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(54) Title: NICOTINE CONJUGATE VACCINE

(57) Abstract: An immunogenic composition comprising a nicotine immunoconjugate and an oil-in-water emulsion adjuvant and a method of producing such compositions are provided. Use of such adjuvanted nicotine immunoconjugates as medicaments and in the treatment or prevention of nicotine addiction is also provided.



WO 2011/127989 A1

## NICOTINE CONJUGATE VACCINE

### **Field of the invention**

5           The present invention relates to adjuvanted nicotine immunoconjugates and their use in the treatment or prevention of nicotine addiction. The invention also relates to methods of producing such adjuvanted immunoconjugates.

### **Background to the invention**

10           Nicotine ((*S*)-(-)-1-methyl-2-(3-pyridyl)pyrrolidine) is an alkaloid derived from the tobacco leaf. The nicotine molecule is formed of an aromatic six-membered ring (pyridine) and an aliphatic five-membered ring (pyrrolidine) linked by a single bond. The pyrrolidine ring is N-methylated and linked through its carbon -2 to the carbon -3 of pyridine. The carbon -2 is chiral and there is virtually free rotation  
15           around the single bond linking the two rings. The absolute configuration of carbon -2 is *S*, and so the natural enantiomer of nicotine is (*S*)-(-)-nicotine.

            Nicotine is widely available in many different forms, such as cigarettes, cigars, pipe tobacco and chewing tobacco, and is highly addictive. Due to significant adverse health effects of tobacco use, in particular smoking, users often try to quit. However,  
20           the poor rates of success in those who try to quit indicate that there is a strong need for additional therapies for nicotine addiction.

            Nicotine conjugate vaccines induce anti-nicotine antibodies that, upon exposure following smoking, bind to nicotine in the blood. Nicotine itself is a small molecule that readily crosses the blood-brain barrier but nicotine bound to an antibody  
25           cannot enter the brain. This mechanism of action has been largely demonstrated in mouse and rat animal models, and was correlated with an IgG dose-dependent activity attenuation. As a result, nicotine sequestered within the blood-stream cannot mediate its addictive effects on the CNS.

### **Summary of the invention**

            The invention provides an immunogenic composition comprising:

- a) a nicotine immunoconjugate; and
- b) an oil-in-water emulsion adjuvant which comprises a metabolisable oil and an emulsifying agent.

The immunogenic composition may be used to induce a specific anti-nicotine immune response in a subject, and so may be used in the treatment or prevention of nicotine addiction.

The invention further provides:

- 5       - an immunogenic composition of the invention for use as a medicament;
- an immunogenic composition of the invention for use in the prevention or treatment of nicotine addiction;
- use of an immunogenic composition of the invention for the manufacture of a medicament for the prevention or treatment of nicotine addiction;
- 10       - a method of raising a specific antibody response against nicotine in an individual, which method comprises administering an effective amount of an immunogenic composition of the invention to said individual;
- a method of preventing or treating nicotine addiction, which method comprises administering an effective amount of an immunogenic composition of the
- 15       invention to an individual in need thereof; and
- a method of producing a composition according to any one of the preceding claims, the method comprising combining a nicotine immunoconjugate with an oil-in-water emulsion adjuvant.

## 20       **Brief description of the drawings**

**Figure 1** shows the averaged quantity of nicotine-binding IgG in the sera of C57B1/6 mice (n = 13 per treatment group) immunized with the adjuvanted conjugate vaccines (NicA6-CRM and NicA4-CRM, adjuvanted with alum, AS04 or AS03).

25       **Figure 2** shows the averaged quantity of nicotine-binding IgG in the sera of BALB/c mice (n = 13 per treatment group) immunized with the adjuvanted conjugate vaccines (NicA6-CRM and NicA4-CRM, adjuvanted with alum, AS04 or AS03).

**Figures 3 and 4** show that antibodies generated in mice immunised with NicA4-CRM + AS03 are poorly cross-reactive with (bind nicotine much better than) cotinine. The results obtained for nicotine are shown as ■ and results for cotinine as  
 30       △.

**Detailed description**

The present invention provides an immunogenic composition which may be used in the prevention or treatment of nicotine addiction. The term "immunogenic composition", as used in the present invention, refers to a composition that comprises an immunogenic component capable of provoking an immune response in an individual, such as a human. Accordingly, in one embodiment the invention provides an immunogenic composition comprising a nicotine immunoconjugate and an oil-in-water emulsion adjuvant which comprises a metabolisable oil and an emulsifying agent. In another embodiment of the invention, the immunogenic composition of the invention is a vaccine, i.e. the immunogenic composition can be administered to a subject to raise an immune response against nicotine that is capable of preventing or treating nicotine addiction in the subject. Accordingly, the term "vaccine" as used herein refers to both therapeutic vaccines (for the treatment of the condition) as well as prophylactic vaccines (for prevention of the condition).

**Nicotine immunoconjugates**

The nicotine immunoconjugate used in the present invention consists of a nicotine hapten and a carrier moiety, which may be connected via a linker molecule. The nicotine hapten is capable of eliciting a specific anti-nicotine antibody response when attached to the carrier moiety, and so is the specificity-determining portion of the immunoconjugate. The nicotine hapten will typically mimic the structure of the native nicotine molecule, although it need not be directly derived or synthesized from nicotine. Any nicotine hapten that is capable of eliciting a specific anti-nicotine antibody response when linked to a carrier moiety may be used in the present invention. In the context of this invention, the term "nicotine hapten" is used to include both the specificity-determining portion (i.e. the nicotine-like structure) and the linker molecule used to attach it to the carrier, if present.

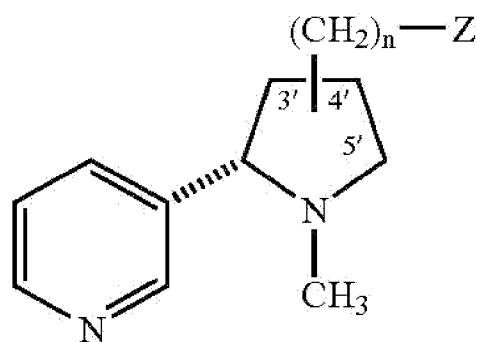
The nicotine hapten is not inherently immunogenic and requires coupling to a carrier moiety to elicit an antibody response. The term "carrier moiety" refers to a conjugation partner that can enable the nicotine hapten to induce a specific anti-nicotine antibody response when administered to a subject. Typical carrier moieties include proteins, peptides, virus-like particles (VLPs) and artificial vehicles such as microspheres. Proteins that are suitable for use as carriers include, but are not limited to, tetanus toxoid (TT), diphtheria toxoid (DT), diphtheria toxin mutant CRM197,

Pseudomonas exoprotein A (ExoA), cholera toxin B (CTB), pertussis toxin, outer membrane protein complex (OMPC) from *Neisseria meningitidis*, the B subunit of heat-labile *E. coli*, keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA).

The nicotine hapten may be covalently or non-covalently linked to the carrier moiety using any suitable method such as, but not limited to, covalent bond formation via amide, carbamate, urea, hydrazone, semicarbazone, thioether, triazole (Click chemistry) or disulfide bond formation. A linker molecule may be used in order to displace the nicotine hapten a certain distance from the carrier molecule to improve its immunogenicity. The linking may take place at any position on the nicotine molecule.

A number of nicotine haptens and immunoconjugates are already known in the art and may be used in the method of the invention. See, for example, the nicotine haptens and immunoconjugates disclosed in the following documents, which are incorporated by reference herein in their entirety: WO98/14216, US5876727 (Xenova); WO 99/61054, US6656469 (Independent Pharmaceutica); WO2004/009116, US6932971 (Cytos); and WO00/32239, US6232082 and US7247502 (Nabi).

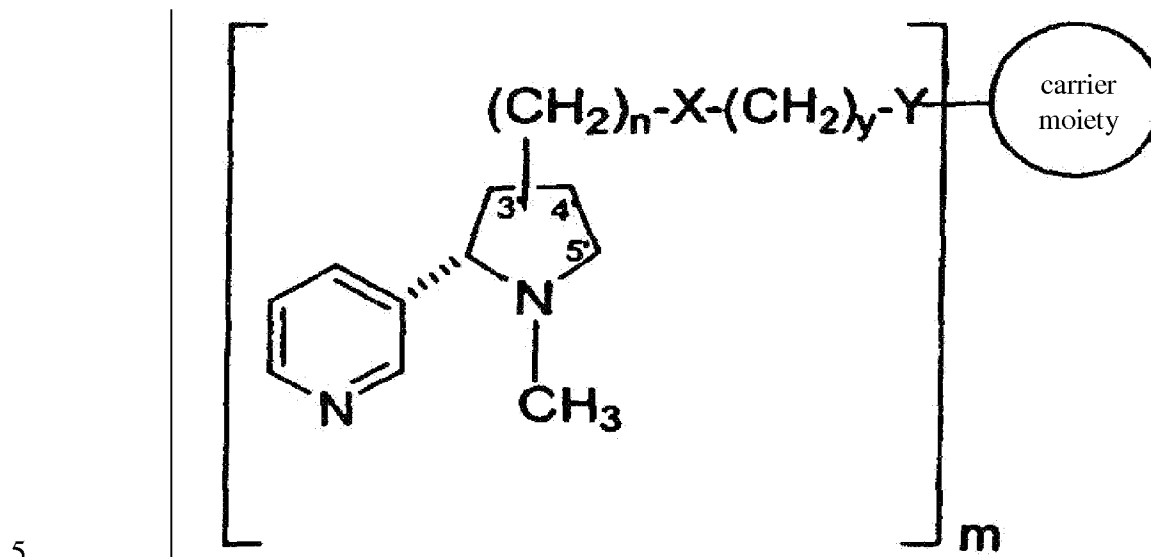
In one embodiment of the invention, the nicotine hapten is represented by formula (I):



wherein  $n$  is 0 to 12,  $Z$  is  $\text{NH}_2$ ,  $\text{COOH}$ ,  $\text{CHO}$  or  $\text{SH}$  and the  $-(\text{CH}_2)_n\text{-Z}$  moiety can be bonded to the 3', 4' or 5' position of nicotine. The  $Z$  moiety is capable of binding to the carrier moiety, directly or via an additional linker molecule. It will be apparent to a person skilled in the art that when the  $Z$  moiety is covalently attached to a linker molecule or directly to a carrier it will be modified by virtue of the covalent linking. For example, when covalently linked to a linker molecule or carrier the  $Z$  moiety may be represented by  $\text{NH-}$ ,  $\text{COO-}$ ,  $\text{CO-}$  or  $\text{S-}$  in the final structure. In a

particular embodiment, Z is NH<sub>2</sub> and the -(CH<sub>2</sub>)<sub>n</sub>-Z moiety is bonded to the 3' position of nicotine

In another embodiment of the invention, the nicotine immunoconjugate is of formula (II):



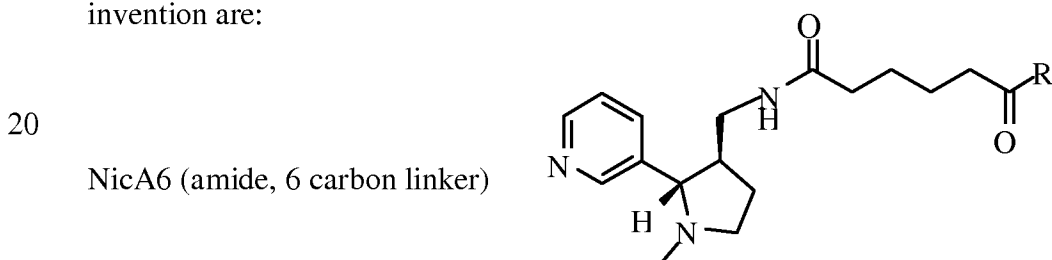
wherein m is 1 to 2500; n is 0 to 12; y is 1 to 12; X is selected from the group consisting of NH-CO, CO-NH, CO-NH-NH, NH-NH-CO, NH-CO-NH, CO-NH-NH-CO, and S-S; Y is selected from the group consisting of NH-CO, CO-NH, CO-NH-NH, NH-NH-CO, NH-CO-NH, CO-NH-NH-CO, and S-S; and the -(CH<sub>2</sub>)<sub>n</sub>-X-(CH<sub>2</sub>)<sub>y</sub>-Y- moiety is bonded to the 3', 4' or 5' position of nicotine. In a particular embodiment of the invention, X is NH-CO, Y is CO-NH and the -(CH<sub>2</sub>)<sub>n</sub>-X-(CH<sub>2</sub>)<sub>y</sub>-Y- moiety is bonded to the 3' position of nicotine .

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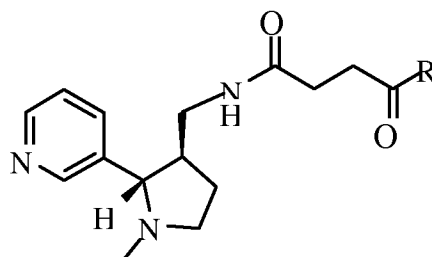
In certain embodiments of the invention, the terminal atom or atom group of the Y moiety (i.e. CO, NH or S) is derived from the carrier molecule. For example, the terminal NH group in the Y moiety of formula (II) may be derived from an amino acid in a carrier protein, for example a lysine residue.

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Two examples of nicotine immunoconjugates that may be used in the present invention are:



NicA4 (amide, 4 carbon linker)



wherein R is a carrier moiety.

In one aspect, the nicotine immunoconjugate is not AM1 (ether link, 6 carbon linker) in conjugation to carrier proteins keyhole limpet hemocyanin (KLH), tetanus toxoid (TT) or diphtheria toxin cross-reactive mutant 197 (CRM) as disclosed in Moreno *et al*: Molecular Pharmaceutics; Vol 7, NO. 2, 431-441.

### Oil-in-water emulsion adjuvant

The present invention is based on the finding that when nicotine immunoconjugates are adjuvanted with an oil-in-water emulsion, they induce significantly higher titer nicotine-specific antibody responses than the same immunoconjugates adjuvanted with alum or alum combined with 3D-MPL (3-de-O-acylated monophosphoryl lipid A). The improved immune response obtained using an oil-in-water adjuvant may mean that fewer doses of the nicotine immunoconjugate are required in order to have the same therapeutic effect as a non-adjuvanted nicotine immunoconjugate or with an aluminium-based adjuvant and/or that the therapeutic effect is longer lasting.

In order for any oil-in-water composition to be suitable for human administration, the oil phase of the emulsion system has to comprise a metabolisable oil. The meaning of the term “metabolisable” is well known in the art, and can be defined as ‘being capable of being transformed by metabolism’ (Dorland’s Illustrated Medical Dictionary, W.B. Sanders Company, 25th edition (1974)). The oil may be any vegetable oil, fish oil, animal oil or synthetic oil, which is not toxic to the recipient, and which is capable of being transformed by metabolism. Nuts, seeds, and grains are common sources of vegetable oils. Synthetic oils may also be used and can include commercially available oils such as NEOBEE® and others.

A suitable metabolisable oil is squalene (2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene), an unsaturated oil which is found in large quantities in shark-liver oil, and in lower quantities in olive oil, wheat germ oil, rice bran oil and yeast. Squalene is a metabolisable oil by virtue of the fact that it is an intermediate in the biosynthesis of cholesterol. The metabolisable oil is typically present in an amount of 0.5% to 10% (v/v) of the total volume of the immunogenic composition.

The oil-in-water emulsion adjuvant further comprises an emulsifying agent. A suitable emulsifying agent is polyoxyethylene sorbitan monooleate (Tween 80<sup>TM</sup>). The emulsifying agent is typically present in the adjuvant composition in an amount of 0.125 to 4% (v/v) of the total volume of the immunogenic composition.

The oil-in-water emulsion of the present invention may further comprise a tocol. Tocols are well known in the art and are described in EP0382271. A suitable tocol is alpha-tocopherol or a derivative thereof such as alpha-tocopherol succinate (also known as vitamin E succinate). The tocol is typically present in the adjuvant composition in an amount of 0.25% to 10% (v/v) of the total volume of the immunogenic composition.

In an oil-in-water emulsion, the oil and emulsifier should be in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline (PBS).

In one embodiment of the invention, the oil-in-water emulsion adjuvant comprises squalene, polyoxyethylene sorbitan monooleate (Tween 80<sup>TM</sup>) and alpha-tocopherol. Typically the oil-in-water emulsion adjuvant will comprise from 2 to 10% squalene, from 0.3 to 3% polyoxyethylene sorbitan monooleate and from 2 to 10% alpha-tocopherol (percentages are of the total volume of the immunogenic composition), and may be produced according to the procedure described in WO 95/17210. The amount of squalene may be equal to or less than the amount of alpha-tocopherol as this provides a more stable emulsion. The oil-in-water emulsion may also contain polyoxyethylene sorbitan trioleate (Span 85) and/or Lecithin, for example at a level of 1% of the total volume of the immunogenic composition.

Methods of producing oil-in-water emulsions are well known to the person skilled in the art. Commonly, the method comprises mixing the oil phase (which may comprise a tocol) with a surfactant such as a PBS/TWEEN80<sup>TM</sup> solution, followed by homogenisation using a homogenizer. A method comprising passing the mixture twice through a syringe needle would be suitable for homogenising small volumes of

liquid. Equally, the emulsification process in microfluidiser (M110S Microfluidics machine, maximum of 50 passes, for a period of 2 minutes at maximum pressure input of 6 bar (output pressure of about 850 bar)) could be adapted by a person skilled in the art to produce smaller or larger volumes of emulsion. The adaptation could be achieved by routine experimentation comprising the measurement of the resultant emulsion until a preparation was achieved with oil droplets of the required diameter.

The oil-in-water emulsion systems of the present invention may have a small oil droplet size in the sub-micron range. Suitably the droplet sizes will be in the range of 120 to 750 nm, for example sizes from 120 to 600 nm in diameter. The oil-in water emulsion may contain oil droplets of which at least 70% by intensity are less than 500 nm in diameter, at least 80% by intensity are less than 300 nm in diameter, or at least 90% by intensity are in the range of 120 to 200 nm in diameter.

The oil droplet size (i.e. diameter) according to the present invention is given by intensity. There are several ways of determining the diameter of the oil droplet size by intensity. Intensity is measured by use of a sizing instrument, suitably by dynamic light scattering such as the Malvern Zetasizer 4000 or the Malvern Zetasizer 3000HS. A first possibility is to determine the z average diameter ZAD by dynamic light scattering (PCS-Photon correlation spectroscopy); this method additionally gives the polydispersity index (PDI), and both the ZAD and PDI are calculated with the cumulants algorithm. These values do not require the knowledge of the particle refractive index. A second means is to calculate the diameter of the oil droplet by determining the whole particle size distribution by another algorithm, either the Contin, or NNLS, or the automatic "Malvern" one (the default algorithm provided for by the sizing instrument). Most of the time, as the particle refractive index of a complex composition is unknown, only the intensity distribution is taken into consideration, and if necessary the intensity mean originating from this distribution.

### **Nicotine specific antibodies**

As used herein, "nicotine-specific" antibodies are those that bind specifically to the nicotine molecule. Specific binding is distinguished from non-specific binding (e.g., binding to surfaces such as ELISA wells or blotting membranes). Nicotine-specific antibodies bind to nicotine with high affinity, compared to nonspecific binding.

For nicotine vaccines to be effective, the antibodies generated to nicotine after immunization must be able to bind nicotine as it moves from the lungs into the blood, in order to prevent nicotine movement into the brain. Cotinine is the major by-product of nicotine metabolism and, because of its long half-life, is present at much higher levels in the blood of smokers than nicotine (approximately 10-fold higher). If the antibodies generated by a vaccine bind to cotinine to a significant degree, the effectiveness of the antibodies for preventing movement of nicotine into the brain may be compromised.

As used herein, a “nicotine specific antibody” is one that binds more avidly to nicotine than to cotinine.

### **Treatment or prevention of nicotine addiction**

Dependence is a state in which there is a compulsive or chronic need for a drug or other substance (Dorlands Illustrated Medical Dictionary, 30<sup>th</sup> Edition, Saunders). Substance dependence is a compulsive use of a substance despite significant problems resulting from such use (*Id.*). Substance dependence may include physiological as well as psychological dependence.

Nicotine addiction can thus be defined as a dependence on the drug nicotine. Nicotine addiction may also be termed “nicotine use disorder” (see *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*).

The DSM-IV-TR provides criteria for the diagnosis of nicotine use disorder. Additionally, questionnaires such as the Fagerstrom Test for Nicotine Dependence (FTND) are also available to aid health care workers in determining the level of a subject’s nicotine dependence.

As used herein, treatment of nicotine addiction does not imply 100% success rate, i.e., does not imply that every subject treated will quit tobacco use indefinitely. A successful treatment for nicotine addiction is one that results in a higher percentage of subjects quitting or reducing tobacco use during or immediately after treatment, compared to a control group (subjects not receiving treatment for nicotine addiction). The effect of nicotine addiction treatment may be assessed by various parameters, such as decrease in intensity of tobacco use, cessation of tobacco use, the total length of time over which the decrease or cessation persists, or the number of subjects who maintain cessation of tobacco use over time.

As used herein, prevention of nicotine addiction does not imply 100% success rate. A preventive treatment for nicotine addiction is one that reduces the number of

subjects who become addicted to nicotine, compared to the number of subjects who become addicted in a control group not receiving the treatment.

### **Immunogenic composition formulation and administration**

5           The amount of the nicotine immunoconjugate of the present invention present in each dose of immunogenic composition is selected as an amount which induces the desired immune response without significant, adverse side effects. Such amount will vary depending upon which specific immunoconjugate is employed and the type and amount of oil-in-water adjuvant used. An optimal amount for a particular  
10 immunoconjugate may be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Generally, it is expected that each human dose will comprise 1-1000 $\mu$ g of immunoconjugate, for example 20-800 $\mu$ g, or 50-600 $\mu$ g, or 100-500 $\mu$ g. A typical human dose may contain approximately 20, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550 or 600 $\mu$ g of immunoconjugate. The  
15 term "human dose" is used to mean a dose which is in a volume suitable for human use. Generally this is between 0.3 and 1.5 ml. In one embodiment, a human dose is 0.5 ml.

Following an initial vaccination, subjects typically receive a boost after a certain interval of time. Additional boosts may also be given, for example a total of 2,  
20 3, 4 or 5 boost vaccinations. The initial and boost vaccinations may be given at 1, 2, 3, 4, 5 or 6 day intervals, at 1, 2, 3 or 4 week intervals, at 1, 2, 3, 4, 5 or 6 month intervals, at 1 year intervals, or at any combination of these time intervals.

In certain embodiments, the initial vaccination may consist of the immunogenic composition of the invention (nicotine immunoconjugate combined  
25 with an oil-in-water emulsion adjuvant) whereas the boosting composition may contain only the nicotine immunoconjugate as an active component, or the nicotine immunoconjugate in combination with a different adjuvant, such as alum.

The immunogenic compositions of the invention may be provided by any of a variety of routes such as oral, topical, subcutaneous, mucosal, intravenous,  
30 intramuscular, intranasal, sublingual, intradermal and via suppository. Typically the immunogenic composition will be administered via intramuscular injection or transdermally, for example using a patch. In some embodiments, the initial vaccination is given by one route of administration (for example by intramuscular

injection) whereas the boosting composition is given by a different route of administration (for example using a transdermal patch).

In another embodiment of the invention, the nicotine immunoconjugate and oil-in-water emulsion adjuvant may be co-administered separately. For example, the immunoconjugate and oil-in-water emulsion adjuvant may be administered simultaneously or within a short period of time (such as within 2, 5, 10 or 15 minutes) by the same or different routes of administration as discussed herein.

Use of the immunogenic composition may be prophylactic or therapeutic. The invention described herein is primarily but not exclusively concerned with therapeutic use of adjuvanted nicotine immunoconjugates for the treatment of nicotine addiction.

Appropriate pharmaceutically acceptable carriers or excipients for use in the invention are well known in the art and include for example water or buffers. Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenum Press New York, 1995. New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978.

The immunogenic compositions of the invention comprise certain components as laid out above. In a further aspect of the invention the immunogenic composition consists essentially of, or consists of, said components.

The present invention is now described with respect to the following examples which serve to illustrate the invention.

### **Example 1**

#### **Materials and methods**

##### *Synthesis of nicotine haptens*

The NicA4 and NicA6 haptens were synthesized according to the methodology disclosed in WO 00/32239 (Nabi).

##### *Conjugation of NicA4 to CRM-197*

NicA4 hapten was dissolved in dimethylformamide (DMF) to a concentration of 30-40 mg/mL. Next, HBTU (O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate) was added at a 1.5 molar excess of HBTU to hapten, followed

by diisopropylethylamine at a 2 molar excess to hapten. The mixture was incubated at room temperature for 30 minutes with shaking. The CRM-197 was diluted in 0.2M phosphate buffer, pH 7.2 to a concentration of 2 mg/mL. Then the diluted CRM-197 was added to the activated hapten at a 400 molar excess of hapten to CRM, mixed well and placed in refrigerator overnight. The NicA4-CRM immunoconjugate was purified using Amicon Ultra centrifugal cartridges with a 10 KD MWCO. The conjugate was washed 5 times with 10mM phosphate buffer, pH = 7.2 and centrifuged at 10 -20°C at ref=1500 for 20 minutes or until retentate volume was reduced to 1-1.5 mL. The conjugate was then reconstituted to approx. 2 mg/mL protein, and sterile filtered using a 0.22µ PVDF filter.

#### *Conjugation of NicA6 to CRM-197*

NicA6 was conjugated to CRM-197 protein carrier using the resin carbodiimide/S-NHS activation method. To prepare the activated ester of NicA6, enough resin carbodiimide (N-cyclohexylcarbodiimide) for a 5 x molar excess relative to hapten was added to a sterile reaction vessel of appropriate volume. A solution of N-hydroxysulfosuccinimide sodium salt (S-NHS) was prepared by dissolving 2 x molar equivalents of S-NHS relative to hapten in 1µL water for every 1mg S-NHS. The NicA6 hapten was prepared as a 20-30 mg/mL solution in dimethylformamide (DMF). The 5 x molar excess of resin carbodiimide was transferred to the hapten/DMF solution, then the entire volume of S-NHS was added to the hapten/DMF/resin solution. Finally 2.0 molar equivalents of diisopropylethylamine (DIEA) relative to hapten were added. The solution was left to react overnight with slow vortexing at room temperature.

To conjugate the activated ester of NicA6 to the protein carrier (CRM-197, diphtheria toxoid), the CRM-197 solution was diluted to 3 mg/ml. After the resin had settled (or had been briefly centrifuged) the supernatant was drawn off and added to the CRM-197 solution. The mixture was allowed to react at room temperature for 20 - 24 hours or until RP-HPLC showed no hapten-SNHS ester remaining. Purification of the conjugate was carried out by either ultrafiltration or small scale dialysis.

*Hapten:carrier ratio*

The nicotine immunoconjugates were analyzed for hapten:carrier ratio with the following results: NicA6-CRM (Lot #1525-161, 17 haptens / carrier, 4276 µg/mL) NicA4-CRM (Lot #1520-173, 14 haptens / carrier, 3155 µg/mL).

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*Preparation of AS03*

The oil-in-water emulsion used in the subsequent examples (AS03) is composed of an organic phase made of two oils (alpha-tocopherol and squalene), and an aqueous phase of phosphate buffered saline (PBS) containing polyoxyethylene sorbitan monooleate (Tween 80<sup>TM</sup>) as emulsifying agent. Unless otherwise stated, the oil-in-water emulsion adjuvant formulations used in the subsequent examples were made comprising the following oil-in-water emulsion component (final concentrations given): 2.5% squalene (v/v), 2.5% alpha-tocopherol (v/v), 0.9% polyoxyethylene sorbitan monooleate (v/v). This emulsion was prepared as follows as a two-fold concentrate.

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The emulsion is made by mixing under strong agitation an oil phase composed of hydrophobic components ( $\alpha$ -tocopherol and squalene) and an aqueous phase containing the water soluble components (polyoxyethylene sorbitan monooleate and PBS mod (modified), pH 6.8). While stirring, the oil phase (1/10 total volume) is transferred to the aqueous phase (9/10 total volume), and the mixture is stirred for 15 minutes at room temperature. The resulting mixture is then subjected to shear, impact and cavitation forces in the interaction chamber of a microfluidizer (15000 PSI – 8 cycles) to produce submicron droplets (distribution between 100 and 200 nm). The resulting pH is between  $6.8 \pm 0.1$ . The emulsion is then sterilised by filtration through a 0.22 µm membrane and the sterile bulk emulsion is stored refrigerated in Cupac containers at 2 to 8°C. Sterile inert gas (nitrogen or argon) is flushed into the dead volume of the emulsion final bulk container for at least 15 seconds.

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*Formulation of adjuvanted immunoconjugates*

Each immunoconjugate (NicA4-CRM and NicA6-CRM) was combined with one of three adjuvants: alum (alhydrogel aluminum hydroxide batch 4095) at 50 µg Al+++ per dose; AS03 (oil-in-water emulsion) at 25 µl per dose; or ASO4 (alum and 3D-MPL) at 50 µg Al+++ combined with 5 µg 3D-MPL per dose. The ASO4-containing preparations were made as follows: 1 or 10 µg of immunoconjugate was

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adsorbed to 30 µg of alum. A preparation of 3D-MPL adsorbed to alum was added to make a final dosage of 1 or 10 µg immunoconjugate, 50 µg alum and 5 µg 3D-MPL in 50 µL.

#### 5 *Immunogenicity of adjuvanted nicotine immunoconjugates in mice*

C57Bl/6 or BALB/c female mice obtained from *The Jackson Laboratory, Bar Harbor, Maine* were fed ad libitum, housed 13 mice per cage, and allowed free access to sterile water and food. The mice were 13 weeks old when the primary vaccination was administered. The adjuvanted nicotine immunoconjugates were administered at 10 µg/dose or 1 µg/dose (see Table 1). Sufficient antigen/alum solution was prepared for both the primary and secondary injections in the case of AS04 solutions. Diluent was 10 mM sodium phosphate buffer, pH 7.2. Two 50 µl injections were performed, 14 days apart via the intramuscular route in alternating (right for primary, left for secondary) biceps femoris muscles. Blood was collected on day 21 via cardiac draw on anesthetized animals. Blood was allowed to clot, and serum was collected and used in the following analyses to determine anti-nicotine antibody concentration and affinity.

TABLE 1: Test groups, each group included 26 mice (n=13 C57Bl/6 mice and n=13 BALB/c mice)

| Group | Hapten | Conjugate | Conjugate Dose (ul) | Adjuvant |
|-------|--------|-----------|---------------------|----------|
| A     | Nic-A6 | CRM197    | 10                  | Alum     |
| B     | Nic-A6 | CRM197    | 10                  | AS04     |
| C     | Nic-A6 | CRM197    | 10                  | AS03     |
| D     | Nic-A6 | CRM197    | 1                   | Alum     |
| E     | Nic-A6 | CRM197    | 1                   | AS04     |
| F     | Nic-A6 | CRM197    | 1                   | AS03     |
|       |        |           |                     |          |
| G     | Nic-A4 | CRM197    | 10                  | Alum     |
| H     | Nic-A4 | CRM197    | 10                  | AS04     |
| I     | Nic-A4 | CRM197    | 10                  | AS03     |
| J     | Nic-A4 | CRM197    | 1                   | Alum     |
| K     | Nic-A4 | CRM197    | 1                   | AS04     |
| L     | Nic-A4 | CRM197    | 1                   | AS03     |

### *Antibody Characterization*

Antibodies were analysed using the Octet Red (ForteBio) instrument and software. NicA4-ubiquitin conjugates were coupled to biotin (Thermo cat#21329  
5 Easy-link PEG4 biotin) 5x excess of biotin. Strep-avidin biosensor (ForteBio) was prepared according to vendor. Briefly, sensors are re-hydrated in PBS/0.05% Tween at room temp, for at least 30 min at 300 rpm. Biotinylated conjugate was allowed to couple to the sensor in excess at room temp, for at least 15 minutes with gentle orbital rotation or overnight at 4°C with no shaking. For concentration and kinetics, serum  
10 was diluted to an appropriate concentration in PBS/0.05% Tween, positive control sera (produced in house) and or monoclonal 9D9 (Scripps Institute Janda, K) and normal mouse sera were included on each plate. Tips were regenerated in 10 mM HCl. Displacement experiments used sera at a 1:25 dilution. Nicotine bitartrate (Sigma) pH 7 in PBS/0.05% Tween was used at 1:10 dilutions from 100mM. S-(-)-  
15 Cotinine (TRC) pH 7 in PBS/0.05% Tween used at 1:10 dilutions from 1 M. Percent displacement is calculated against PBS/0.05% Tween alone.

Competitive radioimmunoassay was based on Journal of Immunologic Methods, 34 (1980)345-352, completed with pooled sera from various groups known to contain high titers of antibodies that bind to nicotine. Briefly, Nicotine, L-(-)-[N-  
20 Methyl-<sup>3</sup>H]- (Perkin Elmer) was added to an appropriate nicotine or cotinine solution, made in RIA buffer at various concentrations. 50 µl of tritiated nicotine / cold nicotine or cotinine mix and 50 µl sera or RIA buffer were combined and incubated 2hr at RT. 100 µl ice cold saturated ammonium sulfate, pH 7, solution was added dropwise to each tube while vortexing, and tubes were vortexed for an additional 5  
25 sec. and incubated 15 min at RT, then spun in microfuge at 12,000 rpm 3 min. 100 µl of supernatant was removed and added directly to vial containing scintillation fluid (National Diagnostics EcoScint). Counts were performed on Packard TriCarb 2900.

### **Results**

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#### *Quantitation of antibodies that bind to nicotine*

The quantity of nicotine-binding IgG in the sera of mice immunized with the conjugate vaccines was determined by extrapolation from a standard curve generated with a monoclonal antibody (mAb 9D9) specific for nicotine. Serum from each of the

thirteen C57bl/6 mice in each of the twelve groups shown in Table 1 was tested, as was serum from each of the thirteen Balb/6 mice in each of the groups.

The data obtained in C57Bl/6 mice is presented in Table 2 and Figure 1, and the data obtained in BALB/c mice is presented in Table 3 and Figure 2. Both

- 5 conjugates induced high levels of nicotine-binding IgG when combined with AS03. In the C57Bl/6 mice, AS03 had the strongest adjuvant effect, evidenced by the fact that the vaccines containing AS03 induced significantly higher levels of nicotine-binding IgG than the vaccines containing alum or AS04 ( $p < 0.05$ ). This difference was apparent at both doses of conjugate tested. The results were essentially the same in
- 10 BALB/c mice, though the difference between vaccines containing AS03 and the AS04 was not statistically significant when NicA4-CRM was used as the antigen.

Table 2 - Antibody Concentration Results for C57bl/6 mice

| Group | Conjugate +<br>adjuvant | Conjugate<br>Dose ( $\mu$ L) | Average<br>( $\mu$ g/mL) | Standard<br>Deviation | Standard Error<br>of Mean |
|-------|-------------------------|------------------------------|--------------------------|-----------------------|---------------------------|
| A     | NicA6-CRM+alum          | 10                           | 99                       | 54                    | 17                        |
| B     | NicA6-CRM+AS03          | 10                           | 1158                     | 344                   | 109                       |
| C     | NicA6-CRM+AS04          | 10                           | 330                      | 127                   | 40                        |
| D     | NicA6-CRM+alum          | 1                            | 67                       | 36                    | 11                        |
| E     | NicA6-CRM+AS03          | 1                            | 462                      | 201                   | 63                        |
| F     | NicA6-CRM+AS04          | 1                            | 237                      | 116                   | 37                        |
|       |                         |                              |                          |                       |                           |
| G     | NicA4-CRM+alum          | 10                           | 112                      | 36                    | 11                        |
| H     | NicA4-CRM+AS03          | 10                           | 794                      | 244                   | 77                        |
| I     | NicA4-CRM+AS04          | 10                           | 320                      | 76                    | 24                        |
| J     | NicA4-CRM+alum          | 1                            | 102                      | 46                    | 14                        |
| K     | NicA4-CRM+AS03          | 1                            | 401                      | 257                   | 81                        |
| L     | NicA4-CRM+AS04          | 1                            | 270                      | 95                    | 30                        |

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Table 3 - Antibody Concentration Results for Balb/c mice

| Group | Conjugate +<br>adjuvant | Conjugate<br>dose | Average<br>( $\mu$ g/mL) | Standard<br>Deviation | Standard Error of<br>Mean |
|-------|-------------------------|-------------------|--------------------------|-----------------------|---------------------------|
| A     | NicA6-CRM+alum          | 10                | 219                      | 129                   | 41                        |
| B     | NicA6-CRM+AS03          | 10                | 393                      | 152                   | 48                        |

|   |                |    |     |     |    |
|---|----------------|----|-----|-----|----|
| C | NicA6-CRM+AS04 | 10 | 308 | 109 | 34 |
| D | NicA6-CRM+alum | 1  | 46  | 36  | 11 |
| E | NicA6-CRM+AS03 | 1  | 403 | 80  | 25 |
| F | NicA6-CRM+AS04 | 1  | 473 | 194 | 61 |
|   |                |    |     |     |    |
| G | NicA4-CRM+alum | 10 | 282 | 154 | 49 |
| H | NicA4-CRM+AS03 | 10 | 984 | 189 | 60 |
| I | NicA4-CRM+AS04 | 10 | 653 | 168 | 53 |
| J | NicA4-CRM+alum | 1  | 178 | 90  | 28 |
| K | NicA4-CRM+AS03 | 1  | 623 | 156 | 49 |
| L | NicA4-CRM+AS04 | 1  | 333 | 142 | 45 |

*Affinity of antibodies that bind to nicotine*

- 5 The affinity data (dissociation rate constant  $K_{off}$ ) for the nicotine-binding IgG in the serum samples is presented in Table 4 (C57Bl/6 mice) and Table 5 (BALB/c mice). In C57Bl/6 mice, AS03 nearly always induced higher affinity antibodies than alum or AS04, and the differences were statistically significant most of the time (Table 2). The findings were similar in the BALB/c mice (Table 3).

10 Table 4

| C57Bl/6 |                  |                   |           | T-test (p value)      |                      |              |
|---------|------------------|-------------------|-----------|-----------------------|----------------------|--------------|
| Group   | Vaccine          | Conjugate Dose ug | AVG Koff  |                       | AS03 vs AS04 or alum | AS04 vs alum |
| A       | NicA6-CRM +alum  | 10                | 4.303E-04 |                       | 0.00073              | 0.00183      |
| B       | NicA6-CRM +AS03  | 10                | 1.994E-04 |                       |                      |              |
| C       | NicA6-CRM + AS04 | 10                | 2.230E-04 |                       | 0.42367              |              |
| D       | NicA6-CRM +alum  | 1                 | 1.734E-04 |                       | 0.69916              | 0.25195      |
| E       | NicA6-CRM +AS03  | 1                 | 1.540E-04 |                       |                      |              |
| F       | NicA6-CRM +AS04  | 1                 | 2.937E-04 |                       | 0.04062              |              |
|         |                  |                   |           | <b>NicA6 v. NicA4</b> |                      |              |
| G       | NicA4-CRM +alum  | 10                | 3.171E-04 | 0.22363               | 0.04612              | 0.57422      |
| H       | NicA4-CRM +AS03  | 10                | 2.083E-04 | 0.73548               |                      |              |
| I       | NicA4-CRM +AS04  | 10                | 3.720E-04 | 0.00412               | 0.00053              |              |
| J       | NicA4-CRM +alum  | 1                 | 3.977E-04 | 0.03919               | 0.14628              | 0.72544      |

|   |                    |   |           |         |         |  |
|---|--------------------|---|-----------|---------|---------|--|
| K | NicA4-CRM<br>+AS03 | 1 | 2.493E-04 | 0.04430 |         |  |
| L | NicA4-CRM<br>+AS04 | 1 | 5.110E-04 | 0.07852 | 0.04280 |  |

Table 5

| BALB/c |                    |                      |             | T-test (p value):         |                            |                 |
|--------|--------------------|----------------------|-------------|---------------------------|----------------------------|-----------------|
| Group  | Vaccine            | Conjugate<br>Dose µg | AVG<br>Koff |                           | AS03 vs<br>AS04 or<br>alum | AS04 vs<br>alum |
| A      | NicA6-CRM<br>+alum | 10                   | 4.556E-04   |                           | 0.00208                    | 0.13464         |
| B      | NicA6-CRM<br>ASO3+ | 10                   | 2.370E-04   |                           |                            |                 |
| C      | NicA6-CRM<br>+AS04 | 10                   | 3.574E-04   |                           | 0.01034                    |                 |
| D      | NicA6-CRM<br>+alum | 1                    | 5.298E-04   |                           | 0.29432                    | 0.51528         |
| E      | NicA6-CRM<br>+ASO3 | 1                    | 4.402E-04   |                           |                            |                 |
| F      | NicA6-CRM<br>+ASO4 | 1                    | 5.919E-04   |                           | 0.04327                    |                 |
|        |                    |                      |             | <b>NicA6 vs<br/>NicA4</b> |                            |                 |
| G      | NicA4-CRM<br>+alum | 10                   | 6.259E-04   | 0.168487                  | 0.00011                    | 0.00242         |
| H      | NicA4-CRM<br>ASO3+ | 10                   | 1.499E-04   | 0.012667                  |                            |                 |
| I      | NicA4-CRM<br>+AS04 | 10                   | 1.929E-04   | 0.001026                  | 0.12711                    |                 |
| J      | NicA4-CRM<br>+alum | 1                    |             |                           |                            |                 |
| K      | NicA4-CRM<br>+ASO3 | 1                    | 2.599E-04   | 0.000000                  |                            |                 |
| L      | NicA4-CRM<br>+ASO4 | 1                    | 4.012E-04   | 0.000001                  | 0.00178                    |                 |

### *Specificity of anti-nicotine antibodies*

- 5 For nicotine vaccines to be effective, the antibodies generated to nicotine after immunization must be able to bind nicotine as it moves from the lungs into the blood in order to prevent its movement into the brain. Cotinine is the major by-product of nicotine metabolism and, because of its long half-life, is present at much higher levels in the blood of smokers than nicotine (~10-fold higher). If the antibodies generated by
- 10 nicotine vaccines bind to cotinine to a significant degree, it is possible that the effectiveness of the antibodies for preventing movement of nicotine into the brain will be compromised. For this reason, it is important to determine the level to which nicotine-specific antibodies generated by candidate vaccines cross-react with cotinine.

To determine the level of cross-reactivity between nicotine and cotinine, antibodies generated after vaccination of mice with NicA4-CRM plus AS03 were allowed to bind to biosensors coated with nicotine immunoconjugate-ubiquitin. The nicotine immunoconjugate used to coat the biosensors contained a different linker molecule to that of NicA4, in order to avoid detection of anti-linker antibodies. The amount of free nicotine or cotinine required to displace the antibodies from the nicotine hapten was then determined and the results compared. Using sera from one of the BALB/c mice immunized with NicA4-CRM plus AS03, we found that more than 500 times more cotinine than nicotine was required to displace 50% of the antibodies bound to nicotine on the biosensor (Figure 3). The data indicate that the vaccine induces antibodies with high specificity for nicotine, and that the quantity of cotinine in smokers blood would probably not be sufficient to interfere with the ability of these antibodies to prevent movement of nicotine from the blood into the brain.

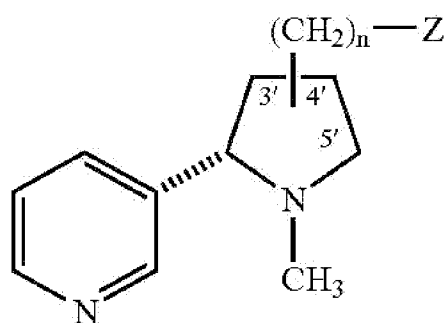
Radioimmunoassay was also used to evaluate the level of antibody cross-reactivity between nicotine and cotinine. Because there is no pre-binding of the antibodies to nicotine tethered to a carrier in this assay, any antibodies induced by the vaccine that bind to determinants on the tether become irrelevant. Thus, the RIA assay allows a more “pure” evaluation of the nicotine binding ability of the antibodies. In this system, radiolabelled (tritium) “hot” nicotine was bound to the antibodies in pooled sera from C57Bl/6 mice immunized with NicA4-CRM plus AS03. The amount of free “cold” nicotine or cotinine required to prevent binding of the “hot” nicotine was determined. As shown in Figure 4, more than 3500 times more “cold” cotinine than nicotine was required to displace the 25% of the “hot” nicotine from the antibodies generated after immunization with NicA4-CRM plus AS03, indicating once again that the antibodies induced by this vaccine do not cross-react with cotinine to a significant degree.

### *Conclusion*

These results indicate that nicotine immunoconjugate vaccines consisting of nicotine haptens coupled to CRM and adjuvanted with AS03 (oil-in-water emulsion) induce significantly higher titre nicotine-specific antibody responses than the same vaccines adjuvanted with alum or AS04 (alum and 3D-MPL). The antibodies induced by the vaccines have moderate affinity for nicotine and appear to be nicotine-specific, since cotinine, the major by-product of nicotine metabolism, is poorly cross-reactive.

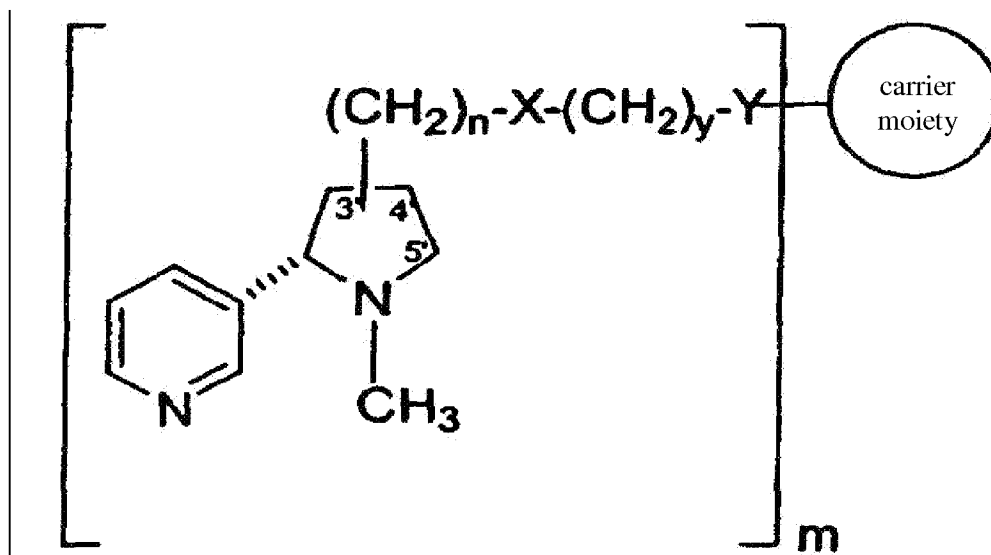
CLAIMS

1. An immunogenic composition comprising:
  - (a) a nicotine immunoconjugate; and
  - (b) an oil-in-water emulsion adjuvant which comprises a metabolisable oil and an emulsifying agent.
2. A composition according to claim 1, wherein the nicotine immunoconjugate consists of a nicotine hapten, optionally containing a linker molecule, and a carrier moiety.
3. A composition according to claim 2, wherein the nicotine hapten is represented by formula (I):



wherein n is 0 to 12; Z is NH<sub>2</sub>, COOH, CHO or SH and is capable of binding to the carrier molecule, either directly or via an additional linker molecule; and the -(CH<sub>2</sub>)<sub>n</sub>-Z moiety is bonded to the 3', 4' or 5' position of nicotine.

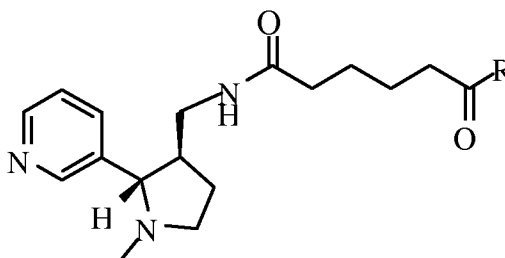
4. A composition according to any one of the preceding claims, wherein the nicotine immunoconjugate is of formula (II):



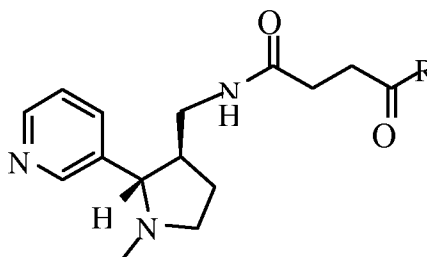
wherein m is 1 to 2500; n is 0 to 12; y is 1 to 12; X is selected from the group consisting of NH-CO, CO-NH, CO-NH-NH, NH-NH-CO, NH-CO-NH, CO-NH-NH-CO, and S-S; Y is selected from the group consisting of NH-CO, CO-NH, CO-NH-NH, NH-NH-CO, NH-CO-NH, CO-NH-NH-CO, and S-S; and the  $-(CH_2)_n-X-(CH_2)_y-Y-$  moiety is bonded to the 3', 4' or 5' position of nicotine.

5. A composition according to any one of the preceding claims, wherein the nicotine immunoconjugate is either of:

NicA6



NicA4



wherein R is a carrier moiety.

6. A composition according to any one of the preceding claims, wherein the carrier moiety is tetanus toxoid (TT), diphtheria toxoid (DT), CRM197, Pseudomonas exoprotein A (ExoA), keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA).
7. A composition according to any one of the preceding claims, wherein the metabolisable oil is squalene.
8. A composition according to any one of the preceding claims, wherein the emulsifying agent is polyoxyethylene sorbitan monooleate.
9. A composition according to any one of the preceding claims wherein the oil-in-water emulsion adjuvant further comprises a tocol, optionally alpha-tocopherol.
10. A composition according to any of the preceding claims, wherein the oil-in-water emulsion adjuvant comprises squalene, polyoxyethylene sorbitan monooleate and alpha-tocopherol.

11. A method of producing a composition according to any one of the preceding claims, the method comprising combining a nicotine immunoconjugate with an oil-in-water emulsion adjuvant.
- 5 12. A composition according to any one of claims 1 to 10 for use as a medicament.
13. A composition according to any one of claims 1 to 10 for use in the prevention or treatment of nicotine addiction.
- 10 14. Use of a composition according to any one of claims 1 to 10 for the manufacture of a medicament for the prevention or treatment of nicotine addiction.
- 15 15. A method of raising a specific antibody response against nicotine in an individual, which method comprises administering an effective amount of an immunogenic composition according to any one of claims 1 to 10 to said individual.
16. A method of preventing or treating nicotine addiction, which method comprises administering an effective amount of an immunogenic composition according to any one of claims 1 to 10 to an individual in need thereof.

Figure 1

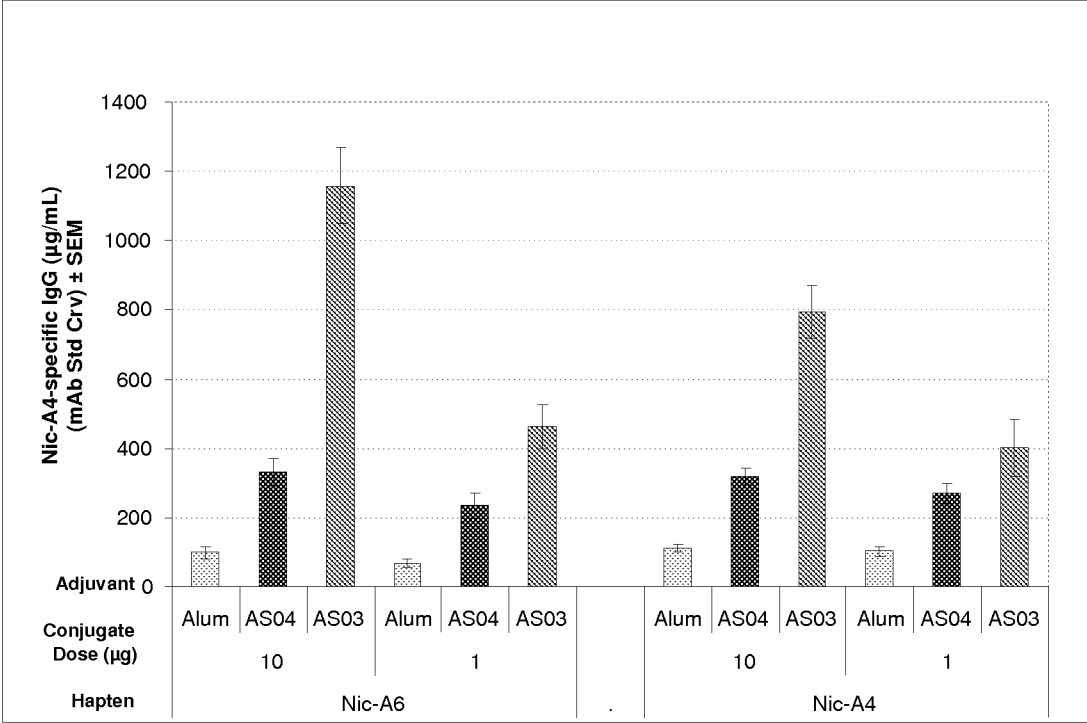


Figure 2

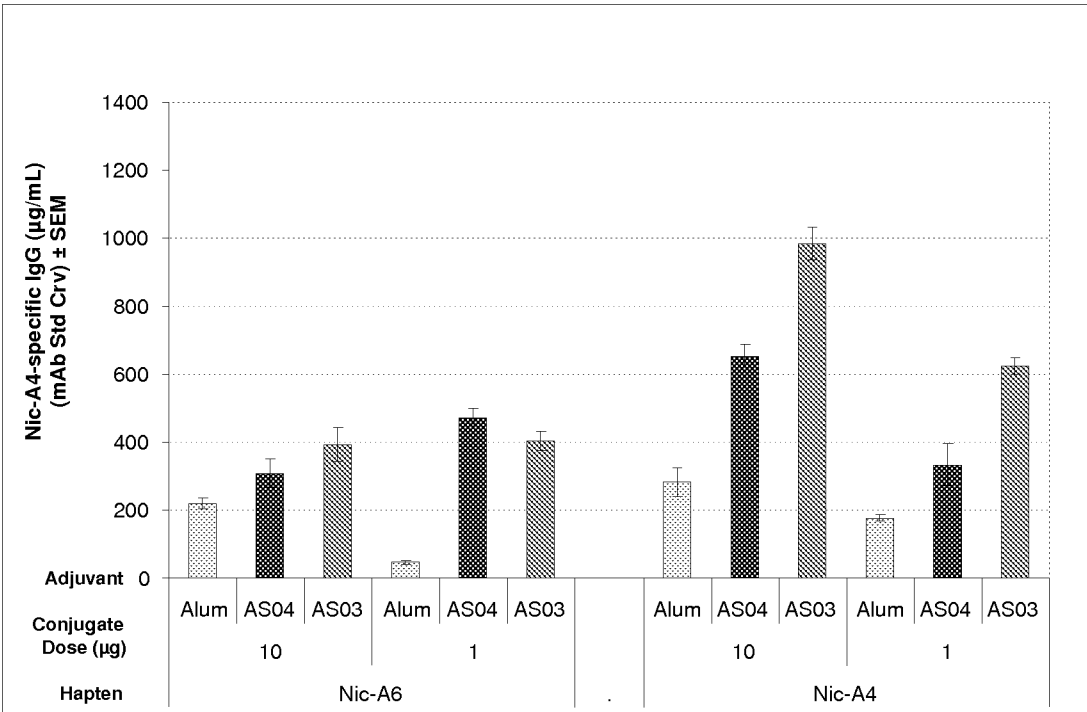


Figure 3

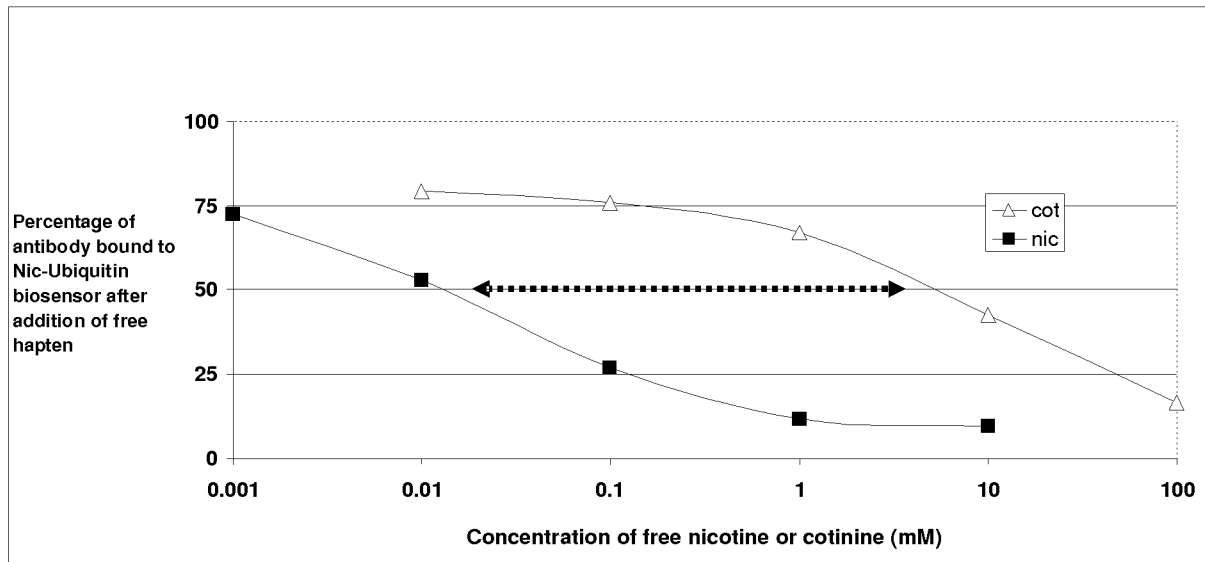
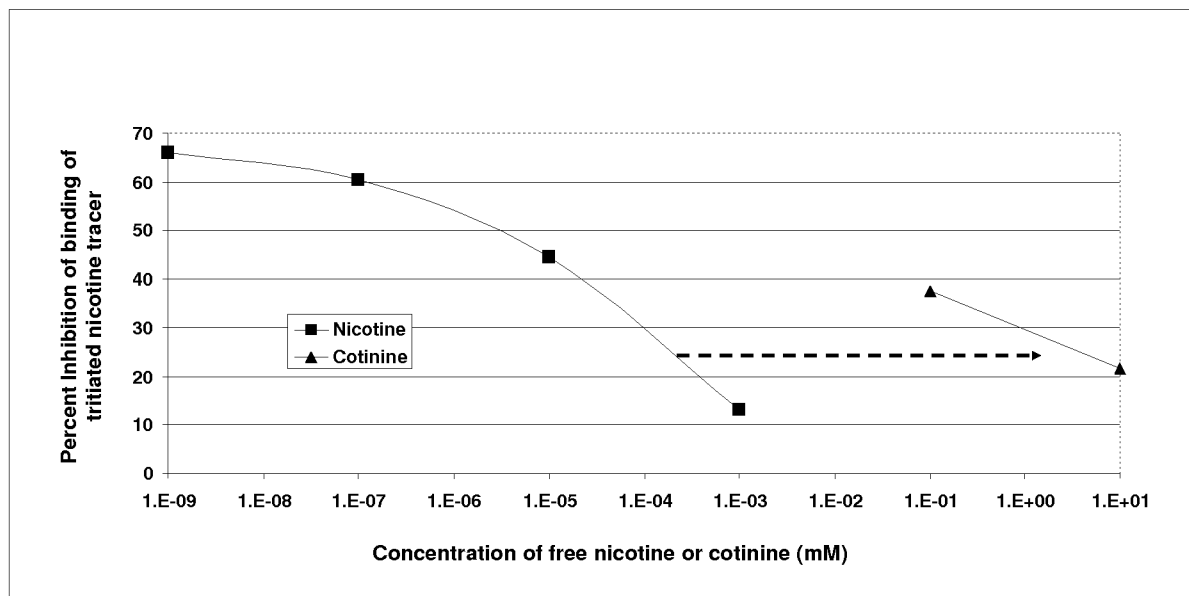


Figure 4



# INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2010/058328

## A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/00 A61K39/39 A61K47/48 A61P25/34 C07K16/16  
ADD.

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)

C07K A61K

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 practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                           | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | MORENO AMIRA Y ET AL: "A Critical Evaluation of a Nicotine Vaccine within a Self-Administration Behavioral Model", MOLECULAR PHARMACEUTICS, vol. 7, no. 2; March 2010 (2010-03), pages 431-441, XP007915865, ISSN: 1543-8384 | 1,2,6-16              |
| Y         | figures 1-5; tables 1-3                                                                                                                                                                                                      | 3-5                   |
| Y         | WO 00/32239 A1 (NABI [US]; ENNIFAR SOFIANE [US]; FATTOM ALI IBRAHIM [US]; NASO ROBERT) 8 June 2000 (2000-06-08)                                                                                                              | 3-5                   |
| A         | page 4, line 10 - page 8, line 14; claims 1-35; examples 1-14                                                                                                                                                                | 1,2,6-16              |
|           | -/--                                                                                                                                                                                                                         |                       |



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

17 January 2011

Date of mailing of the international search report

25/01/2011

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Authorized officer

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# INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/058328

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                             | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | WO 2009/120954 A2 (SCRIPPS RESEARCH INST [US]; JANDA KIM D [US]; DICKERSON TOBIN J [US]) 1 October 2009 (2009-10-01)                                                                                                                                                                                                                                                                           | 1,2,6,<br>11-16       |
| A         | examples 1-7                                                                                                                                                                                                                                                                                                                                                                                   | 7-10                  |
| A         | -----<br>CERNY E H ET AL: "Vaccines against nicotine",<br>HUMAN VACCINES 2009 LANDES BIOSCIENCE USA,<br>vol. 5, no. 4, 2009, pages 200-205,<br>XP007915864,<br>ISSN: 1554-8600<br>page 202, right-hand column, lines 21-41                                                                                                                                                                     | 1-16                  |
| Y         | -----<br>KEYLER D E ET AL: "Enhanced immunogenicity of a bivalent nicotine vaccine",<br>INTERNATIONAL IMMUNOPHARMACOLOGY,<br>ELSEVIER, AMSTERDAM, NL,<br>vol. 8, no. 11,<br>1 November 2008 (2008-11-01), pages<br>1589-1594, XP025408613,<br>ISSN: 1567-5769, DOI:<br>DOI:10.1016/J.INTIMP.2008.07.001<br>[retrieved on 2008-07-24]                                                           | 4,5                   |
| A         | figures 1-4                                                                                                                                                                                                                                                                                                                                                                                    | 1,2,6-16              |
| A         | -----<br>OLIVER ET AL: "Development of an anti-cotinine vaccine to potentiate nicotine-based smoking cessation strategies",<br>VACCINE, ELSEVIER LTD, GB,<br>vol. 25, no. 42,<br>28 September 2007 (2007-09-28), pages<br>7354-7362, XP022277613,<br>ISSN: 0264-410X, DOI:<br>DOI:10.1016/J.VACCINE.2007.08.019<br>figure 1                                                                    | 4-16                  |
| A         | -----<br>CARRERA M R A ET AL: "A SECOND-GENERATION VACCINE PROTECTS AGAINST THE PSYCHOACTIVE EFFECTS OF COCAINE",<br>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES (PNAS),<br>NATIONAL ACADEMY OF SCIENCE, US,<br>vol. 98, no. 4,<br>13 February 2001 (2001-02-13), pages<br>1988-1992, XP008063031,<br>ISSN: 0027-8424, DOI:<br>DOI:10.1073/PNAS.041610998<br>figure 1 | 4-16                  |
|           | -----                                                                                                                                                                                                                                                                                                                                                                                          |                       |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP2010/058328

## 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.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## 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 additional 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 fees.
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.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 3(completely); 1, 2, 6-16(partially)

An immunogenic composition comprising:

- (a) a nicotine immunoconjugate; and
- (b) an oil-in-water emulsion adjuvant which comprises a metabolisable oil and an emulsifying agent, wherein the nicotine immunoconjugate consists of a nicotine hapten, optionally containing a linker molecule, and a carrier moiety, and wherein the nicotine hapten is represented by formula (I), wherein n is 0 to 12; Z is NH<sub>2</sub>, COOH, CHO or SH and is capable of binding to the carrier molecule, either directly or via an additional linker molecule; and the -(CH<sub>2</sub>)<sub>n</sub>-Z moiety is bonded to the 3', 4' or 5' position of nicotine. Subject-matter related thereto.

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2. claims: 4(completely); 1, 2, 6-16(partially)

An immunogenic composition comprising:

- (a) a nicotine immunoconjugate; and
- (b) an oil-in-water emulsion adjuvant which comprises a metabolisable oil and an emulsifying agent, wherein the nicotine immunoconjugate is of formula (II), wherein m is 1 to 2500; n is 0 to 12; y is 1 to 12; X is selected from the group consisting of NH-CO, CO-NH, CO-NH-NH, NH-NH-CO, NH-CO-NH, CO-NH-NH-CO, and S-S; Y is selected from the group consisting of NH-CO, CO-NH, CO-NH-NH, NH-NH-CO, NH-CO-NH, CO-NH-NH-CO, and S-S; and the -(CH<sub>2</sub>)<sub>n</sub>-X-(CH<sub>2</sub>)<sub>y</sub>-Y- moiety is bonded to the 3', 4' or 5' position of nicotine. Subject-matter related thereto.

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3. claims: 5(completely); 1, 2, 6-16(partially)

Idem as item 2, wherein the nicotine immunoconjugate is either of Nica6 or Nica4 depicted in claim 5 Subject-matter related thereto.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2010/058328

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