



No. 06-110710- -00003-0000

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Act. 538 PETICION Folios: 3

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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Re: P1.-GLAXOSMITHKLINE BIOLOGICALS S.A.-Solicitud de Patente de Invención en Fase Nacional PCT para "VACUNA POLIVALENTE BASADA EN MAGE-3 Y NY-ESO-1 PARA INMUNOTERAPIA DE CANCER".-Clase A61K 39/00.-Expediente número 06-110.710.-

1. Me refiero a mi memorial de 6 de Febrero de 2007.
2. De conformidad con lo dispuesto en el Artículo 5.2.12 de la Circular Externa No. 03 de 28 de Abril de 2003, y estando dentro del plazo de seis meses contados desde la fecha de la publicación de la solicitud en la Gaceta de la Propiedad Industrial número **584** de 31 de Diciembre de 2007, solicito a ese Despacho proceder a realizar el examen de patentabilidad de la presente invención.
3. Por otro lado, atentamente me permito manifestar a usted que en el extracto de la solicitud arriba nombrada, publicado en la Gaceta de la Propiedad Industrial número **584** de 31 de Diciembre de 2007, página 483, el título de la invención aparece publicado en forma equivocada, el título correcto es: "VACUNA POLIVALENTE **BASADA** EN MAGE-3 Y NY-ESO-1 PARA INMUNOTERAPIA DE CANCER.
4. En vista de lo anterior, muy comedidamente solicito a usted que las aclaraciones correspondientes sean incluidas en la Fe de Erratas de la próxima Gaceta de la Propiedad Industrial.

5. Me permito recordar a usted, que tal como se informó en el Petitorio de la invención, la presente solicitud corresponde a Capítulo I.
6. Anexo me permito presentar recibo número 08-45.208, expedido por la Superintendencia de Industria y Comercio, que acredita el pago de la tasa que dicho examen causa.
7. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Superintendente,


ALICIA LLOREDA RICAURTE

T.P. 53.215

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 900176089-2

RECIBO OFICIAL DE CAJA : 06 - 45,200
 FECHA : JUNIO 6 DE 2008

**** CONSIGNACION ****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
LOREDA Y CIA S. A	CONSIGNACION	BANCO DE BOGOTA	062754327	2756088	06/06/2008	8,815,200.00

***** C O N C E P T O *****

CANT	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
		85 EXAMEN DE PATENTABILIDAD DE INVEN	420,000.00
		TOTAL :	\$ 420,000.00

SON: CUATROCIENTOS VEINTE MIL PESOS

RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

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DIRECCIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 6-110710

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **584** DE

FECHA **31 DE DICIEMBRE DE 2007** EL TÉRMINO HÁBIL SEÑALADO POR LA

LEY EXPIRA EL **31 DE MARZO DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____ NO X



US 2003/0044813 A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2003/0044813 A1
(43) Pub. Date: Mar. 6, 2003

(54) CANCER-TESTIS ANTIGENS

(52) U.S. Cl. 435/6; 435/7.23

(76) Inventors: Lloyd J. Old, New York, NY (US);
Matthew J. Scanlan, New York, NY
(US); Yao-Tseng Chen, New York, NY
(US)

(57) ABSTRACT

Correspondence Address:

WOLF GREENFIELD & SACKS, PC
FEDERAL RESERVE PLAZA
600 ATLANTIC AVENUE
BOSTON, MA 02210-2211 (US)

(21) Appl. No.: 10/054,683

(22) Filed: Jan. 22, 2002

Related U.S. Application Data

(60) Provisional application No. 60/280,718, filed on Mar. 30, 2001. Provisional application No. 60/285,154, filed on Apr. 20, 2001. Provisional application No. 60/327,432, filed on Oct. 5, 2001.

Publication Classification

(51) Int. Cl.⁷ C12Q 1/68; G01N 33/574

Cancer-testis (CT) antigens have been identified by screening public databases for transcripts that are expressed in tumor tissues and a limited set of normal tissues, or by screening for genes that are expressed in cancer and testis tissues (but not other normal tissues). The invention relates to nucleic acids and encoded polypeptides which are CT antigens expressed in patients afflicted with cancer. The invention provides, inter alia, isolated nucleic acid molecules, expression vectors containing those molecules and host cells transfected with those molecules. The invention also provides isolated proteins and peptides, antibodies to those proteins and peptides and cytotoxic T lymphocytes which recognize the proteins and peptides. Fragments of the foregoing including functional fragments and variants also are provided. Kits containing the foregoing molecules additionally are provided. The molecules provided by the invention can be used in the diagnosis, monitoring, research, or treatment of conditions characterized by the expression of one or more CT antigens.

US 2003/0044813 A1

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Mar. 6, 2003

[0043] Also provided in accordance with a further aspect of the invention are compositions of matter useful in stimulating an immune response to a plurality of a proteins comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:19, 21 and 23. The compositions include a plurality of peptides that are fragments of the proteins, wherein the peptides bind to one or more MHC molecules presented on the surface of non-testis cells. In certain embodiments of the compositions, at least a portion of the plurality of peptides bind to MHC molecules and elicit a cytolytic response thereto. In other embodiments, at least one of the proteins is encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:18, 20 and 22. Preferably, the compositions also include an adjuvant. Preferred adjuvants include saponins, GM-CSF, interleukins, and immunostimulatory oligonucleotides.

[0044] In another aspect of the invention, isolated antibodies are provided which selectively binds to a complex of: (i) a peptide that is a fragment of a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:19, 21 and 23, and (ii) a MHC molecule to which binds the peptide to form the complex, wherein the isolated antibody does not bind to (i) or (ii) alone. Preferably the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, or an antigen-binding fragment thereof.

[0045] According to a further aspect of the invention, methods are provided for treating or diagnosing or monitoring a subject having a cancer characterized by expression of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:18, 20, and 22, in cells or tissues other than testis. The methods include administering to the subject the foregoing antibodies, in an amount effective to treat, diagnose or monitor the condition. Preferably the antibodies are coupled to one or more therapeutically or diagnostically useful agents.

[0046] The invention also involves the use of the genes, gene products, fragments thereof, agents which bind thereto, and so on in the preparation of medicaments. A particular medicament is for treating cancer.

[0047] These and other aspects of the invention will be described in further detail in connection with the detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] FIG. 1 shows a digitized image of RT-PCR expression analysis of cancer/testis-associated Unigene clusters in normal adult tissues and cancer.

[0049] FIG. 1A shows the expression of mRNA transcripts in normal adult tissue following 35 cycles of PCR. Lane 1, brain; 2, kidney; 3, liver; 4, pancreas; 5, placenta; 6, testis; 7, small intestine; 8, heart; 9, prostate; 10, adrenal gland; 11, spleen; 12, colon; 13, stomach; 14, lung; 15, bladder; 16, ovary; 17, mammary gland; 18, cervix; 19, skeletal muscle. The majority of transcripts are testis-specific, with the exceptions of Hs.183009, Hs.293317, Hs.128836, and Hs.130926. Expression of Hs.130926 was used as a positive control for cDNA template integrity of the various tissue samples.

[0050] FIG. 1B shows RT-PCR analysis of CT15/Hs.177959 mRNA expression in renal cancer (RCC1, RCC6, RCC5), CT16/Hs.245431 mRNA expression in melanoma (Mel-1 and Mel-11) and breast cancer; and CT17/Hs.178062 mRNA expression in breast cancer (BR-297), renal cancer (RCC5) and melanoma (Mel-1).

DETAILED DESCRIPTION OF THE INVENTION

[0051] As a consequence of T cell epitope cloning and SEREX analysis, a growing number of cancer-testis (CT) antigens have now been defined. See Table 1 and references cited therein. There are now 14 genes or gene families identified that code for presumptive cancer-testis antigens.

TABLE 1

Cancer-testis (CT) antigens						
CT*	System	# Genes	Chromosomal Location	Detection System**	Refs.	
1	MAGE	16	Xq28/Xp21	T, Ab	7, 10, 12, 13	14
2	BAGE	2	Unknown	T	15, 16	
3	GAGE	9	Xp11	Ab	10, 17	
4	SSX	>5	Xp11	Ab	18, 19	
5	NY-BRO-1 LAGE-1	2	Xq28	Ab, T, RDA		
6	SCP-1	3	1p12-p13	Ab	20	
7	CT17/MAGE-C1	1	Xq26	Ab, RDA	21, 22	
8	CT8	1	Unknown	Ab	23	
9	CT9	1	1p	Ab	24	
10	CT10/MAGE-C2	1	Xq27	RDA, Ab	25, 26	
11	CT11p	1	Xq26-Xq27	***	27	
12	SAGE	1	Xq28	RDA	28	
13	cTAGF-1	1	18p11	Ab	29	
14	OY-TES-1	2	12p12-p13	Ab	30	

*Numbered according to the CT nomenclature proposed by Old & Chen (11).

**Ab = Antibody, T = CD8+ T cell, RDA = representational difference analysis.

***Defined by differential mRNA expression in a parental vs. metastatic melanoma cell variant.

[0052] A thorough analysis of these gene reveals that they encode products with the following characteristics.

[0053] i) mRNA expression in normal tissues is restricted to testis, fetal ovary, and placenta, with little or no expression detected in adult ovary.

[0054] ii) mRNA expression in cancers of diverse origin is common—up to 30-40% of a number of different cancer types, e.g., melanoma, bladder cancer, sarcoma express one or more CT antigens.

[0055] iii) The X chromosome codes for the majority of CT antigens, but a number of more recently defined CT coding genes have a non-X chromosomal locus.

[0056] iv) In normal adult testis, expression of CT antigens is primarily restricted to immature germ cells—, e.g., spermatogonia (31). However, a recently defined CT antigen, OY-TES-1, is clearly involved in late stages of sperm maturation (see below). In fetal ovary, immature germ cells (oogonia/primary oocytes) express CT antigens, whereas oocytes in the resting primordial follicles do not (32). In fetal placenta, both cytotrophoblast and

generating antibodies or determining binding of the polypeptide fragments, or for generating immunoassay components. Likewise, fragments can be employed to produce nonfused fragments of the CT antigen polypeptides, useful, for example, in the preparation of antibodies, and in immunoassays. Fragments further can be used as antisense molecules to inhibit the expression of CT antigen nucleic acids and polypeptides, particularly for therapeutic purposes as described in greater detail below.

[0078] As mentioned above, this disclosure intends to embrace each and every fragment of each sequence, beginning at the first nucleotide, the second nucleotide and so on, up to 8 nucleotides short of the end, and ending anywhere from nucleotide number 8, 9, 10 and so on for each sequence, up to the entire length of the disclosed sequence. Preferred fragments are those useful as amplification primers, e.g., typically between 12 and 32 nucleotides (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32) in length.

[0079] Those skilled in the art are well versed in methods for selecting such sequences, typically on the basis of the ability of the fragment to selectively distinguish the sequence of interest from other sequences in the human genome of the fragment to those on known databases typically is all that is necessary, although in vitro confirmatory hybridization and sequencing analysis may be performed.

[0080] Especially preferred fragment include nucleic acids encoding a series of epitopes, known as "polytopes". The epitopes can be arranged in sequential or overlapping fashion (see, e.g., Thomson et al., *Proc. Natl. Acad. Sci. USA* 92:5845-5849, 1995; Gilbert et al., *Nature Biotechnol.* 15:1280-1284, 1997), with or without the natural flanking sequences, and can be separated by unrelated linker sequences if desired. The polytope is processed to generated individual epitopes which are recognized by the immune system for generation of immune responses.

[0081] Thus, for example, peptides derived from a polypeptide having an amino acid sequence encoded by one of the nucleic acid disclosed herein, and which are presented by MHC molecules and recognized by CTL or T helper lymphocytes, can be combined with peptides from other CT antigens (e.g., by preparation of hybrid nucleic acids or polypeptides) to form "polytopes". The two or more peptides (or nucleic acids encoding the peptides) can be selected from those described herein, or they can include one or more peptides of previously known CT antigens. Immunization with such polytopes can be administered to induce or enhance an immune response. The polytopes can be derived from tumor associated antigens including MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9, GAGE-10, GAGE-11, GAGE-12, GAGE-13, GAGE-14, GAGE-15, GAGE-16, GAGE-17, GAGE-18, GAGE-19, GAGE-20, GAGE-21, GAGE-22, GAGE-23, GAGE-24, GAGE-25, GAGE-26, GAGE-27, GAGE-28, GAGE-29, GAGE-30, GAGE-31, GAGE-32, GAGE-33, GAGE-34, GAGE-35, GAGE-36, GAGE-37, GAGE-38, GAGE-39, GAGE-40, GAGE-41, GAGE-42, GAGE-43, GAGE-44, GAGE-45, GAGE-46, GAGE-47, GAGE-48, GAGE-49, GAGE-50, GAGE-51, GAGE-52, GAGE-53, GAGE-54, GAGE-55, GAGE-56, GAGE-57, GAGE-58, GAGE-59, GAGE-60, GAGE-61, GAGE-62, GAGE-63, GAGE-64, GAGE-65, GAGE-66, GAGE-67, GAGE-68, GAGE-69, GAGE-70, GAGE-71, GAGE-72, GAGE-73, GAGE-74, GAGE-75, GAGE-76, GAGE-77, GAGE-78, GAGE-79, GAGE-80, GAGE-81, GAGE-82, GAGE-83, GAGE-84, GAGE-85, GAGE-86, GAGE-87, GAGE-88, GAGE-89, GAGE-90, GAGE-91, GAGE-92, 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with CD2 expressed on T cells. This interaction induces T cell IL-2 and IFN-gamma production and can thus complement but not substitute, the B7/CD28 costimulatory interaction (Parra et al., *J. Immunol.*, 158:637-642 (1997), Fenton et al., *J. Immunother.*, 21:2:95-108 (1998)).

[0163] Lymphocyte function associated antigen-1 (LFA-1) is expressed on leukocytes and interacts with ICAM-1 expressed on APCs and some tumor cells. This interaction induces T cell IL-2 and IFN-gamma production and can thus complement but not substitute, the B7/CD28 costimulatory interaction (Fenton et al., *J. Immunother.*, 21:2:95-108 (1998)). LFA-1 is thus a further example of a costimulatory molecule that could be provided in a vaccination protocol in the various ways discussed above for B7.

[0164] Complete CTL activation and effector function requires Th cell help through the interaction between the Th cell CD40L (CD40 ligand) molecule and the CD40 molecule expressed by DCs (Ridge et al., *Nature*, 393:474 (1998), Bennett et al., *Nature*, 393:478 (1998), Schoenberger et al., *Nature*, 393:480 (1998)). This mechanism of this costimulatory signal is likely to involve upregulation of B7 and associated IL-6/IL-12 production by the DC (APC). The CD40-CD40L interaction thus complements the signal 1 (antigen/MHC-TCR) and signal 2 (B7-CD28) interactions.

[0165] The use of anti-CD40 antibodies to stimulate DC cells directly, would be expected to enhance a response to tumor antigens which are normally encountered outside of an inflammatory context or are presented by non-professional APCs (tumor cells). In these situations Th help and B7 costimulation signals are not provided. This mechanism might be used in the context of antigen pulsed DC based therapies or in situations where Th epitopes have not been defined within known TRA precursors.

[0166] A CT antigen polypeptide, or a fragment thereof, also can be used to isolate their native binding partners. Isolation of such binding partners may be performed according to well-known methods. For example, isolated CT antigen polypeptides can be attached to a substrate (e.g., chromatographic media, such as polystyrene beads, or a filter), and then a solution suspected of containing the binding partner may be applied to the substrate. If a binding partner which can interact with CT antigen polypeptides is present in the solution, then it will bind to the substrate-bound CT antigen polypeptide. The binding partner then may be isolated.

[0167] It will also be recognized that the invention embraces the use of the CT antigen cDNA sequences in expression vectors, as well as to transfect host cells and cell lines, be these prokaryotic (e.g., *E. coli*), or eukaryotic (e.g., dendritic cells, B cells, CHO cells, COS cells, yeast expression systems and recombinant baculovirus expression in insect cells). Especially useful are mammalian cells such as human, mouse, hamster, pig, goat, primate, etc. They may be of a wide variety of tissue types, and include primary cells and cell lines. Specific examples include keratinocytes, peripheral blood leukocytes, bone marrow stem cells and embryonic stem cells. The expression vectors require that the pertinent sequence, i.e., those nucleic acids described supra, be operably linked to a promoter.

[0168] The invention also contemplates delivery of nucleic acids, polypeptides or peptides for vaccination

Delivery of polypeptides and peptides can be accomplished according to standard vaccination protocols which are well known in the art. In another embodiment, the delivery of nucleic acid is accomplished by ex vivo methods, i.e., by removing a cell from a subject, genetically engineering the cell to include a CT antigen, and reintroducing the engineered cell into the subject. One example of such a procedure is the use of dendritic cells as delivery and antigen presentation vehicles for the administration of CT antigens in vaccine therapies. Another example of such a procedure is outlined in U.S. Pat. No. 5,399,346 and in exhibits submitted in the file history of that patent, all of which are publicly available documents. In general, it involves introduction in vitro of a functional copy of a gene into a cell(s) of a subject, and returning the genetically engineered cell(s) to the subject. The functional copy of the gene is under operable control of regulatory elements which permit expression of the gene in the genetically engineered cell(s). Numerous transfection and transduction techniques as well as appropriate expression vectors are well known to those of ordinary skill in the art, some of which are described in PCT application WO95/00654. In vivo nucleic acid delivery using vectors such as viruses and targeted liposomes also is contemplated according to the invention.

[0169] In preferred embodiments, a virus vector for delivering a nucleic acid encoding a CT antigen is selected from the group consisting of adenoviruses, adeno-associated viruses, poxviruses including vaccinia viruses and attenuated poxviruses, Semliki Forest virus, Venezuelan equine encephalitis virus, retroviruses, Sindbis virus, and Ty virus-like particle. Examples of viruses and virus-like particles which have been used to deliver exogenous nucleic acids include: replication-defective adenoviruses (e.g., Xiang et al., *Virology* 219:220-227, 1996; Elloit et al., *J. Virol.* 71:5375-5381, 1997; Chengalvala et al., *Vaccine* 15:335-339, 1997), a modified retrovirus (Townsend et al., *J. Virol.* 71:3365-3374, 1997), a nonreplicating retrovirus (Irwin et al., *J. Virol.* 68:5036-5044, 1994), a replication defective Semliki Forest virus (Zhao et al., *Proc. Natl. Acad. Sci. USA* 92:3009-3013, 1995), canarypox virus and highly attenuated vaccinia virus derivative (Paoletti, *Proc. Natl. Acad. Sci. USA* 93:11349-11353, 1996), non-replicative vaccinia virus (Moss, *Proc. Natl. Acad. Sci. USA* 93:11341-11348, 1996), replicative vaccinia virus (Moss, *Dev. Biol. Stand.* 82:55-63, 1994), Venezuelan equine encephalitis virus (Davis et al., *J. Virol.* 70:3781-3787, 1996), Sindbis virus (Pugachev et al., *Virology* 212:587-594, 1995), and Ty virus-like particle (Allsopp et al., *Eur. J. Immunol.* 26:1951-1959, 1996). In preferred embodiments, the virus vector is an adenovirus or an alphavirus.

[0170] Another preferred virus for certain applications is the adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus is capable of infecting a wide range of cell types and species and can be engineered to be replication-deficient. It further has advantages, such as heat and lipid solvent stability, high transduction frequencies in cells of diverse lineages, including hematopoietic cells, and lack of superinfection inhibition thus allowing multiple series of transductions. The adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the

absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

[0171] In general, other preferred viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Adenoviruses and retroviruses have been approved for human gene therapy trials. In general, the retroviruses are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., "Gene Transfer and Expression, A Laboratory Manual," W.H. Freeman Co., New York (1990) and Murry, E. J. Ed., "Methods in Molecular Biology," vol. 7, Humana Press, Inc., Clifton, N.J. (1991).

[0172] Preferably the foregoing nucleic acid delivery vectors: (1) contain exogenous genetic material that can be transcribed and translated in a mammalian cell and that can induce an immune response in a host, and (2) contain on a surface a ligand that selectively binds to a receptor on the surface of a target cell, such as a mammalian cell, and thereby gains entry to the target cell.

[0173] Various techniques may be employed for introducing nucleic acids of the invention into cells, depending on whether the nucleic acids are introduced in vitro or in vivo in a host. Such techniques include transfection of nucleic acid-CaPO₄ precipitates, transfection of nucleic acids associated with DEAE, transfection or infection with the foregoing viruses including the nucleic acid of interest, liposome mediated transfection, and the like. For certain uses, it is preferred to target the nucleic acid to particular cells. In such instances, a vehicle used for delivering a nucleic acid of the invention into a cell (e.g., a retrovirus, or other virus; a liposome) can have a targeting molecule attached thereto. For example, a molecule such as an antibody specific for a surface membrane protein on the target cell or a ligand for a receptor on the target cell can be bound to or incorporated within the nucleic acid delivery vehicle. Preferred antibodies include antibodies which selectively bind a CT antigen, alone or as a complex with a MHC molecule. Especially preferred are monoclonal antibodies. Where liposomes are employed to deliver the nucleic acids of the invention, proteins which bind to a surface membrane protein associated with endocytosis may be incorporated into the liposome formulation for targeting and/or to facilitate uptake. Such proteins include capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half life, and the like. Polymeric delivery systems also have been used

successfully to deliver nucleic acids into cells, as is known by those skilled in the art. Such systems even permit oral delivery of nucleic acids.

[0174] When administered, the therapeutic compositions of the present invention can be administered in pharmaceutically acceptable preparations. Such preparations may contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents.

[0175] The therapeutics of the invention can be administered by any conventional route, including injection or by gradual infusion over time. The administration may, for example, be oral, intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal. When antibodies are used therapeutically, a preferred route of administration is by pulmonary aerosol. Techniques for preparing aerosol delivery systems containing antibodies are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the antibodies, such as the paratope binding capacity (see, for example, Sciarra and Cutie, "Aerosols," in *Remington's Pharmaceutical Sciences*, 18th edition, 1990, pp. 1694-1712, incorporated by reference). Those of skill in the art can readily determine the various parameters and conditions for producing antibody aerosols without resort to undue experimentation. When using antisense preparations of the invention, slow intravenous administration is preferred.

[0176] The compositions of the invention are administered in effective amounts. An "effective amount" is that amount of a CT antigen composition that alone, or together with further doses, produces the desired response, e.g. increases an immune response to the CT antigen. In the case of treating a particular disease or condition characterized by expression of one or more CT antigens, such as cancer, the desired response is inhibiting the progression of the disease. This may involve only slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. This can be monitored by routine methods or can be monitored according to diagnostic methods of the invention discussed herein. The desired response to treatment of the disease or condition also can be delaying the onset or even preventing the onset of the disease or condition.

[0177] Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

[0178] The pharmaceutical compositions used in the foregoing methods preferably are sterile and contain an effective

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Thr Tyr Asp Asp Ile Asn Lys Cys Gln Cys Ser Gly Ala Val Cys Ile
325 330 335
Met Asn Pro Glu Ala Ile His Phe Ser Gly Val Lys Ile Phe Ser Asn
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Cys Ser Phe Glu Asp Phe Ala His Phe Ile Ser Lys Gln Lys Ser Gln
355 360 365
Cys Leu His Asn Gln Pro Arg Leu Asp Pro Phe Phe Lys Gln Gln Ala
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Val Cys Gly Asn Ala Lys Leu Glu Ala Gly Glu Glu Cys Asp Cys Gly
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Pro Glu Asn His Tyr Val Gln Thr Gly His Pro Cys Gly Leu Asn Gln
465 470 475 480
Trp Ile Cys Ile Asp Gly Val Cys Met Ser Gly Asp Lys Gln Cys Thr
485 490 495
Asp Thr Phe Gly Lys Glu Val Glu Phe Gly Pro Ser Glu Cys Tyr Ser
500 505 510
His Leu Asn Ser Lys Thr Asp Val Ser Gly Asn Cys Gly Ile Ser Asp
515 520 525
Ser Gly Tyr Thr Gln Cys Glu Ala Asp Asn Leu Gln Cys Gly Lys Leu
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Ile Cys Lys Tyr Val Gly Lys Phe Leu Leu Gln Ile Pro Arg Ala Thr
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Val Cys Asn Asn Lys Lys His Cys His Cys Ser Ala Ser Tyr Leu Pro
625 630 635 640
Pro Asp Cys Ser Val Gln Ser Asp Leu Trp Pro Gly Gly Ser Ile Asp
645 650 655
Ser Gly Asn Phe Pro Pro Val Ala Ile Pro Ala Arg Leu Pro Glu Arg
660 665 670
Arg Tyr Ile Glu Asn Ile Tyr His Ser Lys Pro Met Arg Trp Pro Phe
675 680 685
Phe Leu Phe Ile Pro Phe Phe Ile Ile Phe Cys Val Leu Ile Ala Ile
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ggactctgcc cacttttgat cccactaaag tgctggagac aggtgaaggc caactatag 420
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35 40 45
Ala Pro Ser Gly Glu Ile Lys Asn Glu Gly Ala Pro Ala Val Gln Gly
50 55 60
Thr Asp Val Glu Ala Phe Gln Gln Glu Leu Ala Leu Leu Lys Ile Glu
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atcatgtctc cggctggggt atcaagccaa gctattttaa ggtgttttaa aagaaggat 180
ggaagggaaga cctcttagga ccaactgtgt tttggataca agtggtacat atocattctg 240
taactattat ttgtttctca gtataattgt tccagataaa actatgatgg atggctcgtt 300

amount of CT antigen or nucleic acid encoding CT antigen for producing the desired response in a unit of weight or volume suitable for administration to a patient. The response can, for example, be measured by determining the immune response following administration of the CT antigen composition via a reporter system by measuring downstream effects such as gene expression, or by measuring the physiological effects of the CT antigen composition, such as regression of a tumor or decrease of disease symptoms. Other assays will be known to one of ordinary skill in the art and can be employed for measuring the level of the response.

[0179] The doses of CT antigen compositions (e.g., polypeptide, peptide, antibody, cell or nucleic acid) administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of administration used and the state of the subject. Other factors include the desired period of treatment. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits.

[0180] In general, for treatments for eliciting or increasing an immune response, doses of CT antigen are formulated and administered in doses between 1 ng and 1 mg, and preferably between 10 ng and 100 μ g, according to any standard procedure in the art. Where nucleic acids encoding CT antigen or variants thereof are employed, doses of between 1 ng and 0.1 mg generally will be formulated and administered according to standard procedures. Other protocols for the administration of CT antigen compositions will be known to one of ordinary skill in the art, in which the dose amount, schedule of injections, sites of injections, mode of administration (e.g., intra-tumoral) and the like vary from the foregoing. Administration of CT antigen compositions to mammals other than humans, e.g. for testing purposes or veterinary therapeutic purposes, is carried out under substantially the same conditions as described above.

[0181] Where CT antigen peptides are used for vaccination, modes of administration which effectively deliver the CT antigen and adjuvant, such that an immune response to the antigen is increased, can be used. For administration of a CT antigen peptide in adjuvant, preferred methods include intradermal, intravenous, intramuscular and subcutaneous administration. Although these are preferred embodiments, the invention is not limited by the particular modes of administration disclosed herein. Standard references in the art (e.g., *Remington's Pharmaceutical Sciences*, 18th edition, 1990) provide modes of administration and formulations for delivery of immunogens with adjuvant or to a non-adjuvant carrier.

[0182] When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptable compositions. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-phar-

maceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

[0183] A CT antigen composition may be combined, if desired, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

[0184] The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; and phosphoric acid in a salt.

[0185] The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

[0186] The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

[0187] Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

[0188] Compositions suitable for parenteral administration conveniently comprise a sterile aqueous or non-aqueous preparation of CT antigen polypeptides or nucleic acids, which is preferably isotonic with the blood of the recipient. This preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or

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tgtgatttta atttgaccag aatatatgat atatatgat aatttcacag ataatttact 2460
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Arg	Ser	Ile	Ile	Lys	Glu	Gly	Ile	Glu	Ser	Gln	Ala	Ser	Tyr	Lys	Ile	35	40	45	
Val	Ile	Glu	Gly	Lys	Pro	Tyr	Thr	Val	Asn	Leu	Met	Gln	Lys	Asn	Phe	50	55	60	
Leu	Pro	His	Asn	Phe	Arg	Val	Tyr	Ser	Tyr	Ser	Gly	Thr	Gly	Ile	Met	65	70	75	80
Lys	Pro	Leu	Asp	Gln	Asp	Phe	Gln	Asn	Phe	Cys	His	Tyr	Gln	Gly	Tyr	85	90	95	
Ile	Glu	Gly	Tyr	Pro	Lys	Ser	Val	Val	Met	Val	Ser	Thr	Cys	Thr	Gly	100	105	110	
Leu	Arg	Gly	Val	Leu	Gln	Phe	Glu	Asn	Val	Ser	Tyr	Gly	Ile	Glu	Pro	115	120	125	
Leu	Glu	Ser	Ser	Val	Gly	Phe	Glu	His	Val	Ile	Tyr	Gln	Val	Lys	His	130	135	140	
Lys	Lys	Ala	Asp	Val	Ser	Leu	Tyr	Asn	Glu	Lys	Asp	Ile	Glu	Ser	Arg	145	150	155	160
Asp	Leu	Ser	Phe	Lys	Leu	Gln	Ser	Ala	Glu	Pro	Gln	Gln	Asp	Phe	Ala	165	170	175	
Lys	Tyr	Ile	Glu	Met	His	Val	Ile	Val	Glu	Lys	Gln	Leu	Tyr	Asn	His	180	185	190	
Met	Gly	Ser	Asp	Thr	Thr	Val	Val	Ala	Gln	Lys	Val	Phe	Gln	Leu	Ile	195	200	205	
Gly	Leu	Thr	Asn	Ala	Ile	Phe	Val	Ser	Phe	Asn	Ile	Thr	Ile	Ile	Leu	210	215	220	
Ser	Ser	Leu	Glu	Leu	Trp	Ile	Asp	Glu	Asn	Lys	Ile	Ala	Thr	Thr	Gly	225	230	235	240
Glu	Ala	Asn	Glu	Leu	Leu	His	Thr	Phe	Leu	Arg	Trp	Lys	Thr	Ser	Tyr	245	250	255	
Leu	Val	Leu	Arg	Pro	His	Asp	Val	Ala	Phe	Leu	Leu	Val	Tyr	Arg	Glu	260	265	270	
Lys	Ser	Asn	Tyr	Val	Gly	Ala	Thr	Phe	Gln	Gly	Lys	Met	Cys	Asp	Ala	275	280	285	
Asn	Tyr	Ala	Gly	Gly	Val	Val	Leu	His	Pro	Arg	Thr	Ile	Ser	Leu	Glu	290	295	300	
Ser	Leu	Ala	Val	Ile	Leu	Ala	Gln	Leu	Leu	Ser	Leu	Ser	Met	Gly	Ile	305	310	315	320

expression product thereof, or a fragment of an expression product thereof complexed with an HLA molecule, wherein the nucleic acid molecule comprises a nucleotide sequence set forth as SEQ ID NO:34, and

determining the interaction between the agent and the nucleic acid molecule or the expression product to diagnose the cancer in the subject.

6. A method of diagnosing a cancer, comprising:

contacting a non-testis, non-ovary, non-lung, non-breast, non-prostate, non-colon biological sample isolated from a subject with an agent that specifically binds to a nucleic acid molecule, an expression product thereof, or a fragment of an expression product thereof complexed with an HLA molecule, wherein the nucleic acid molecule comprises a nucleotide sequence set forth as SEQ ID NO:36, and

determining the interaction between the agent and the nucleic acid molecule or the expression product to diagnose the cancer in the subject.

7. A method for determining regression, progression or onset of a cancer characterized by expression of abnormal levels of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, comprising

monitoring a plurality of non-testis samples obtained at different times from a subject who has or is suspected of having the cancer, for a parameter selected from the group consisting of

- (i) the protein,
- (ii) a peptide derived from the protein,
- (iii) an antibody which selectively binds the protein or peptide,
- (iv) cytolytic T cells specific for a complex of the peptide derived from the protein and an MHC molecule, and
- (v) a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22; and

comparing the parameters from the plurality of samples to determine regression, progression or onset of the cancer.

8. The method of claim 7, wherein the sample is a body fluid, a body effusion, cell or a tissue.

9. The method of claim 7, wherein the step of monitoring comprises contacting the sample with a detectable agent selected from the group consisting of

- (a) an antibody which selectively binds the protein of (i), or the peptide of (ii),
- (b) a protein or peptide which binds the antibody of (iii),
- (c) a cell which presents the complex of the peptide and MHC molecule of (iv), and
- (d) at least one nucleic acid probe or primer that hybridizes to the nucleic acid molecule of (v) or its complement.

10. The method of claim 9, wherein the antibody, the protein, the peptide, the cell or the nucleic acid probe or primer is labeled with a radioactive label or an enzyme.

11. The method of claim 7, wherein the protein is a plurality of proteins, the parameter is a plurality of parameters, each of the plurality of parameters being specific for a different of the plurality of proteins, at least one of which is a CT antigen protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22.

12. A pharmaceutical preparation for a human subject comprising

an agent which when administered to the subject enriches selectively the presence of complexes of an HLA molecule and a human CT antigen peptide, and

a pharmaceutically acceptable carrier, wherein the human CT antigen peptide is a fragment of a human CT antigen encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22.

13. The pharmaceutical preparation of claim 12, wherein the agent comprises a plurality of agents, each of which enriches selectively in the subject complexes of an HLA molecule and a different human CT antigen peptide, wherein at least one of the human CT antigens is encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22.

14. The pharmaceutical preparation of claim 12, wherein the agent is selected from the group consisting of

- (1) an isolated polypeptide comprising the human CT antigen peptide,
- (2) an isolated nucleic acid operably linked to a promoter for expressing the isolated polypeptide,
- (3) a host cell expressing the isolated polypeptide, and
- (4) isolated complexes of the polypeptide, and an HLA molecule.

15. The pharmaceutical preparation of claims 12-14, further comprising an adjuvant.

16. The pharmaceutical preparation of claim 12, wherein the agent is a cell expressing an isolated polypeptide comprising the human CT antigen peptide, and wherein the cell is nonproliferative.

17. The pharmaceutical preparation of claim 12, wherein the agent is a cell expressing an isolated polypeptide comprising the human CT antigen peptide, and wherein the cell expresses an HLA molecule that binds the polypeptide.

18. The pharmaceutical preparation of claim 17, wherein the cell expresses one or both of the polypeptide and HLA molecule recombinantly.

19. The pharmaceutical preparation of claim 17, wherein the cell is nonproliferative.

20. A composition comprising

an isolated agent that binds selectively a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:21, 23, 25, 27, 29, 31, 35 and 37.

21. The composition of matter of claim 20, wherein the agent is an antibody or an antigen-binding fragment thereof.

22. The composition of claim 21, wherein the antibody is a monoclonal antibody, a chimeric antibody or a humanized antibody.

23. A composition of matter comprising a conjugate of the agent of claim 20 or 21 and a therapeutic or diagnostic agent.

24. The composition of matter of claim 23, wherein the conjugate is of the agent and a therapeutic or diagnostic that is a toxin.

25. A pharmaceutical composition comprising

an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, and

a pharmaceutically acceptable carrier.

26. The pharmaceutical composition of claim 25, wherein the isolated nucleic acid molecule comprises at least two isolated nucleic acid molecules coding for two different polypeptides, each polypeptide comprising a different human CT antigen.

27. The pharmaceutical composition of claim 25 or 26 further comprising an expression vector with a promoter operably linked to the isolated nucleic acid molecule.

28. The pharmaceutical composition of claim 25 or 26 further comprising a host cell recombinantly expressing the isolated nucleic acid molecule.

29. A pharmaceutical composition comprising

an isolated polypeptide comprising a polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, and

a pharmaceutically acceptable carrier.

30. The pharmaceutical composition of claim 29, wherein the isolated polypeptide comprises at least two different polypeptides, each comprising a different human CT antigen.

31. The pharmaceutical composition of claim 29 or 30, further comprising an adjuvant.

32. A protein microarray comprising at least one polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, or an antigenic fragment of the polypeptide.

33. The microarray of claim 32, wherein the at least one polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:19, 21 and 23.

34. A protein microarray comprising at least one polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:24, 26, 28 and 30, or an antigenic fragment of the polypeptide.

35. The microarray of claim 34, wherein the at least one polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:25, 27, 29 and 31.

36. A protein microarray comprising at least one polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:32, 34 and 36, or an antigenic fragment of the polypeptide.

37. The microarray of claim 36, wherein the at least one polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:33, 35 and 37.

38. A protein microarray comprising a plurality of antibodies or antigen-binding fragments thereof that specifically bind at least one polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, or an antigenic fragment of the polypeptide.

39. The microarray of claim 38, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:19, 21 and 23.

40. A protein microarray comprising a plurality of antibodies or antigen-binding fragments thereof that specifically bind at least one polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:24, 26, 28 and 30, or an antigenic fragment of the polypeptide.

41. The microarray of claim 40, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:25, 27, 29 and 31.

42. A protein microarray comprising a plurality of antibodies or antigen-binding fragments thereof that specifically bind at least one polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:32, 34 and 36, or an antigenic fragment of the polypeptide.

43. The microarray of claim 42, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:33, 35 and 37.

44. A nucleic acid microarray comprising at least one nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, or a fragment thereof of at least 20 nucleotides that selectively hybridizes to its complement in a biological sample.

45. A nucleic acid microarray comprising at least one nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:24, 26, 28 and 30, or a fragment thereof of at least 20 nucleotides that selectively hybridizes to its complement in a biological sample.

46. A nucleic acid microarray comprising at least one nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:32, 34 and 36, or a fragment thereof of at least 20 nucleotides that selectively hybridizes to its complement in a biological sample.

47. An isolated fragment of a human CT antigen which, or a portion of which, binds a HLA molecule or a human antibody, wherein the CT antigen is encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22.

48. The fragment of claim 47, wherein the fragment is part of a complex with the HLA molecule.

49. The fragment of claim 47, wherein the fragment is between 8 and 12 amino acids in length.

50. A kit for detecting the expression of two or more human CT antigens comprising

two or more pairs of isolated nucleic acid molecules, each of which consists essentially of a nucleic acid molecule selected from the group consisting of (a) a 12-32 nucleotide contiguous segment of the nucleotide sequence of any of SEQ ID NOs:18, 20 or 22, and (b) complements of (a), wherein the contiguous segments are nonoverlapping, and wherein the nucleic acid molecules in each of the pairs are specific for a human CT antigen.

51. The kit of claim 50, wherein the pair of isolated nucleic acid molecules is constructed and arranged to selectively amplify at least a fragment of an isolated nucleic acid molecule selected from the group consisting of SEQ ID NOs:18, 20 and 22.

52. A method for treating a subject with a cancer characterized by expression of a human CT antigen, comprising

administering to the subject an amount of an agent, which enriches selectively in the subject the presence of complexes of a HLA molecule and a human CT antigen peptide, effective to ameliorate the disorder, wherein the human CT antigen peptide is a fragment of a human CT antigen encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22.

53. The method of claim 52, wherein the cancer is characterized by expression of a plurality of human CT antigens and wherein the agent is a plurality of agents, each of which enriches selectively in the subject the presence of complexes of an HLA molecule and a different human CT antigen peptide, wherein at least one of the human CT antigens is encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22.

54. The method of claim 52, wherein the agent is an isolated polypeptide encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22.

55. A method for treating a subject having a cancer characterized by expression of a human CT antigen in cells of the subject, comprising:

- (i) removing an immunoreactive cell containing sample from the subject,
 - (ii) contacting the immunoreactive cell containing sample to the host cell under conditions favoring production of cytolytic T cells against a human CT antigen peptide that is a fragment of the human CT antigen,
 - (iii) introducing the cytolytic T cells to the subject in an amount effective to lyse cells which express the human CT antigen, wherein the host cell is transformed or transfected with an expression vector comprising an isolated nucleic acid molecule operably linked to a promoter, wherein the isolated nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22.
56. The method of claim 55, wherein the host cell recombinantly expresses an HLA molecule which binds the human CT antigen peptide.
57. The method of claim 55, wherein the host cell endogenously expresses an HLA molecule which binds the human CT antigen peptide.
58. A method for treating a subject having a cancer characterized by expression of a human CT antigen in cells of the subject, comprising:
- (i) identifying a nucleic acid molecule expressed by the cells of the cancer, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, or a fragment thereof;
 - (ii) transfecting a host cell with a nucleic acid molecule selected from the group consisting of (a) the nucleic acid molecule identified, (b) a fragment of the nucleic acid identified which includes a segment coding for a human CT antigen, (c) degenerates of (a) or (b);
 - (iii) culturing said transfected host cells to express the transfected nucleic acid molecule, and;

(iv) introducing an amount of said host cells or an extract thereof to the subject effective to increase an immune response against the cells of the subject associated with the condition.

59. The method of claim 58, further comprising identifying an MHC molecule which presents a portion of an expression product of the nucleic acid molecule, wherein the host cell expresses the same MHC molecule as identified and wherein the host cell presents an MHC binding portion of the expression product of the nucleic acid molecule.

60. The method of claim 58, wherein the immune response comprises a B-cell response or a T cell response.

61. The method of claim 60, wherein the response is a T-cell response which comprises generation of cytolytic T-cells specific for the host cells presenting the portion of the expression product of the nucleic acid molecule or cells of the subject expressing the human CT antigen.

62. The method of claim 58, wherein the nucleic acid molecule is selected from the group consisting of SEQ ID NOs:18, 20 and 22.

63. The method of claim 58 or 59, further comprising treating the host cells to render them non-proliferative.

64. A method for treating or diagnosing or monitoring a subject having a cancer characterized by expression of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20, and 22, in cells or tissues other than testis, comprising

administering to the subject an antibody which specifically binds to the protein or a peptide derived therefrom, the antibody being coupled to a therapeutically or diagnostically useful agent, in an amount effective to treat, diagnose or monitor the condition.

65. The method of claim 64, wherein the antibody is a monoclonal antibody or an antigen-binding fragment thereof.

66. The method of claim 65, wherein the monoclonal antibody is a chimeric antibody or a humanized antibody.

67. A method for treating a cancer characterized by expression of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, in cells or tissues other than testis, comprising

administering to a subject a pharmaceutical composition of any one of claims 12-19 and 25-31 in an amount effective to prevent, delay the onset of, or inhibit the condition in the subject.

68. The method of claim 67, further comprising first identifying that the subject expresses abnormal amounts of the protein in a non-testis tissue.

69. A method for treating a subject having a cancer characterized by expression of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, in cells or tissues other than testis, comprising

- (i) identifying cells from the subject which express abnormal amounts of the protein;
- (ii) isolating a sample of the cells;
- (iii) cultivating the cells, and

(iv) introducing the cells to the subject in an amount effective to provoke an immune response against the cells.

70. The method of claim 69, further comprising rendering the cells non-proliferative, prior to introducing them to the subject.

71. A method for treating a pathological cell condition characterized by expression of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22, in cells or tissues other than testis, comprising

administering to a subject in need thereof an effective amount of an agent which inhibits the expression or activity of the protein.

72. The method of claim 71, wherein the agent is an inhibiting antibody which selectively binds to the protein and wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody or an antibody fragment.

73. The method of claim 71, wherein the agent is an antisense nucleic acid molecule which selectively binds to the nucleic acid molecule which encodes the protein.

74. A composition of matter useful in stimulating an immune response to a plurality of proteins comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 21 and 23 comprising

a plurality of peptides that are fragments of the proteins, wherein the peptides bind to one or more MHC molecules presented on the surface of non-testis cells.

75. The composition of matter of claim 74, wherein at least a portion of the plurality of peptides bind to MHC molecules and elicit a cytolytic response thereto.

76. The composition of matter of claim 74, wherein at least one of the proteins is encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20 and 22.

77. The composition of matter of claim 74, further comprising an adjuvant.

78. The composition of matter of claim 77, wherein said adjuvant is selected from the group consisting of saponins, GM-CSF, interleukins, and immunostimulatory oligonucleotides.

79. An isolated antibody which selectively binds to a complex of:

(i) a peptide that is a fragment of a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:19, 21 and 23, and

(ii) a MHC molecule to which binds the peptide to form the complex, wherein the isolated antibody does not bind to (i) or (ii) alone.

80. The antibody of claim 79, wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, or an antigen-binding fragment thereof.

81. A method for treating or diagnosing or monitoring a subject having a cancer characterized by expression of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20, and 22, in cells or tissues other than testis, comprising

administering to the subject the antibody of claim 79, the antibody being coupled to a therapeutically or diagnostically useful agent, in an amount effective to treat, diagnose or monitor the condition.

82. A method for treating or diagnosing or monitoring a subject having a cancer characterized by expression of a protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:18, 20, and 22, in cells or tissues other than testis, comprising

administering to the subject the antibody of claim 80, the antibody being coupled to a therapeutically or diagnostically useful agent, in an amount effective to treat, diagnose or monitor the condition.

* * * * *

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant (for all designated States except US): SMITHKLINE BEECHAM BIOLOGICALS S.A. (BE/BE); Rue de l'Institut 89, B-1330 Rixensart (BE).		
(72) Inventors; and (75) Inventors/Applicants (for US only): CABEZON SILVA, Teresa (BE/BE); SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). COHEN, Joseph (US/BE); SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). SLAOUL, Moncef, Mohamed (BE/BE); SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). VINALS BASSOLS, Carlota (BE/BE); SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).		
(54) Title: TUMOR-ASSOCIATED ANTIGEN DERIVATIVES FROM THE MAGE FAMILY, AND NUCLEIC ACID SEQUENCES ENCODING THEM, USED FOR THE PREPARATION OF FUSION PROTEINS AND OF COMPOSITIONS FOR VACCINATION		
(57) Abstract The present invention relates to novel proteins and to their production, from the MAGE family. In particular, to a MAGE protein fused to an immunological fusion partner, such as Lipoprotein D. Such antigens may be formulated to provide vaccines for the treatment of a range of tumours. Novel methods for purifying MAGE proteins are also provided.		

TUMOR-ASSOCIATED ANTIGEN DERIVATIVES FROM THE MAGE FAMILY, AND NUCLEIC ACID SEQUENCES ENCODING THEM, USED FOR THE PREPARATION OF FUSION PROTEINS AND OF COMPOSITIONS FOR VACCINATION

The present invention relates to protein derivatives, comprising a tumor-associated antigen, that find utility in cancer vaccine therapy. In particular the derivatives of the invention include fusion proteins comprising an antigen encoded by the family of MAGE genes (e.g. MAGE-3, MAGE-1), linked to an immunological fusion partner which provides T helper epitopes, such as, for example the lipidated form of protein D from Haemophilus influenzae B; chemically modified MAGE proteins wherein the antigen's disulphide bridges are reduced and the resulting thiols blocked and genetically modified MAGE proteins provided with an affinity tag and/or genetically modified to prevent disulphide bridge formation. Methods are also described for purifying MAGE proteins and for formulating vaccines for treating a range of cancers, including, but not limited to Melanoma, breast, bladder, lung, NSCLC, head and squamous cell carcinoma, colon carcinoma and oesophagus carcinoma.

Antigens encoded by the family of MAGE genes are predominately expressed on melanoma cells (including malignant melanoma) and some other cancers including NSCLC (non small cell lung cancer), head and neck squamous cell carcinoma, bladder transitional cell carcinoma and oesophagus carcinoma, but are not detectable on normal tissues except in the testis and the placenta (Gaugler, 1994; Weynants, 1994; Patard, 1995). MAGE-3 is expressed in 69% of melanomas (Gaugler, 1994), and can also be detected in 44% of NSCLC (Yoshimatsu 1988), 48% of head and neck squamous cell carcinoma, 34% of bladder transitional cell carcinoma 57% of oesophagus carcinoma 32% of colon cancers and 24% of breast cancers (Van Pel, 1995); Inoue, 1995 Fujie 1997; Nishimura 1997). Cancers expressing MAGE proteins are known as Mage associated tumours.

The immunogenicity of human melanoma cells has been elegantly demonstrated in experiments using mixed cultures of melanoma cells and autologous lymphocytes. These culture often generate specific cytotoxic T lymphocytes (CTLs) able to lyse exclusively the autologous melanoma cells but neither autologous fibroblasts, nor autologous EBV-transformed B lymphocytes (Knuth, 1984;

In one embodiment of the present invention, the derivative is a fusion proteins comprising an antigen from the MAGE protein family linked to a heterologous partner. The proteins may be chemically conjugated, but are preferably expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared to non-fused protein. Thus the fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

In a preferred form of the invention, the immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium, *Haemophilus influenza B* (WO91/18926). Preferably the protein D derivative comprises approximately the first 1/3 of the protein, in particular approximately the first N-terminal 100-110 amino acids. Preferably the protein D derivative is lipidated. Preferably the first 109 residues of the Lipoprotein D fusion partner is included on the N-terminus to provide the vaccine candidate antigen with additional exogenous T-cell epitopes and increase expression level in *E. coli* (thus acting also as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells.

Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemagglutinin). Typically the N terminal 81 amino acids are utilised, although different fragments may be used provided they include T-helper epitopes.

In another embodiment the immunological fusion partner is the protein known as LYTA. Preferably the C terminal portion of the molecule is used. Lyta is derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L-alanine amidase, amidase LYTA, (coded by the *lytA* gene {Gene, 43 (1986) page 265-272}) an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-

The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as *E. coli* may be treated with a solution of CaCl_2 (Cohen *et al.*, Proc. Nat. Acad. Sci., 1973, 69, 2110) or with a solution comprising a mixture of RbCl , MnCl_2 , potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbCl and glycerol. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells. The invention also extends to a host cell transformed with a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, Maniatis *et al.* and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 50°C.

The product is recovered by conventional methods according to the host cell and according to the localisation of the expression product (intracellular or secreted into the culture medium or into the cell periplasm). Thus, where the host cell is bacterial, such as *E. coli* it may, for example, be lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. Where the host cell is mammalian, the product may generally be isolated from the nutrient medium or from cell free extracts. Conventional protein isolation techniques include selective precipitation, adsorption chromatography, and affinity chromatography including a monoclonal antibody affinity column.

The proteins of the present invention are provided either soluble in a liquid form or in a lyophilised form.

It is generally expected that each human dose will comprise 1 to 1000 µg of protein, and preferably 30 - 300 µg.

The present invention also provides pharmaceutical composition comprising a protein of the present invention in a pharmaceutically acceptable excipient.

A preferred vaccine composition comprises at least Lipoprotein D - MAGE-3. Such vaccine may optionally contain one or more other tumor-associated antigen. For example other members belonging to the MAGE and GAGE families. Suitable other tumour associated antigen include MAGE-1, GAGE-1 or Tyrosinase proteins.

Vaccine preparation is generally described in Vaccine Design ("The subunit and adjuvant approach" (eds. Powell M.F. & Newman M.J). (1995) Plenum Press New York). Encapsulation within liposomes is described by Fullerton, US Patent 4,235,817.

5 The proteins of the present invention are preferably adjuvanted in the vaccine formulation of the invention. Suitable adjuvants include an aluminium salt such as aluminium hydroxide gel (alum) or aluminium phosphate, but may also be a salt of calcium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatised polysaccharides, 10 or polyphosphazenes. Other known adjuvants include CpG containing oligonucleotides. The oligonucleotides are characterised in that the CpG dinucleotide is unmethylated. Such oligonucleotides are well known and are described in, for example WO96/02555.

In the formulation of the inventions it is preferred that the adjuvant 15 composition induces an immune response preferentially of the TH1 type. Suitable adjuvant systems include, for example a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL) together with an aluminium salt. CpG oligonucleotides also preferentially induce a TH1 response,

An enhanced system involves the combination of a monophosphoryl lipid A 20 and a saponin derivative particularly the combination of QS21 and 3D-MPL as disclosed in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol as disclosed in WO 96/33739.

A particularly potent adjuvant formulation involving QS21 3D-MPL & tocopherol in an oil in water emulsion is described in WO 95/17210 and is a 25 preferred formulation.

Accordingly in one embodiment of the present invention there is provided a vaccine comprising a protein of the present invention, more preferably a Lipoprotein D (or derivative thereof) - MAGE-3 adjuvanted with a monophosphoryl lipid A or derivative thereof.

30 Preferably the vaccine additionally comprises a saponin, more preferably QS21.

Preferably the formulation additionally comprises an oil in water emulsion and tocopherol. The present invention also provides a method for producing a vaccine formulation comprising mixing a protein of the present invention together with a pharmaceutically acceptable excipient, such as 3D-MPL.

5 In one aspect of the invention there is provided a process for purifying a recombinantly produced MAGE-protein. The process comprises solubilising the protein, for example in a strong chaotropic agent (such as for example, urea, guanidium hydrochloride), or in a Zwitterionic detergent, e.g. (Empigen BB - n-dodecyl- N,N-dimethylglycine), reducing the protein's intra and inter molecular 10 disulphide bonds, blocking the resulting thiols to prevent oxidative recoupling, and subjecting the protein to one or more chromatographic steps.

Preferably, the blocking agent is an alkylating agent. Such blocking agents include but are not limited to alpha haloacids or alpha haloamides. For example iodoacetic acid and iodoacetamide which results in carboxymethylation or 15 carboxyamidation (carbamidomethylation) of the protein. Other blocking agents may be used and are described in the literature (See for example, The Proteins Vol II Eds H neurath, RL Hill and C-L Boeder, Academic press 1976, or Chemical Reagents for Protein modification Vol I eds. RL Lundblad and CM Noyes, CRC Press 1985). Typical examples of such other blocking agents include N - 20 ethylmaleimide, chloroacetyl phosphate, O-methylisourea and acrylonitrile. The use of the blocking agent is advantageous as it prevents aggregation of the product, and ensure stability for downstream purification.

In an embodiment of the invention the blocking agents are selected to induce a stable covalent and irreversible derivative (eg alpha halo acids or alpha 25 haloamides). However other blocking agents maybe selected such that after purification the blocking agent may be removed to release the non derivatised protein.

MAGE proteins having derivatised free thiol residues are new and form an aspect of the invention. In particular carboxyamidated or carboxymethylated 30 derivatives are a preferred embodiment of the invention.

In a preferred embodiment of the invention the proteins of the present invention is provided with an affinity tag, such as CLYTA or a polyhistidine tail.

EXAMPLE IV:**1. Vaccine preparation using LPD-MAGE-3-His protein:**

5 The vaccine used in these experiments is produced from a recombinant DNA, encoding a Lipoprotein D 1/3-MAGE-3-His, expressed in *E. coli* from the strain AR58, either adjuvanted or not. As an adjuvant, the formulation comprises a mixture of 3 de -O-acylated monophosphoryl lipid A (3D-MPL) and QS21 in an oil/water emulsion. The adjuvant system SBAS2 has been previously described WO
10 95/17210.

3D-MPL: is an immunostimulant derived from the lipopolysaccharide (LPS) of the Gram-negative bacterium *Salmonella minnesota*. MPL has been deacylated and is lacking a phosphate group on the lipid A moiety. This chemical treatment
15 dramatically reduces toxicity while preserving the immunostimulant properties (Ribi, 1986). Ribi Immunochemistry produces and supplies MPL to SB-Biologicals. Experiments performed at Smith Kline Beecham Biologicals have shown that 3D-MPL combined with various vehicles strongly enhances both the humoral and a
20 TH1 type of cellular immunity.

QS21: is a natural saponin molecule extracted from the bark of the South American tree *Quillaja saponaria* Molina. A purification technique developed to separate the individual saponines from the crude extracts of the bark, permitted the isolation of the particular saponin, QS21, which is a triterpene glycoside
25 demonstrating stronger adjuvant activity and lower toxicity as compared with the parent component. QS21 has been shown to activate MHC class I restricted CTLs to several subunit Ags, as well as to stimulate Ag specific lymphocytic proliferation (Kensil, 1992). Aquila (formally Cambridge Biotech Corporation) produces and supplies QS21 to SB-Biologicals.

30 Experiments performed at SmithKline Beecham Biologicals have demonstrated a clear synergistic effect of combinations of MPL and QS21 in the induction of both humoral and TH1 type cellular immune responses.

The oil/water emulsion is composed an organic phase made of of 2 oils (a tocopherol and squalene), and an aqueous phase of PBS containing Tween 80 as emulsifier. The emulsion comprised 5% squalene 5% tocopherol 0.4% Tween 80
5 and had an average particle size of 180 nm and is known as SB62 (see WO 95/17210).

Experiments performed at SmithKline Beecham Biologicals have proven that the adjunction of this O/W emulsion to 3D-MPL/QS21 (SBAS2) further increases the
10 immunostimulant properties of the latter against various subunit antigens.

2. Preparation of emulsion SB62 (2 fold concentrate):

Tween 80 is dissolved in phosphate buffered saline (PBS) to give a 2% solution in the PBS. To provide 100 ml two fold concentrate emulsion 5g of DL
15 alpha tocopherol and 5ml of squalene are vortexed to mix thoroughly. 90ml of PBS/Tween solution is added and mixed thoroughly. The resulting emulsion is then passed through a syringe and finally microfluidised by using an M110S microfluidics machine. The resulting oil droplets have a size of approximately 180
20 nm.

3. Preparation of Lipoprot. D1/3 - MAGE-3-His QS21/3D MPL oil in water (SBAS2) formulation:

25 The adjuvant is formulated as a combination of MPL and QS21, in an oil/water emulsion. This preparation is delivered in vials of 0.7 ml to be admixed with the lyophilised antigen (vials containing from 30 to 300 µg antigen).

The composition of the adjuvant diluent for the lyophilised vaccine is as follows :

CLAIMS:

1. A tumour-associated antigen derivative from the MAGE family.
2. An antigen as claimed in claim 1, when the derivative is a MAGE protein linked
- 5 to an immunological fusion or expression enhancer partner.
3. An antigen as claimed in claim 1 or 2 wherein the derivative comprises an affinity tag.
4. An antigen as claimed in any of claims 1 to 3 which contains a derivatised free thiol.
- 10 5. An antigen as claimed in claim 4 which is a carboxyamide or carboxymethylated derivative.
6. A protein as claimed in claim 2, 3, 4 or 5 wherein the fusion partner is protein D or fragment thereof from *Haemophilus influenzae* B, NS1 protein from influenza or a fragment thereof or Lyta from *Streptococcus pneumoniae* or fragment thereof.
- 15 7. A protein as claimed in claim 2, 3, 4 or 5 wherein the fusion partner is the lipidated form of protein D or fragment thereof from *Haemophilus influenzae* B.
8. A protein as claimed in claim 1 to 7 wherein the MAGE protein is selected from
- 20 the group MAGE A1, MAGE A2, MAGE A3, MAGE A4, MAGE A5, MAGE A6, MAGE A7, MAGE A8, MAGE A9, MAGE A10, MAGE A11, MAGE A12, MAGE B1, MAGE B2, MAGE B3 and MAGE B4, MAGE C1, MAGE C2.
9. A nucleic acid sequence encoding a protein as claimed herein.
- 25 10. A vector comprising a nucleic acid of claim 9.
11. A host transformed with a vector of claim 10.

12. A vaccine containing a protein as claimed in any of claims 1 to 8 or a nucleic acid as claimed in claim 9.
13. A vaccine as claimed in claim 12 additionally comprising an adjuvant, and/or
- 5 immunostimulatory cytokine or chemokine.
14. A vaccine as claimed in claim 12 or 13 wherein the protein is prescated in an oil in water or a water in oil emulsion vehicle.
- 10 15. A vaccine as claimed in claim 13 or 14 wherein the adjuvant comprises 3D-MPL, QS21 or a CpG oligonucleotide.
16. A vaccine as claimed herein additionally comprising one or more other
- 15 antigens.
17. A vaccine as claimed herein for use in medicine.
18. Use of a protein or nucleic acid as claimed herein for the manufacture of a vaccine for immunotherapeutically treating a patient suffering from melanomas or
- 20 other MAGE-associated tumours.
19. A process for the purification of a MAGE protein or derivative thereof, comprising reducing the disulphide bonds, blocking the resulting free thiol group with a blocking group, and subjecting the resulting derivative to one or more
- 25 chromatographic purification steps.
20. A process for the production of a vaccine, comprising the steps of purifying a MAGE protein or a derivative thereof, by the process of claim 19 and formulating the resulting protein as a vaccine.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham Biologicals

(ii) TITLE OF THE INVENTION: Vaccine

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SmithKline Beecham

(B) STREET: 2 New Horizons Court, Great West Road, B

(C) CITY: Middx

(D) STATE:

(E) COUNTRY: UK

(F) ZIP: TW8 9EP

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

(D) SOFTWARE: FastSEQ for Windows Version 2.0

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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Dalton, Marcus J

(B) REGISTRATION NUMBER:

(C) REFERENCE/DOCKET NUMBER: B45126

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 0181 9756348

(B) TELEFAX: 0181 9756177

(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 452 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Asp Pro Lys Thr Leu Ala Leu Ser Leu Ala Ala Gly Val Leu
 1 5 10 15
 Ala Gly Cys Ser Ser His Ser Ser Asn Met Ala Asn Thr Gln Met Lys
 20 25 30
 Ser Asp Lys Ile Ile Ala His Arg Gly Ala Ser Gly Tyr Leu Pro

35 40 45
 Glu His Thr Leu Glu Ser Lys Ala Leu Ala Phe Ala Gln Ala Asp
 50 55 60
 Tyr Leu Glu Gln Asp Leu Ala Met Thr Lys Asp Gly Arg Leu Val Val
 65 70 75 80
 Ile His Asp His Phe Leu Asp Gly Leu Thr Asp Val Ala Lys Lys Phe
 85 90 95
 Pro His Arg His Arg Lys Asp Gly Arg Tyr Tyr Val Ile Asp Phe Thr
 100 105 110
 Leu Lys Glu Ile Gln Ser Leu Glu Met Thr Glu Asn Phe Glu Thr Met
 115 120 125
 Asp Leu Glu Gln Arg Ser Gln His Cys Lys Pro Glu Glu Gly Leu Glu
 130 135 140
 Ala Arg Gly Glu Ala Leu Gly Leu Val Gly Ala Gln Ala Pro Ala Thr
 145 150 155 160
 Glu Glu Gln Glu Ala Ala Ser Ser Ser Ser Thr Leu Val Glu Val Thr
 165 170 175
 Leu Gly Glu Val Pro Ala Ala Glu Ser Pro Asp Pro Pro Gln Ser Pro
 180 185 190
 Gln Gly Ala Ser Ser Leu Pro Thr Thr Met Asn Tyr Pro Leu Trp Ser
 195 200 205
 Gln Ser Tyr Glu Asp Ser Ser Asn Gln Glu Glu Glu Gly Pro Ser Thr
 210 215 220
 Phe Pro Asp Leu Glu Ser Glu Phe Gln Ala Ala Leu Ser Arg Lys Val
 225 230 235 240
 Ala Glu Leu Val His Phe Leu Leu Leu Lys Tyr Arg Ala Arg Glu Pro
 245 250 255
 Val Thr Lys Ala Glu Met Leu Gly Ser Val Val Gly Asn Trp Gln Tyr
 260 265 270
 Phe Phe Pro Val Ile Phe Ser Lys Ala Ser Ser Ser Leu Gln Leu Val
 275 280 285
 Phe Gly Ile Glu Leu Met Glu Val Asp Pro Ile Gly His Leu Tyr Ile
 290 295 300
 Phe Ala Thr Cys Leu Gly Leu Ser Tyr Asp Gly Leu Leu Gly Asp Asn
 305 310 315 320
 Gln Ile Met Pro Lys Ala Gly Leu Leu Ile Val Leu Ala Ile Ile
 325 330 335
 Ala Arg Glu Gly Asp Cys Ala Pro Glu Glu Lys Ile Trp Glu Glu Leu
 340 345 350
 Ser Val Leu Glu Val Phe Glu Gly Arg Glu Asp Ser Ile Leu Gly Asp
 355 360 365
 Pro Lys Lys Leu Leu Thr Gln His Phe Val Gln Glu Asn Tyr Leu Glu
 370 375 380
 Tyr Arg Gln Val Pro Gly Ser Asp Pro Ala Cys Tyr Glu Phe Leu Trp
 385 390 395 400
 Gly Pro Arg Ala Leu Val Glu Thr Ser Tyr Val Lys Val Leu His His
 405 410 415
 Met Val Lys Ile Ser Gly Gly Pro His Ile Ser Tyr Pro Pro Leu His
 420 425 430
 Glu Trp Val Leu Arg Glu Gly Glu Glu Thr Ser Gly Gly His His His
 435 440 445
 His His His
 450

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1353 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(71) Applicant (for all designated States except US): SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).		
(72) Inventors; and (75) Inventors/Applicants (for US only): BOON, Thierry [BE/BE]; Ludwig Institute for Cancer Research, Avenue Hippocrate 74 UCL 7459, B-1200 Brussels (BE). SILLA, Silvia [IT/BE]; Ludwig Institute for Cancer Research, Avenue Hippocrate 74 UCL 7459, B-1200 Brussels (BE). UYTENHOVE, Catherine [BE/BE]; Ludwig Institute for Cancer Research, Avenue Hippocrate 74 UCL 7459, B-1200 Brussels (BE).		
(74) Agent: DALTON, Marcus, Jonathan, William; SmithKline Beecham, Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).		
(54) Title: ADJUVANT COMPOSITIONS FOR VACCINES		
(57) Abstract The present invention provides improved adjuvant compositions comprising QS21/3DMPL and Interleukin 12. These find utility in a range of prophylactic and therapeutic vaccines, including cancer vaccines.		

ADJUVANT COMPOSITIONS FOR VACCINES

The present invention relates to improved adjuvant compositions, for the stimulation of an immune response suitable for immunotherapy applications. In

5 particular the present invention relates to compositions comprising mixture of a saponin adjuvant with monophosphoryl lipid A' or derivative thereof and interleukin 12. In particular, the invention relates to compositions comprising 3 de-o-acylated monophosphoryl lipid A, QS21, and IL12. Such compositions are particularly useful in the immunotherapy of tumours.

10 Cancer is a disease developing from a single cell due to genetic changes. Clinical detection of these tumours occurs mostly in a relatively late stage of disease, when the primary tumour can be removed by surgery, and the existence of micro metastases settled in different organs has often already occurred. Chemotherapy does often not completely eliminate these cells, which then remain as

15 a source for recurrent disease.

Immune cells are able to control all different tissues (with the exception of the brain) and, due to their memory function, can also eliminate hidden cells re-entering the circulation (metastasis). Therefore, an activated immune response to tumour cells is expected to be of clinical benefit. Despite their undifferentiated

20 growth, tumour cells are in many aspects indistinguishable from normal cells, and over-expression of certain proteins or expression of mutated proteins is in most cases not sufficient to activate the immune response. This situation results in failure of immune surveillance. Thus, strategies for therapy of disseminated tumours need to specifically activate the immune response to tumour cells and to trigger migratory

25 activity of cytotoxic T cells for example leading to elimination of most and possibly every single tumour cell. Genetic mutation in tumour cells is intense, and strong immune responses are therefore required to prevent further genetic changes of the tumour cells (escape variants) under the pressure of the immune system.

It is now well established that cellular antigens which are not cell surface

30 proteins *per se* can be the targets of immune rejection through their recognition by immune regulatory and cytotoxic T cells. New potential target antigens for

immune-mediated tumour rejection are being identified, based on their recognition by immune T cells, rather than by antibodies. Such antigens may or may not induce antibody formation. It is now recognized that the expression of tumour antigens by a cell is in itself not sufficient for *induction* of an immune response to these antigens. Initiation of a tumour rejection response requires a series of immune amplification phenomena dependent on the intervention of antigen presenting cells, which are responsible for delivery of a series of activation signals which ultimately leads to the rejection of the tumour.

Tumour rejection antigens which are presented on tumour cells and which are recognised by cytotoxic T cells can lead to lysis of the cell. To achieve this, in a clinical setting a vaccine composition comprising a tumour rejection antigen needs to be presented in a suitable adjuvant system to enable a suitable immune response to be mounted. However, activation of the immune systems requires activation signals which are initiated by antigen presenting cells and are not activated by the tumour cells themselves.

Vaccination with isolated tumour rejection antigens has been envisaged either by recombinant proteins, by the use of live recombinant vectors or by DNA vectors. Preferably subunit antigens will be used. However, to ensure these are effective, powerful adjuvant systems are required.

Accordingly, the present invention provides an adjuvant composition comprising a combination of a saponin adjuvant in combination with monophosphoryl lipid A or derivative thereof together with the cytokine Interleukin 12.

Immunologically active saponin fractions having adjuvant activity derived from the bark of the South American tree *Quillaja Saponaria Molina* are known in the art. For example QS21, also known as QA21, is an Hplc purified fraction from the *Quillaja Saponaria Molina* tree and its method of its production is disclosed (as QA21) in US patent No. 5,057,540. *Quillaja saponin* has also been disclosed as an adjuvant by Scott et al, Int. Archs. Allergy Appl. Immun., 1985, 77, 409.

Monophosphoryl lipid A and derivatives thereof are known in the art. A preferred derivative is 3 de-o-acylated monophosphoryl lipid A, and is known from British Patent No. 2220211.

Interleukin 12 (IL-12) is known. For a review see Trinchieri G. Interleukin-12 - A proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Immunology* 13: 251.276, 1995. It is a heterodimeric cytokine produced mostly by phagocytic cells in response to bacteria, bacterial products, and intracellular parasites, and to some degree by B lymphocytes. In particular, IL-12 is produced by antigen presenting cells and instrumental in induction of TH-1 cell responses. IL-12 induces IFN-gamma from NK and T cells, acts as a growth factor for activated NK and T cells, enhances the cytotoxic activity of NK cells, and induces cytotoxic T lymphocyte generation.

IL-12 and IL-12-induced IFN-gamma favor Th1 cell differentiation by priming CD4 (+) T cells for high IFN-gamma production. However, we surprisingly found that other cytokines, such as IFN- γ , IL-2, IL-6, IL-7, GM-CSF or MCP were unable to enhance the effect of the QS21/MPL adjuvant.

Preferably the compositions of the invention contain the immunologically active saponin fraction in substantially pure form. Preferably the compositions of the invention contain QS21 in substantially pure form, that is to say, the QS21 is at least 90% pure, preferably at least 95% pure and most preferably at least 98% pure. Other immunologically active saponin fractions useful in compositions of the invention include QA17/QS17.

In a preferred embodiment the composition also comprises a sterol such as cholesterol wherein the sterol is present in an excess ratio to that of the saponin. These show decreased reactogenicity when compared to compositions in which the cholesterol is absent, while the adjuvant effect is maintained. In addition it is known that QS21 degrades under basic conditions where the pH is about 7 or greater. Thus a further advantage is that the stability of QS21 to basemediated hydrolysis is enhanced in formulations containing cholesterol.

Although the adjuvant compositions can be utilised for the treatment or prophylaxis of a range of disease, they find particular utility in the field of cancer immunotherapy.

In particular, the adjuvant formulation finds utility particularly with tumour rejection antigens such as those for prostate, breast, colorectal, pancreatic, renal or melanoma cancers. Exemplary antigens include MAGE 1 and MAGE 3 or other MAGE antigens for the treatment of melanoma, BAGE or GAGE LAGE (NY-eso-1) PRAME or Her-2/neu; Robbins and Kawakami (1996), Current Opinions in Immunology 8, pps 628-636; Van den Eynde *et al.*, International Journal of Clinical & Laboratory Research (submitted 1997); Correale *et al.* (1997), Journal of the National Cancer Institute 89, p293. Indeed these antigens are expressed in a wide range of tumour types such as melanoma, lung carcinoma, sarcoma and bladder carcinoma. Other classes of antigens useful in the context of the present invention include tissue specific antigens such as Prostate Specific antigen (PSA); Prostate Specific Membrane antigen (PMSA), Melan A/Mart 1, gp100, tyrosinase TRP1 or TRP2.

Accordingly in one aspect of the present invention there is provided a vaccine comprising an adjuvant composition according to the invention and a tumour rejection antigen, or tissue specific antigen.

Other antigens or antigenic compositions include for example, polysaccharide antigens, protein antigens or DNA encoding antigens or antigenic compositions derived from HIV-1, (such as gp120 or gp160), any of Feline Immunodeficiency virus, human or animal herpes viruses, such as gD or derivatives thereof or Immediate Early protein such as ICP27 from HSV1 or HSV2, cytomegalovirus (especially human) (such as gB or derivatives thereof), Varicella Zoster Virus (such as gpI, II or III), or from a hepatitis virus such as hepatitis B virus for example Hepatitis B Surface antigen or a derivative thereof, hepatitis A virus, hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as Respiratory Syncytial virus (for example RSV F and G proteins or immunogenic fragments thereof disclosed in US Patent 5,149,650 or chimeric polypeptides containing immunogenic fragments from HSRV proteins F and G, eg GF

glycoprotein disclosed in US Patent 5,194,595), antigens derived from meningitis strains such as meningitis A, B and C, Streptococcus Pneumonia, human papilloma virus, in particular from strains HPV6, 11, 16 and 18, Influenza virus, Haemophilus Influenza B (Hib), Epstein Barr Virus (EBV), or derived from bacterial pathogens such as Salmonella, Neisseria, Borrelia (for example OspA or OspB or derivatives thereof), or Chlamydia, or Bordetella for example P.69, PT and FHA, or derived from parasites such as plasmodium or toxoplasma.

The P815 tumour is a mastocytoma, induced in a D BA/2 mouse with methylcholanthrene and cultured as both an in vitro tumour and cell line. This represents an excellent model system for the human.

The model system described in this application is a murine system whereby a murine tumour antigen, P1A, expressed in the mouse mastocytoma P815, is being tested for its ability to stimulate CTL in the mouse with and without adjuvant. The significance of this system is that P1A is a true tumour rejection antigen in that its gene is the same in both normal and tumour cells but the gene is silent in normal cells and only expressed in tumour cells. This is in comparison to other P815 antigens that were previously found and which are created by mutation of normal alleles. These are called tum- variants or tum- antigens. Mutations in the tum- antigens create new antigenic peptides which can then be recognised by CTL.

Tum- antigens are likely to be tumour-specific whereas true tumour antigens will be shared between different tumours and patients and therefore the latter will be better candidates for vaccine formulations. Human tumour rejection antigens analogous to P1A include the MAGE, BAGE, GAGE etc. families as described earlier. These genes are found in both normal and tumour tissues but the corresponding proteins are expressed only in tumours and in normal testis. As the testis is an immune privileged site it is unlikely to be affected by any vaccine.

P1A is a true murine TRAs. Therefore, one can test a large number of adjuvants with a variety of different assays in mice giving a good indication as to which formulations should be used with human tumour rejection antigens in human clinical trials.

Preferred compositions of the invention are those forming a liposome structure. Compositions where the sterol/immunologically active saponin fraction forms an ISCOM structure also form an aspect of the invention.

The ratio of QS21 : sterol will typically be in the order of 1 : 100 to 1 : 1 weight to weight. Preferably excess sterol is present, the ratio of QS21 : sterol being at least 1 : 2 w/w. Typically for human administration QS21 and sterol will be present in a vaccine in the range of about 1 ng to about 100µg, preferably about 10µg to about 50µg per dose.

The liposomes preferably contain a neutral lipid, for example phosphatidylcholine, which is preferably non-crystalline at room temperature, for example egg yolk phosphatidylcholine, dioleoyl phosphatidylcholine or dilauryl phosphatidylcholine. The liposomes may also contain a charged lipid which increases the stability of the liposome-QS21 structure for liposomes composed of saturated lipids. In these cases the amount of charged lipid is preferably 1-20% w/w, most preferably 5-10%. The ratio of sterol to phospholipid is 1-50% (mol/mol), most preferably 20-25%.

The compositions of the invention also contain a 3 deacylated monophosphoryl lipid A derivative (3-de-O-acylated monophosphoryl lipid A, also known as 3D-MPL) and is manufactured by Ribi Immunochem, Montana. A preferred form is disclosed in International Patent Application 92/116556.

Suitable compositions of the invention are those wherein liposomes are initially prepared without 3D-MPL, and 3D-MPL is then added, preferably as 100nm particles. The 3D-MPL is therefore not contained within the vesicle membrane (known as 3D-MPL out). Compositions where the 3D-MPL is contained within the vesicle membrane (known as 3D-MPL in) also form an aspect of the invention. The antigen can be contained within the vesicle membrane or contained outside the vesicle membrane or encapsulated. Preferably soluble antigens are outside and hydrophobic or lipidated antigens are either contained inside or outside the membrane. The 3D-MPL will be present in the range of about 1Ug to 100Ug and preferably about 10 to 50 µg per dose of human vaccine.

Often the vaccines of the invention will not require any specific carrier and formulated in an aqueous or other pharmaceutically acceptable buffer. In some cases it may be advantageous that the vaccines of the present invention will further contain alum.

5 Example 1:

a) - Immunisation of DBA/2 mice with peptide + 3 D-MPL + QS21 + Lipids ± IL12

Human tumours express antigens that can be recognized by autologous CTL. These antigens constitute useful targets for cancer immunotherapy. We decided to evaluate in the P815 murine mastocytoma model the efficacy of an immunization method that could be applied to human patients. Syngeneic DBA/2 mice were injected with antigenic peptides mixed with adjuvant and murine IL12.

An adjuvant composition comprising QS21, lipids (DQ) and 3 de-o-acylated monophosphoryl lipid A (3D-MPL) was prepared.

Briefly a mixture of lipid (such as phosphatidylcholine either from egg-yolk or synthetic) and cholesterol in organic solvent, is dried down under vacuum (or alternatively under a stream of inert gas). An aqueous solution (such as phosphate buffered saline) is then added, and the vessel agitated until all the lipid is in suspension. This suspension is then microfluidised until the liposome size is reduced to 100 nm, and then sterile filtered through a 0.2 µm filter. Extrusion or sonication could replace this step.

The cholesterol phosphatidylcholine ratio is 1:4 (w/w), and the aqueous solution is added to give a final cholesterol concentration of 5 to 50 ng/ml. The liposomes have a defined size of 100 nm and are referred to as SUV (for small unilamellar vesicles). If this solution is repeatedly frozen and thawed the vesicles fuse to form large multilamellar structures (MLV) of size ranging from 500nm to 15 µm.

QS21 in aqueous solution is added to the liposomes. This mixture is then added to 50 µg of P198 peptide (KYQAVTTTL) and 3D-MPL.

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**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

Abogado **ALICIA LLOREDA RICAURTE**

ASUNTO Radicación 06-110710
 Trámite 011
 Evento 378
 Actuación 519
 Oficio
 Folios 016 239448

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional	☒	Cap. I	☒
Vía PCT (fase nacional)	☒	Cap. II	☐

No. Solicitud Internacional PCT/EP2005/004956
Fecha de Presentación Internacional 02 Mayo 2005

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 37 a 61	31 Octubre 2006
Reivindicaciones:	Folios 62 a 63	31 Octubre 2006
Secuencias:	Folios 64 a 65	31 Octubre 2006
Prioridad:	GB 0409940.4	04 Mayo 2004

2. Objeto de la invención

Título de la solicitud: "Vacuna polivalente basada en MAGE-3 y NY-ESO-1 para inmunoterapia del cáncer".

El problema planteado en esta solicitud consiste en la necesidad de proporcionar terapias de tipo inmunológico para el tratamiento del cáncer.

Para resolver este problema técnico la solicitud en estudio proporciona una vacuna polivalente que comprende la combinación de antígenos MAGE-3 y NY-ESO-1 y un adyuvante inmunoestimulador.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 39/00

3. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

- La reivindicación 1 contiene los términos "antígeno MAGE-3 modificado" "baja dosis de aceite en agua", "oligonucleótido inmunoestimulador", "Saponina",

“lipopolisacarido” y “ISCOMS” los cuales no son claros y no definen a que tipo de compuestos hace referencia ni su naturaleza ó estructura. En el caso de los términos “antígeno MAGE-3 modificado” y “oligonucleótido inmunoestimulador” se deben definir sus secuencias y el tipo de modificación realizada, esto con el fin de definir claramente a que tipo de sustancias se refiere. Establecer el tipo de modificación realizada al antígeno MAGE ya que puede generarse una modificación de cualquier tipo lo que genera múltiples posibilidades de antígeno.

- La reivindicación 1 reclama composiciones en las que se emplean diferentes tipos de adyuvantes inmunoestimuladores, (sin embargo en la descripción no se encuentra sustento para todas las composiciones que resultarían de emplear los anteriores tipos de adyuvantes.
- La reivindicación 5 se encuentra mal redactada ya que hace mención a “la saponina es QS21 formulada en un colesterol que contiene liposoma” y teniendo en cuenta el capítulo descriptivo esta composición de saponina esta combinada con colesterol y dentro de un liposoma (folio 48). Se sugiere hacer la corrección correspondiente.
- Las reivindicaciones 2 y 6 hace referencia a la presencia de un oligonucleótido inmunoestimulador el cual es CpG, para caracterizarlo de mejor manera se sugiere establecer la secuencia que identifica dicho CpG.
- El capítulo reivindicatorio no presenta soporte en la descripción debido a que las composiciones reclamadas que combinan los dos antígenos MAGE-3 modificado y NY-ESO-1 no se encuentran divulgadas, por el contrario se enseñan composiciones que se formulan por separado cada uno de los antígenos con una composición adyuvante y estas dos composiciones independientes se administran de forma secuencial o concomitante.

Descripción (Art 28 D 486)

La Descripción no divulga la invención de manera suficientemente completa para que el la persona del oficio normalmente versada en la materia pueda ejecutarla ó reproducirla ya que no se divulga como las vacunas de la presente invención pueden mejorar el efecto antitumoral de las vaunas de cáncer en comparación con una vacuna que contiene solamente uno de los antígenos expresados por una célula tumoral. Asimismo, no divulga una composición única que contenga todos y cada uno de los componentes, sino por el contrario se enseña que los antígenos MAGE-3 modificado y NY-ESO-1 se formulan por separado con una composición adyuvante y estas dos composiciones independientes se proporcionan para la administración secuencial o concomitante (ver folio 41).

4. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de presentación de la solicitud de prioridad (04 Mayo 2004) y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Item	Documento	Título	Fecha de Publicación
D1	US2003/0044813	Cáncer testis antigens	06 Marzo 2003

D2	WO99/40188	Tumor-associated antigen derivatives from the mage family, and nucleic acid sequences encoding them, used for the preparation of fusion proteins and of compositions for vaccination	12 Agosto 1999
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D1 (folios 95 a 98) divulga antígenos de cáncer de testículo (CT) y composiciones que estimulan la respuesta inmune (vacunas) que comprenden proteínas aisladas, péptidos y anticuerpos para el tratamiento de trastornos caracterizados por la expresión de uno o más antígenos CT.
URL:http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&adjacent=true&locale=en_gb&FT=D&date=20030306&CC=US&NR=2003044813A1&KC=A1

D2 (folios 99 a 102) divulga nuevas proteínas de la familia MAGE y su producción. En particular, una proteína MAGE fusionada a una lipoproteína de antígenos de D. dichas proteínas pueden ser formuladas para proporcionar vacunas para el tratamiento de una amplia gama de tumores.
URL:http://worldwide.espacenet.com/publicationDetails/biblio?CC=WO&NR=9940188A2&KC=A2&FT=D&date=19990812&DB=EPODOC&locale=en_gb

5. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La **reivindicación 1** consiste en una composición de vacuna comprendiendo (a) un componente de antígeno que comprende una combinación de un antígeno MAGE-3 modificado y un antígeno NY-ESO-1 o derivado de los mismos, y (b) un componente de adyuvante que comprende un adyuvante inmunoestimulador que comprende uno o más de: sal de alumbre, colesterol; emulsión de aceite en agua (emulsión A/A); baja dosis de emulsión de aceite en agua; un oligonucleótido inmunoestimulador, tocoferol; liposoma, una saponina; ISCOMS; y un lipopolisacarido.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
Composición de vacuna	Composición que estimulan la respuesta inmune (vacunas)
a) Un componente de antígeno que comprende una combinación de un antígeno MAGE-3 modificado y un antígeno NY-ESO-1 o derivado de los mismos	Combinación de al menos 2 péptidos antigénicos de tipo CT (MAGE-3 / NY-ESO-1)
b) un componente de adyuvante que comprende un adyuvante inmunoestimulador (uno o más) - sal de alumbre – colesterol - emulsión de aceite en agua (emulsión A/A) - baja dosis de emulsión de aceite en agua - un oligonucleótido inmunoestimulador – tocoferol – liposoma - una saponina - ISCOMS; y un lipopolisacarido	Adyuvante - un oligonucleótido inmunoestimulador una saponina - ISCOMS; y un lipopolisacarido.

Las características **esenciales** de la composición de vacuna de la reivindicación 1 habían sido divulgadas previamente en D1, el cual da a conocer composiciones que estimulan la respuesta inmune que comprenden al menos la combinación de dos diferentes péptidos antigénicos de tipo CT (folio 95, paágina 5, párrafo 0043; folio 98, pagina 56, reivindicación 30) dentro de los que se encuentran MAGE-3 y NY-ESO-1 y derivados de estos (folio 95, pagina 5, párrafo 0051; folio 96, página 8, párrafo 0081), y un adyuvante que comprende un oligonucleótido inmunoestimulador, una saponina, etc. (folio 95, página 5, párrafo 0043; folio 98, página 56, reivindicación 31 - 78).

En consecuencia, la reivindicación 1 no es nueva, según lo divulgado en el documento D1.

La reivindicación dependiente 2 no menciona alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no es nueva.

Nivel Inventivo (Art18 D486)

La **reivindicación 3** consiste en una vacuna según se reivindica en la reivindicación 1 o 2, en donde el antígeno MAGE-3 modificado tiene una secuencia de polipéptido según se describe en SEC ID No. 1.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
Composición de vacuna	Composición que estimulan la respuesta inmune (vacunas)	Composiciones de vacuna
a) Un componente de antígeno que comprende una combinación de un antígeno MAGE-3 modificado y un antígeno NY-ESO-1 o derivado de los mismos Donde MAGE-3 modificado tiene SEC ID No. 1	Combinación de al menos 2 péptidos antigénicos de tipo CT (MAGE-3 / NY-ESO-1)	Un componente de antígeno de MAGE-3 proteína D 1/3 que tiene SEC ID No. 1 Junto con uno o más antígenos de la familia GAGE
b) un componente de adyuvante que comprende un adyuvante inmunoestimulador (uno o más) - sal de alumbre - colesterol - emulsión de aceite en agua (emulsión A/A) - baja dosis de emulsión de aceite en agua - un oligonucleótido inmunoestimulador - tocoferol - liposoma - una saponina - ISCOMS; y un lipopolisacárido	Adyuvante inmunoestimulador - un oligonucleótido inmunoestimulador una saponina - ISCOMS; y un lipopolisacárido.	Adyuvante -sal de alumbre - emulsión de aceite en agua (emulsión A/A) - un oligonucleótido inmunoestimulador (CpG) - tocoferol una saponina - y un lipopolisacárido

El estado de la técnica cercano corresponde a los documentos D1 a D2 en donde:

D1 divulga composiciones que estimulan la respuesta inmune, dichas composiciones comprenden al menos la combinación de dos diferentes péptidos antigénicos de tipo CT (folio 95, pagina 5, párrafo 0043; folio 98, pagina 56, reivindicación 30) dentro de los que se encuentran MAGE-3 y NY-ESO-1 y derivados de estos (folio 95, pagina 5, párrafo 0051; folio 96, pagina 8, párrafo 0081), y un adyuvante que comprende un oligonucleótido inmunoestimulador, una saponina, etc. (folio 95, pagina 5, párrafo 0043; folio 98, pagina 56, reivindicación 31 - 78).

La diferencia entre la invención, definida en la reivindicación 3 y las composiciones antigénicas divulgadas en D1 consiste en que el antígeno de MAGE-3 modificado corresponde al antígeno de SEC ID No. 1.

Por otro lado **D2** describe una composición de vacuna que tiene como componente principal el antígeno MAGE-3 unido como proteína de fusión a la proteína D 1/3 (folio 99, página 4, línea 11 a 15; pagina 8, línea 26 a 28; folio 101, reivindicación 12) con secuencia SEC ID No. 1, la cual es idéntica a la reclamada en la presente solicitud (folio 102). Dicha composición de vacuna además de contener MAGE-3 proteína D 1/3 puede contener uno o más antígenos asociados a tumores tales como antígenos GAGE perteneciente a la familia CT (antígenos de cáncer de testículos) (folio 99, pagina 8, línea 29 a 31; folio 101, reivindicación 16), asimismo, la composición

contiene un adyuvante en un vehículo tipo emulsión (folio 101, reivindicación 14) que comprende lipopolisacaridos (3D-MLP) (folio 100, pagina 9, línea 16 a 17; folio 101, reivindicación 15), saponinas (QS21) (folio 100, pagina 9, línea 30 a 31; folio 101, reivindicación 15), oligonucleótido inmunoestimulador (secuencias CpG) (folio 100, pagina 9, línea 10 a 13; folio 101, reivindicación 15) y combinaciones de los mismos.

La diferencia entre la invención, definida en la reivindicación 3 y las composiciones antigénicas divulgadas en D2 consiste en que el antígeno adicional de las composiciones que contienen MAGE-3 proteína D 1/3 de SEC ID No. 1 es el antígeno NY-ESO-1.

El hecho de combinar dos diferentes péptidos antigénicos de tipo CT como son el MAGE-3 proteína D 1/3 de SEC ID No. 1 y el NY-ESO-1 no evidencia un efecto técnico sorprendente con respecto a lo divulgado en el estado de la técnica puesto que no se observa que las composiciones de vacuna reclamadas presenten actividad sinérgica o aditiva con respecto a otras vacunas que contienen otros antígenos de similar naturaleza. De igual manera es conocido en el estado de la técnica la combinación de diferentes tipos de adyuvantes inmunoestimuladores los cuales mejoran la respuesta inmune en formulaciones de vacunas.

Es así que el problema técnico objetivo de esta solicitud consiste en proporcionar composiciones de vacunas que estimulan la respuesta inmune para el tratamiento del cáncer alternativas a las dadas a conocer en D1 y D2 que contengan el antígeno de MAGE-3 modificado de SEC ID No. 1 y el antígeno NY-ESO-1 en combinación.

A partir de lo expuesto anteriormente, una persona del oficio normalmente versada en la materia que pretenda obtener composiciones de vacunas par el tratamiento de cáncer, estaria motivado a combinar dos diferentes péptidos antigénicos de tipo CT como los divulgados en D1 y escoger como uno de dichos péptidos un antígeno como el MAGE-3 proteína D 1/3 de SEC ID No. 1 divulgado en D2 en llegando de esta manera al contenido de la reivindicación 3

Asimismo una persona del oficio normalmente versada podria incluir en las composiciones de vacuna divulgadas en D2 que contienen el antígeno MAGE-3 proteína D 1/3 de SEC ID No. 1 otro agente antigénico de tipo CT como el NY-ESO-1 llegando de esta manera al contenido de la reivindicación 3

Por todo lo anterior y teniendo en cuenta lo divulgado en el estado de la técnica (D1 y D2) el objeto reivindicado no tiene nivel inventivo.

Puesto que las reivindicaciones dependientes 4 a 7 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

6. Conclusión

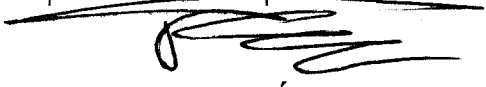
Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US2003/0044813	1-2 / 3 a 7	Novedad / Nivel Inventivo
D2	WO99/40188	3 a 7	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días

contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o sí a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha
_____ **15 DIC. 2011.**

Elaborado por: Ana Delia Pinzón García
Revisado Por: Jacqueline Alfonso 
Aprobado por: José Luis Salazar López