTCCCACCGGTCCTACAGCTGCCAAGTGACCCACGAGGGCTCCA
CCGTGGAAAAGACCGTGGCTCCTACCGAGTGCTCCTGATAA

Fig.5 (continued)

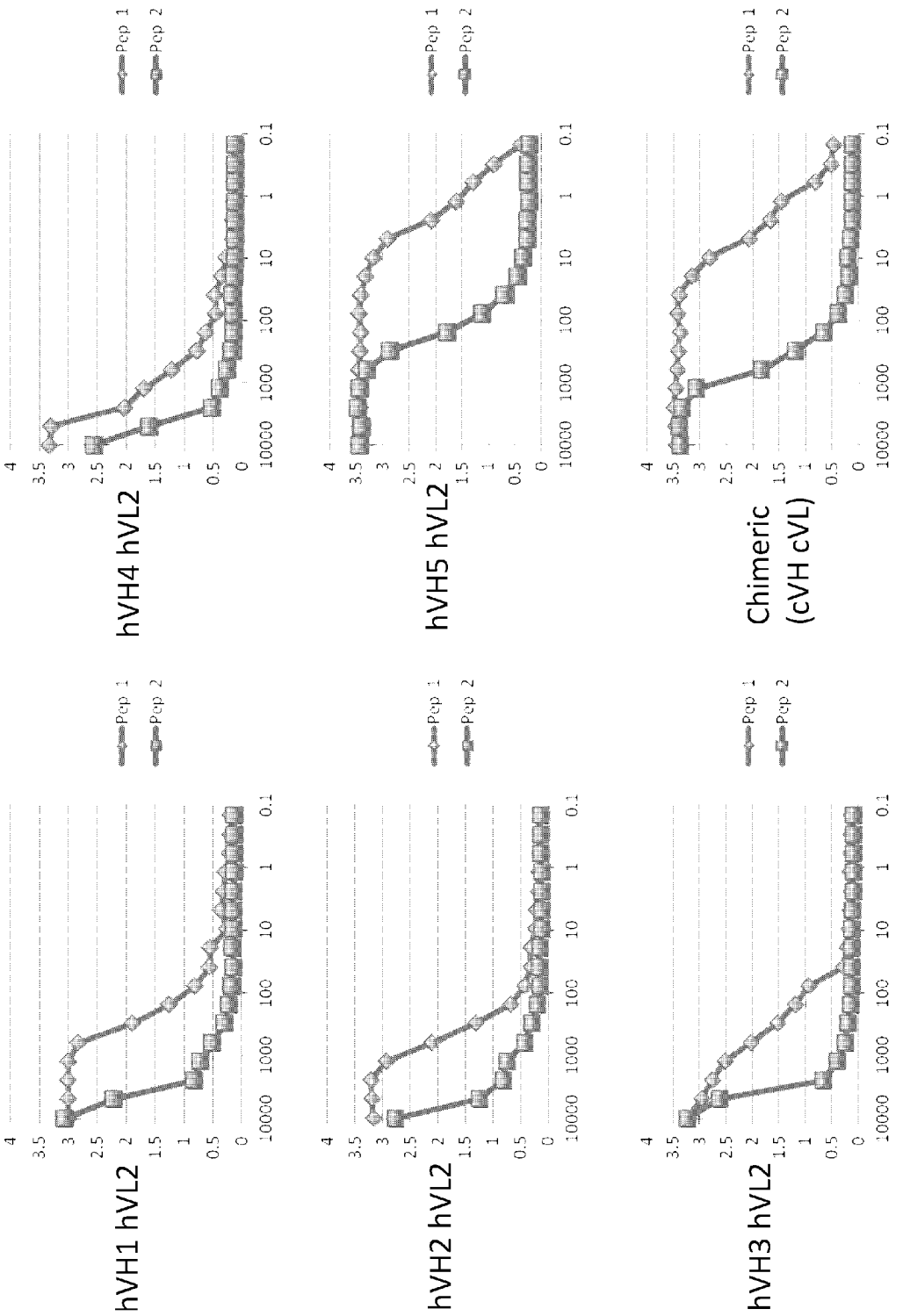
hVH1 hVL2
hVH2 hVL2
hVH3 hVL2
hVH4 hVL2
hVH5 hVL2
Chimeric (cVH cVL)
Pep 1
Pep 2
4
3.5
3
2.5
2
1.5
1
0.5
0
10000
1000
100
10
1
0.1

Fig.6

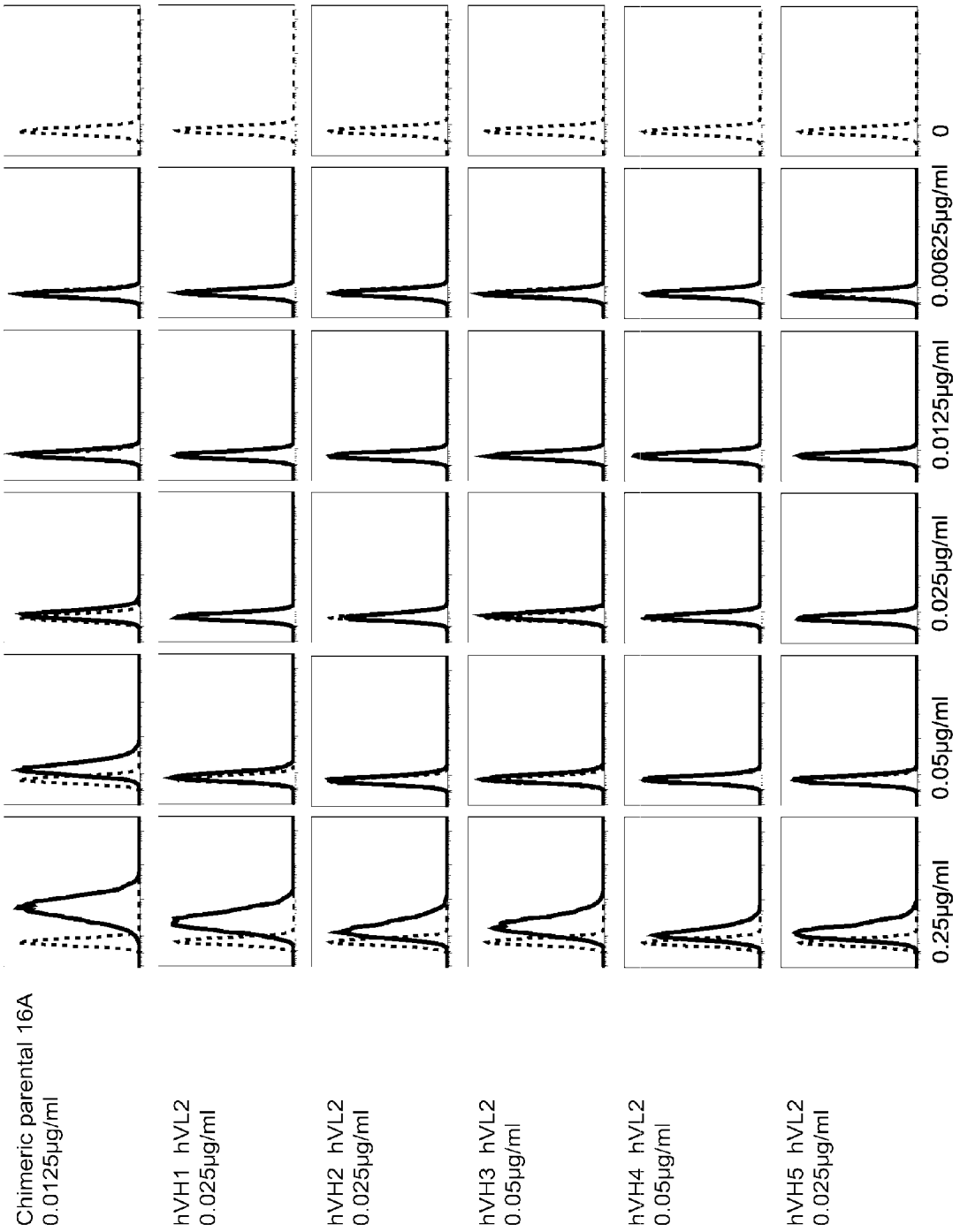
Chimeric parental 16A
0.0125μg/ml
hVH1 hVL2
0.025μg/ml
hVH2 hVL2
0.025μg/ml
hVH3 hVL2
0.05μg/ml
hVH4 hVL2
0.05μg/ml
hVH5 hVL2
0.025μg/ml
0.25μg/ml
0.05μg/ml
0.025μg/ml
0.0125μg/ml
0.00625μg/ml
0

Fig.7

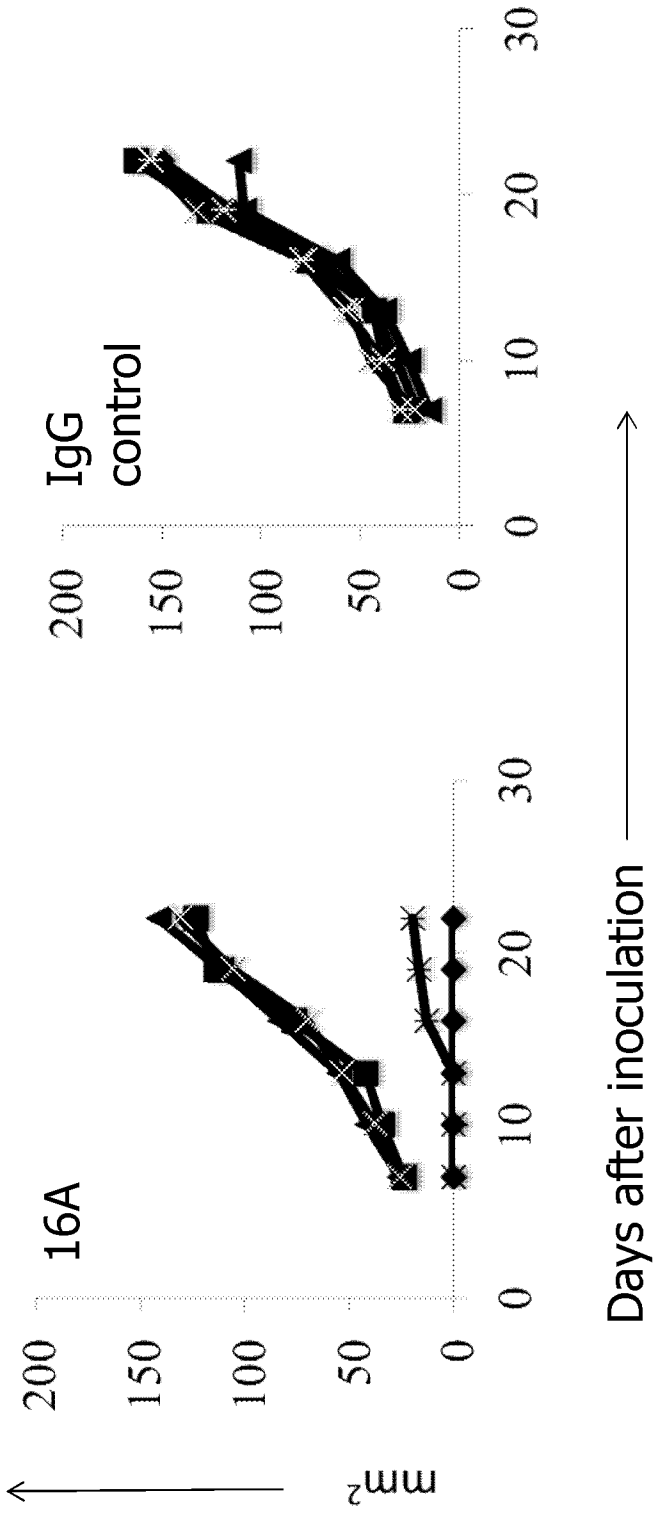
16A
IgG control
200
150
100
50
0
0
10
20
30
mm²
Days after inoculation

Fig.8

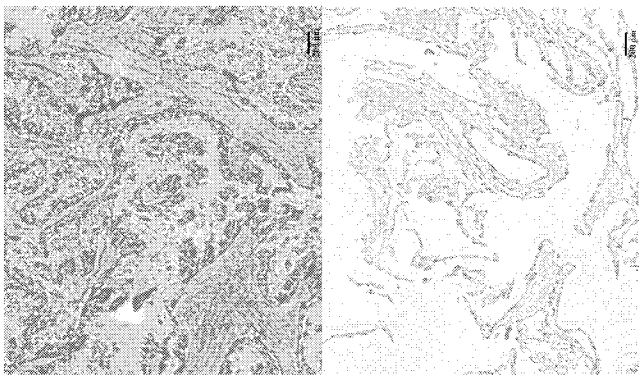

Fig.9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/071546

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395(2006.01)i; A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K; A61P

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

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

CNKI,CNABS,WANFANG,CNTXT,TWTXT,DWPI,EPTXT,WOTXT,USTXT,NCBI,ISI-WEB OF KNOWLEDGE, GOOGLE SCHOLAR:MUC1,antibody,humanized,constant region,VH,VL,CDR,IgG1,cancer,tumor,16A,murine,hybridoma, heavy chain,light chain,RPAPGS,SEQ ID NO:1,2,5-33

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WEI, Song et al. "MUC1 glycopeptide epitopes predicted by computational glycomics" *International Journal of Oncology,* Vol. 41, No. 6, 31 December 2012 (2012-12-31), pages 1977-1984 see the whole document, especially the abstract, pages 1981 and 1983	1, 6, 7, 12-14
Y	PICHINUK, Edward et al. "Antibody targeting of cell-bound MUC1 SEA domain kills tumor cells" *Cancer Research,* Vol. 72, No. 13, 01 July 2012 (2012-07-01), pages 3324-3336 see the whole document, especially the abstract, page 3326	1, 6, 7, 12-14
A	CN 103880956 A (UNIV FOURTH MILITARY MEDICAL) 25 June 2014 (2014-06-25) see the whole document	1-14

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 October 2017	**24 October 2017**
Name and mailing address of the ISA/CN **STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China** Facsimile No. **(86-10)62019451**	Authorized officer **TIAN,Yuan** Telephone No. **(86-10)62411047**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/071546

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

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

1. ☑ Claims Nos.: **13-14**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] The subject matter of claims 13-14 is a method for treatment of the human or animal body according to Rule 39.1 (iv). The search has been carried out and based on the alleged effects of the compositions.

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

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

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2017/071546

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
CN 103880956 A	25 June 2014	CN 103880956 B	30 December 2015