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(54) **IMMUNOGENIC GLYCOPEPTIDES, COMPOSITION COMPRISING THE GLYCOPEPTIDES AND USE THEREOF**

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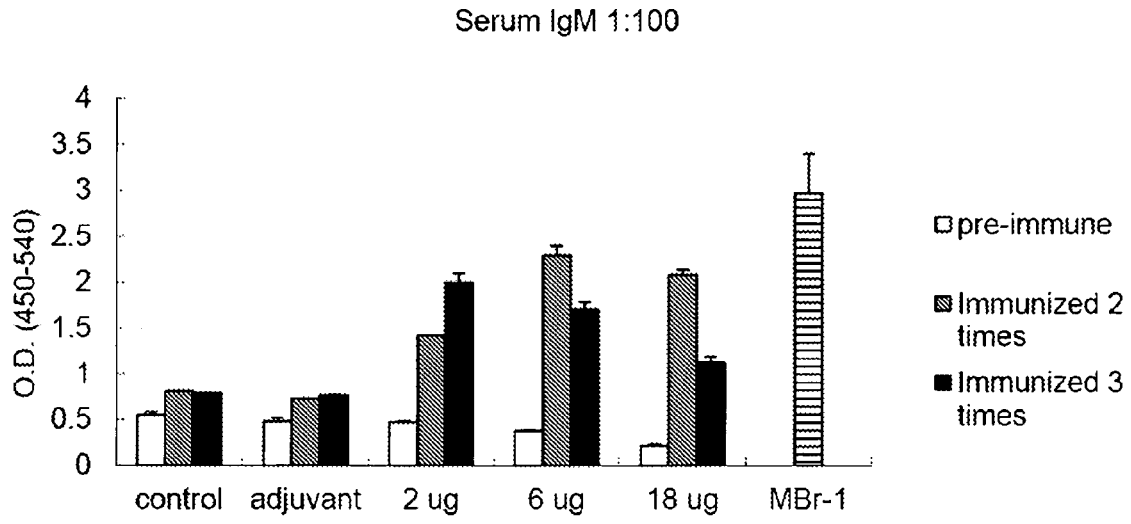
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(57) **ABSTRACT**

Disclosed herein are an immunogenic glycopeptide for inducing immune response to treat cancer. Other aspects of the present disclosure are pharmaceutical composition comprising the immunogenic glycopeptide; and method for preventing and/or treating a cancer.

(A)


Serum IgM 1:100
O.D. (450-540)
4
3.5
3
2.5
2
1.5
1
0.5
0
control
adjuvant
2 ug
6 ug
18 ug
MBr-1
pre-immune
Immunized 2 times
Immunized 3 times

(B)

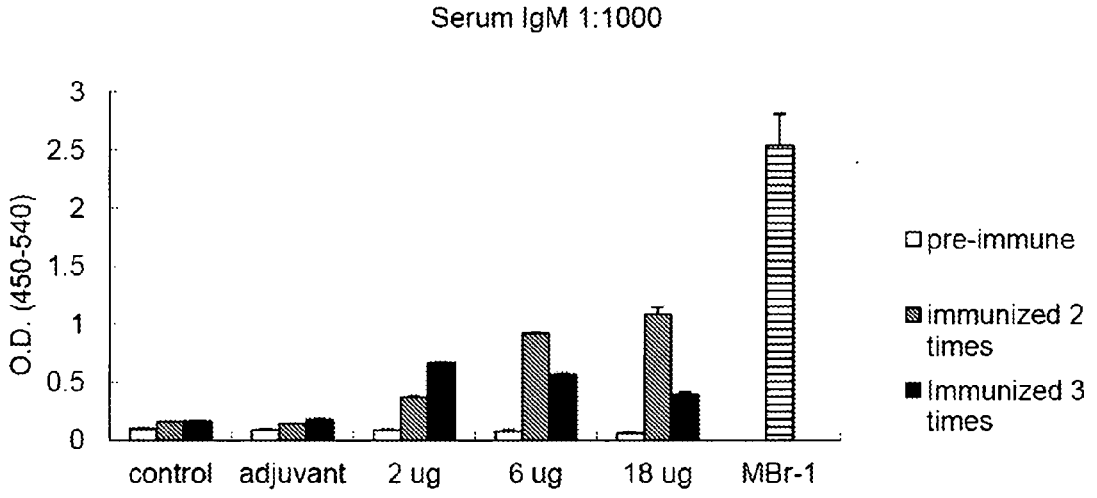
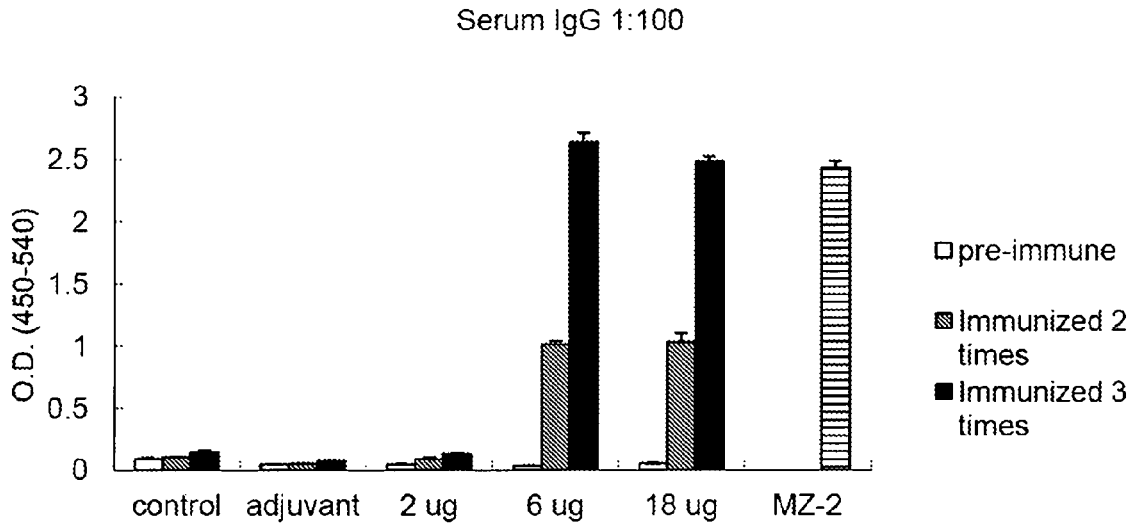

Serum IgM 1:1000
O.D. (450-540)
3
2.5
2
1.5
1
0.5
0
control
adjuvant
2 ug
6 ug
18 ug
MBr-1
pre-immune
immunized 2 times
Immunized 3 times

Fig. 1

(A)


Serum IgG 1:100
O.D. (450-540)
3
2.5
2
1.5
1
0.5
0
control
adjuvant
2 ug
6 ug
18 ug
MZ-2
pre-immune
Immunized 2 times
Immunized 3 times

(B)

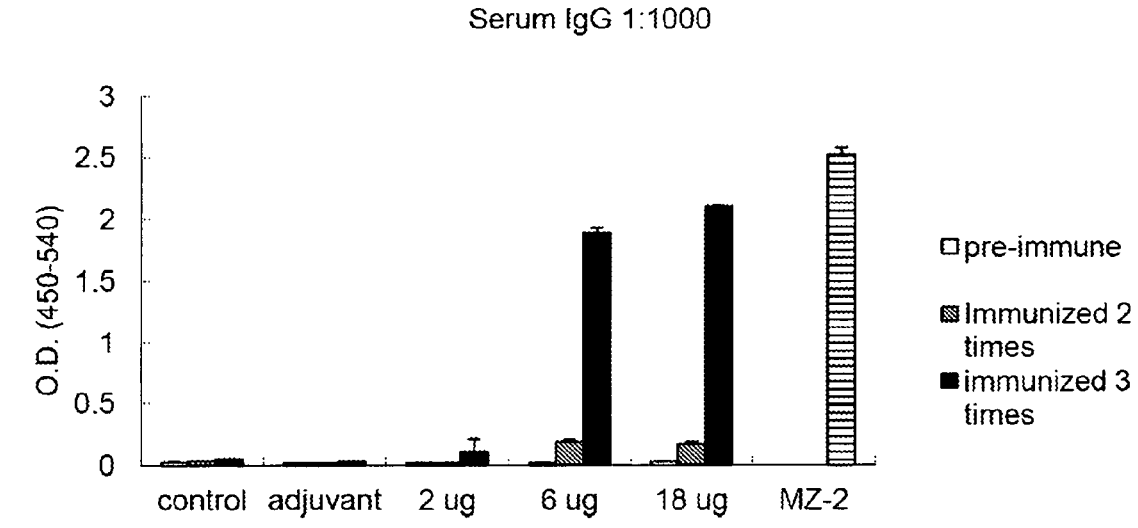
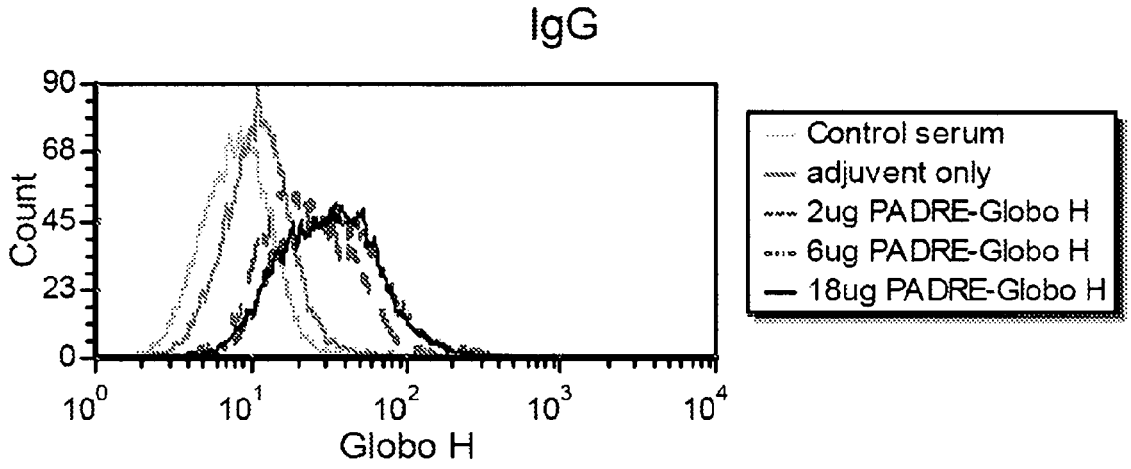

Serum IgG 1:1000
O.D. (450-540)
3
2.5
2
1.5
1
0.5
0
control
adjuvant
2 ug
6 ug
18 ug
MZ-2
pre-immune
Immunized 2 times
immunized 3 times

Fig. 2

(A)


IgG
Count
90
68
45
23
0
10^0
10^1
10^2
10^3
10^4
Globo H
Control serum
adjuvent only
2ug PADRE-Globo H
6ug PADRE-Globo H
18ug PADRE-Globo H

(B)

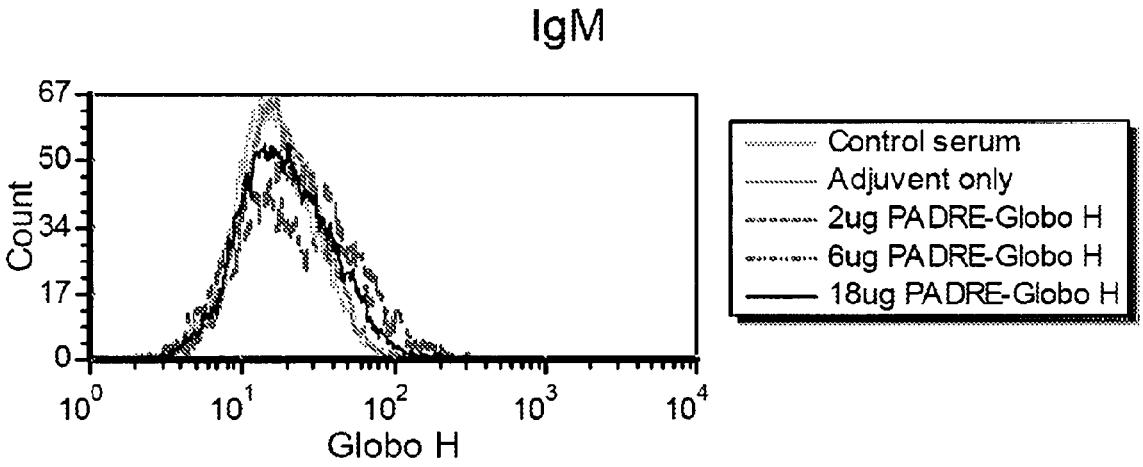
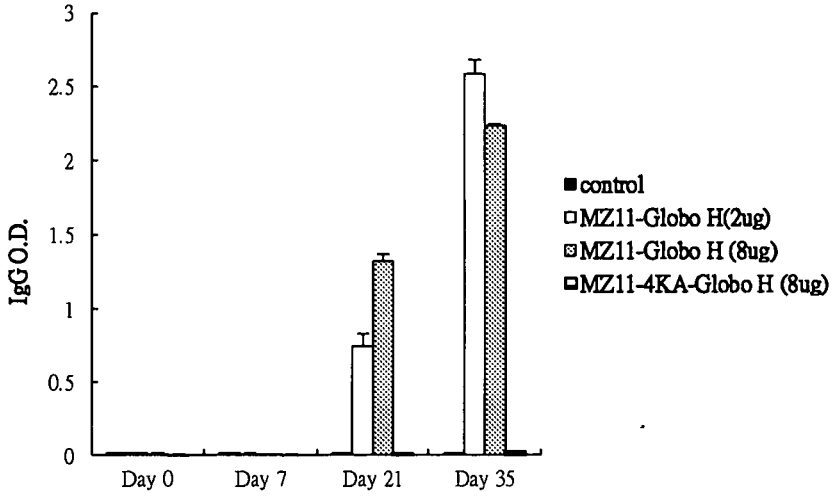

IgM
Count
67
50
34
17
0
10^0
10^1
10^2
10^3
10^4
Globo H
Control serum
Adjuvent only
2ug PADRE-Globo H
6ug PADRE-Globo H
18ug PADRE-Globo H

Fig. 3

(A)


IgG O.D.
3
2.5
2
1.5
1
0.5
0
Day 0
Day 7
Day 21
Day 35
control
MZ11-Globo H(2ug)
MZ11-Globo H (8ug)
MZ11-4KA-Globo H (8ug)

(B)

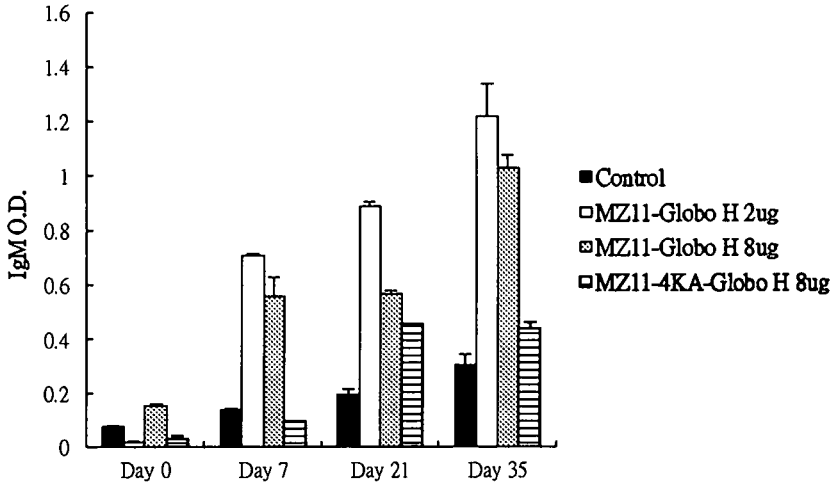

IgM O.D.
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Day 0
Day 7
Day 21
Day 35
Control
MZ11-Globo H 2ug
MZ11-Globo H 8ug
MZ11-4KA-Globo H 8ug

Fig. 4

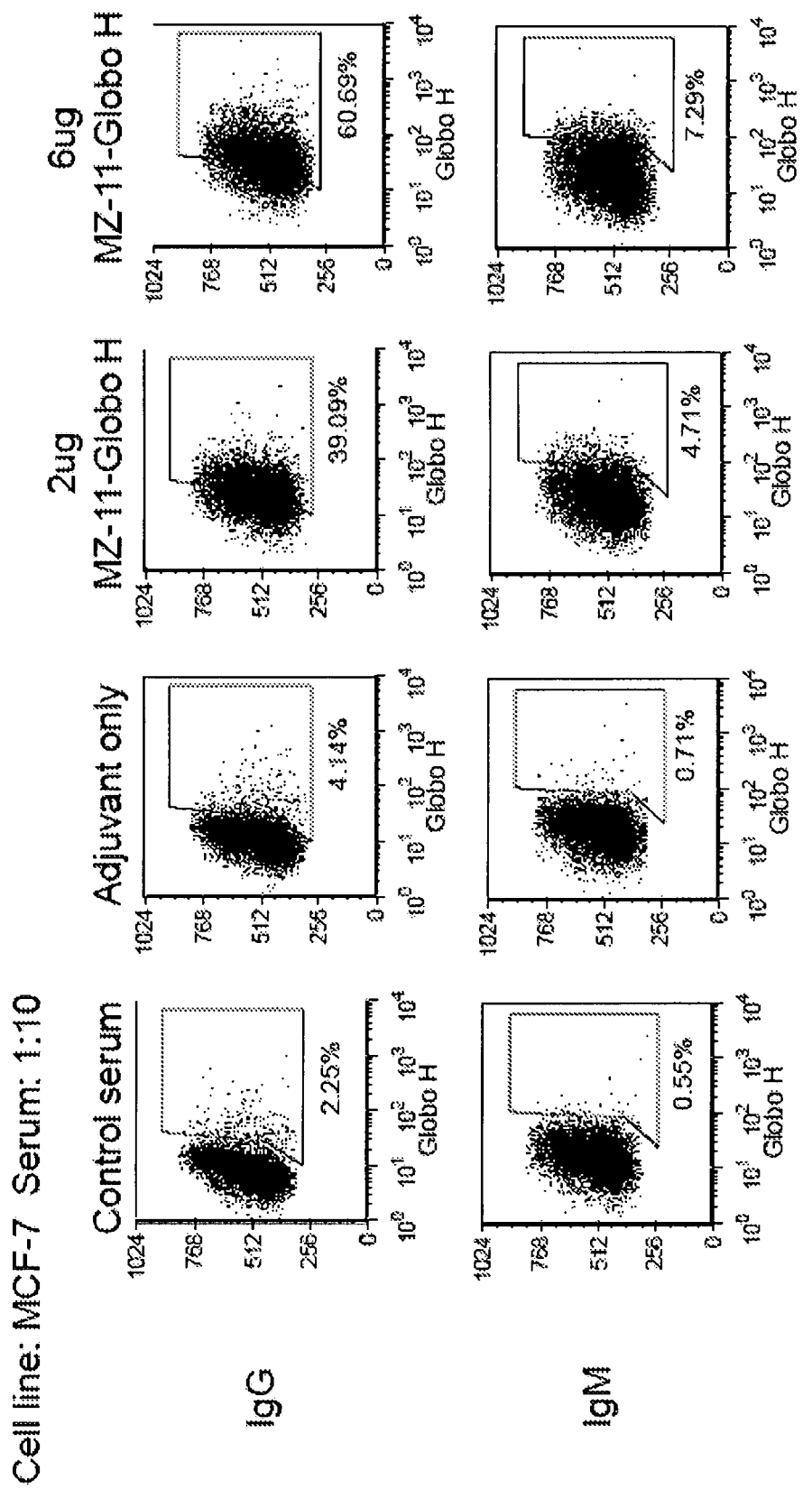

Cell line: MCF-7 Serum: 1:10
Control serum
Adjuvant only
2ug
MZ-11-Globo H
6ug
MZ-11-Globo H
IgG
IgM
2.25%
4.14%
39.09%
60.69%
0.55%
0.71%
4.71%
7.29%
Globo H

Fig. 5

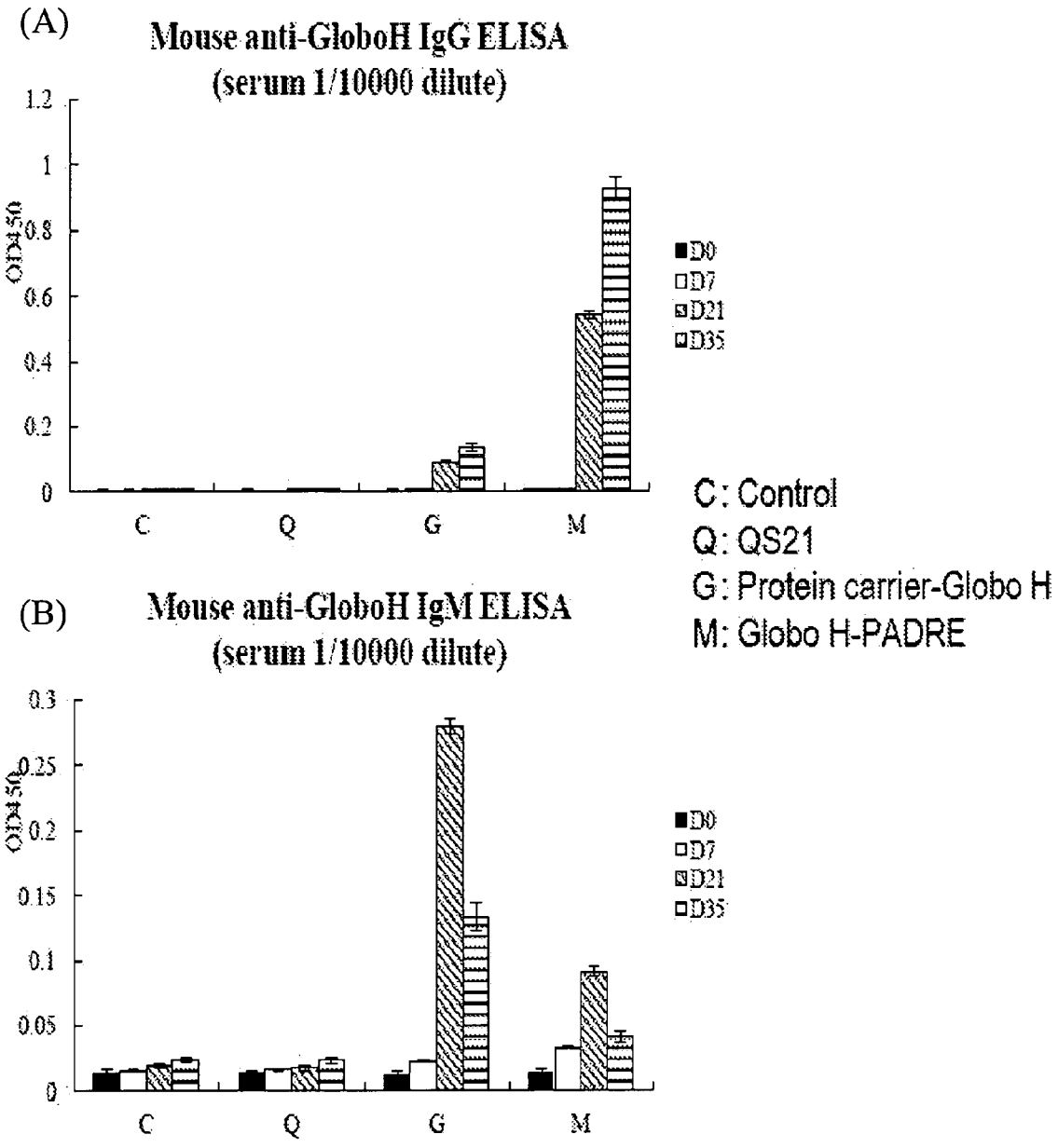

(A)
Mouse anti-GloboH IgG ELISA
(serum 1/10000 dilute)
OD450
1.2
1
0.8
0.6
0.4
0.2
0
C
Q
G
M
D0
D7
D21
D35
C: Control
Q: QS21
G: Protein carrier-Globo H
M: Globo H-PADRE
(B)
Mouse anti-GloboH IgM ELISA
(serum 1/10000 dilute)
OD450
0.3
0.25
0.2
0.15
0.1
0.05
0
C
Q
G
M
D0
D7
D21
D35

Fig. 6

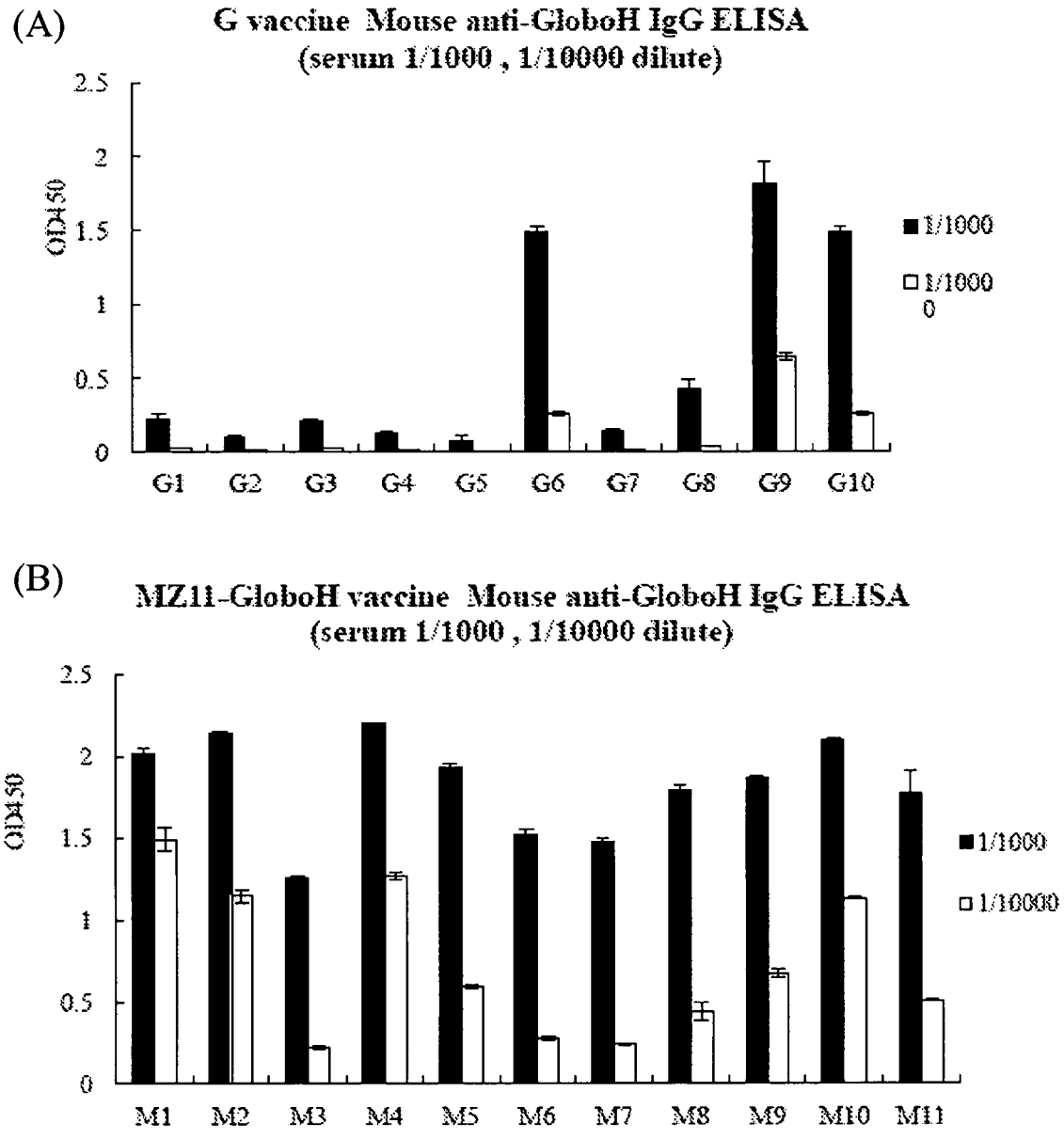
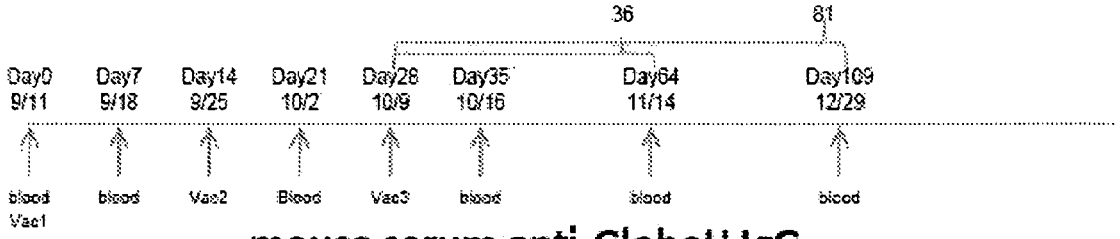
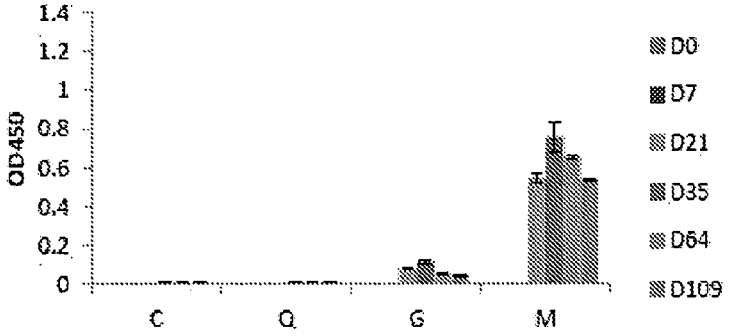

(A)
G vaccine Mouse anti-GloboH IgG ELISA
(serum 1/1000 , 1/10000 dilute)
OD450
2.5
2
1.5
1
0.5
0
G1 G2 G3 G4 G5 G6 G7 G8 G9 G10
1/1000
1/10000
(B)
MZ11-GloboH vaccine Mouse anti-GloboH IgG ELISA
(serum 1/1000 , 1/10000 dilute)
OD450
2.5
2
1.5
1
0.5
0
M1 M2 M3 M4 M5 M6 M7 M8 M9 M10 M11
1/1000
1/10000

Fig. 7


36
81
Day0 9/11
Day7 9/18
Day14 9/25
Day21 10/2
Day28 10/9
Day35 10/16
Day64 11/14
Day109 12/29
blood Vac1
blood
Vac2
Blood
Vac3
blood
blood
blood

(A)


mouse serum anti-GloboH IgG
1/10000 dilute
OD450
1.4
1.2
1
0.8
0.6
0.4
0.2
0
C
Q
G
M
D0
D7
D21
D35
D64
D109

(B)

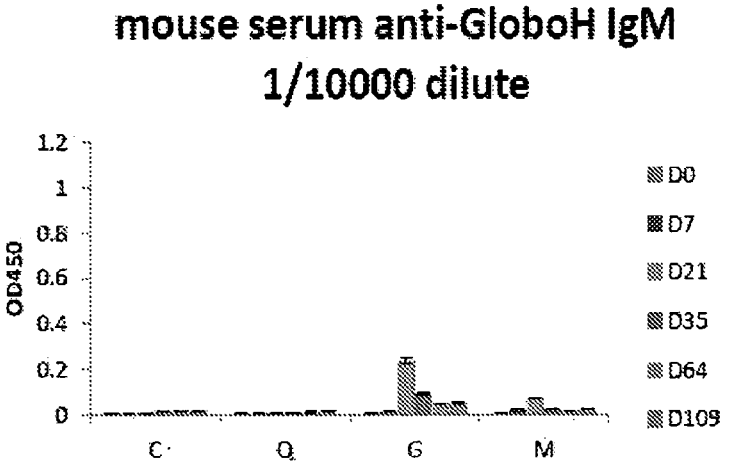
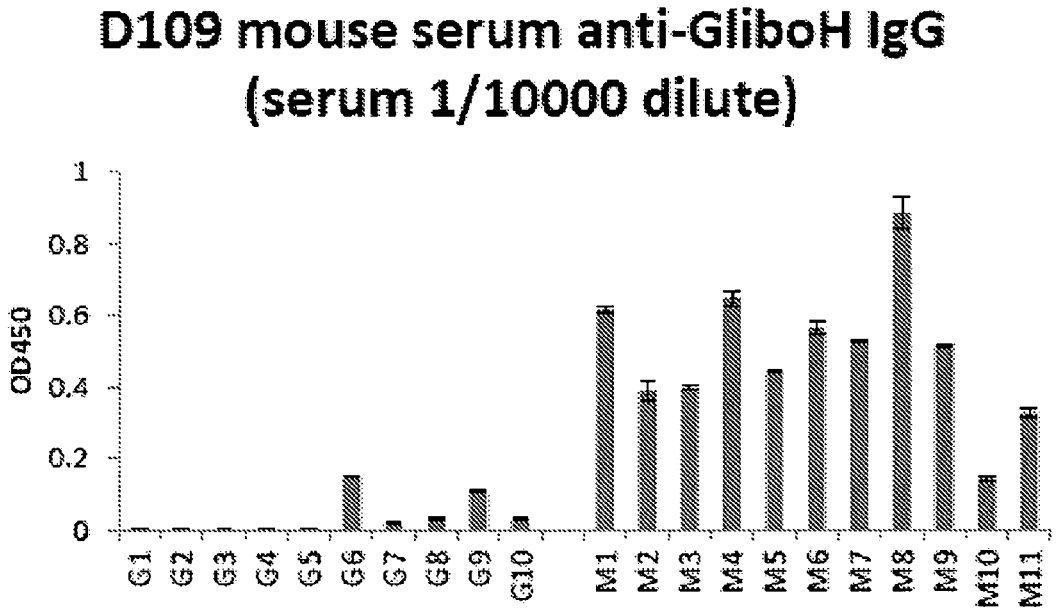

mouse serum anti-GloboH IgM
1/10000 dilute
OD450
1.2
1
0.8
0.6
0.4
0.2
0
C
Q
G
M
D0
D7
D21
D35
D64
D109

Fig. 8

(A)


D109 mouse serum anti-GliboH IgG
(serum 1/10000 dilute)
OD450
1
0.8
0.6
0.4
0.2
0
G1
G2
G3
G4
G5
G6
G7
G8
G9
G10
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10
M11

(B)

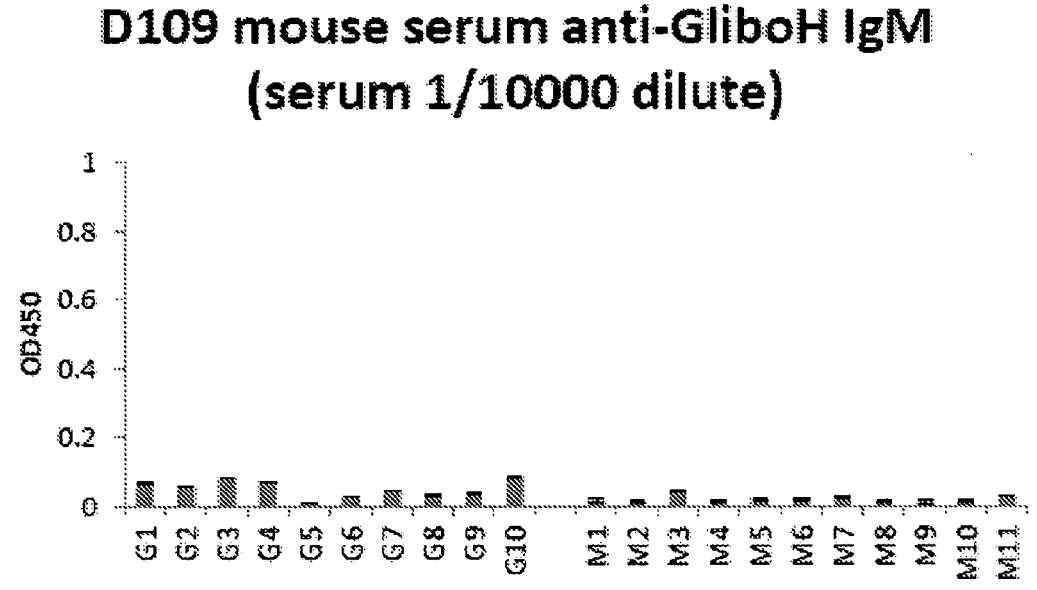
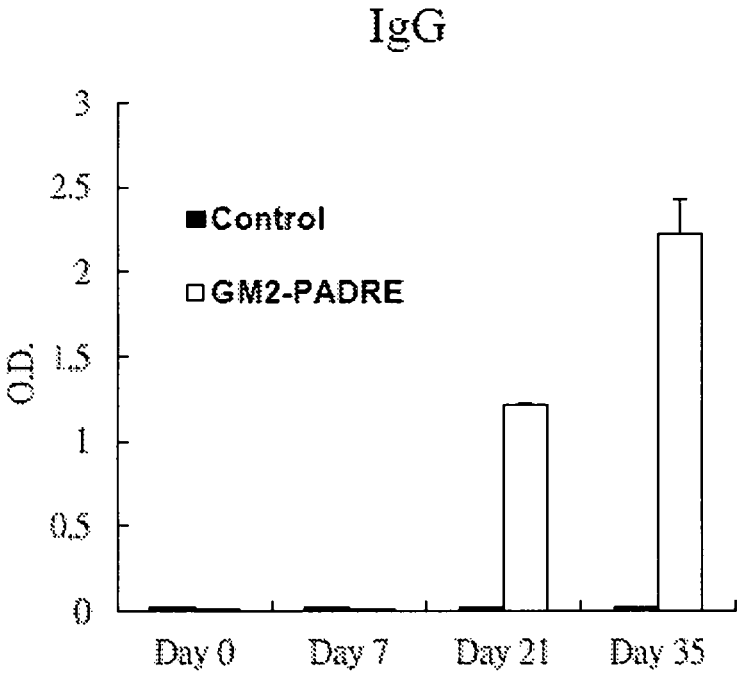

D109 mouse serum anti-GliboH IgM
(serum 1/10000 dilute)
OD450
1
0.8
0.6
0.4
0.2
0
G1
G2
G3
G4
G5
G6
G7
G8
G9
G10
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10
M11

Fig. 9

(A)


IgG
3
2.5
2
1.5
1
0.5
0
O.D.
Control
GM2-PADRE
Day 0
Day 7
Day 21
Day 35

(B)

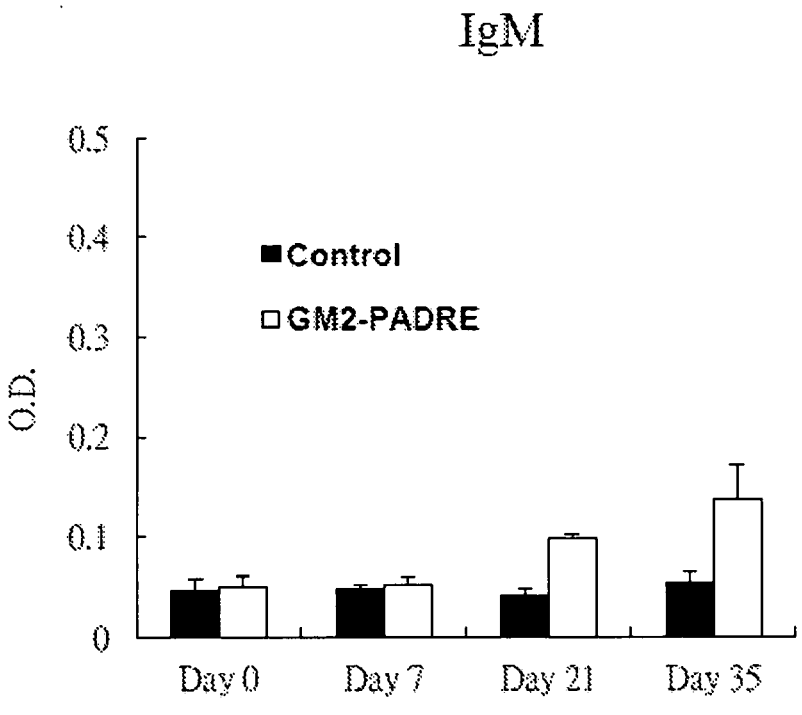

IgM
0.5
0.4
0.3
0.2
0.1
0
O.D.
Control
GM2-PADRE
Day 0
Day 7
Day 21
Day 35

Fig. 10

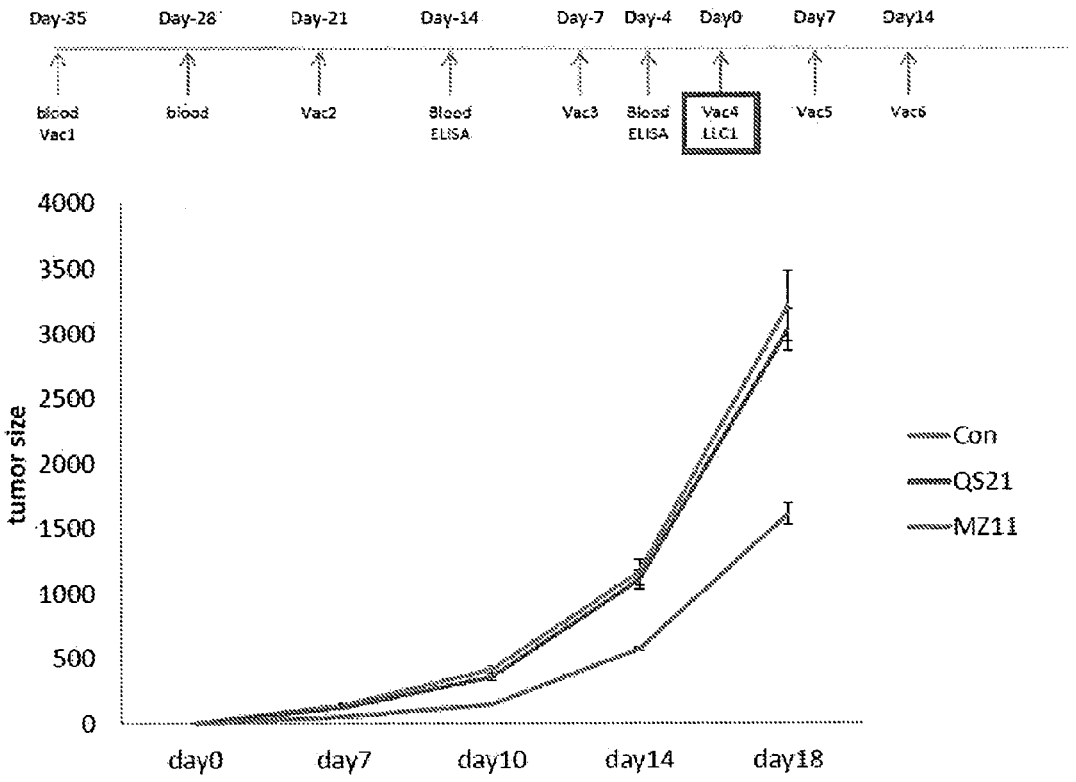

Day-35
Day-28
Day-21
Day-14
Day-7
Day-4
Day0
Day7
Day14
blood
Vac1
blood
Vac2
Blood
ELISA
Vac3
Blood
ELISA
Vac4
LLC1
Vac5
Vac6
4000
3500
3000
2500
2000
1500
1000
500
0
tumor size
day0
day7
day10
day14
day18
Con
QS21
MZ11

Fig. 11

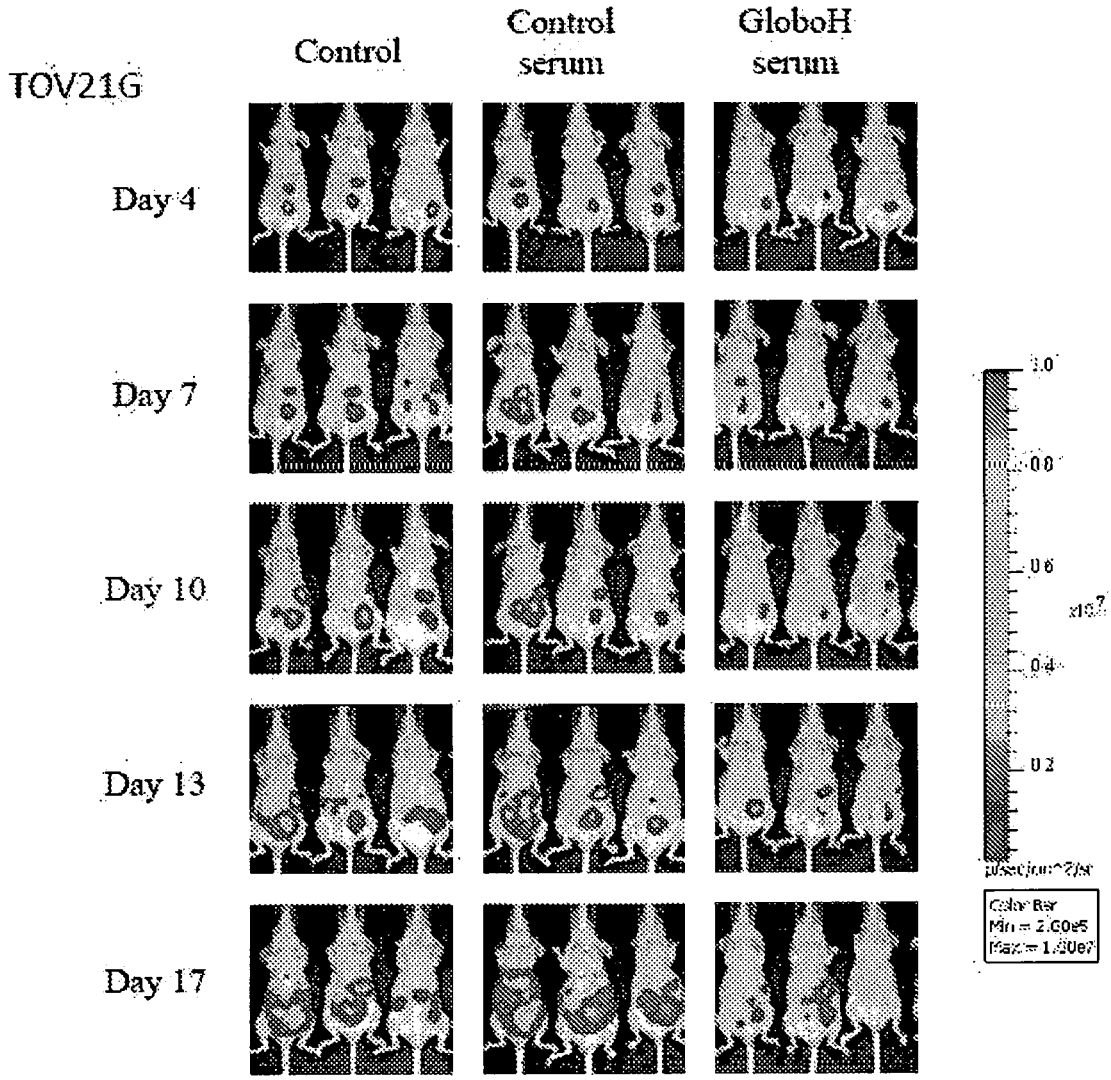
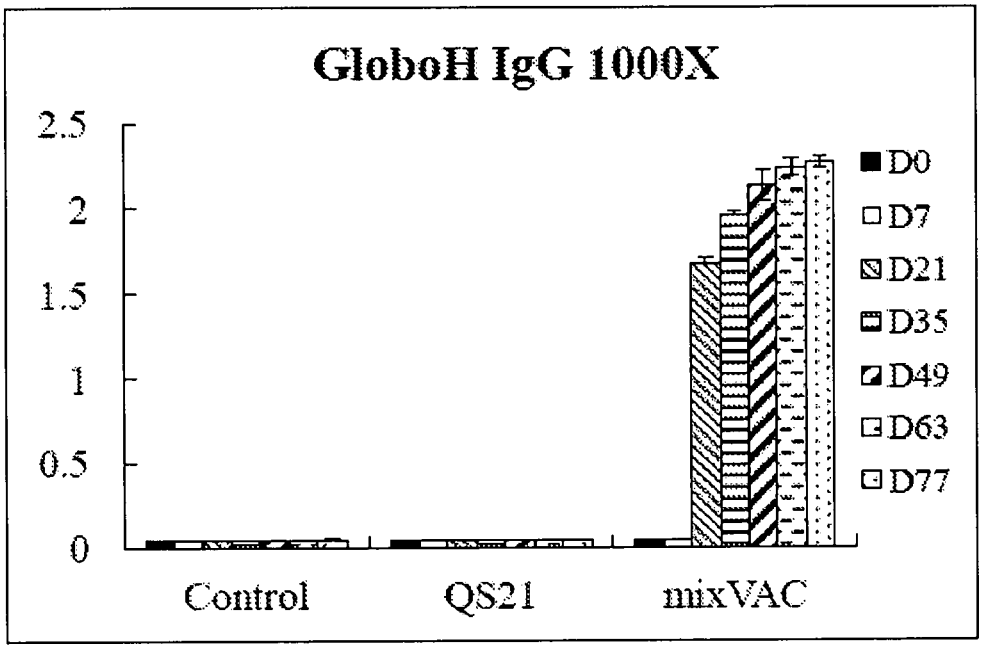

TOV21G
Control
Control serum
GloboH serum
Day 4
Day 7
Day 10
Day 13
Day 17
1.0
0.8
0.6
0.4
0.2
Color Bar
Min = 2.60e5
Max = 1.80e7

Fig. 12

(A)


GloboH IgG 1000X
2.5
2
1.5
1
0.5
0
Control
QS21
mixVAC
D0
D7
D21
D35
D49
D63
D77

(B)

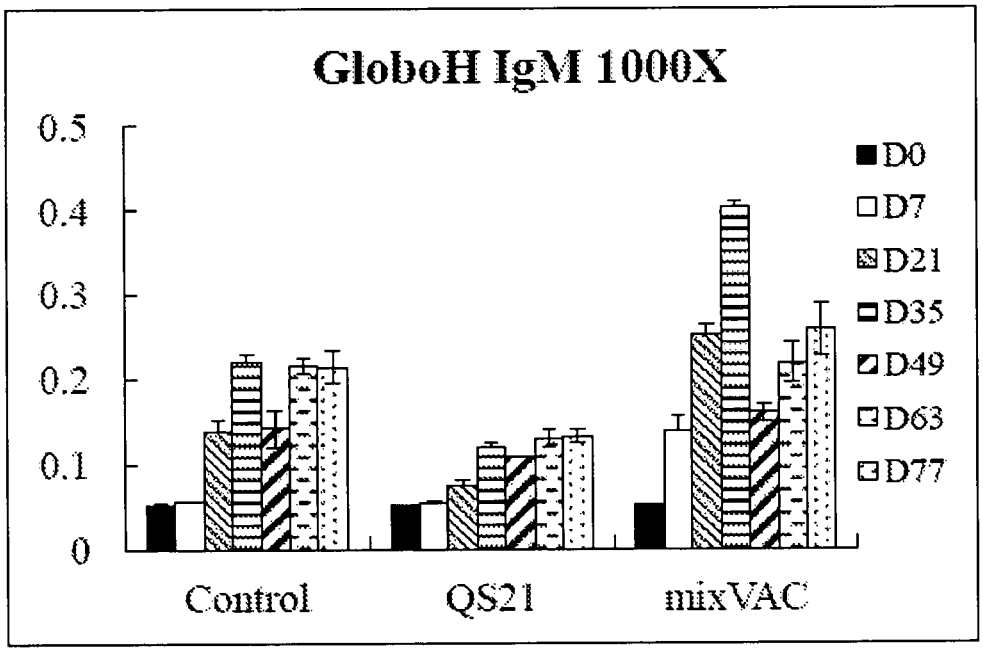
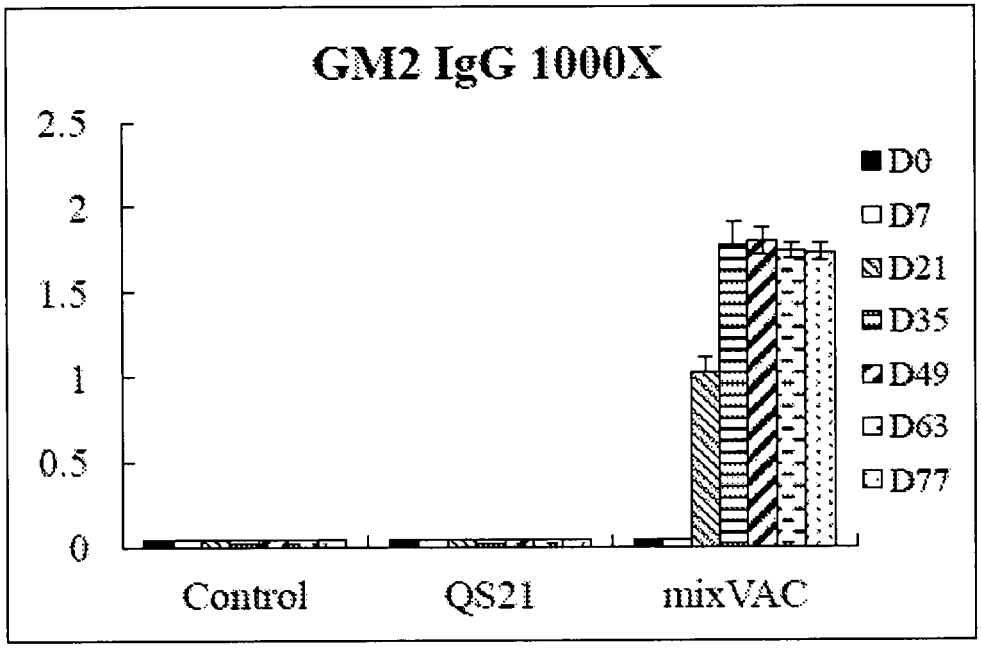

GloboH IgM 1000X
0.5
0.4
0.3
0.2
0.1
0
Control
QS21
mixVAC
D0
D7
D21
D35
D49
D63
D77

Fig. 13

(C)



(D)

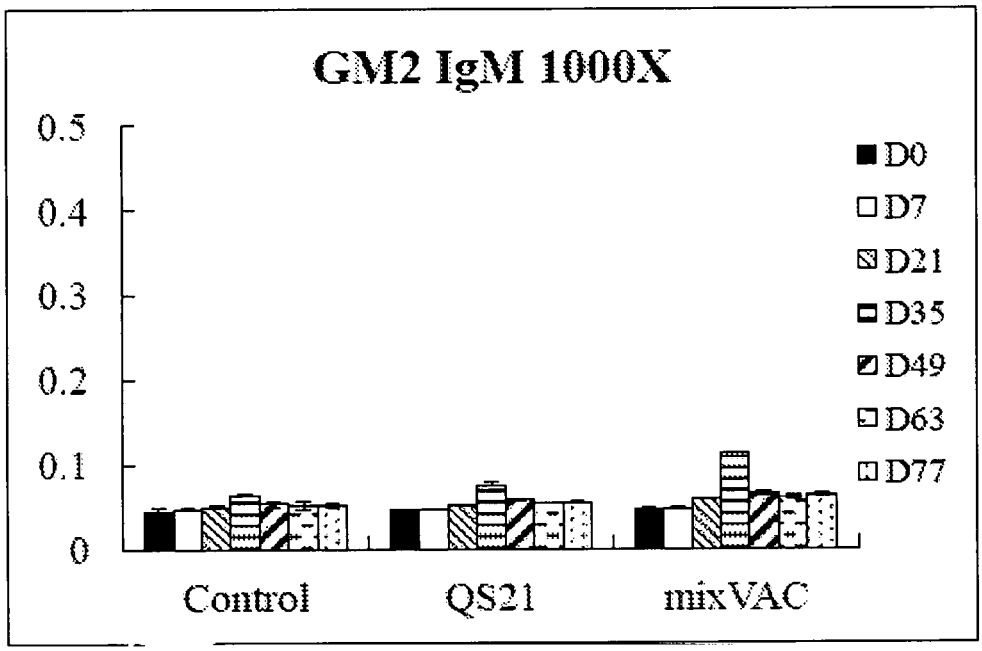
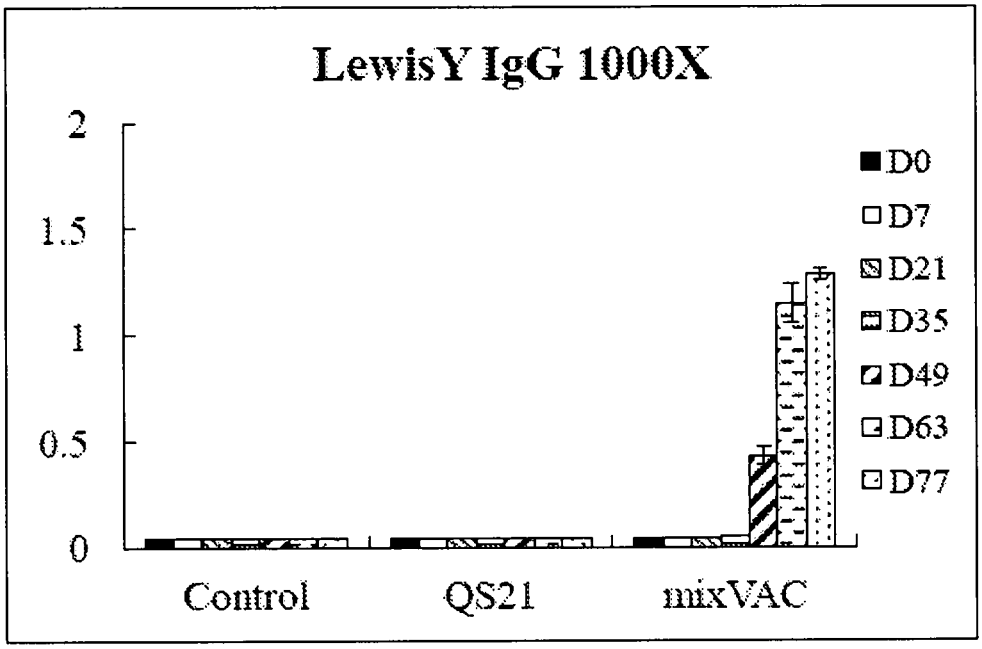


Fig. 13 (continued)

(E)



(F)

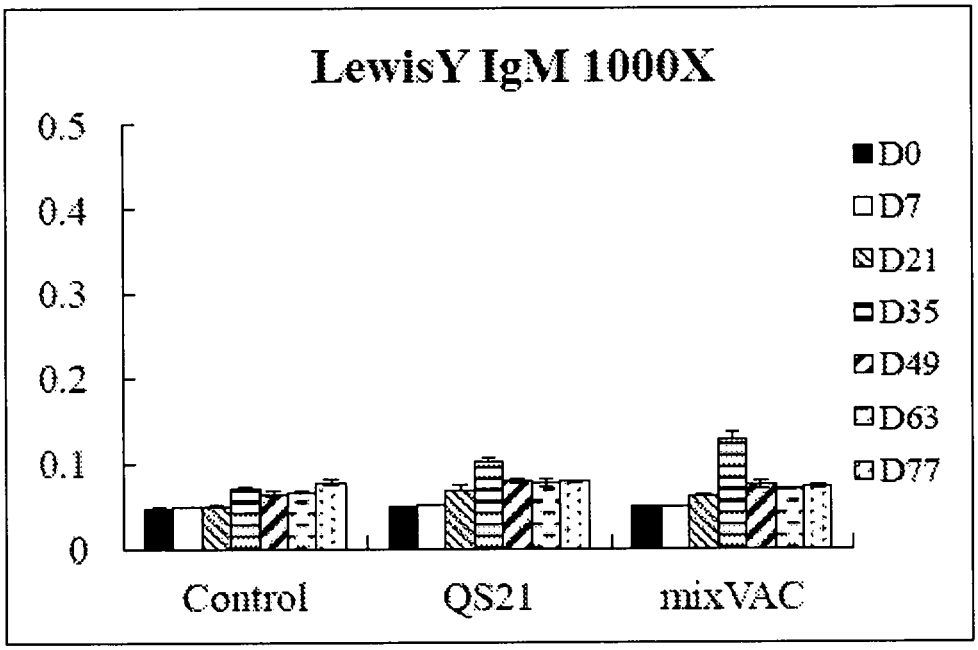
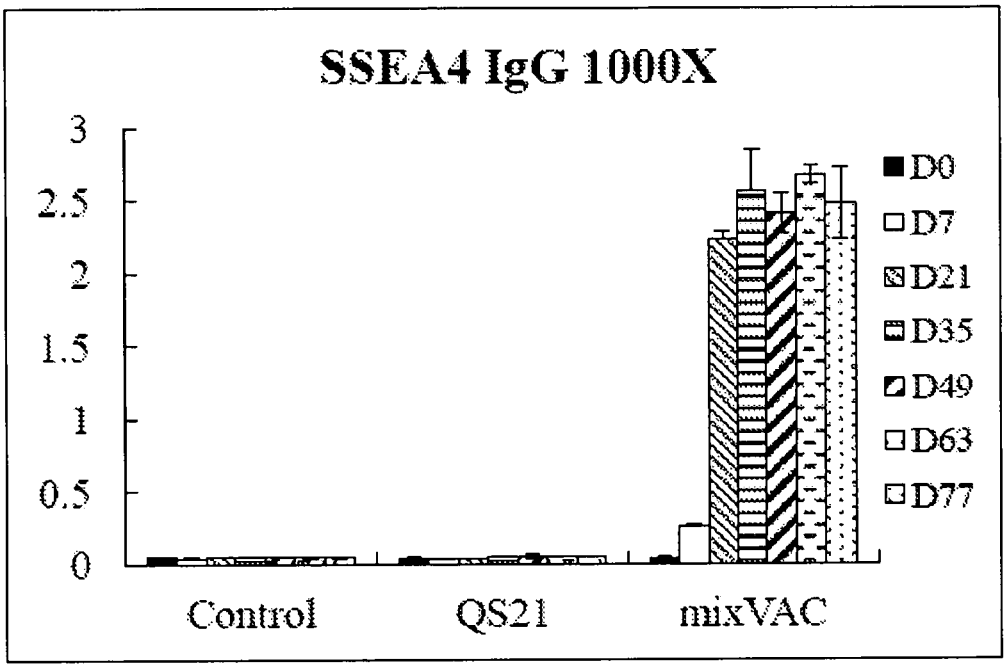


Fig. 13 (continued)

(G)



(H)

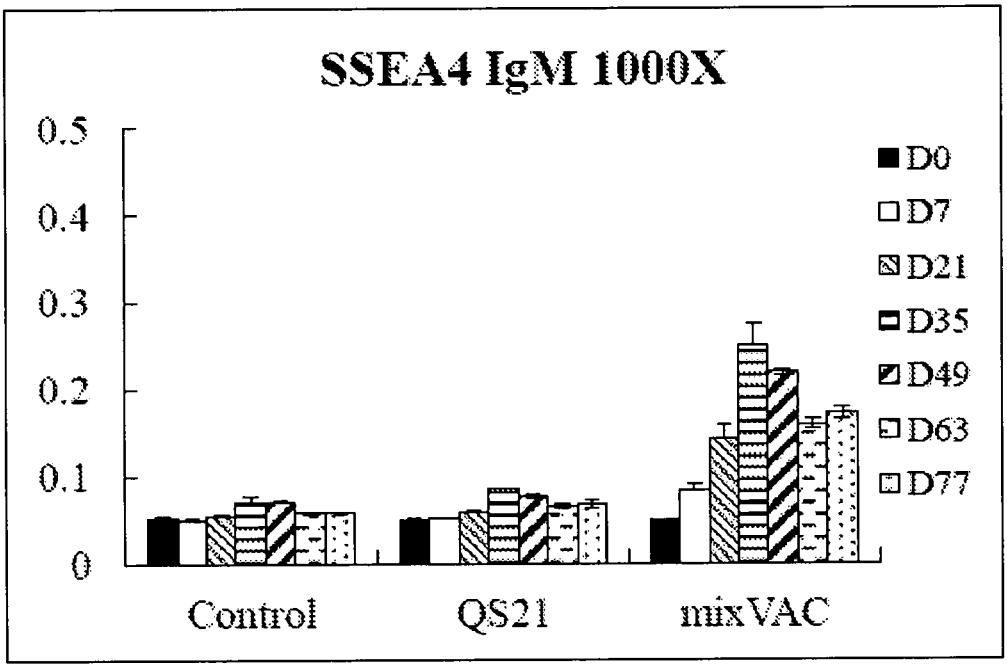


Fig. 13 (continued)

IMMUNOGENIC GLYCOPEPTIDES, COMPOSITION COMPRISING THE GLYCOPEPTIDES AND USE THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to the field of immunotherapy of cancer. In particular, the disclosed invention relates to an immunogenic glycopeptide, a pharmaceutical composition comprising the glycopeptide and to the use thereof for enhancing the immune response and notably in cancer therapy.

BACKGROUND OF THE INVENTION

[0002] Globo H (Fucα1→2Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→O-cer) 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-expressing cancers. In addition to Globo H, other known carbohydrate antigens including GM2, GD2, GD3, fucosyl-GM1, Globo-H, Lewis^y (Le^y, Fucα1→2Galβ1→4[Fucα1-3] GlcNAcβ1→3Galβ1→O-cer, Tn (GalNAcα-O-Ser/Thr), TF (Galβ1→3GalNAcα-O-Ser/Thr and STn (NeuAcα2→6GalNAcα-O-Ser/Thr are also used as target antigens for cancer immunotherapy (Susan F Slovin et al., *Carbohydrate Vaccines as Immunotherapy for Cancer, Immunology and Cell Biology* (2005) 83, 418-428; Zhongwu Guo and Qianli Wang, *Recent Development in Carbohydrate-Based Cancer Vaccines, Curr Opin Chem Biol.* 2009 December; 13(5-6): 608-617; Therese Buskas et al., Immunotherapy for Cancer: Synthetic Carbohydrate-based Vaccines, *Chem Commun* (*Camb*). 2009 Sep. 28; (36): 5335-5349).

[0003] However, most carbohydrate antigens are often tolerated by the immune system, and consequently, the immunogenicity induced by them 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. For example, 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. U.S. 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, Le$_Y$ or STn. U.S. 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. U.S. 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 October;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 S D, 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.

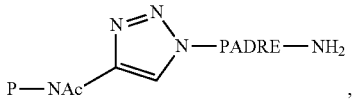
[0007] In view of the foregoing, there exists a need in the art for developing alternative strategies for improving the immunization and/or therapeutic efficacy of carbohydrate-based vaccines.

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] In one aspect, the present disclosure is directed to an immunogenic glycopeptide or a derivative thereof, wherein the immunogenic glycopeptide or a derivative thereof may elicit high titers of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against Globo H in vivo.

[0010] According to one embodiment of the present disclosure, the immunogenic glycopeptide has the structure of:



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wherein the PADRE is a pan-DR epitope and has at least 10 consecutive amino acid residues that is at least 90% identical to the amino acid sequence of AKXVAAWTLKAAA (SEQ ID NO: 1), where X is a cyclohexylalanine residue; and wherein P is Globo H, GD2, GM2, SSEA 4, Lewis Y or STn.

[0011] According to another embodiment, the amino acid sequence of the PADRE is identical to the amino acid sequence of SEQ ID NO: 1.

[0012] In 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 immunogenic glycopeptide according to any of the above-mentioned aspect/embodiments of the present disclosure and (2) a pharmaceutically acceptable carrier.

[0014] According to certain embodiments of the present disclosure, the cancer is any of tumor-associated carbohydrate-expressing cancers; preferably, the cancer is breast cancer, ovarian cancer, pancreatic cancer, prostate cancer, colorectal cancer or lung cancer.

[0015] In yet another aspect, the present disclosure is directed to a method of treating a tumor-associated carbohydrate-expressing cancer in a subject in need thereof.

[0016] According to embodiments of the present disclosure, the method includes administering to the subject the immunogenic glycopeptide or pharmaceutical composition according to any of the aspects/embodiments described in the present disclosure.

[0017] Many of the attendant features and advantages of the present disclosure will becomes better understood with reference to the following detailed description considered in connection with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWING

[0018] FIGS. **1** (A) and (B) provide bar graphs illustrating the IgM titers of mice immunized with the Globo H-PADRE glycopeptide according to one working example of the present disclosure (FIG. **1**(A) for diluted serum IgM 1:100 and FIG. **1**(B) for diluted serum IgM 1:1000).

[0019] FIGS. **2** (A) and (B) provide bar graphs illustrating the IgG titers of mice immunized with the Globo H-PADRE glycopeptide according to one working example of the present disclosure (FIG. **2**(A) for diluted serum IgG 1:100 and FIG. **2**(B) for diluted serum IgG 1:1000).

[0020] FIGS. **3** (A) and (B) show the binding affinity of the anti-Globo H IgG and IgM antibodies with Globo H (FIG. **3**(A) for binding affinity of anti-Globo H IgG antibodies with Globo H and FIG. **3**(B) for binding affinity of anti-Globo H IgM antibodies with Globo H).

[0021] FIGS. **4** (A) and (B) show that mMouse immunized with 2 μg of glycopeptide by direct conjugation of PADRE to Globo and QS**21** adjuvant (2 μg) exhibits high-titer of anti-Globo H IgG and IgM with immune boost effect. MZ-11-Globo H: Globo H-PADRE, MZ-11-4KA-Globo H: PADRE-branched Globo H.

[0022] FIG. **5** shows that antibodies in serum from mice vaccinated with Globo H-PADRE (+adjuvant QS21) bind to Globo H-expressing MCF-7 cells. MZ-11-Globo H: Globo H-PADRE.

[0023] FIGS. **6** (A) and (B) show that glycopeptide Globo H-PADRE (M) induces higher titer of anti-Globo H IgG antibody than general carrier protein-Globo H conjugation (C) does. C: control; Q: adjuvant QS21. FIG. **6**(A) refers to the results of mouse-anti-Globo IgG ELISA and FIG. **6**(B) refers to the results of mouse-anti-Globo IgM ELISA.

[0024] FIGS. **7**(A) and **7**(B) shows that antibody titers in individual mouse receiving glycopeptide Globo H-PADRE are constantly high, whereas antibody titers in mouse receiving carrier protein-Globo H conjugation are variable and most are low. FIG. **7**(A) refers to the results of G vaccine Mouse anti-GloboH IgG ELISA and FIG. **7**(B) refers to the results of MZ-11-Globo vaccine Mouse anti-Globo IgG ELISA. G1-G10 represent mouse No.1-No.10 receiving carrier protein-Globo H vaccine. M1-M10 represent mouse No.1-N.10 receiving glycopeptide Globo H-PADRE vaccine.

[0025] FIGS. **8** (A) and (B) show that glycopeptide Globo H-PADRE (M) induces long-term anti-Globo H IgG, whereas general carrier protein-Globo H conjugation (G) does not. FIG. **8**(A) refers to the results of mouse serum anti-GloboH IgG and FIG. **8**(B) refers to the results of mouse serum anti-GloboH IgM.

[0026] FIGS. **9**(A) and (B) show that dissection of individual mouse receiving glycopeptide Globo H-PADRE shows constantly long-lived high-titer anti-Globo H IgG antibody (FIG. **9**(A) for D**109** mouse serum anti-GliboH IgG and FIG. **9**(B) for D109 mouse serum anti-GliboH IgM). G1-G10 represent mouse No.1-No.10 receiving carrier protein-Globo H vaccine. Ml-M10 represent mouse No.1-N.10 receiving glycopeptide Globo H-PADRE vaccine.

[0027] FIGS. **10**(A) and (B) show that GM**2**-PADRE glycopeptide induces high-titer anti-carbohydrate IgG antibody (FIG. **10**(A) for the induction of IgG and FIG. **10**(B) for the induction of IgM).

[0028] FIG. **11** shows that mouse treated by Globo H-PADRE vaccine demonstrated slower tumor growth.

[0029] FIG. **12** shows that mouse treated by adoptive transfer of serum from mice immunized by Globo H-PADRE vaccine showed small tumor burden.

[0030] FIGS. **13**(A) to (H) shows polyvalent vaccines composed of Globo H-, GM2-, Lewis Y-PADRE conjugation mixtures or SSEA4, GM2, Lewis Y-PADRE conjugation mixtures can induce high-titer of IgG against each of respective carbohydrate antigen (FIG. **13**(A) for Globo IgG; FIG. **13**(B) for Globo IgM; FIG. **13**(C) for GM 2IgG; FIG. **13**(D) for GM2 IgM; FIG. **13** (E) for LewisY IgG; FIG. **13**(F) for LewisY IgM; FIG. **13**(G) for SSEA4 IgG and FIG. **13**(H) for SSEA4 IgM).

DETAILED DESCRIPTION OF THE INVENTION

[0031] The present invention is based, at least, on the finding that the glycopeptide conjugate of a tumor-associated carbohydrate antigen and the PADRE sequence is capable of eliciting an immune response in a mammal. The glycopeptide facilitates the activation of both B cells and T cells, thereby resulting in the production of IgM and IgG that specifically bind to the carbohydrate antigen. Particularly, the glycopeptide conjugate can be used as a vaccine capable of inducing high-titer anti-carbohydrate IgG antibody for treating cancer expression tumor-associated carbohydrate antigens. More particularly, polyvalent glycopeptide conjugate vaccine elicites high-titer polyvalent anti-carbohydrate IgG antibodies for treating cancer expressing tumor-associated carbohydrate antigens.

[0032] Therefore, in one aspect, the present disclosure is directed to an immunogenic glycopeptide. Moreover, the immunogenic glycopeptide according to the present disclosure can be provided for use in treating (including preventing) cancer; for example, it shall be manufactured as a medicament, e.g., comprised in a pharmaceutical composition. The present immunogenic glycopeptide and the pharmaceutical composition comprising the same can also be applied in a method for treating and/or preventing cancer. Accordingly, the present disclosure also contemplates a method for treating cancer in a subject suffering therefrom comprising administering to said subject a therapeutically effective amount of the glycopeptide or pharmaceutical composition as defined herein.

Definition

[0033] 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.

[0034] 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.

[0035] 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.

[0036] The term "epitope" refers to a unit of structure conventionally bound by an immunoglobulin V_H/V_L pair. An epitope defines the minimum binding site for an antibody, and thus represent the target of specificity of an antibody.

[0037] As used herein, the term "glycopeptide" refers to a compound in which carbohydrate is covalently attached to a peptide or oligopeptide.

[0038] 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.

[0039] "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.

[0040] 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 90%, such as at least 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.

[0041] 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.

[0042] 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.

[0043] 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.

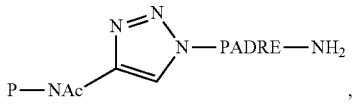
[0044] As used herein, a "pharmaceutically acceptable carrier" is one that is suitable for use with the subjects without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio. Also, each carrier must be "acceptable" in the sense 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.

[0045] As used herein, the term "adjuvant" refers to an immunological agent that modifies the effect of an immunogen, while having few if any direct effects when administered by itself It is often included in vaccines to enhance the recipient's immune response to a supplied antigen, while keeping the injected foreign material to a minimum. Adjuvants are added to vaccines to stimulate the immune system's response to the target antigen, but do not in themselves confer immunity.

[0046] As used herein, 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.

Immunogenic Glycopeptide of the Invention

[0047] In one aspect, the present invention provides an immunogenic glycopeptide having the following structure:



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wherein the PADRE is a pan-DR epitope and has at least 10 consecutive amino acid residues that is at least 80% identical to the amino acid sequence of AKXVAAWTLKAAA (SEQ ID No. **1**), where X is a cyclohexylalanine residue; and wherein P is Globo H, GD2, GM2, SSEA 4, Lewis, LewisY or STn. 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%.

[0048] The PADRE sequence is a non-natural sequence engineered to introduce anchor residues for different known DR-binding motifs. For example, X (cyclohexylalanine) in position 3 is an aliphatic residue corresponding to the position 1 of DR-binding motif, T in position 8 is a non-charged hydroxylated residue corresponding to position 6 of DR-binding; while A in position 11 is a small hydrophobic residue corresponding to position 9 of the DR-binding motif Generally, substituting one residue with another residue of substantially the same chemical and/or structural property, e.g., substituting X (cyclohexylalanine) with aromatic F (phenylalanine), will not significantly affect the binding affinity of the PADRE sequence.

[0049] According to another embodiment, the amino acid sequence of the PADRE is identical to the amino acid sequence of SEQ ID No. 1.

[0050] According to various embodiments of the present disclosure, the PADRE sequence has 10 to 20 amino acid residues. In one embodiment, the last residue (K) can be omitted. In certain embodiments, the first residue (A) or the first two residues (A and K) are omitted. In one embodiment, the PADRE sequence is lack of the first two residues and the last residue.

Compositions and Applications of Immunogenic Glycopeptide of Antibodiy of the Invention

[0051] To prevent a subject from contracting cancer, the present immunogenic glycopeptide or a pharmaceutical composition comprising the same is administered to the subject in a therapeutically (or immunogenically) effective amount. Accordingly, the pharmaceutical composition and treating method also fall within the scope of the present invention.

[0052] In one aspect, the invention provides a pharmaceuticala composition comprising one or more immunogenic glycopeptide of the present invention as described herein. In one embodiment, the composition is a vaccine. In one embodiment, the composition is a vaccine. In a further embodiment, the composition is a polyvalent vaccine comprising one or more Globo H- , GM2-, Lewis Y, or SSEA4-PADRE glycopeptide as described herein. In another further embodiment, the composition is a polyvalent vaccine comprises Globo H- , GM2- and Lewis Y-PADRE glycopeptides or SSEA4- , GM2 and Lewis Y-PADRE glycopeptides, as described herein.

[0053] In addition to the immunogenic glycopeptide, said pharmaceutical composition can further comprises a pharmaceutically acceptable carrier. The pharmaceutical composition may further comprises one or more pharmaceutically acceptable additives, including binders, flavorings, buffering agents, thickening agents, coloring agents, anti-oxidants, diluents, stabilizers, buffers, emulsifiers, dispersing agents, suspending agents, antiseptics and the like.

[0054] In addition to the immunogenic glycopeptide, said pharmaceutical composition further comprises a pharmaceutically acceptable carrier. The pharmaceutical composition may further comprises one or more pharmaceutically acceptable additives, including binders, flavorings, buffering agents, thickening agents, coloring agents, anti-oxidants, diluents, stabilizers, buffers, emulsifiers, dispersing agents, suspending agents, antiseptics and the like.

[0055] The choice of a pharmaceutically-acceptable carrier to be used in conjunction with the present glycopeptide is basically determined by the way the composition is to be administered. The pharmaceutical composition of the present invention may be administered orally or subcutaneous, intravenous, intrathecal or intramuscular injection.

[0056] Injectables for administration can be prepared in sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents include, but are not limited to, propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Illustrative examples of aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Common parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils; whereas intravenous vehicles often include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like.

[0057] Optionally, the pharmaceutically acceptable carrier may be an immunogenic adjuvant. Alternatively, the present pharmaceutical composition may optionally comprise an immunogenic adjuvant. An immunogenic adjuvant is a compound that, when combined with an antigen, increases the immune response to the antigen as compared to the response induced by the antigen alone. For example, an adjuvant may augment humoral immune responses, cell-mediated immune responses, or both. Exemplary immunogenic adjuvants include, but are not limited to mineral salts, polynucleotides, polyarginines, ISCOMs, saponins, monophosphoryl lipid A, imiquimod, CCR-5 inhibitors, toxins, polyphosphazenes, cytokines, immunoregulatory proteins, immunostimulatory fusion proteins, co-stimulatory molecules, and combinations thereof. Mineral salts include, but are not limited to, $AIK(SO_4)_2$, $AINa(SO_4)_2$, $AINH(SO_4)_2$, silica, alum, $Al(OH)_3$, $Ca_3(PO4)_2$, kaolin, or carbon. Useful immunostimulatory polynucleotides include, but are not limited to, CpG oligonucleotides with or without immune stimulating complexes (ISCOMs), CpG oligonucleotides with or without polyarginine, poly IC or poly AU acids. Toxins include cholera toxin. Saponins include, but are not limited to, QS21, QS17 or QS7. Also, examples of are muramyl dipeptides, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-DMP), N-acetyl-nornuramyl-L-alanyl-D-isoglutamine, N-acetylmuramyul-L-alanyl-D-isoglutaminyl-L-alanine-2-(1 '2'-dipalmitoyl-sn-glycero-3-hydroxphosphoryloxy)-ethylamine RIBI (MPL+TDM+CWS) in a 2 percent squalene/TWEEN 80 emulsion, lipopolysaccharides and its various derivatives, including lipid A, Freund's Complete Adjuvant (FCA), Freund's Incomplete Adjuvants, Merck Adjuvant 65, polynucleotides (e.g. poly IC and poly AU acids), wax D from Mycobacterium tuberculosis, substances found in Corynebacterium parvum, Bordetella pertussis, and members of the genus Brucella, Titermax, Quil A, ALUN, Lipid A derivatives, choleratoxin derivatives, HSP derivatives, LPS derivatives, synthetic peptide matrixes or GMDP, Montanide ISA-51 and QS-21, CpG oligonucleotide, poly I:C, and GMCSF. Combinations of adjuvants can also be used. Preferably, the adjuvant is aluminum salts (such as aluminum phosphate and aluminum hydroxide), calcium phosphate, polyinosinic-polycytidylic acid (poly I:C), CpG motif, and saponins (such as Quil A or QS21). Preferably, the adjuvant is aluminum hydroxide or QS21.

[0058] In another aspect, the present invention provides a method for preventing and/or treating a cancer, comprises administering an effective amount of the immunogenic glycopeptide described herein or a derivative thereof to a subject.

[0059] According to various working examples presented below, adult C57BL/6 mice (weight 20-25 grams) immunized with about 2 μg to 54 μg of the immunogenic glycopeptide elicited desired immune response. Hence, in certain embodiments of the present disclosure, the therapeutically effective amount of the immunogenic glycopeptide for mice could be expressed as 0.08-27 mg/kg body weight.

[0060] The therapeutically Effective amount for a human subject can be estimated from the animal doses according to various well-established standards or conversion means. For

example, the "Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" by Food and Drug Administration of U.S. Department of Health and Human Services provides several conversion factors for converting animal doses to human equivalent doses (HEDs). For mice weighted between 11 to 34 grams, to convert the mice dose (in mg/kg) to HED (in mg/kg) for a 60 kg adult human, the mice dose is multiplied by 0.081. In the instant case, the therapeutically effective amount of the present immunogenic glycopeptide for an adult human subject is 0.06-2.2 mg/kg body weight. According to various embodiments of the present disclosure, when the subject is human, the therapeutically effective amount of the immunogenic glycopeptide can be at least 1 mg/kg.

[0061] According to various embodiments of the present disclosure, the cancer treatable by the immunogenic glycopeptide, the pharmaceutical composition comprising the same or the treating method described herein is tumor-associated carbohydrate-expressing cancers; preferably, the cancer is breast cancer, ovarian cancer, pancreatic cancer, prostate cancer, colorectal cancer or lung cancer.

[0062] 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 Preparation of Immunogenic Globo H-PADRE Glycopeptide

[0063] 5.5 mg of customly synthesized PADRE-azide was dissolved in 110 μl of DMSO, and 5 mg of Globo H-b-N-acetyl propargyl (Carbosynth) was dissolved in 1 ml of distilled water, wherein PADRE has the sequence as shown in SEQ ID NO: **1**. For click reaction, 1 μmole of both PADRE-azide and Globo H-b-acetyl propargyl were first mixed and added with distilled water to a final volume of 500 μl, and then 500 μl of t-butanol (Sigma), 200 μl of 100 mM $CuSO_4.5H_2O$ (Sigma) and 160 μl of 500 mM fresh prepared Na-L-ascorbate (Sigma) were sequentially added under magnetic stirring. The mixture was incubated overnight under stir at room temperature, followed by addition of 50 μl of 27% ammonium hydroxide (Sigma). The product, the Globo H-PADRE glycopeptide, was further diluted with one volume of distilled water and stored at 4° C.

Example 2 Production of Anti-Globo H IgG and IgM antibodies

[0064] Adult female C57BL/6 mice (5 in each group at 5 weeks old, average weight 16-20 gm; Biolasco, Taiwan, R.O.C.) were injected subcutaneously to abdomen region with the Globo H-PADRE glycopeptide of Example 1, above, together with the complete Freund's adjuvant (CFA; from Sigma) as the adjuvant. Three immunizations were given at a 2-week interval; each vaccination contained 2, 6 or 18 μg Globo H-PADRE glycopeptide with 50 μl adjuvant. Serum was collected one week after the last immunization, and then subjected to enzyme-linked immunosorbent assay (ELISA) to measure the production of the anti-Globo H antibody. Serum from naïve mice injected with PBS and serum from mice immunized with the adjuvant only were used as negative controls. Sera raised against the MBr1 antibodies (Enzo Life Science; 0.5 μg/ml) or MZ-2 antibodies (produced in Example 3 below; 1 μg/ml) were used as positive controls.

[0065] For ELISA, diluted serum (1:100 or 1:1000) from mice immunized with Globo H-PADRE was added into designated wells of a 96-well ELISA plate and incubated at room temperature for one hour. Wells were then washed six times with 0.1% Tween-20 in 1XPBS. Thereafter, 1:2500 diluted anti-mouse IgG-HRP or anti-mouse IgM-HRP (Jackson Immuno Research) was added to the wells and incubated at room temperature for another one hour, and washed six times with 0.1% Tween-20 in 1XPBS. Color development was performed by incubation of the washed wells with DMT ELISA kit, and stopped by adding 2N H_2SO_4. Signals were read and recorded by ELISA reader at O.D. 450 nm (reference: 540 nm). Elisa results are depicted in FIG. **1** (FIG. (A) for diluted serum IgM 1:100 and FIG. (B) for diluted serum igM 1:1000) and FIG. **2** (FIG. (A) for diluted serum IgG 1:100 and FIG. (B) for diluted serum IgG 1:1000).

[0066] The data in FIG. **1** indicate that Globo H-PADRE glycopeptide induced the production of anti-Globo H IgM. For mice immunized with 2 μg Globo H-PADRE glycopeptide, the anti-Globo H IgM titers increased as immunization proceeded.

[0067] A cell binding assay was performed to elucidate the binding affinity of the anti-Globo H IgG and IgM antibodies with Globo H. Briefly, 100 μl of 1:10 diluted serum or 10 μg/ml of monoclonal antibodies in 1× PBS were incubated with 2×10^5 of cells at room temperature for 20 minutes. The cells were washed once with 2 ml of 1×PBS. After centrifugation, the wash buffer was discarded and cells were resuspended in 100μl 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 1× PBS after centrifugation. The binding of antibodies with cells were detected by flow cytometry. Results of cell binding assay are summarized in FIG. **3** (FIG. **3**(A) for binding affinity of anti-Globo H IgG antibodies with Globo H and FIG. **3**(B) for binding affinity of anti-Globo H IgM antibodies with Globo H). As can be seen in FIG. **3**, anti-Globo H IgG antibodies obtained from immunizations with the present Globo H-PADRE glycopeptide displayed excellent recognition of MCF-7 cells which express the Globo H antigen.

Example 3 Direct Conjugation of PADRE with Globo H Induces High-titer of Anti-Globo H IgG with Boost effect

[0068] C57BL/6 mice were immunized 3 times with 2 ug or 8 ug of single Globo H conjugated vaccine (MZ-**11**-Globo H) or 8 ug of 4 Globo H conjugated vaccine (MZ-11-4KA-Globo H) plus QS-21 as adjuvant at a 2-week interval. Serum was harvested before and 7 days after each immunization. For ELISA assay, 1 ug of streptavidin (21135, Thermo) was dissolved in 100 uL of **1**× PBS and coated on 96-well Costar assay plate (9018, Coming) before loading of biotin-Globo H (0.1 ug/well). The wells were then blocked with 1% BSA in 1× PBS, and incubated with serum 1:1000

diluted in the same blocking solution, followed by washing with **1**× PBS-0.1% Tween 20. The bound mouse IgG and IgM were detected using HRP-conjugated goat anti-mouse IgG-Fc (1:5000; 115-035-071, Jackson Immunoresearch) and HRP-conjugated goat-anti-mouse IgM μ chain (1:5000; AP128P, MILLIPORE). The color development was performed by adding 100 uL of NeA-Blue solution (010116-1, Clinical Science Products) and stopped with 50 uL of 2N sulfuric acid. The O.D. was read at 450 nm subtracted 540 nm as reference. FIG. **4** shows that mMouse immunized with 2 μg of glycopeptide by direct conjugation of PADRE to Globo and QS**21** adjuvant (2 μg) exhibits high-titer of anti-Globo H IgG and IgM with immune boost effect. MZ-**11**-Globo H: Globo H-PADRE, MZ-11-4KA-Globo H: PADRE-branched Globo H.

Example 4 IgG in Sera from Mice Immunized with Globo H-PADRE Efficiently Bind to Globo H-expression Breast Cancer Cell Line (MCF-7)

[0069] C57BL/6 mice were immunized 3 times with adjuvant alone or 2, 6, or 18 ug of Globo H-PADRE (MZ-11-Globo H) at a 2-week interval. Anti-serum were harvested 7 days after last immunization. Serum from mice without immunization was collected as control. For FACS, 5×10^5 of MCF-7 cells were stained with 100 uL of 1:10 diluted serum in flow tube followed by 100 uL of 1:100 diluted PE-conjugated goat anti-mouse IgG-Fc antibody (115-116-071, Jackson immunoresearch) and 1:100 diluted APC-conjugated rat anti-mouse IgM (17-5790-82, eBioscience). The stained cells were analyzed using BD FACSCalibur. FIG. **5** shows that antibodies in serum from mice vaccinated with Globo H-PADRE (+adjuvant QS21) bind to Globo H-expressing MCF-7 cells. MZ-11-Globo H: Globo H-PADRE.

Example 5 Globo H-PADRE (M) Induces Much Higher Titer Anti-Globo H IgG Than Carrier Protein-Globo H (G) with Class Switch

[0070] C57BL/6 mice were immunized with adjuvant (QS**21** 20 ug/mice), 2 ug of general carrier protein-Globo H conjugation (G) vaccine, or Globo H-PADRE conjugation (MZ11-GloboH) vaccine at a 2-week interval. Anti-Globo H serum was harvested before and 7 days after each vaccination. The titer of anti-Globo H serum in pooled serum or each mice were detected by ELISA assay with appropriated secondary antibody. FIG. **6** shows that glycopeptide Globo H-PADRE (M) induces higher titer of anti-Globo H IgG antibody than general carrier protein-Globo H conjugation (C) does. C: control; Q: adjuvant QS21. FIG. **7** shows that antibody titers in individual mouse receiving glycopeptide Globo H-PADRE are constantly high, whereas antibody titers in mouse receiving carrier protein-Globo H conjugation are variable and most are low and it represents that Globo H-PADRE (M) stably induces high titer of anti-Globo H IgG in individual mouse.

Example 8 Globo H-PADRE (M) Induces Long-Lived Anti-Globo H IgG Antibody and B cell Memory Responses

[0071] Anti-Globo H serum was harvested on 36 and 81 days after last Vaccination (D64 and D109). The titer of anti-Globo H antibodies The titers of anti-Globo H serum in pooled serum or each mouse were detected by ELISA assay with appropriated secondary antibody with $1/_{10000}$ dilution. FIG. **8** shows that glycopeptide Globo H-PADRE (M) induces long-term anti-Globo H IgG, whereas general carrier protein-Globo H conjugation (G) does not and FIG. **9** shows that dissection of individual mouse receiving glycopeptide Globo H-PADRE shows constantly long-lived high-titer anti-Globo H IgG antibody. Very low level of anti-Globo H IgG antibody is noted in mouse receiving carrier protein-Globo H conjugation.

Example 9 Carbohydrate-PADRE Glycopeptide Induces High-titer Anti-Carbohydrate IgG Antibody (GM2 as example)

[0072] C57BL/6 mice were immunized with adjuvant (QS21 20 ug/mice) or GM2-PADRE conjugation vaccine with adjuvant (QS-21 20 ug/mice) at a 2-week interval. Anti-GM2 serum was harvested before and 7 days after each vaccination. The titer of anti-GM2 serum in pooled serum or each mice were detected by ELISA assay with appropriated secondary antibody. FIG. **10** shows that GM**2**-PADRE glycopeptide induces high-titer anti-carbohydrate IgG antibody.

Example 10 Anti-tumor Effect of Globo H-PADRE Glycopeptide Vaccine in Immunocompetent Mouse Model

[0073] Mice were divided into 3 groups and subcutaneously (s.c.) administered with 1× PBS (control), 20 ug of QS-21 alone or 6 ug of Globo H-PADRE (MZ11-Globo H) plus 20 ug of QS-21 at a 2-week interval. Seven days after third vaccination, mice were s.c. implanted 1×10^5 of LLC1 cells and were concomitantly vaccinated again. The vaccination interval was changed to 7 days after tumor inoculation. Tumor size was measured by caliper at day 7, 10, 14 and 18 after tumor implantation and calculated at length× width×height. FIG. **11** shows that mouse treated by Globo H-PADRE vaccine demonstrated slower tumor growth (LLC1 cells subcutaneous tumor model in immuno-competent mice).

Example 11 Adoptive Transfer of Immunized Serum to Intra-Peritoneal Ovarian Tumor Model Showed Obvious Anti-tumor Efficacy

[0074] Mice were divided into 2 groups. Serum was collected from group 1 mice without immunization as control. Serum was also collected from group 2 mice vaccinated with Globo H-PADRE (MZ11-Globo H) 3 times at a 2-week interval as anti-Globo H serum. One million TOV21G cells were intra-peritoneal (i.p.) implanted into 5-week-old female NU/NU mice (BioLASCO Taiwan). After 4 days, mice were administered with 200 μL of control serum or anti-GloboH serum 3 times a week through i.p. route. Untreated mice were set as control. For monitoring tumor growth, tumor bearing mice were i.p. injected 2004 of luciferin (3.9 mg/ml). The chemoluminescent intensity of each mouse was detected by a non-invasive IVIS system (Xenogen) with fixed exposure condition per batch of experiment.

SEQUENCE LISTING

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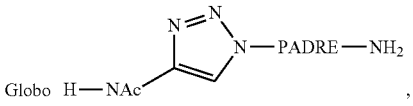
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What is claimed is:

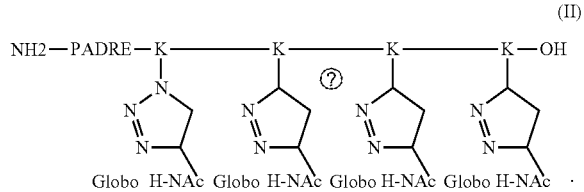
1. An immunogenic glycopeptide or a derivative thereof, wherein the immunogenic glycopeptide has the structure of formula I:



wherein the PADRE is a pan-DR epitope and has at least **10** consecutive amino acid residues that is at least 90% identical to the amino acid sequence of SEQ ID No. 1.

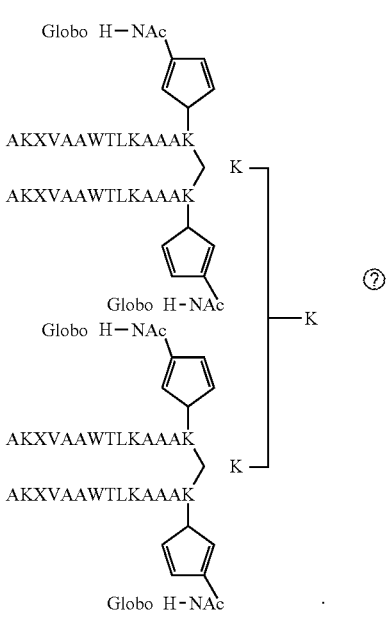
2. The immunogenic glycopeptide or a derivative thereof of claim **1**, wherein the amino acid sequence of the PADRE is identical to the amino acid sequence of SEQ ID No. 1.

3. The immunogenic glycopeptide or a derivative thereof of claim **1**, wherein the derivative has the structure of formula II,



(?) indicates text missing or illegible when filed

4. The immunogenic glycopeptide or a derivative thereof of claim **1**, wherein the derivative has the structure of formula III,



(?) indicates text missing or illegible when filed

5. A pharmaceutical composition for treating Globo H-positive cancer in a subject in need thereof, comprising a therapeutically effective amount of the immunogenic glycopeptide of claim **1** and a pharmaceutically acceptable carrier.

6. The pharmaceutical composition of claim **5**, wherein the PADRE has the amino acid sequence that is identical to the amino acid sequence of SEQ ID No. 1.

7. The pharmaceutical composition of claim **5**, wherein the Globo H-positive cancer is breast cancer, ovarian cancer, pancreatic cancer, prostate cancer, colorectal cancer or lung cancer.

8. The pharmaceutical composition of claim **7**, wherein the ovarian cancer is primary ovarian cancer.

9. An antibody which specifically binds to at least one epitope defined by the immunogenic glycopeptide of claim **1**.

10. The antibody of claim **9**, wherein the PADRE has the amino acid sequence that is identical to the amino acid sequence of SEQ ID No. 1.

11. The antibody of claim **9**, wherein the antibody is a humanized antibody and has a heavy chain and a light chain respectively having, in a variable region, an amino acid sequence at least 90% identical to SEQ ID No. 2 and SEQ ID No. 3.

12. The antibody of claim **11**, wherein the amino acid sequence of the variable region of the heavy chain is identical to SEQ ID No. 2.

13. The antibody of claim **11**, wherein the amino acid sequence of the variable region of the light chain is identical to SEQ ID No. 3 or SEQ ID No. 4.

14. A pharmaceutical composition for treating Globo H-positive cancer in a subject in need thereof, comprising a therapeutically effective amount of the antibody of claim **9** and a pharmaceutically acceptable carrier.

15. The pharmaceutical composition of claim **14**, wherein the PADRE has the amino acid sequence that is identical to the amino acid sequence of SEQ ID No. 1.

16. The pharmaceutical composition of claim **14**, wherein the antibody is a humanized antibody and has a heavy chain and a light chain respectively having an amino acid sequence at least 90% identical to SEQ ID No. 2 and SEQ ID No. 3.

17. The pharmaceutical composition of claim **16**, wherein the amino acid sequence of the heavy chain is identical to the amino acid sequence of SEQ ID No. 2.

18. The pharmaceutical composition of claim **16**, wherein the amino acid sequence of the light chain is identical to the amino acid sequence of SEQ ID No. 3 or SEQ ID No. 4.

19. The pharmaceutical composition of claim **14**, wherein the Globo H-positive cancer is breast cancer, ovarian cancer, pancreatic cancer, prostate cancer, colorectal cancer or lung cancer.

20. The pharmaceutical composition of claim **19**, wherein the ovarian cancer is primary ovarian cancer.

21. A method of treating Globo H-positive cancer in a subject in need thereof, comprising the step of administering to the subject the pharmaceutical composition of claim **12**.

22. The method of claim **21**, wherein the Globo H-positive cancer is breast cancer, ovarian cancer, pancreatic cancer, prostate cancer, colorectal cancer or lung cancer.

23. The method of claim **22**, wherein the ovarian cancer is primary ovarian cancer.

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