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## (72) Inventor; and

(71) Applicant : ZHOU, Dapeng [CN/US]; 2222 Maroneal Street, Unit 642, Houston, TX 77030 (US).

## (72) Inventor; and

(75) Inventor/Applicant (for US only): LI, Chun [US/US]; 6807 Spanish Bay Court, Missouri City, TX 77459 (US).

(74) Agent: SHANGHAI ACTION INTELLECTUAL PROPERTY AGENCY CO., LTD.; c/o Guo Chunyuan, Room 1220, Yongsheng Building, No. 2025 Zhongshan West Road, Xuhui District, Shanghai 200235 (CN).

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## Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

## Published:

— with international search report (Art. 21(3))

## (54) Title: NANOPARTICLE FORMULATED GLYCOLIPID ANTIGENS FOR IMMUNOTHERAPY

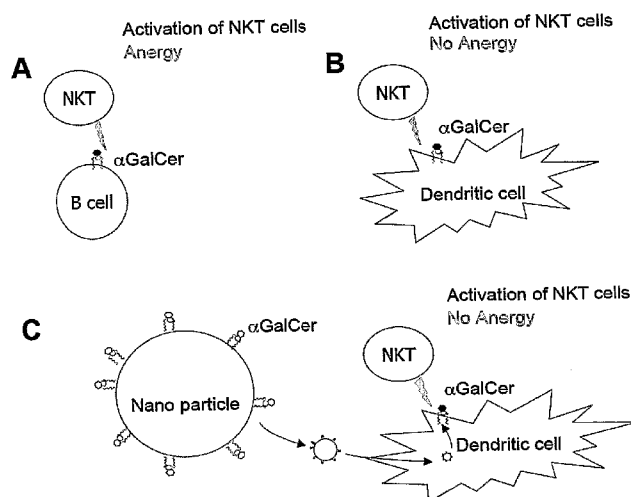


Fig.1

(57) Abstract: A nanoparticle formulated glycolipid antigen contains 2 components: 1). Nanoparticles at size of 10 to 2000 nm, composed by biodegradable polymers, such as poly(lactic acid), poly(glycolic acid), polycaprolactone, and other polyester species; polythoesters species; polyanhydrides species and polyphosphazene species, as well as any copolymers composed by 2 or more than 2 polymers as mentioned. 2) 6-aminoethoxy-6-deoxy-alpha-galactosylceramide. The key technology applied to this invention is, component 2 is chemically conjugated to the surface of component 1, through amide linkage. The objection of this invention is to provide a novel surface conjugated formulation to a glycolipid antigen, which can stimulate immune system repeatedly in long term treatment of cancer and chronic viral infection status. The glycolipid conjugated to surface of nanoparticles can repeatedly stimulate NKT cells to produce IFN-g, a cytokine with potent anticancer and antiviral activities. This novel drug has been found to have anticancer effect in a mouse model of tumor metastasis.

## Description

### NANOPARTICLE FORMULATED GLYCOLIPID ANTIGENS FOR IMMUNOTHERAPY

[1]        **FIELD OF THE INVENTION**

[2]        The present invention relates to a novel nanoparticle formulated glycolipid antigen which has a strong activity to trigger the immune system to release anticancer and antiviral cytokines. In contrast to previous invented glycolipid antigens, such novel nanoparticle formulation does not cause the anergy (non-responsiveness after stimulation) of the immune system, and can be used repeatedly to treat long term illness such as cancer and chronic viral diseases.

[3]        **BACKGROUND OF THE INVENTION**

[4]        **Alpha-galactosylceramide (KRN7000), a potent immunotherapeutic.**

alpha-galactosylceramide (also named as KRN7000), is a marine-sponge derived glycosphingolipid (US patent 5,780,441). It is a super-agonist antigen for NKT cells, limits melanoma metastasis and inhibits the replication of hepatitis B virus in mouse models through a Th1 cytokine (IFN- $\gamma$ ) mediated mechanism. In several clinical trials involving cancer and hepatitis C patients, alpha-galactosylceramide has been demonstrated to be safe, and to induce strong Th1 cytokine response.

[5]        Natural Killer T cells are a unique subset of lymphocytes that have markers and functions of T cells and NK cells. NKT cells are activated within 2 hr of antigen (alpha-galactosylceramide) stimulation, and produce large amount of Th1 and Th2 cytokines. In addition to secreting cytokines, NKT cells play extremely important role in initiating the cell-to-cell contact and crosstalk between several types of immune cells, including dendritic cells, macrophages, CD4 T cells and CD8 T cells. In contrast to conventional T cells which recognize peptide antigens, NKT cells recognize lipid antigens presented by the non-polymorphic MHC-like molecule CD1d.

[6]        **Soluble alpha-galactosylceramide induces anergy of NKT cells.** A major problem for alpha-galactosylceramide is, it causes NKT non-responsiveness (anergy) after one dose of treatment, because alpha-galactosylceramide can be presented by CD1d expressing B cells in the peripheral blood (Figure 1A), and stimulate the NKT cells without proper co-stimulatory molecules.

[7]        To overcome the anergy induced by soluble alpha-galactosylceramide, Dhodapkar and Steinmann have developed a cell therapy approach (Figure 1B), by intravenously injecting alpha-galactosylceramide pulsed, *ex vivo* generated dendritic cells from patients' peripheral blood mononuclear cells. The cell therapy method avoided the NKT anergy mechanism, and showed potent efficacy in cancer patients in eliciting tumor antigen-specific CD8 responses. However, cell therapy is expensive, and impracticable for virus infected patients since their tissues are excluded from GMP processing. Hence, new methods are needed to stimulate NKT cells in vivo repeatedly and effectively.

[8]        **Nanoparticle formulated alpha-galactosylceramide overcomes NKT anergy.**

Based on the above progress, we have formed the hypothesis that alpha-galactosylceramide, packed in nanoparticles, will be preferentially taken up by dendritic cells and released into lysosomes (Figure 1C). Alpha-galactosylceramide is loaded to the antigen presenting molecule, CD1d (a non-MHC antigen presenting molecule), in the lysosome, and recycled to cell surface, which activates NKT cells. We have invented a technology through chemical synthesis, to conjugate alpha-galactosylceramide to the surface of polymer based nanoparticles.

[9] **Rationale to use surface conjugating of alpha-galactosylceramide, instead of encapsulating approach.** There are two approaches for our consideration to load alpha-galactosylceramide in nanoparticles: 1) surface coating, which means we synthesize nanoparticles first, and conjugate alpha-galactosylceramide to the surface of nanoparticles; 2) direct encapsulating approach, which means to mix alpha-galactosylceramide and polymer together, and form the nanoparticles with alpha-galactosylceramide evenly distributed inside the nanoparticles. We have decided to use the former approach, due to the biological behavior of NKT cells, the cell type which respond to alpha-galactosylceramide drug.

[10] As demonstrated in Figure 2, the stimulation of NKT cells includes the expansion, contraction, and memory phases. In the expansion phase, the NKT cells produce anti-tumor and anti-viral cytokines. In contraction phase and memory phase, most NKT cells die, while 5 to 10% of "memory" NKT cells begin to "rest" and re-program themselves for the next stimulation. Thus a continuous release of alpha-galactosylceramide will not cause effective stimulation of NKT cells, instead, continuous release of alpha-galactosylceramide has been shown to cause the non-responsiveness (anergy) of NKT cells. In view of the above mechanism, we designed the method of surface coating, which causes rapid release of alpha-galactosylceramide upon being phagocytosed by dendritic cells and macrophages, but not consistent release of alpha-galactosylceramide to the serum. In contrast, it is known that encapsulating method is suitable for long term, constant release of encapsulated drugs. Consistent release of encapsulated drugs can last for up to 10 days after intravenous injection of encapsulating nanoparticles, which accumulate in liver and spleen. Thus, encapsulating method does not fit for the purpose of releasing NKT ligand.

[11] **Rationale to choose biodegradable polymer based nanoparticles.** Polymers such as PLGA has excellent biocompatibility, biodegradability, commercial availability, and previous application to deliver drugs such as proteins, peptide vaccines, and hydrophobic anticancer drugs.

[12] The acyl groups of polymers, exposed on the surface of nanoparticles, can be utilized to conjugate with amine groups. However, alpha-galactosylceramide does not contain amine groups. Since the 6-OH group of galactose is known to be dispensable for the antigenic activity of alpha-galactosylceramide, we have invented a glycolipid analog, 6-aminoethoxy-6-deoxy-alpha-galactosylceramide, which contains amine group in 6-OH group of galactose. This allows the formation of amide linkage (Figure 3). To avoid antibody response to any additional protein (such as streptavidin-biotin system), we will directly conjugate alpha-galactosylceramide to nanopolymer materials. Both alpha-galactosylceramide and polymers are not antigenic in mouse and human, and the

chemical conjugates of polymers and alpha-galactosylceramide do not create immuno-reactive epitopes.

[13] **SUMMARY OF THE INVENTION**

[14] The present invention relates to a nanoparticle formulated glycolipid antigen, containing 2 components:

[15] 1). Nanoparticles at size of 10 to 2000 nm, composed by biodegradable polymers, such as, poly (lactic acid), poly(glycolic acid), polycaprolactone, and other polyester species; polyorthoesters species; polyanhydrides species and polyphosphazene species, as well as any copolymers composed by 2 or more than 2 species of polymers as mentioned above.

[16] 2) 6-aminoethoxy-6-deoxy-alpha-galactosylceramide

[17] The key technology applied to this invention is, component 2 is chemically conjugated to the surface of component 1, through amide linkage (Figure 3)

[18] The object of this invention is to provide a novel surface conjugated formulation to a glycolipid antigen, which can stimulate immune system repeatedly in long term treatment of cancer and chronic viral infection status.

[19] The present inventors found the glycolipid conjugated to surface of nanoparticles can repeatedly stimulate NKT cells to produce IFN- $\gamma$ , a cytokine with potent anticancer and antiviral activities. This novel drug has been found to have anticancer effect in a mouse model of tumor metastasis.

[20] **BEST MODE OF CARRYING OUT THE INVENTION**

[21] **Preparation of nanoparticles, with poly(lactic-co-glycolic acid) as example (abbreviated as PLGA).** The nanospheres will be prepared using nanoprecipitation method in the presence of the stabilizing copolymer. The PLGA, from Absorbable Polymers International (Pelham, AL, USA), will be dissolved in acetone (0.5% w/v). The polymer solution will be added drop-wise to an aqueous solution containing poloxamine 904 (BASF Wyandotte Corporation, Parsippany, NJ, USA). The mixture will be stirring at room temperature until the organic solvent is completely dried. The resulting nanoparticle dispersions will be passed through a 1- $\mu$ m filter, and purified by centrifugation. Nanoparticles will be re-dispersed in water, flash-frozen in liquid nitrogen and subject to lyophilization. The dry powder formulation will be stored in -20 °C. This protocol will provide nanoparticles with the diameter of about 50 nm to 2000 nm, depending on the molecular weight of PLGA, stirring rate, and the concentration of surfactant.

[22] **Preparation of 6-aminoethoxy-6-deoxy-alpha-galactosylceramide, which contains amine group for direct conjugating to PLGA nanoparticles (Figures 4&5).**

[23] **Conjugation of 6-aminoethoxy-6-deoxy-alpha-galactosylceramide to PLGA nanoparticles.** 6-aminoethoxy-6-deoxy-alpha-galactosylceramide will be conjugated to PLGA particles at 1% and 10% (w/w ratio).

[24] **EXPERIMENTAL EXAMPLE 1: IMMUNOSTIMULATORY ACTIVITY OF THE DRUG OF THE PRESENT INVENTION**

[25] In vivo stimulation of NKT cells by nanoparticle formulated  $\alpha$ GalCer:  
Nanoparticle containing 1  $\mu$ g alpha-galactosylceramide was injected to C57BL6 mice,

with non-conjugated nanoparticles as negative control. In parallel, we studied soluble form of alpha-galactosylceramide.

[26] The repeated in vivo NKT cell stimulation: C57/BL6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed in M.D. Anderson Cancer Center animal facilities under standard pathogen free conditions abiding institutional guidelines. 6 weeks old C57BL/6 mice were used for all experiments. 3 mice per group were used for each experiment. 1  $\mu$ g alpha-galactosylceramide, or nanoparticle formulated alpha-galactosylceramide in 200  $\mu$ L PBS was intravenously injected (through tail vein) to each mouse. Mice were treated every 10 days for 3 times. 200  $\mu$ L of PBS/1%DMSO was used as control.

[27] Measurement of IFN- $\gamma$  secretion after each stimulation: Mice were bled at 24 h after each drug treatment. Serum IFN- $\gamma$  was measured by ELISA using a kit from BD Biosciences (San Jose, CA).

[28] Results: Nanoparticle overcomes the anergy of NKT cells: Similarly to alpha-galactosylceramide, the first injection of nanoparticle formulated alpha-galactosylceramide elicited cytokine release, peaked at 24–48 h post injection, as measured by serum IFN- $\gamma$  concentration. As shown in Table 1, nanoparticle formulated alpha-galactosylceramide induced IFN- $\gamma$  secretion after each stimulation. In contrast, soluble alpha-galactosylceramide caused IFN- $\gamma$  secretion only at the first treatment and failed to induce upon subsequent stimulations. Thus our new formulation of alpha-galactosylceramide can repeatedly stimulate NKT cells and induce IFN- $\gamma$  production without leading to anergy.

Table 1.  $\alpha$ GalCer-nanoparticles repeatedly activate NKT cells to produce IFN- $\gamma$  (pg/mL).

[31]

|              | $\alpha$ GalCer-nanoparticles | Nanoparticles w/o $\alpha$ GalCer | $\alpha$ GalCer |
|--------------|-------------------------------|-----------------------------------|-----------------|
| ×1 injection | 286 $\pm$ 39 <sup>a</sup>     | 48 $\pm$ 1                        | 2601 $\pm$ 257  |
| ×2 injection | 252 $\pm$ 62                  | 42 $\pm$ 5                        | 65 $\pm$ 2      |
| ×3 injection | 261 $\pm$ 102                 | 45 $\pm$ 3                        | 40 $\pm$ 11     |

<sup>a</sup> Data were average of three mice per group along with standard deviation.

[34] **EXPERIMENTAL EXAMPLE 2: ANTITUMOR ACTIVITY OF THE DRUG OF THE PRESENT INVENTION**

[35] **Anti-metastasis effect.** The B16F10 melanoma lung metastasis tumor model was employed to evaluate the anticancer function of the present invention. 48 hours after drug treatment,  $1 \times 10^5$  B16F10 melanoma cells were injected intravenously through

tail vein. 14 days after tumor injection, the mice were sacrificed and the tumor nodes formed through metastasis (primary end point) were counted in mouse lung.

[36] **Results:** As showed in Figure 6, the present invention has significant anticancer effect.

[37] **BRIEF DESCRIPTION OF THE DRAWINGS**

[38] The structure and the technical means adopted by the present invention to achieve the above and other objects can be best understood by referring to the following detailed description of the preferred embodiments and the accompanying drawings, wherein

[39] Fig. 1 is a perspective view of 3 forms of alpha-galactosylceramide, according to a first embodiment of the present invention;

[40] Fig. 2 is a perspective view of the expansion, contraction, and memory phases of NKT cells after alpha-galactosylceramide according to the present invention, explaining why the alpha-galactosylceramide must be conjugated to the surface of nanoparticles, instead of being encapsulated inside, according to conventional wisdom.

[41] Fig.3 is a perspective view of the chemical linkage binding alpha-galactosylceramide to the surface of nanoparticles.

[42] Fig.4 is a perspective view of a newly invented structure, 6-aminoethoxy-6-deoxy-alpha-galactosylceramide, a novel glycolipid analog of alpha-galactosylceramide, which contains amine group for chemical conjugation to the surface of nanoparticles.

[43] Fig.5 is a perspective view of the chemical synthesis route for producing 6-aminoethoxy-6-deoxy-alpha-galactosylceramide, a novel glycolipid analog of alpha-galactosylceramide.

[44] Fig.6 is a perspective view of an example of the anticancer effect of this invention.

[45] **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

[46] The present invention will now be described with some preferred embodiments thereof. For the purpose of easy to understand, elements that are the same in the preferred embodiments are denoted by the same reference numerals.

[47] Please refer to Fig. 1.

[48] A. Soluble form of  $\alpha$ GalCer is mainly presented by B cells, which stimulates NKT cells without co-stimulatory signals, and cause NKT anergy after once drug injection.

[49] B.  $\alpha$ GalCer loaded dendritic cells stimulate NKT cells without causing anergy, but generation of dendritic cells for cell therapy is a tedious and expensive procedure.

- [50] C. Nanoparticle formulated  $\alpha$ GalCer is designed to be preferentially taken up by dendritic cells. The  $\alpha$ GalCer will be released in the lysosome and recycled to cell surface, which stimulate NKT cells without causing anergy.
- [51] Fig. 2 shows the stimulation of NKT cells include expansion, contraction, and memory phase.  $\alpha$ GalCer can not be delivered in a continuously released form, because continuous exposure of NKT cells to  $\alpha$ GalCer will cause non-responsiveness (anergy).
- [52] Fig. 3 shows Component 2 (containing amine group) is conjugated to the surface of component 1 (containing acyl group) through amide linkage.
- [53] Fig.4 shows 6-aminoethoxy-6-deoxy-alpha-galactosylceramide, a novel structure we have invented, which with structural similarity to alpha-galactosylceramide (KRN7000), but can be directly conjugated to PLGA nanoparticles through its amide group (red color).
- [54] Fig. 5 shows the chemical synthesis route we have invented for producing 6-aminoethoxy-6-deoxy-alpha-galactosylceramide.
- [55] Fig.6 shows anticancer effect of nanoparticle-formulated alpha-galactosylceramide. The B16F10 melanoma lung metastasis tumor model was used to test the tumor preventing activity of nanoparticle formulated alpha-galactosylceramide drugs in mice. 48 hours after nanoparticle drug treatment,  $1 \times 10^5$  B16F10 melanoma cells were injected intravenously through tail vein. 14 days after tumor injection, the mice were sacrificed and the tumor nodes formed through metastasis were counted in mouse lung, after being fixed by Fekete's solution.
- [56] **PBS:** control mice treated by PBS.
- [57]  **$\alpha$ GalCer:** 2  $\mu$ g soluble form of  $\alpha$ GalCer.
- [58]  **$\alpha$ GalCer 200 nm:** 2  $\mu$ g  $\alpha$ GalCer bound to 200 nm size of polylactic acid nanoparticles.
- [59]  **$\alpha$ GalCer 2000 nm:** 2  $\mu$ g  $\alpha$ GalCer bound to 2000 nm size of polylactic acid nanoparticles.
- [60] Each group contained 5 mice ( $\times \blacktriangle \blacksquare \blacklozenge *$ ).

## Claims

1. A product comprising any feature described herein, either individually or in combination with any feature, in any configuration.
2. A composition comprising an alpha-galactosylceramide conjugated nanoparticle.
3. A composition comprising 6-aminoethoxy-6-deoxy-alpha-galactosylceramide.
4. A composition of claim 2 wherein the nanoparticle comprises polylactic acid, poly(glycolic acid), polycaprolactone, and other polyester species; polyorthoesters species; polyanhydrides species and polyphosphazene species, as well as any copolymers composed by 2 or more than 2 species of polymers as mentioned.
5. A process of making a conjugated nanoparticle alpha-galactosylceramide formulation wherein alpha-galactosylceramide or its analogs are chemically conjugated to the surface of nanoparticles through amide linkage or other chemical linkages.
6. A pharmaceutical composition comprising a conjugated nanoparticle alpha-galactosylceramide.
7. A method of preventing anergy of NKT cells comprising the step of administering a therapeutically effective amount of a conjugated nanoparticle formulated with alpha-galactosylceramide to a patient in need thereof.
8. A method of preventing or treating cancer, chronic viral disease, chronic bacteria infections, autoimmune diseases, asthma, allergy, diabetes, atherosclerosis, and other chronic disease conditions, comprising administering a therapeutically effective amount of a conjugated nanoparticle alpha-galactosylceramide in combination with a protein/peptide antigen to a patient in need thereof.
9. An adjuvant for protein/peptide antigen comprising a conjugated alpha-galactosylceramide nanoparticle.

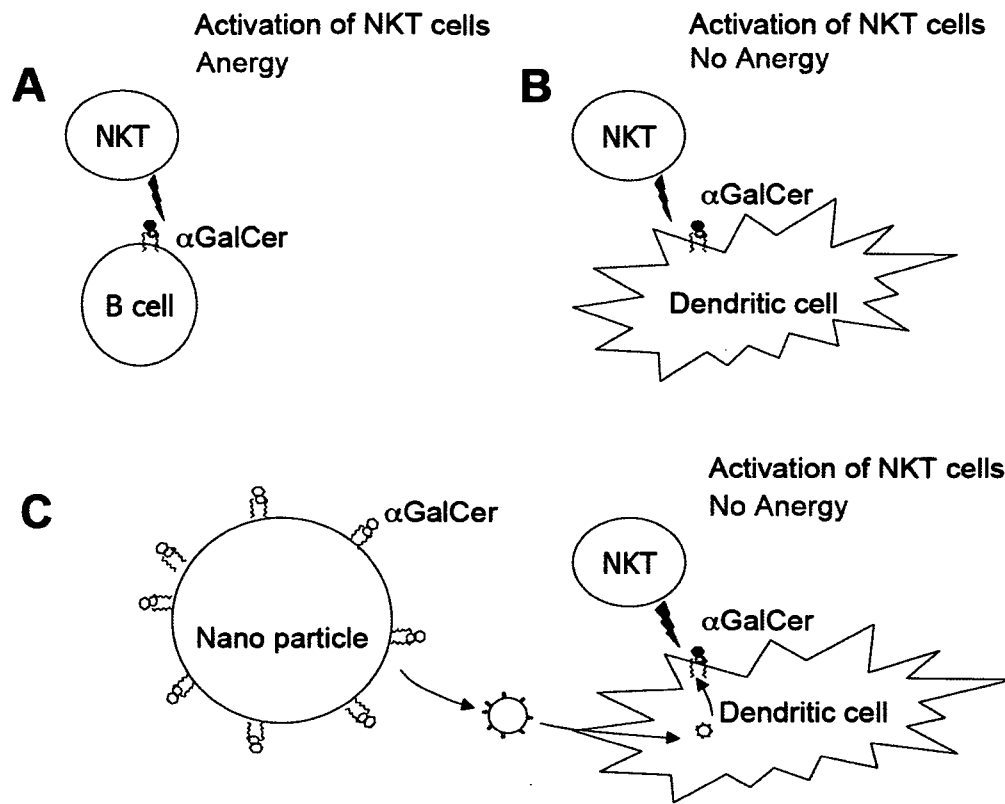


Fig.1

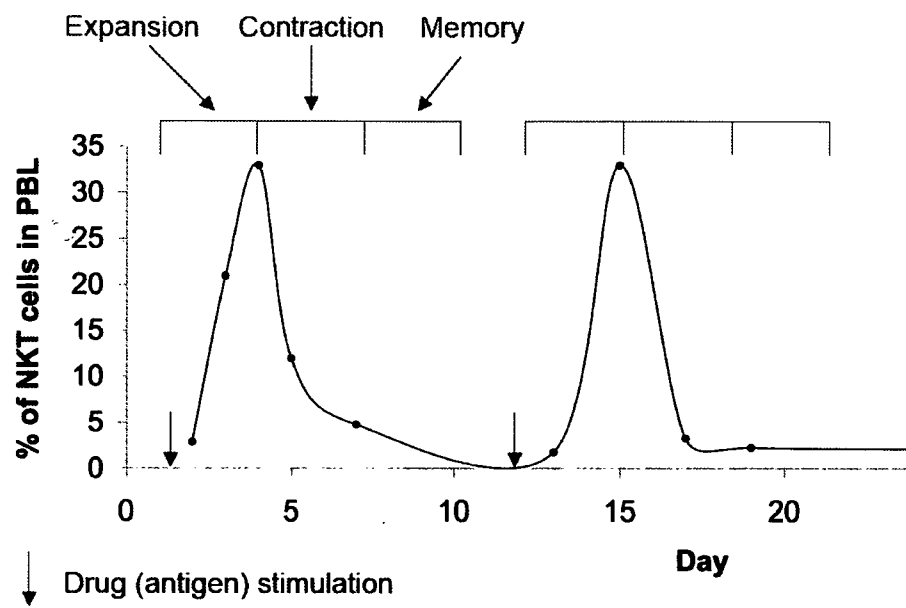


Fig. 2

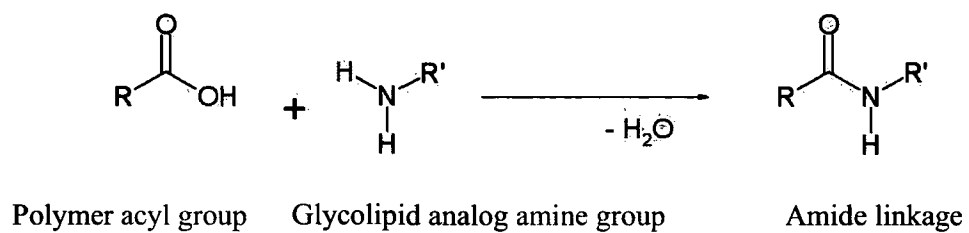


Fig. 3

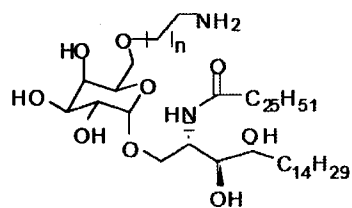
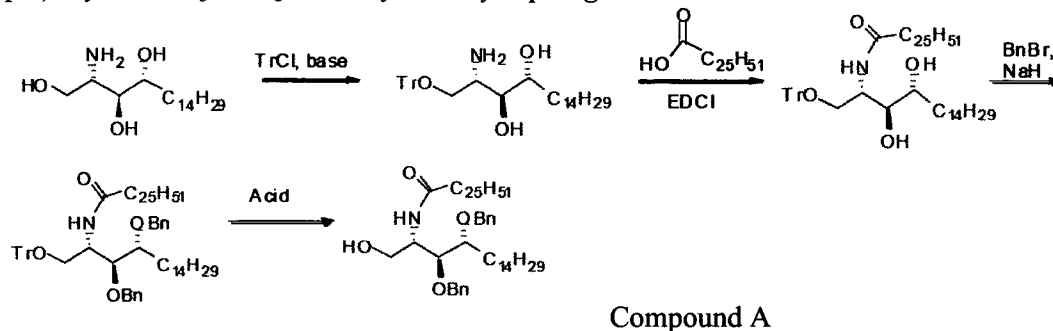
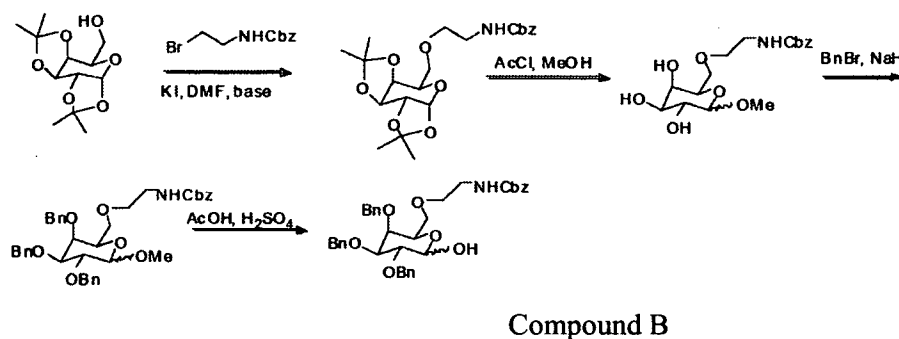


Fig. 4

Step 1): Synthesis of Compound A from Phytosphingosine.



Step 2): Synthesis of Compound B from 1,2,3,4-DI-O-ISOPROPYLIDENE- $\alpha$ -D-GALACTOPYRANOSE.



Step 3): Synthesis of 6-aminoethoxy-6-deoxy- $\alpha$ -galactosylceramide from Compound A and B.

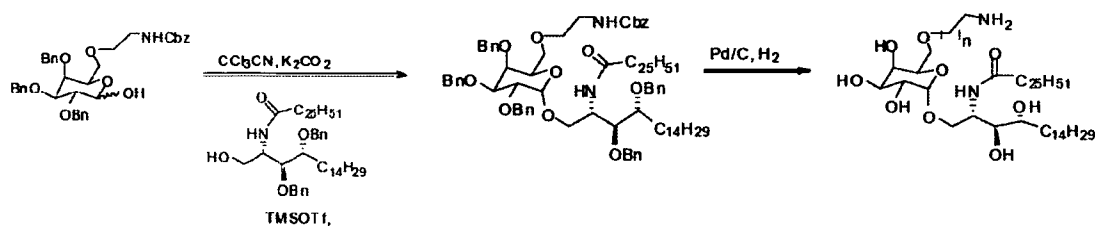


Fig. 5

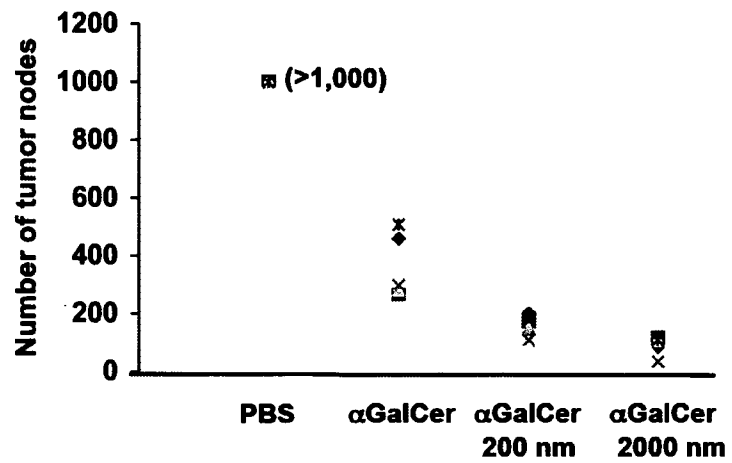


Fig. 6

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/CN2010/000100

**A. CLASSIFICATION OF SUBJECT MATTER**

See extra sheet

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)

IPC: A61K31/385, A61K31/70, C07H15/04

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)

WPI, EPODOC, CNKI, CNPAT, REG, galactosylceramide, nano+, km7000, structure search according to the formula shown in Fig. 4

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                         | Relevant to claim No. |
|-----------|----------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | WO 2008115641 A2 (UNIV YALE) 25 Sep. 2008(25.09.2008)<br>The whole document                                                | 2-6, the last claim   |
| X         | US 20080206319 A1 (Thomas EBENSEN ET-AL) 28 Aug. 2008 (28.08.2008)<br>Paragraphs [0021], [0045]-[0046], [[0074] and [0094] | 2-6, the last claim   |

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

|                                                                                                                                                                          |                                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents:                                                                                                                                 | “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                                              |
| “A” document defining the general state of the art which is not considered to be of particular relevance                                                                 | “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                                                                     |
| “E” earlier application or patent but published on or after the international filing date                                                                                | “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 |
| “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) | “&” document member of the same patent family                                                                                                                                                                                                    |
| “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                                                                   |                                                                                                                                                                                                                                                  |

|                                                                                                                                                                                                              |                                                                                       |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Date of the actual completion of the international search<br>18 Apr. 2010 (18.04.2010)                                                                                                                       | Date of mailing of the international search report<br><b>06 May 2010 (06.05.2010)</b> |
| Name and mailing address of the ISA/CN<br>The State Intellectual Property Office, the P.R.China<br>6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China<br>100088<br>Facsimile No. 86-10-62019451 | Authorized officer<br><b>CHEN, Junxia</b><br>Telephone No. (86-10)62086343            |

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2010/000100

### 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.: 7-8

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

**The subject matter of claims 7-8 relates to the methods of treatment for diseases of human or animal bodies.**

2. ☒ Claims Nos.: 1

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:

**Since there is no concrete technical feature described in claim 1, claim 1 is so unclear that no meaningful international search can be carried out.**

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).

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

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

**See extra sheet**

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2010/000100

### A. CLASSIFICATION OF SUBJECT MATTER

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

A61K 39/385 (2006.01)i

A61P35/00 (2006.01)i

A61P31/12 (2006.01)i

A61P37/00 (2006.01)i

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

This application contains the following groups of inventions:

Group I comprises claims 2, 4-6 and the last claim not being numbered, which respectively relate to a composition comprising an alpha-galactosylceramide conjugated nanoparticle (claims 2 and 4) or a composition comprising a conjugated nanoparticle alpha-galactosylceramide (claim 6), a process of making a conjugated nanoparticle alpha-galactosylceramide formulation (claim 5) or an adjuvant for protein/peptide antigen comprising a conjugated nanoparticle alpha-galactosylceramide (the last claim not being numbered).

Group II comprises claim 3 which relates to a composition comprising 6-aminoethoxy-6-deoxy-alpha-galactosylceramide.

The same feature in the above groups of inventions is alpha-galactosylceramide, which could not make a contribution over the prior art (please see paragraph [4] in the description) and then could not be considered as a special technical feature within the meaning of PCT Rule 13.2.

Therefore, the above-said groups of inventions are not so linked as to form a single inventive concept under PCT Rule 13.1.

International application No.  
PCT/CN2010/000100

Form PCT/ISA /210 (patent family annex) (July 2009)