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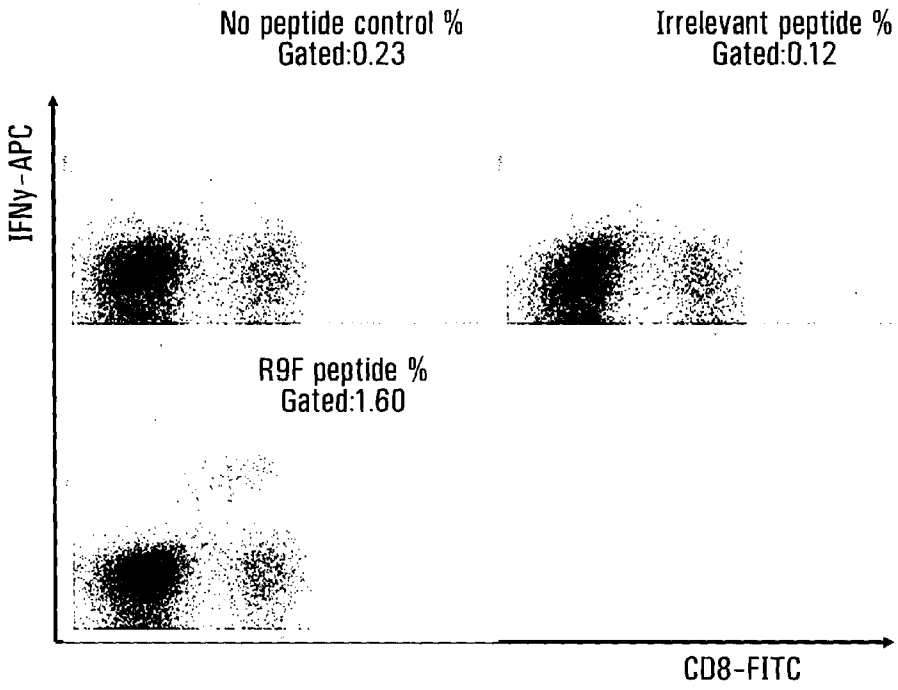
(54) Title: USE OF LIPOSOMES IN A CARRIER COMPRISING A CONTINUOUS HYDROPHOBIC PHASE AS A VEHICLE FOR CANCER TREATMENT



(57) Abstract: The invention compositions comprising a continuous hydrophobic phase and liposomes as a vehicle for delivery of an antigen capable of inducing a cytotoxic T lymphocyte (CTL) response and methods for their use in the treatment of cancer.

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Use of liposomes in a carrier comprising a continuous hydrophobic phase as a vehicle for cancer treatment

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority from Canadian Patent Application Nos. 2,523,032; 2,533,705; and 2,542,212 filed October 7, 2005; January 13, 2006 and April 7, 2006, respectively, and further claims the benefit and priority from U.S. Provisional Patent Application No.60/806,573, filed July 5, 2006, all of which are incorporated by reference herein.

FIELD OF THE INVENTION

The present application relates to the use of a composition comprising liposomes and a continuous hydrophobic phase as a vehicle for delivery of an antigen capable of inducing a cytotoxic T lymphocyte (CTL) response in the treatment of cancer.

BACKGROUND OF THE INVENTION

Long lasting vaccines comprising liposomes and a variety of antigens have been previously described in the art. These vaccine compositions have been shown to be effective in inducing an enhanced humoral immune response (determined by increased antibody production) against a specific antigen, which is dependent on T helper 2 (Th2) function. However, for a composition to adversely affect cancer, it must be able to induce a cell-mediated (cytotoxic T lymphocyte (CTL)) response. A CTL is a sub-group of T lymphocytes that is capable of inducing the death of infected somatic or tumor cells; they kill (lyse) cells that are infected with viruses (or other pathogens), or are

otherwise damaged or dysfunctional. A CTL response is mediated through T helper 1 (Th1) cytokines.

In general, CTL responses are short-lived, lasting only several weeks (Knutson *et al.*, *Clin. Cancer. Res.* 8(5):1014-1018, 1990; Dudley *et al.*, *J. Immunother.* 24(4):363-73, 2002; and Fernando *et al.*, *Scand. J. Immunol.* 47(5):459-65, 1998). Recurrence of cancer is always of concern, thus the induction of a long-lasting CTL response is necessary to ensure that cancers do not reoccur.

Thus, there remains a need for the development of long-lasting immuno-therapeutic compositions for use in the treatment of cancer, without the need for multiple booster treatments.

SUMMARY OF THE INVENTION

In one embodiment, there is provided a composition comprising: a carrier comprising a continuous phase of a hydrophobic substance; liposomes; and at least one antigen capable of inducing a $CD8^+$ cytotoxic T lymphocyte (CTL) response. The composition preferably also comprises at least one T helper epitope.

The present application in a further aspect provides a method for treating cancer in a subject comprising administering the compositions as described herein.

According to another aspect, the present application provides a kit useful for treating cancer in subject comprising a composition as described herein, and instructions for its use thereof

Other aspects and features of the present invention will become apparent to those of ordinary skill in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

In the figures, which illustrate embodiments of the invention by way of example only:

Figure 1 illustrates *ex vivo* intracellular IFN-γ staining of splenocytes from mice exposed to an E7 epitope of HPV 16 (R9F peptide; SEQ ID NO: 1), an irrelevant peptide, or no peptide 14 days post-treatment with a composition comprising CpG oligodeoxynucleotide (CpG ODN)(SEQ ID NO: 12), and R9F peptide fused with PADRE encapsulated in liposomes contained in a PBS/FIA water-in-oil emulsion. None of the control treatments caused expansion of $CD8_+$/IFN-γ T cells above background levels when exposed to R9F post-treatment (data not shown). In contrast, a 12.9 fold increase of R9F-responsive CTLs occurred in splenocytes of mice exposed to R9F peptide as compared to mice exposed to irrelevant peptide.

Figure 2 illustrates lysis of R9F peptide (SEQ ID NO: 1) loaded (squares) and irrelevant peptide loaded (diamonds) EL-4 cells by splenocytes from mice treated 30 days prior with a composition comprising R9F peptide fused with PADRE (SEQ ID NO: 10), and encapsulated with CpG ODN (SEQ ID NO: 12) in liposomes suspended in a PBS/FIA water-in-oil emulsion.

Figure 3 illustrates lysis of EL-4 cells by splenocytes from mice treated with one of the following

compositions: (i) a fusion peptide (R9F peptide (SEQ ID NO: 1) fused with PADRE (SEQ ID NO: 10)) and CpG ODN (SEQ ID NO: 12) encapsulated in liposomes suspended in a PBS/FIA water-in-oil emulsion (diamonds); (ii) unencapsulated fusion peptide and CpG ODN (SEQ ID NO: 12) in a PBS/FIA water-in-oil emulsion (triangles and crosses), or (iii) liposome encapsulated fusion peptide in a PBS/FIA water-in-oil emulsion (closed circles). The splenocytes were then exposed to either R9F peptide (crosses, diamonds and closed circles) or an irrelevant peptide (SEQ ID NO: 13) (triangles and open circles) 130 days post-treatment. Splenocytes from mice treated with fusion peptide alone showed base levels of lysis of EL-4 cells exposed to either R9F or an irrelevant peptide 130 days post-treatment (data not shown).

Figure 4 illustrates the effect of a single administration of a composition comprising CpG ODN, a fusion peptide (R9F peptide (SEQ ID NO: 1) fused with PADRE (SEQ ID NO: 10)) encapsulated in liposomes suspended in a PBS/FIA water-in-oil emulsion (open squares) on the size of 14-day C3 tumors in mice. Tumors were undetectable by palpation by day 30(16 days post-treatment). In contrast, C3 tumors in mice given a control treatment comprising the composition as described above in the absence of a fusion peptide (closed squares) continued to increase in size. All mice (n=10) were challenged with 0.5×10^6 C3 tumour cells 14 days prior to treatment. The difference in size of tumors in the two treatment groups was statistically significant ($p = 0.002$ at day 25).

Figure 5 illustrates the effect of prophylactic treatment on the percentage of mice that are tumor-free 61 days post-challenge with 0.5×10^6 C3 cells. Treatment groups consisted of mice treated with one of the following

compositions comprising: (1) PBS; (2) CpG in PBS; (3) fusion peptide (R9F peptide (SEQ ID NO: 1) fused with PADRE (SEQ ID NO: 10)) in PBS; (4) fusion peptide encapsulated in liposomes suspended in a PBS/FIA water-in-oil emulsion; (5) fusion peptide and CpG ODN in a water-in-oil emulsion; (6) fusion peptide and CpG ODN encapsulated in liposomes; (7) fusion peptide and the lipopeptide, Pam3Cys-SKKKK (Pam3c) encapsulated in liposomes suspended in a water-in-oil emulsion.

Figure 6 illustrates *ex vivo* detection of TRP-2 specific IFN-γ producing splenocytes (spot forming cells, SFC) in mice 8 days following a single treatment with one of the following: (A) a composition comprising a tyrosine-related protein-2 (TRP-2) peptide (S9L; SEQ ID NO: 7) with PADRE (SEQ ID NO: 10) and CpG ODN (SEQ ID NO: 12) encapsulated in liposomes suspended in a PBS/ISA51 water-in-oil emulsion; (B) a composition comprising S9L with PADRE in liposomes suspended in a water-in-oil emulsion; or (C) a composition comprising an irrelevant peptide (SEQ ID NO: 13) with CpG in liposomes suspended in PBS/ISA51 a water-in-oil emulsion.

Figure 7 illustrates *ex vivo* detection of p53 specific IFN-γ producing splenocytes (SFC) in mice following a single treatment with (A) a modified p53 peptide (mK9M; SEQ ID NO: 8) with PADRE (SEQ ID NO: 10) and CpG ODN (SEQ ID NO: 12) encapsulated in liposomes and suspended in a PBS/ISA51 water-in-oil emulsion, or one of the following control compositions: (B) mK9M with PADRE in a water-in-oil emulsion; (C) mK9M with PADRE encapsulated in liposomes and suspended in a water-in-oil emulsion; and (D) an irrelevant peptide (SEQ ID NO: 13) with CpG ODN encapsulated in liposomes and suspended in a water-in-oil emulsion.

Figure 8 illustrates ex *vivo* detection of TRP-2- and p53-specific IFN-γ producing splenocytes (SFC) in mice following a single treatment with a composition comprising (A) p53 (mK9M peptide; SEQ ID NO: 8) and TRP-2 (V8L; SEQ ID NO: 6) peptides with PADRE (SEQ ID NO: 10) and CpG ODN (SEQ ID NO: 12) encapsulated in liposomes and suspended in a PBS/ISA51 water-in-oil emulsion or one of the following control compositions: (B) p53 and TRP-2 peptides with PADRE encapsulated in liposomes and suspended in a PBS/ISA51 water-in-oil emulsion; (C) p53 and TRP-2 peptides with PADRE and CpG ODN; (D) p53 and TRP-2 peptides with PADRE.

Figure 9 illustrates the effect of a single treatment with HLA-A2 E6/7 CTL epitope-containing peptides on 19-day-old TC1/A2 established tumors. A single treatment of a composition comprising a mixture of four HPV E6/7 peptides (Y10T (SEQ ID NO: 2), L9V (SEQ ID NO: 3), T81 (SEQ ID NO: 4), and T10V (SEQ ID NO: 5)) encapsulated in liposomes with CpG ODN (SEQ ID NO: 12) and PADRE (SEQ ID NO: 10) then suspended in a PBS/ISA51 water-in-oil emulsion (squares) or a long peptide containing the four HPV E6/7 peptides described above linked together with "aay" (alanine-alanine-tyrosine) linkers (AB2 peptide; SEQ ID NO: 14) encapsulated in liposomes with CpG ODN and PADRE then suspended in a water-in-oil emulsion (diamonds) eradicated TC1/A2 tumors by 21 days post-treatment. Treatment with one E7 peptide of HPV (L9V; SEQ ID NO: 3) encapsulated in liposomes with CpG ODN and PADRE then suspended water-in-oil emulsion significantly reduced tumor size (triangles). Treatment with PBS alone (crosses) did not prevent tumor growth.

Figure 10 illustrates tumor growth in five mice injected with PBS alone 19 days post-tumor implantation.

Figure 11 illustrates the effect of a single treatment with a composition comprising a mixture of four individual HLA-A2 E6/7 CTL epitope-containing peptides (Y10T, L9V, T81, and T10V; SEQ ID NOs: 2, 3, 4 and 5, respectively) encapsulated in liposomes with CpG ODN (SEQ ID NO: 12) and PADRE (SEQ ID NO: 10) then suspended in a PBS/ISA51 water-in-oil emulsion on the growth of 19-day-old TC1/A2 established tumors in five mice.

Figure 12 illustrates the effect of a single treatment with a composition comprising one long peptide (AB2; SEQ ID NO: 14) encapsulated in liposomes with CpG ODN (SEQ ID NO: 12) and PADRE (SEQ ID NO: 10) then suspended in a PBS/ISA51 water-in-oil emulsion on the growth of 19-day-old TC1/A2 established tumors in five mice.

Figure 13 illustrates the effect of a single treatment with a composition comprising a HLA-A2 E7 CTL epitope (L9V; SEQ ID NO: 3) encapsulated in liposomes with CpG ODN (SEQ ID NO: 12) and PADRE (SEQ ID NO: 10) then suspended PBS/ISA51 water-in-oil emulsion on tumor growth of 19-day-old TC1/A2 established tumors in five mice.

Figure 14 illustrates *ex vivo* detection of IFN-γ producing splenocytes in mice 9 days post-immunization by a single administration of a composition comprising (A) four short unlinked HPV-A2 HPV E6/E7 CTL epitope-containing peptides (T8l, Y10T, L9V or T10V; SEQ ID Nos: 2, 3, 4 and 5, respectively) or (B) two medium length dipeptides linked together with a "kkp" linker (Y10T-kkp-L9V (SEQ ID NO: 15) and T81-kkp-T10V (SEQ ID NO: 16)). Both compositions were encapsulated in liposomes with CpG ODN (SEQ ID NO: 12) and PADRE (SEQ ID NO: 10) then suspended in a PBS/ISA51 water-in-oil emulsion. Spleens from control mice (C) contained

background numbers of splenocytes that could be stimulated with each individual short peptide or a mixture of the four short peptides to produce IFN-γ. In contrast, spleens from mice in the group immunized with either the four short unlinked peptides or two medium dipeptides contained larger numbers of splenocytes that were stimulated with these peptides to produce IFN-γ. Stimulation of splenocytes from mice immunized with either formulation of the invention and stimulated with the mixture of the four short peptides produced approximately five times as many IFNγ-producing splenocytes compared to control splenocytes indicting the linking peptides with "kkp" had no effect on the immune response.

Figure 15 shows the growth of melanoma tumors in mice treated with one of the following compositions comprising at least one peptide derived from a melanoma-associated protein (TRP-2 or p53) and encapsulated with CpG ODN and PADRE in liposomes suspended in a water-in-oil emulsion: (i) V8L peptide (SEQ ID NO: 6) (diamonds); (ii) S9L peptide (SEQ ID NO: 7) (squares); (iii) mK9M peptide (SEQ ID NO: 8) (triangles); (iv) V8L and mK9M peptides (crosses); and (v) S9L and mK9M peptides (stars). Control mice were treated with PBS alone (circle). Mice were administered the treatment 5 days post-tumor implantation. Each data point is the average size of tumors in five mice.

Figure 16 illustrates the percentage of mice with melanoma tumors after treatment with one of the following compositions comprising at least one peptides derived from a melanoma-associated protein (TRP-2 or p53) and encapsulated with CpG ODN (SEQ ID NO: 12) and PADRE (SEQ ID NO: 10) in liposomes suspended in a PBS/ISA51 water-in-oil emulsion: (i) V8L peptide (SEQ ID NO: 6) (diamonds); (ii) S9L peptide

(SEQ ID NO: 7) (squares); (iii) mK9M peptide (SEQ ID NO: 8) (triangles); (iv) V8L and mK9M peptides (crosses); and (v) S9L and mK9M peptides (stars). Control mice were treated with PBS alone (circles). Mice were administered the treatment 5 days post-tumor implantation. Each data point is the average size of tumors in five mice.

Figure 17 illustrates the eradication or reduction of 6-day-old established B16 tumors in mice upon a single treatment with a composition comprising CpG ODN (SEQ ID NO: 12), PADRE and a TRP-2 and/or a p53 CTL epitope, encapsulated in liposomes which were suspended in PBS then emulsified in ISA51. A single treatment with a mixture of TRP-2 and p53 CTL epitopes rendered all mice tumor-free by 21 days post-administration (triangles). A single treatment with TRP-2 (diamonds) or p53 (squares) alone rendered 40% of the mice tumor-free 33 days post treatment. Treatment with PBS in controls mice had no effect on the progression of tumors (crosses).

Figure 18 illustrates that C3 tumors were reduced in size in mice treated with a composition comprising CpG ODN (SEQ ID NO: 12) and an E7 epitope of HPV 16 (R9F peptide; SEQ ID NO: 1) encapsulated in liposomes and suspended in a PBS/ISA51 water-in-oil emulsion, followed by dermal application of Aldara™ within 15-20 hours (squares). In contrast, C3 tumors were not reduced in size in control mice that received PBS (crosses) or PBS followed by dermal application of Aldara within 15-20 hours to the site of PBS injection (triangles). Treatment was administered 5 days post tumor implantation in all treatment groups. Tumor size is the average size of tumors in ten mice.

Figure 19 illustrates the percentage of mice having a palpable tumor after treatment five days post tumor implantation in all treatment groups. Only 20% of mice 20 days post-tumor implantation had a palpable tumor after treatment with a composition comprising CpG ODN (SEQ ID NO: 12) and an E7 epitope of HPV 16 (R9F peptide; SEQ ID NO: 1) encapsulated in liposomes and suspended in a PBS/ISA51 water-in-oil emulsion, followed by dermal application of Aldara™ ointment (5% imiquimod) to the site of administration within 15-20 hours (diamonds). In contrast, 90% of mice had palpable tumors 20 days after treatment with PBS alone (triangles) and 100% of mice had a palpable tumor after treatment with PBS followed by a dermal application of Aldara (squares).

Figure 20 illustrates *ex vivo* detection of IFN-γ producing splenocytes exposed to the melanoma-related antigen, TRP-2 (S9L peptide; SEQ ID NO: 7), or B16F10 cells, 8 days post-immunization with a single administration of a treatment composition comprising S9L peptide and a tetanus toxoid epitope (F21E; SEQ ID NO: 11) encapsulated together in liposomes with CpG ODN (SEQ ID NO: 12) in a PBS/ISA51 water-in-oil emulsion (B). Control mice were immunized with the above-described composition formulated without the tetanus toxoid T helper epitope (A). Five times as many splenocytes that were stimulated with B16-F10 cells to produce IFN-γ were present in spleens from the group immunized with the treatment composition of the invention compared to the control group.

DETAILED DESCRIPTION

The present application provides compositions comprising at least one antigen capable of inducing a $CD8^+$

cytotoxic T lymphocyte (CTL) response together with at least one T helper epitope and liposomes suspended in a carrier comprising a continuous phase of a hydrophobic substance. Further, the invention teaches the use of said compositions in a method for treating cancer in a subject.

The compositions as described herein are useful for treating a broad range of cancers, including, without limitation: cancers caused by human papilloma virus (HPV), such as, for example, cervical and/or vulvar cancer; cancers involving expression of tyrosinase, such as, for example, melanoma; cancers involving mutations or overexpression of the p53 gene product, such as, for example, breast cancer or lymph node metastases; and other cancers like melanoma that express more than one tumor-associated protein simultaneously. In another embodiment, the compositions described herein are useful for treating cancers, including, without limitation: lung, ovarian, multiple myeloma, B cell lymphoma, hepatoma, sarcoma, bladder, prostate, thyroid, H/N tumors, colon, rectum, renal, pancreas, gastric, adenocarcinoma, T cell leukemia, lymphosarcoma, uterine, esophageal, non-Hodgkin's lymphomas, endometrial, and RCC tumors. Any cancer that has a cell surface component that is different in quantity or substance from the cell type from which the cancer is derived is a candidate for treatment by the invention. In particular, p53 is a candidate target for broadly applicable cancer treatments (DeLeo, A.B., *Crit. Rev. Immunol.*, 18:29, 1998; Vierboom, M.P.M. et al., *Peptide-Based Cancer Vaccines*. W. M. Kast, ed. Landes Bioscience, Georgetown, 2000).

As used herein, the terms "tumor", "tumor cells", "cancer" and "cancer cells", (used interchangeably) refer to cells that exhibit abnormal growth, characterized by a

significant loss of control of cell proliferation or cells that have been immortalized. The term "cancer" or "tumor" includes metastatic as well as non-metastatic cancer or tumors. A cancer may be diagnosed using criteria generally accepted in the art, including the presence of a malignant tumor.

"Treating" or "treatment of" cancer refers to an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilisation of the state of disease, prevention of development of disease, prevention of spread of disease, delay or slowing of disease progression, delay or slowing of disease onset, amelioration or palliation of the disease state, and remission (whether partial or total). "Treating" can also mean prolonging survival of a patient beyond that expected in the absence of treatment. "Treating" can also mean inhibiting the progression of disease temporarily, although more preferably, it involves halting the progression of disease permanently in a subject.

The subject to be treated may be any vertebrate, preferably a mammal, more preferably a human.

Antigens

Suitable antigens of the composition are those that are capable of inducing a cell-mediated (CTL) immune response in a subject.

Cell-mediated immunity is an immune response that does not involve antibodies but rather involves the

activation of macrophages and natural killer cells, the production of antigen-specific cytotoxic T lymphocytes and the release of various cytokines in response to an antigen. Cytotoxic T lymphocytes are a sub-group of T lymphocytes (a type of white blood cell) which are capable of inducing the death of infected somatic or tumor cells; they kill cells that are infected with viruses (or other pathogens), or are otherwise damaged or dysfunctional.

Most cytotoxic T cells express T-cell receptors that can recognise a specific peptide antigen bound to Class I MHC molecules. These CTLs also express CD8 ($CD8^+$ T cells), which is attracted to portions of the Class I MHC molecule. This affinity keeps the CTL and the target cell bound closely together during antigen-specific activation.

Cellular immunity protects the body by, for example, activating antigen-specific cytotoxic T-lymphocytes that are able to lyse body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens; activating macrophages and natural killer cells, enabling them to destroy intracellular pathogens; and stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

In one embodiment, the antigen may be, for example, a peptide, a suitable native, non-native, recombinant or denatured protein or polypeptide, or a fragment thereof, or an epitope that is capable of producing a CTL immune response in a subject.

The term "polypeptide" encompasses any chain of amino acids, regardless of length (e.g., at least 6, 8, 10, 12, 14, 16, 18, or 20 amino acids) or post-translational modification (e.g., glycosylation or phosphorylation), and includes, for example, natural proteins, synthetic or recombinant polypeptides and peptides, epitopes, hybrid molecules, variants, homologs, analogs, peptoids, peptidomimetics, etc. A variant or derivative therefore includes deletions, including truncations and fragments; insertions and additions, for example conservative substitutions, site-directed mutants and allelic variants; and modifications, including peptoids having one or more non-amino acyl groups (for example, sugar, lipid, etc.) covalently linked to the peptide and post-translational modifications. As used herein, the term "conserved amino acid substitutions" or "conservative substitutions" refers to the substitution of one amino acid for another at a given location in the peptide, where the substitution can be made without substantial loss of the relevant function. In making such changes, substitutions of like amino acid residues can be made on the basis of relative similarity of side-chain substituents, for example, their size, charge, hydrophobicity, hydrophilicity, and the like, and such substitutions may be assayed for their effect on the function of the peptide by routine testing.

Polypeptides, peptides or epitopes that have substantial identity to those disclosed herein may be used. Two sequences are considered to have substantial identity if, when optimally aligned (with gaps permitted), they share at least approximately 50% sequence identity, or if the sequences share defined functional motifs. In alternative embodiments, optimally aligned sequences may be considered to be substantially identical (i.e., to have substantial

identity) if they share at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity over a specified region. The term "identity" refers to sequence similarity between two polypeptides molecules. Identity can be determined by comparing each position in the aligned sequences. A degree of identity between amino acid sequences is a function of the number of identical or matching amino acids at positions shared by the sequences, for example, over a specified region. Optimal alignment of sequences for comparisons of identity may be conducted using a variety of algorithms, as are known in the art, including the ClustalW program, available at http://clustalw.genome.ad.jp, the local homology algorithm of Smith and Waterman, 1981, Adv. Appl. Math 2: 482, the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, the search for similarity method of Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85:2444, and the computerised implementations of these algorithms (such as GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI, U.S.A.). Sequence identity may also be determined using the BLAST algorithm, described in Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10 (using the published default settings). Software for performing BLAST analysis is available through the National Center for Biotechnology Information (through the internet at http://www.ncbi.nlm.nih.gov/).

The amount of antigen used in a single treatment with a composition as described herein may vary depending on the type of antigen and the size of the subject. One skilled in the art will be able to determine, without undue experimentation, the effective amount of antigen to use in a particular application. The term "effective amount" as used

herein means an amount effective, at dosages and for periods of time necessary, to achieve the desired result.

In one embodiment, the antigen may be at least one CTL epitope capable of inducing a CTL response. For example, the antigen may be a CTL epitope derived from a virus, such as HPV.

In another embodiment, the antigen may be a CTL epitope selected from the group consisting of an epitope derived from the E6 or E7 protein of HPV.

In a further embodiment, the epitope of E6 protein of HPV comprises the peptide sequence TIHDIILECV (T10V) (SEQ ID NO: 5). In another embodiment, the epitope of the E7 protein of HPV comprises a peptide sequence selected from the group consisting of RAHYNIVTF (R9F) (SEQ ID NO: 1), YMLDLQPETT (Y10T) (SEQ ID NO: 2), LLMGTLGIV (L9V) (SEQ ID NO: 3), and TLGIVCPI (T81) (SEQ ID NO: 4).

In another embodiment, the CTL epitope may be an epitope of a tumor-associated protein, such as for example, a melanoma-associated protein. In a further embodiment, the melanoma-associated protein is a tyrosine related protein-2 (TRP-2) or p53, which can be obtained by various methods including recombinant technology or chemical synthesis.

In one embodiment an epitope of a TRP-2 derived protein comprises the peptide sequence, for example, SVYDFFVWL (S9L; SEQ ID NO: 7). In another embodiment, an epitope of a TRP-2 derived protein comprises the peptide sequence VYDFFVWL (V8L; SEQ ID NO: 6). In another embodiment, an epitope of a p53 derived protein comprises a peptide sequence selected from KYMCNSSCM (K9M; wild type p53; SEQ ID NO: 9), KYICNSSCM (mK9M; modified p53; SEQ ID

NO: 8), and AKXVAAWTLKAAAKYICNSSCM (mK9M (SEQ ID NO: 9) coupled to PADRE (SEQ ID NO: 10)).

In one embodiment, the composition may comprise a mixture of CTL epitopes as antigens for inducing a CTL response.

In a further embodiment, the antigen may be any peptide or polypeptide that is capable of inducing a specific CTL response that is able to effectively recognise a specific conformation on targeted tumor cells and cause their destruction.

In still a further embodiment, the antigen may comprise a peptide sequence selected from the following table:

Table 1.

Antigen	Sequence	HLA	Patent
Mart-1/ Melan-A	AAGIGILTV (SEQ ID NO:18)	A2	US 5,844,075
	EAAGIGILTV (SEQ ID NO: 19)	A2	US 5,844,075
	ILTVILGVL (SEQ ID NO: 20)	A2	US 5,844,075
	AEEAAGIGIL (SEQ ID NO: 21)	B45	US 7,037,509
	AEEAAGIGILT (SEQ ID NO: 22)	B45	Unknown
MCIR	TILLGIFFL (SEQ ID NO: 23)	A2	Unknown
	FLALIICNA (SEQ ID NO: 24)	A2	Unknown
Gp100	KTWGQYWQV (SEQ ID NO: 25)	A2	US 5,844,075

Antigen	Sequence	HLA	Patent
Gp100	AMLGTHTMEV (SEQ ID NO: 26)	A2	Unknown
	MLGTHTMEV (SEQ ID NO: 27)	A2	Unknown
	SLADTNSLAV (SEQ ID NO: 28)	A2	US 5,844,075
	ITDQVPFSV (SEQ ID NO: 29)	A2	US 5,844,075
	LLDGTATLRL (SEQ ID NO: 30)	A2	US 5,844,075
	YLEPGPVTA (SEQ ID NO: 31)	A2	US 5,844,075
	VLYRYGSFSV (SEQ ID NO: 32)	A2	US 5,844,075
	RLPRIFCSC (SEQ ID NO: 33)	A2	Unknown
	LIYRRRLMK (SEQ ID NO: 34)	A3	Unknown
	ALNFPGSQK (SEQ ID NO: 35)	A3	Unknown
	SLIYRRRLMK (SEQ ID NO: 36)	A3	Unknown
	ALLAVGATK (SEQ ID NO: 37)	A3	US 6,558,671
	ALLAVGATK (SEQ ID NO: 38)	A3	US 6,977,074
	VYFFLPDHL (SEQ ID NO: 39)	A24	Unknown
	SNDGPTLI (SEQ ID NO: 40)	Cw8	Unknown
PSA	VSHSFPHPLY (SEQ ID NO: 41)	A1	US 6,037,135
	FLTPKKLQCV (SEQ ID NO: 42)	A2	US 6,881,405
	VISNDVCAQV (SEQ ID NO: 43)	A2	Unknown
PSM	HSTNGVTRIY (SEQ ID NO: 44)	A1	Unknown
Tyrosinase	KCDICTDEY (SEQ ID NO: 45)	A1	US 7,019,112
	SSDYVIPIGTY (SEQ ID NO: 46)	A1	Unknown

Antigen	Sequence	HLA	Patent
Tyrosinase	YMDGTMSQV (SEQ ID NO: 47)	A2	US 6,096,313
	MLLAVLYCL (SEQ ID NO: 48)	A2	US 6,291,430
	AFLPWHRLF (SEQ ID NO: 49)	A24	US 6,291,430
	SEIWRDIDF (SEQ ID NO: 50)	B44	US 6,291,430
	MSLQRQFLR (SEQ ID NO: 51)	A31	US 5,831,016
TRP1	SVYDFFVWL (SEQ ID NO: 52)	A2	US 7,067,120
TRP2	TLDSQVMSL (SEQ ID NO: 53)	A2	Unknown
	LLGPGRPYR (SEQ ID NO: 54)	A31	US 5,831,016
p53	ANDPIFVVL (SEQ ID NO: 55)	Cw8	Unknown

As indicated above in Table 1, proteins (polypeptides) vary in the number of peptide sequences that may serve as CTL-epitopes and consequently can be used in the present invention. The following genes, without limitation, code for tumor-associated proteins that have peptide sequences that can be incorporated as antigens in the invention: p53, HPV E6 and E7, ART-4, CAMEL, CEA, Cyp-B, HER2/neu, hTERT, hTRT, iCE, MUC1, MUC2, PRAME, P15, RUI, RU2, SART-1, SART-3, WT1, PSA, tyrosinase, TRP-1, TRP-2, gp100, MART-1/Melan A, MAGE-A1,MAGE-A2, MAGE-A3, MAGE-A6, MAGE-A10, MAGE-A12, BAGE, DAM-6, DAM-10, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7B, GAGE-8, NA88-A, NY-ESO-1, NY-ESO-1a (CAG-3), AFP, ß-catenin/m, Caspase-8/m, CDK-4/m, ELF2M, GnT-V, G250, Ras, HSP70-2M, HST-2, KIAA0205, MUM-1, MUM-2, MUM-3, Myosin/m, RAGE, SART-2, TRP-2/INT2, and 707-AP.

T helper epitopes

T helper epitopes are a sequence of amino acids (natural or non-natural amino acids) that have T helper activity. T helper epitopes are recognised by T helper lymphocytes, which play an important role in establishing and maximising the capabilities of the immune system, and are involved in activating and directing other immune cells, such as cytotoxic T lymphocytes.

A T helper epitope can consist of a continuous or discontinuous epitope. Hence not every amino acid of a T helper is necessarily part of the epitope. Accordingly, T helper epitopes, including analogs and segments of T helper epitopes, are capable of enhancing or stimulating an immune response. Immunodominant T helper epitopes are broadly reactive in animal and human populations with widely divergent MHC types (Celis *et al.* (1988) *J. Immunol.* 140:1808-1815; Demotz *et al.* (1989) *J. Immunol.* 142:394-402; Chong *et al.* (1992) *Infect. Immun.* 60:4640-4647). The T helper domain of the subject peptides has from about 10 to about 50 amino acids and preferably from about 10 to about 30 amino acids. When multiple T helper epitopes are present, then each T-helper epitope acts independently.

In one embodiment, the composition described herein also comprises at least one T helper epitope. In some instances, the T-helper epitope may form part of the antigen. In particular, if the antigen is of sufficient size, it may contain an epitope that functions as a T-helper epitope. In other embodiments, the T-helper epitope is a separate molecule from the antigen.

In another embodiment, T helper epitope analogs may include substitutions, deletions and insertions of from

one to about 10 amino acid residues in the T helper epitope. T helper segments are contiguous portions of a T helper epitope that are sufficient to enhance or stimulate an immune response. An example of T-helper segments is a series of overlapping peptides that are derived from a single longer peptide.

Sources of T helper epitopes for use in the present invention include, for example, hepatitis B surface antigen helper T cell epitopes, pertussis toxin helper T cell epitopes, measles virus F protein helper T cell epitope, *Chlamydia trachomitis* major outer membrane protein *helper* T cell epitope, diphtheria toxin *helper* T cell epitopes, *Plasmodium falciparum* circumsporozoite helper T cell epitopes, *Schistosoma mansoni* triose phosphate isomerase helper T cell epitopes, *Escherichia coli* TraT helper T cell epitopes and immune-enhancing analogs and segments of any of these T helper epitopes.

In one embodiment, the T helper epitope is a universal T helper epitope. A universal T helper epitope as used herein refers to a peptide or other immunogenic molecule, or a fragment thereof, that binds to a multiplicity of MHC class II molecules in a manner that activates T-cell function in a class II ($CD4^+$ T cells) or class I ($CD8^+$ T cells) -restricted manner.

In another embodiment, the T helper epitope may be a universal T helper epitope such as PADRE (pan-DR epitope) comprising the peptide sequence AKXVAAWTLKAAA (SEQ ID NO: 10), wherein X may be cyclohexylalanyl. PADRE specifically has a $CD4^+$ T-helper epitope, that is, it stimulates induction of a PADRE-specific $CD4^+$ T helper response.

Tetanus toxoid has T helper epitopes that work in the similar manner as PADRE. Tetanus and diphtheria toxins have universal epitopes for human $CD4^+$ cells. (Diethelm-Okita, B.M. *et al.*, Universal epitopes for human $CD4^+$ cells on tetanus and diphtheria toxins. *J. Infect. Diseases,* 181:1001-1009, 2000). In another embodiment, the T helper epitope may be a tetanus toxoid peptide such as F21E comprising the peptide sequence FNNFTVSFWLRVPKVSASHLE (amino acids 947-967; SEQ ID NO: 11).

In another embodiment, the T helper epitope is fused to at least one antigen (i.e., a peptide), or a mixture of antigens, to make a fusion peptide.

Carriers

The carrier of the composition comprises a continuous phase of a hydrophobic substance, preferably a liquid hydrophobic substance. The continuous phase may be an essentially pure hydrophobic substance or a mixture of hydrophobic substances. In addition, the carrier may be an emulsion of water in a hydrophobic substance or an emulsion of water in a mixture of hydrophobic substances, provided the hydrophobic substance constitutes the continuous phase. Further, in another embodiment, the carrier may function as an adjuvant.

Hydrophobic substances that are useful in the compositions as described herein are those that are pharmaceutically and/or immunologically acceptable. The carrier is preferably a liquid but certain hydrophobic substances that are not liquids at atmospheric temperature may be liquefied, for example by warming, and are also useful in this invention. In one embodiment, the hydrophobic carrier may be a PBS/FIA emulsion.

Oil or water-in-oil emulsions are particularly suitable carriers for use in the present invention. Oils should be pharmaceutically and/or immunologically acceptable. Preferred examples of oils are mineral oil (especially light or low viscosity mineral oil), vegetable oil (e.g., corn or canola oil), nut oil (e.g., peanut oil) and squalene. A low viscosity mineral oil is most preferred. Animal fats and artificial hydrophobic polymeric materials, particularly those that are liquid at atmospheric temperature or that can be liquefied relatively easily, may also be used.

Liposomes

Liposomes are completely closed lipid bilayer membranes containing an entrapped aqueous volume. Liposomes may be unilamellar vesicles (possessing a single bilayer membrane) or multilamellar vesicles characterized by multimembrane bilayers, each bilayer may or may not be separated from the next by an aqueous layer. A general discussion of liposomes can be found in Gregoriadis G. *Immunol. Today,* 11:89-97, 1990; and Frezard, F., *Braz. J. Med. Bio. Res.,* 32:181-189, 1999.

Although any liposomes may be used in this invention, including liposomes made from archaebacterial lipids, particularly useful liposomes use phospholipids and unesterified cholesterol in the liposome formulation. The cholesterol is used to stabilize the liposomes and any other compound that stabilizes liposomes may replace the cholesterol. Other liposome stabilizing compounds are known to those skilled in the art. For example, saturated phospholipids produce liposomes with higher transition temperatures indicating increased stability. To avoid

limiting the electrostatic association between the antigen and the liposomes, the antigen may be sequestered in the interior of the liposomes.

Phospholipids that are preferably used in the preparation of liposomes are those with at least one head group selected from the group consisting of phosphoglycerol, phosphoethanolamine, phosphoserine, phosphocholine and phosphoinositol. More preferred are liposomes that comprise lipids in phospholipon 90 G. When unesterified cholesterol is also used in liposome formulation, the cholesterol is used in an amount equivalent to about 10% of the amount of phospholipid. If a compound other than cholesterol is used to stabilize the liposomes, one skilled in the art can readily determine the amount needed in the composition.

Liposome compositions may be obtained, for example, by using natural lipids, synthetic lipids, sphingolipids, ether lipids, sterols, cardiolipin, cationic lipids and lipids modified with poly (ethylene glycol) and other polymers. Synthetic lipids may include the following fatty acid constituents; lauroyl, myristoyl, palmitoyl, stearoyl, arachidoyl, oleoyl, linoleoyl, erucoyl, or combinations of these fatty acids.

Adjuvants

The composition may further comprise one or more pharmaceutically acceptable adjuvants, excipients, etc., as are known in the art. See, for example, Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985) and The United States Pharmacopoeia: The National Formulary (USP 24 NF19) published in 1999. In one embodiment, suitable adjuvants include a CpG-containing oligodeoxynucleotide (CpG

ODN). For example, 5'-TCCATGACGTTCCTGACGTT-3'. The skilled person may select an appropriate CpG on the basis of the target species and efficacy. In place of CpG, a lipopeptide, such as Pam3Cys-SKKK)(EMC Microcollections, Germany) or variants, homologs and analogs thereof may be used. In this regard, the Pam2 family of lipopeptides has been shown to be an effective alternative to the Pam3 family of lipopeptides.

The amount of adjuvant used depends on the amount of antigen and on the type of adjuvant. One skilled in the art can readily determine the amount of adjuvant needed in a particular application.

Compositions

In one embodiment, compositions as described herein may be formulated by encapsulating an antigen (defined as a substance that interacts specifically with free antibody and/or with antigen-binding receptors on lymphocytes) or an antigen/adjuvant complex in liposomes to form a liposome-encapsulated antigen and mixing the liposome-encapsulated antigen with a carrier comprising a continuous phase of a hydrophobic substance. If an antigen/adjuvant complex is not used in the first step, a suitable adjuvant may be added to the liposome-encapsulated antigen, to the mixture of liposome-encapsulated antigen and carrier, or to the carrier before the carrier is mixed with the liposome-encapsulated antigen. The order of the process may depend on the type of adjuvant used. The resulting liposome-encapsulated antigen is then mixed with the carrier. (It should be noted that the term "liposome-encapsulated antigen" may refer to liposome-encapsulation of the antigen alone or to the

encapsulation of the antigen/adjuvant complex depending on the context.) This promotes intimate contact between the adjuvant and the antigen and may, at least in part, account for the good immune response. To facilitate use of some adjuvants, the antigen may be first encapsulated in liposomes and the resulting liposome-encapsulated antigen is then mixed with the adjuvant in a carrier comprising a continuous phase of hydrophobic substance.

In formulating a composition that is substantially free of water, the antigen or antigen/adjuvant complex may be encapsulated with liposomes, which may or may not be freeze-dried, and suspended in a hydrophobic substance. In formulating a composition in an emulsion of water in a hydrophobic substance, the antigen or antigen/adjuvant complex may be encapsulated in liposomes, suspended in an aqueous medium followed by the mixing of the aqueous medium with a hydrophobic substance to form an emulsion. In the case of the emulsion, to maintain the hydrophobic substance in the continuous phase, the aqueous medium containing the liposomes may be added in aliquots with mixing to the hydrophobic substance.

In one embodiment, the antigen or the liposome-encapsulated antigen may be freeze-dried before being mixed with the hydrophobic substance or with the aqueous medium as the case may be. In another embodiment, an antigen/adjuvant complex may be encapsulated by liposomes followed by freeze-drying. In a further embodiment, the antigen may be encapsulated in liposomes followed by the addition of adjuvant then freeze-drying to form a freeze-dried liposome-encapsulated antigen with external adjuvant. In yet another instance, the antigen may be encapsulated by liposomes followed by freeze-drying before the addition of

adjuvant. Freeze-drying may promote better interaction between the adjuvant and the antigen.

In another embodiment, formulation of the liposome-encapsulated antigen into a hydrophobic substance may also involve the use of an emulsifier to promote more even distribution of the liposomes in the hydrophobic substance. Typical emulsifiers are well-known in the art and include mannide oleate (Arlacel™ A), lecithin, Tween™ 80, and Spans™ 20, 80, 83 and 85. The emulsifier is used in an amount effective to promote even distribution of the liposomes. Typically, the volume ratio (v/v) of hydrophobic substance to emulsifier is in the range of about 5:1 to about 15:1 with a ratio of about 10:1 being preferred.

Alternatively, the antigen or antigen/adjuvant complex may be associated with, in contact with or separate from liposomes and not encapsulated in liposomes. The efficiency of liposome encapsulation of some hydrophilic antigens or hydrophilic antigen/adjuvant complexes may be poor so that upon being placed in a hydrophobic environment or freeze-drying most of the antigen becomes associated with the external surface of the liposomes. This represents another embodiment of the invention.

In a further embodiment, an antigen (peptide or polypeptide) having a CTL epitope and PADRE (fused to the antigen or separate) may be encapsulated together in liposomes. In another embodiment, more than one antigen may be placed together in the same liposomes. In a further embodiment, other substances may be in used instead of PADRE that have a T-helper epitope, for example, tetanus toxoid peptide(s). In a another embodiment, an adjuvant, preferably a CpG-containing ODN, may be encapsulated in the liposomes

as well. The liposomes are preferably suspended in PBS. This suspension is then emulsified in a hydrophobic carrier, for example, ISA51 or mineral oil. The result is that liposomes containing the antigen(s) and adjuvant(s), preferably PADRE and CpG) are suspended in PBS which in turn is emulsified in a hydrophobic carrier, for example, ISA51 or mineral oil.

Recurrence of cancer is always of concern, thus the induction of a long-lasting CTL response is important to ensure that cancers do not reoccur. In general, CTL responses are short-lived lasting only several weeks, however, the compositions as described herein are capable of inducing a potent CTL response that lasts for at least 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 or 130 days.

In one embodiment, splenocytes isolated from mice treated 130 days prior with a composition comprising a CpG ODN and a CTL epitope of E7 protein of HPV fused to PADRE encapsulated in liposomes suspended in a water-in-oil emulsion retained the ability to lyse mouse lymphoma EL-4 cells (Figure 3). These results indicate that compositions as described herein are able to induce a long-lasting CTL response, which is desirable in cancer treatment.

In one embodiment, treatment with compositions comprising CpG ODN adjuvant and TRP-2 and/or p53 peptides as antigens were able to increase the number of antigen-specific interferon-gamma (IFN-γ) producing splenocytes needed to combat cancer cells (Figures 6-8). Antigens were fused to a universal T helper epitope (PADRE) and encapsulated in liposomes suspended in a water-in-oil emulsion. Both the peptide and tumor cells expressing the protein from which the peptide was derived were able to induce IFN-γ production, demonstrating that use of the

composition of invention to deliver peptide antigens resulted in an immune response relevant to the intended target.

In another embodiment, treatment of established tumors with a single treatment with compositions as described herein was effective in significantly reducing tumor size and the percentage of mice with tumors post-treatment (Figures 9-13 and 15).

Treatment with compositions as described herein may be followed by a dermal application to the site of administration of a suitable composition comprising imiquimod (1-(2-methylpropyl)-1 H-imidazo[4,5-c]quinolin-4-amine) or analogues thereof that are members of a class of non-nucleoside imidazoquinolinamines (hetero-cyclic amine) that activate the immune system through localised induction of cytokines. Imiquimod is a ligand for TLR7 and activates a Th1-like cytokine milieu that includes IFN-α, TNF-α, IL-1α, IL-6, and IL-8. In a further embodiment, treatment with compositions as described herein may be followed by a dermal application to the site of administration of Aldara™ ointment (imiquimod 5%) (3M, St. Paul, MN, U.S.A.) to the site of treatment administration.

In one embodiment, tumor size, and the percentage of tumor-bearing mice, was reduced in mice treated with a single administration of a composition comprising CpG ODN and a fusion peptide encapsulated in liposomes with suspended in a water-in-oil emulsion, followed by a dermal application of Aldara ointment at the site of treatment administration (Figure 18 and 19).

The compositions as described herein may be formulated in a form that is suitable for oral, nasal,

rectal or parenteral administration. Parenteral administration includes intravenous, intraperitoneal, intradermal, subcutaneous, intramuscular, transepithelial, intrapulmonary, intrathecal, and topical modes of administration. The preferred routes are intramuscular, subcutaneous and intradermal to achieve a depot effect.

The compositions as described herein may be effective when administered in a single application.

In another embodiment, the compositions as described herein may be used in combination, before or after, other cancer therapies such as radiotherapy and chemotherapy. It has been previously shown that melanoma recurrence was prevented when patients diagnosed with stage II or III melanoma were treated surgically, then given a vaccine composition comprising a composition to induce a CTL response to melanoma-specific antigens. (Antonia, S.J. *et al.*, *Clin. Cancer Res.* 12:878-887, 2006; Allegra, C.J. and R.W. Childs., *J. National Cancer Inst.* 97:1396-1397, 2005; Cassarino, D.S. *et al.*, *J. Cutaneous Path.* 33:335-342, 2006; Correale, P. *et al.*, *J. National Cancer Inst.* 97:1437-1445, 2005; Gulley, J.L. *et al.*, *Clin. Cancer Res.* 11: 3353-3362, 2005; and Chakraborty, M. *et al.*, *Cancer Res.* 64:4328-4337, 2004).

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1

Cellular response

(a) Activation

To examine the specificity and rapidity of the CTL response, mice were treated once with a composition comprising CpG ODN adjuvant, a CTL epitope of human papilloma virus (HPV) 16, namely R9F (E7 (H2-Db) peptide RAHYNIVTF, amino acids 49-57; SEQ ID NO: 1) fused to PADRE (AKXVAAWTLKAAA-OH (SEQ ID NO: 10); 50 μg/dose), which is a universal T helper epitope, and encapsulated in liposomes (0.2 g lecithin and 0.02 g cholesterol/dose) suspended in a PBS/FIA (phosphate buffered saline/Freund's incomplete adjuvant) emulsion (100 μl/dose). Fourteen days post-treatment, splenocytes (effector cells) were co-cultured for 6 hours with R9F or an irrelevant peptide (KIMCNSSCM; SEQ ID NO: 13). An ex vivo intracellular IFN-γ staining of splenocytes demonstrated that the proportion of IFN-γ positive $CD8_+$ T cells (CTLs) was 13 fold higher (1.6 % of splenocytes) when splenocytes were exposed to R9F peptide than when splenocytes were exposed to the irrelevant peptide (0.12 % of splenocytes or no peptide; Figure 1). As demonstrated in Figure 1, treatment of mice with the above-described composition comprising R9F peptide caused a significant expansion of CTLs that exhibit a specific response to stimulation by HPV epitope, as compared to mice treated with a composition comprising irrelevant peptide.

Intracellular lymphokine staining demonstrated the presence of IFN-γ positive CTLs. To demonstrate the protective function of IFN-γ producing CTLs, mice were

treated with a composition comprising CpG ODN and R9F peptide fused with PADRE and encapsulated in liposomes suspended in a water-in-oil emulsion. Thirty days post-treatment, R9F peptide (RAHYNIVTF; SEQ ID NO: 1) loaded E4 cells (target cells; mouse lymphoma cell line) and an irrelevant peptide (KIMCNSSCM; SEQ ID NO: 13) loaded E4 cells were stimulated *in vitro* for 6 days with splenocytes from treated mice. Cytotoxicity was measured by JAM assay (Figure 2). After the 6-day *in vitro* stimulation, approximately 50 % of R9F peptide loaded EL-4 cells (Effector to Target ratio 10) were lysed by splenocytes from mice treated with the composition comprising R9F peptide (squares). In contrast only approximately 5% of irrelevant peptide loaded EL-4 cells were lysed by the same splenocytes (diamonds; $p < 0.009$).

(b) Duration

The duration of the memory response induced by a single treatment with a particular embodiment of the invention was demonstrated (Figure 3) by the lysis of EL-4 cells by splenocytes obtained from mice 130 days post-treatment with the following compositions: (i) fused peptide (R9F peptide fused with PADRE) and CpG ODN encapsulated in liposomes contained in a PBS/FIA water-in-oil emulsion (diamonds); (ii) unencapsulated fused peptide and CpG ODN in a PBS/FIA water-in-oil emulsion (triangles and crosses); or (iii) liposome encapsulated fused peptide in a PBS/FIA water-in-oil emulsion without a CpG ODN adjuvant (closed circles). Splenocytes treated with liposome-encapsulated fused peptide, liposome-encapsulated CpG ODN, unencapsulated-fused peptide and CpG ODN, and unencapsulated-fused peptide alone served as control splenocytes.

The JAM assay used a six-day *in vitro* stimulation followed by co-culturing splenocytes (effector cells) with R9F or irrelevant peptide-loaded EL-4 cells (target cells) that had been preloaded with ^{3}H-labelled thymidine. Splenocytes from mice immunized with fused peptide and CpG ODN encapsulated in lipsomes suspended in a water-in-oil emulsion lysed 30% of the R9F peptide loaded target cells when the effector to target ratio was 25:1 and 5:1 and 15% of the target cells when the ratio was 1:1 (Figure 3). Splenocytes from mice given the control treatment demonstrated cytotoxicity at background levels. Duration of a CTL response for >130 days following a single treatment is remarkable in relation to the duration of CTL responses reported in the literature.

Example 2

Eradication of cervical cancer

Despite the development of preventative vaccines for human papillomavirus (HPV) induced cervical and vulvar cancer, for example, Gardasil™ and Cervarix™, a therapeutic treatment for cervical and vulvar cancer remains a high priority. In this example, a treatment composition comprising a CTL epitope of human papilloma virus (HPV) 16, namely R9F (E7 (H2-Db) peptide RAHYNIVTF, amino acids 49-57; SEQ ID NO: 1) was used to induce CTLs. These CTLs need $CD4^{+}$ T cell help for their differentiation and expansion, as well as their maturation into functional memory CTLs. To achieve a potent CTL response through $CD4^{+}$ T cell help, R9F peptide was fused to the universal T helper epitope, PADRE (SEQ ID NO: 10), yielding a fusion peptide. The fused peptide was encapsulated in liposomes together with synthetic oligdeoxynucleotides containing CpG ODN motifs or lipopeptide (Pam3Cys-SKKKK). The therapeutic composition

used a PBS/FIA water-in-oil emulsion to deliver the therapeutic formulation in a single treatment. Efficacy of the therapeutic treatment was demonstrated using HPV 16-expressing C3 tumor cells to challenge C57BL/6 mice (10 mice/group), then treating the mice on day 14 post-challenge with the treatment composition described above or a control composition. By day thirty (i.e., 16 days post-treatment), complete eradication of palpable tumors was demonstrated in all 10 mice in the group challenged with the C3 tumor then given treatment (Figure 4; open squares). In contrast, tumors in all 10 control mice treated with a composition comprising all the components of the therapeutic treatment described above except fused peptide, continued to increase in size (closed squares).

Example 3

Prophylaxis

To further demonstrate the ability of a composition of the invention to protect against an *in vivo* challenge with C3 tumor cells, female C57BL/6 mice were injected subcutaneously at the base of the tail with a composition comprising R9F peptide (RAHYNIVTF; SEQ ID NO: 1) fused with PADRE (SEQ ID NO: 10) (referred to as fused peptide) and encapsulated with CpG ODN in liposomes suspended in a water-in-oil emulsion. To determine if the composition would be as protective as a composition comprising a replacement adjuvant to CpG ODN, mice were administered a composition as described above wherein CpG ODN was replaced by an alternative CpG adjuvant, namely, Pam3c (Pam3Cys-sKKKK). Control groups were injected with PBS, CpG ODN in PBS, fused peptide in PBS, fused peptide

suspended in PBS with CpG ODN, or fused peptide encapsulated in liposomes with no adjuvant.

Fifteen days after a single treatment, 0.5×10^6 C3 cells were implanted subcutaneously in the left flank of treated mice as a primary challenge (Figure 5). All mice injected with PBS or CpG ODN in PBS developed tumors within 2 weeks and had to be removed from the study by day 30 based on tumor size as required by animal care protocols. Treatment with fused peptide in PBS protected only 20% of mice (treatment group 3). Fused peptide encapsulated in liposomes in a PBS emulsion prevented 50% of the mice from developing tumors (treatment group 4), suggesting that liposome encapsulated fused peptide offers some protection from the C3 challenge. Treatment with fused peptide and CpG ODN in a PBS emulsion prevented 60% of mice from developing tumors (treatment group 5). In comparison, 100% of mice treated with fused peptide encapsulated in liposomes with CpG ODN remained tumor-free during the 61 day post-challenge monitoring period (treatment group 6). To determine the duration and magnitude of the memory response directed against the tumors, mice in treatment group 6 were given a secondary challenge of $6x10^6$ C3 tumor cells. All mice remained tumor-free for a further 73 days, demonstrating that a single treatment of a composition of the invention provides a robust and long-lasting cellular immune response. Similarly, replacement of CpG with Pam3c resulted in all mice remaining tumor free for 61 days (treatment group 7). These mice were not re-challenged with C3 cells.

Therapy

To evaluate treatment of established palpable C3 tumors, mice were implanted with 0.5×10^6 C3 cells

subcutaneously in the left flank. On either day 4, 5, 6 or 9 post-tumor implantation, mice (n=10) were treated with a composition comprising CpG ODN and R9F peptide (RAHYNIVTF; SEQ ID NO: 1) fused with PADRE (SEQ ID NO: 10) (fused peptide) and encapsulated in liposomes and suspended in a PBS/FIA water-in-oil emulsion a placebo (fused peptide and CpG ODN in a PBS emulsion). A single immunization eradicated tumors by day 40 in all 10 mice in the treatment groups that were immunized 4, 5 or 6 days post-tumor implantation and all 30 mice in the group that was immunized 9 days post-tumor implantation. Only one mouse maintained a tumor until day 40 in the group treated on day 5 post-tumor implantation (Table 1). In contrast, 9/10 mice developed tumors in the groups treated with the placebo composition on day 4 or 6 days post-tumor implantation. In the group of mice administered with the placebo 5 days post-challenge, 10/10 mice developed tumors and 27/30 mice developed tumors in the group treated with the placebo vaccine on day 9 post-tumor implantation.

To evaluate whether replacement of CpG ODN with Pam3c would alter the ability of a composition to eradicate C3 tumors, ten mice were treated with a composition comprising fused peptide encapsulated with Pam3c in liposomes and suspended in a PBS/FIA water-in-oil emulsion or a placebo treatment comprising the same composition, but without liposomes was used to treat a second group of 10 mice. Mice in the two treatment groups were subsequently challenged with 1 x 10^6 C3 cells in the left flank. Therapeutic treatment of established C3 tumors using fused peptide encapsulated with an Pam3c in liposomes was repeated twice with similar results as reported in Table 1.

Treatment (Number of days post-tumor implantation)	Number of mice with tumors (day 40 post-challenge	
	Placebo	Treated
4	9/10	0/10
5	10/10	1/10
6	9/10	0/10
9	27/30	0/30
9* (with Pam3c)	9/10	0/10

Table 2. Eradication of tumors in mice treated with a composition comprising R9F peptide (SEQ ID NO: 1) fused with PADRE (SEQ ID NO: 10) (fused peptide) and in liposomes and suspended in a water-in-oil emulsion containing CpG ODN or Pam3c (*) as adjuvants or a placebo vaccine comprising all of the above components except liposomes.

EXAMPLE 4

A therapeutic treatment of melanoma

Tyrosinase is a protein overexpressed in melanoma. Peptides from tyrosinase protein are generally poor antigens for treatment of melanoma. As described herein, V8L, a peptide from tyrosinase-related protein (TRP-2)(amino acids 181-188; VYDFFVWL; SEQ ID NO: 6) that binds to murine MHC, $H2K^2$ and human HLA-A2.1 was used in a therapeutic treatment to stimulate production of IFN-γ producing cells. Stimulation of the number of TRP-2 specific IFN-γ producing cells indicates that a therapeutic effect directed specifically against melanoma can be anticipated.

C57BL mice were treated once with a composition of the invention comprising CpG ODN and TRP-2 peptide fused to PADRE encapsulated in liposomes suspended in a water-in-oil emulsion. Control treatments were carried out with a composition comprising liposome encapsulated TRP-2 peptide with PADRE in the absence of CpG ODN, and a composition

comprising CpG ODN and an irrelevant peptide (KIMCNSSCM; SEQ ID NO: 13) with PADRE encapsulated in liposomes. In both control treatments, the liposomes were suspended in a PBS/ISA51 water-in-oil emulsion. *Ex vivo* detection of IFN-γ producing splenocytes by ELISPOT indicated that the treatment composition produced the greatest number of TRP-2 specific IFN-γ producing cells (Figure 6, group A). The control treatment (Figure 6, group B) produced about half as many TRP-2 specific IFN-γ producing cells and replacement of TPR-2 by an irrelevant peptide produced background levels of TRP-2 specific IFN-γ producing cells (Figure 6; group C), showing that the treatment composition of the invention produces the largest number of TRP-2 specific IFN-γ producing cells that are required to combat melanoma cancer.

EXAMPLE 5

A therapeutic treatment of breast cancer

The p53 gene product is an ideal and widely expressed target for therapy of malignancies, in particular, breast cancer. A large portion of human cancers exhibits p53 mutations as an early event in tumorigenesis. Overexpression of p53 is an independent predictor of more aggressive cancer, lymph node metastases, failure of standard therapeutic regimens, and ultimately of cancer-related mortality.

Mice treated with a single treatment with a composition of the invention comprising a modified p53 CTL epitope, mK9M, (KYICNSSCM; SEQ ID NO: 8) with CpG ODN and PADRE (SEQ ID NO: 10) encapsulated in liposomes in a PBS/ISA51 water-in-oil emulsion produced approximately 10 to 40 times more p53 peptide specific IFN-γ producing cells (Figure 7; group A) than mice treated with a composition

comprising the peptide fused to PADRE and CpG ODN in the absence of liposomes (Figure 7; group B); a composition comprising fused peptide encapsulated in liposomes without CpG ODN; or a composition in which the fused peptide was replaced by an irrelevant peptide (Figure 7; group D). Increased production of tumor specific IFN-γ producing cells is correlated with a reduction/eradication of cervical cancer tumors (see Examples 2 and 3), therefore, one skilled in the art would predict a similar result for p53 bearing tumors.

EXAMPLE 6

Therapeutic cancer treatment against more than one target

Some cancers express more than one tumor-associated protein simultaneously. Such cancers offer more than one target for therapeutic treatment. For example, melanoma cells overexpress both p53 and TRP raising the possibility that treatments aimed at both p53 and TRP simultaneously could be more effective and specific since cells expressing both p53 and TRP targets would be more vulnerable to treatment.

Mice treated with a single administration of a composition comprising a mixture of p53 (mK9M; KYICNSSCM; SEQ ID NO: 8) and TRP-2 (V8L; VYDFFVWL; SEQ ID NO: 6) peptides with CpG ODN and PADRE (AKXVAAWTLKAAA, wherein X= cyclohexylalanyl); SEQ ID NO: 10) and encapsulated in liposomes suspended in a water-in-oil emulsion produced approximately equal numbers of both p53 and TRP specific IFN-γ producing cells (Figure 8; group A). In contrast, control mice treated with a mixture of p53 fused to PADRE and TRP-2 CTL peptides with PADRE together with CpG ODN without liposomes produced more TRP-2- than p53- specific

IFN-γ producing cells (Figure 8; group C), even in the absence of CpG ODN (Figure 8; group B). Production of p53-specific IFN-γ producing cells was at levels obtained with the control treatments (i.e., treatment composition without CpG ODN (group B) or without CpG ODN and liposomes (group D)). These results indicate that mice administered the treatment composition mount a two-pronged attack against tumors bearing TRP-2 and p53 tumor-associated proteins. Without encapsulation of the fused peptides with CpG ODN in liposomes suspended in a water-in-oil emulsion, treated mice attack only one target, the TRP-2 tumor-associated protein, despite being treated with both TRP-2 and p53 peptides.

EXAMPLE 7

HLA A2 transgenic mice were used, which have a human HLA A2 major histocompatability complex (MHC) gene, and therefore express human MHC, which better mimics human cervical cancer. To be compatible with HLA A2 MHC, CTL epitopes different than those used in the previous examples were utilized. HLA A2 Mice were treated one of the following compositions:

(1) a mixture of four E6/7 human papilloma virus (HPV) derived peptides (MP), the sequence of each peptide being as follows:

Y10T (E7: amino acids 11-20; YMLDLQPETT; SEQ ID NO: 2);

L9V (E7: amino acids 82-90; LLMGTLGIV; SEQ ID NO: 3);

T81 (E7: amino acids 86-93; TLGIVCPI; SEQ ID NO: 4); and

T10V (E6: amino acids 29-38; TIHDIILECV; SEQ ID NO: 5);

2) The above 4 peptides joined together with "aay" linkers into a long peptide (AB2; SEQ ID NO: 14), the sequence of which is as follows:

TIHDIILECVaayYMLDLQPETTaayLLMGTLGIVaayTLGIVCPI;

or

3) A single peptide selected from the four peptides listed above, namely L9V (E7: amino acids 82-90; LLMGTLGIV; SEQ ID NO: 3).

All treatment compositions comprised PADRE (25 μg/dose) and CpG ODN (50 μg/dose) as adjuvants, and were delivered in liposomes suspended in a PBS/ISA51 water-in-oil emulsion. The mixture of 4 peptides contained 25 μg of each peptide/treatment. The long peptide (AB2) was administered at 100 μg/treatment. The composition comprising L9V alone contained 25 μg/treatment. Control mice were injected with PBS (100 μL/treatment).

Mice were challenged with TC1/A2 tumor cells (1 X 10^5 cells/mouse) implanted subcutaneously in the left flank and tumor size was measured every 5 days. Nineteen days post-challenge, mice (5 mice/group) were treated with one of the above-described compositions, or injected with PBS (controls).

It is shown in Figure 9 that a single treatment of the composition comprising mixture of the above-described four HPV E6/7 peptides (squares) or the AB2 long peptide (diamonds) eradicated TC1/A2 tumors 21 days post-treatment. Treatment with the composition comprising the E7 peptide L9V

significantly reduced tumor size (triangles). Treatment with PBS alone (crosses) did not prevent tumor growth. Tumor growth was similar in all five mice in the control group that received PBS (Figure 10). All five mice were removed from the study on day 35 as mandated by excessive tumor size. Tumor size is reported as the average tumor size in five mice.

Reductions in tumor size in mice treated with composition comprising the mixture of peptides was variable (Figure 11). For example, tumor size in mouse 2 was eradicated by day 6 post-treatment. Tumors in the remaining mice were not eradicated until at least day 11 post-treatment. Reductions in tumor size in mice treated with the composition comprising the long peptide (AB2) were also variable (Figure 12). However, tumors were completely eradicated by day 21 post-treatment in all 5 mice.

Reductions in tumor size in mice treated with the composition comprising a single HPV E7 peptide (L9V; SEQ ID NO: 3) was similar in 4/5 mice (Figure 13). Treatment of mouse 3 did not result in tumor size reduction, suggesting that treatment with a composition comprising than one HPV peptide either as a mixture of individual peptides or fused peptides will protect more individuals in a population than immunization against a single peptide.

EXAMPLE 8

In example 7, four HPV 16 E6/E7 peptides were joined together to form one long peptide using the linker "aay" (-alanine-alanine-tyrosine-). This linker is hydrophobic in nature and adds to the hydrophobicity of the fused long peptide making peptide manufacture difficult and

requiring use of dimethyl sulphoxide to solubilize the long peptide.

In this example, a "kkp" linker (-lysine-lysine-proline-) was used in place of the "aay" linker to form 2 dipeptides. One dipeptide was Y10T-kkp-L9V (TIHDIILECVkkpLLMGTLGIV; SEQ ID NO: 15) and the other dipeptide was T81-kkp-T10V (TLGIVCPIkkpYMLDLQPETT; SEQ ID NO: 16). Use of the "kkp" linker resulted in hydrophilic fused peptides that facilitate vaccine manufacture. Using "kkp" to link peptides produced approximately the same number of IFNγ-producing splenocytes as obtained when the same four peptides were used individually (i.e., unlinked (Figure 14). These results demonstrate that use of kkp linker facilitates manufacture of the vaccine antigen without altering antigen processing and induction of peptide-specific IFNγ-producing splenocytes. Production of these cells is a good indicator of effective eradication of cancer cells.

EXAMPLE 9

A therapeutic treatment against melanoma

Examples 4, 5 and 6 demonstrate the ability of composition of the invention to increase production of TRP-2 and p53 specific IFN-γ producing splenocytes, thereby, establishing stimulation of a cellular immune responses against melanoma-associated proteins. B16-F10 cells (10×10^3 cells/mouse) were implanted subcutaneously in the left flank of pathogen-free C57BL/6 mice. Mice were 6-8 weeks of age at the time of implantation and were housed under filter top conditions with water and food ad libitum. Five days after implantation of melanoma cells, mice received a single treatment by subcutaneous injection of a composition

comprising one of two peptides (V8L or S9L (SEQ ID NO: 6 or 7); 25 μg/mouse) derived from TRP-2, one modified peptide (mK9M (SEQ ID NO: 8); 25 μg/mouse) derived from p53 or mixtures of these peptides (25 μg of each peptide/mouse). All compositions also comprised both PADRE (25 μg/mouse) and CpG ODN (50 μg/mouse), and delivered in liposomes suspended in a PBS/ISA51 water-in-oil emulsion Control mice received a single administration of PBS alone. All injections were administered at the base of the tail. Tumor size was determined every 4-5 days using the following formula: longest measurement x (shortest measurement) (Pilon-Thomas *et al.*, *J. of Immunother.*, 29(4), 2006).

Figure 15 demonstrates that mice treated with a composition containing either the V8L (SEQ ID NO: 6)(diamonds) or mK9M peptide (SEQ ID NO: 8) (triangles) inhibited growth of melanoma cells initially, but the melanoma cells overcame the initial inhibition of growth to produce tumors that increased in size to 1200 mm^3. Treatment with compositions comprising S9L peptide (SEQ ID NO: 7) (squares) or mixtures of peptides V8L, and mK9M (KYICNSSCM)(crosses), or S9L and mK9M stars) inhibited growth of melanoma for the entire monitoring period. PBS alone (circles) did not have an effect on tumor growth.

Consideration of the percentage of mice that have tumors at the end of the study indicated that vaccine of the invention that contained either the peptide mK9M (SEQ ID NO: 8) (triangles), V8L (SEQ ID NO: 6)(diamonds) or S9L (SEQ ID NO: 7) (squares) cured only 0, 40, and 40%, respectively, of the mice of their tumors (Figure 16). In contrast, treatment with a composition comprising a mixture S9L and mK9M (stars) cured 80 % of the mice of their tumors and or a mixture of

V8L and mK9M (crosses) cured 100 % of the mice of their tumors.

EXAMPLE 10

B16 melanoma tumor model

In previous examples, the efficacy of compositions of the invention was demonstrated in established tumors in two independent HPV-cervical cancer models (C3 and TC1/A2). HPV-bearing tumors were eradicated by targeting CTL epitopes of HPV presented on the surface of tumor cells. This strategy is particularly effective when treating virally induced cancers. Tumors presenting over-expressed "self" antigens, however, are more difficult to treat as they are invisible to the immune system. Self antigens are tightly guarded by the tolerance mechanism. An effective therapeutic cancer treatment must have the ability to induce immune responses against over-expressed tumor-associated self antigens. Melanoma (including the B16 tumor model) is believed to down-regulate MHC class I expression and presentation of self antigens. A therapeutic composition for treatment of melanoma must activate low-affinity T cell clonotypes that are capable of targeting self epitopes on the surface of the tumor.

A robust and specific CTL response is required for successful treatment of melanoma by vaccination. In pre-clinical studies, it has been shown that a B16-specific CTL activity was not sufficient to protect against B16 tumor growth in vivo (Bellone et al., *J. of Immunol.*, 165(5):2651-2656, 2000). Immunotherapy with CpG-matured dendritic cells pulsed with the melanoma-associated self epitope from TRP-2 failed to achieve tumor regression (Pilon-Thomas et al., *J. of Immunother.*, 29(4), 2006). In two other studies,

treatment of 5-day old established B16 tumors resulted in tumor eradication in less than 50% of treated mice and tumors reappeared in all treated animals (Pilon-Thomas et al., *J. of Immunother.*, 29(4), 2006; and Bronte et al., *Cancer Res.*, 60:253-258, 2000).

The ability of compositions of the invention to raise effective CTL responses against multiple peptide antigens simultaneously was tested. Mice (5 mice/group) were implanted with 10^4 B16 cells and treated once 6 days post-implantation with a composition comprising (0.1ml/dose) 25 μg of a TRP-2 CTL epitope (S9L; SVYDFFVWL SEQ ID NO: 7), 25 μg of a p53 CTL epitope (mK9M; KYICNSSCM SEQ ID NO: 8), 25 μg of PADRE and 50 μg of CpG ODN per treatment. For comparison, a second group of mice (5 mice/group) were treated a composition comprising either 25 μg of the same TRP-2 CTL epitope or 25 μg of the p53 CTL epitope (K9M; KYMCNSSCM SEQ ID NO: 9), 25 μg of a modified p53 CTL epitope, mK9M (SEQ ID NO: 8), 25 μg of PADRE (AKXVAAWTLKAAA, wherein X= cyclohexylalanyl; SEQ ID NO: 10) and 50 μg of CpG ODN per treatment. All components of the composition were incorporated in the liposomes before emulsification in the hydrophobic carrier, ISA51. Control mice were treated with PBS alone.

A single treatment with the composition containing a mixture of the TRP-2 and p53 epitope eradicated tumors in all mice 21 days post-treatment (Figure 17; triangles), whereas treatment with a composition containing TRP-2 (diamonds) or p53 alone (squares) cleared tumors in only 40% of mice. All control mice injected with PBS developed tumors (crosses).

EXAMPLE 11

Mice (C57BL/6) were challenged with C3 tumors that developed into palpable size by 8 days post-challenge. On day 8 post-challenge, mice were divided into two control groups (10 mice/group), and a treatment group that was treated with a single administration of a composition comprising CpG ODN and a fusion peptide (R9F peptide (SEQ ID NO: 1) fused to PADRE (SEQ ID NO: 10) encapsulated in liposomes with suspended in a water-in-oil emulsion.

Within 15-20 hours following treatment, mice received a dermal application of Aldara™ ointment (25 mg (equivalent to 10-12 μl Aldara)) at the site of treatment administration. The active ingredient in Aldara is imiquimod at a concentration of 5%. Imiquimod (1-(2-methylpropyl)-1 H-imidazo[4,5-c]quinolin-4-amine) is a novel synthetic compound that is a member of the imidazoquinolone family of drugs known to have the properties of topical immune response modifiers and stimulators. Imiquimod is a ligand for TLR7 and activates a Th1-like cytokine milieu that includes IFN-α, TNF-α, IL-1α, IL-6, and IL-8. In contrast, the two control groups received either PBS alone (100 μL/mouse) or PBS followed by a dermal application of Aldara ointment (25 mg). Tumour size was reduced in mice given the treatment composition followed by dermal application of Aldara (Figure 18; crosses), but not reduced in controls given PBS alone (crosses) or PBS followed by application of Aldara (triangles). Figure 19 shows that the percentage of tumor-bearing mice was reduced in mice given the treatment composition followed by dermal application of Aldara (diamonds), but not reduced in controls given PBS alone (triangles) or PBS followed by application of Aldara (squares).

EXAMPLE 12

Treatment of melanoma using tetanus a toxoid peptide F21E as a T-helper epitope

A melanoma-related antigen, TRP-2, in combination with a T-helper epitope derived from tetanus toxoid, were encapsulated together in a composition comprising CpG ODN in a PBS/ISA51 water-in-oil emulsion. Tetanus toxoid peptide replaced PADRE, the T-helper epitope used in previous examples, to demonstrate that a variety of T-helper epitopes can be used. Stimulation of the number of TRP-2 peptide-specific IFN-γ producing cells indicates that a therapeutic effect directed specifically against melanoma can be anticipated.

C57BL mice were immunized with a composition comprising a TRP-2 peptide (S9L; SEQ ID NO: 7) and the tetanus toxoid epitope F21E (amino acids 947-967, FNNFTVSFWLRVPKVSASHLE; SEQ ID NO: 11) encapsulated together in liposomes with CpG ODN. Control mice were immunized with the above-described composition formulated without the tetanus toxoid T helper epitope. *Ex-vivo* detection of IFN-γ was performed by ELISPOT on splenocytes isolated from spleens collected 8 days post-immunization. Splenocytes of control and treated mice were plated at $5x10^5$ cells per well and were stimulated *in vitro* with the TRP-2 peptide (S9L), or with the melanoma cancer cell line B16-F10 ($5x10^4$ cells per well, 1:10 effector to target ratio). Splenocytes of mice immunized with the treatment composition contained the largest number of TRP-2 specific IFN-γ producing cells. The immune response was observed when splenocytes were stimulated with the TRP-2 peptide or with the B16-F10 cells (Figure 20). Spleens from control mice demonstrated

background levels of TRP-2 specific IFN-γ producing cells. Thus, a strong anti-melanoma CTL immune response can be detected when the splenocytes are stimulated using the TRP-2 peptide in the presence of a tetanus toxoid epitope, but also using the melanoma antigens present on the surface of B16-F10 cells.

Methods

Cell Lines

The C3 cell line was maintained in Iscove Modified Dulbecco's Medium (IMDM; Sigma, St Louis, MO) supplemented with 10 % heat-inactivated fetal calf serum (Sigma, St Louis, MO), 2 mM L-glutamine (Gibco, Burlington, ON), 50 mM 2-mercaptoethanol (Gibco, Burlington, ON), 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, Burlington, ON). Cells were incubated at 37°C / 5 % CO_2.

The EL-4 cell line is a lymphoma cell line that originated in mice. The EL-4 cell line was maintained in Dulbecco's Modified Eagle Medium (DMEM; Sigma, St Louis, MO) with high glucose content containing 2 mM L-glutamine, and supplemented with 10% heat-inactivated fetal calf serum (Sigma, St Louis, MO), 50 mM 2-mercaptoethanol (Gibco, Burlington, ON), 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, Burlington, ON). Cells were incubated at 37°C / 5 % CO_2.

The B16F1 (B16) melanoma cell line was obtained from American Type Culture Collection (ATCC), Manassas, VA.

Peptides

The HPV 16 E7 (H-2D^b) peptide RAHYNIVTF$^{49-57}$ (R9F) containing a CTL epitope was fused to PADRE containing a CD4$^+$

helper epitope by Dalton Chemical Laboratories Inc. (Toronto, ON). This fusion peptide was used at 50 μg/dose. Where indicated, R9F was used as an antigen (25 μg/dose) or in cytotoxicity assays. The peptide KYMCNSSCM (SEQ ID NO: 13) (Dalton) was used as an irrelevant control peptide.

The tyrosinase-related protein (TRP-2) peptides S9L (amino acids 180-188; SVYDFFVWL; SEQ ID NO: 7) and V8L (amino acids 181-188; VYDFFVWL; SEQ ID NO: 6), as well as the p53 peptides (wild type p53 (K9M), amino acids 232-240; KYMCNSSCM; SEQ ID NO: 9), modified p53 peptide mK9M (amino acids 232-240; KYICNSSCM; SEQ ID NO: 8) and mK9M coupled to PADRE (AKXVAAWTLKAAAKYICNSSCM; SEQ ID NO: 17) were purchased from Dalton Chemical Laboratories, Inc. (Toronto, ON, Canada). These peptides are presented by the murine MHC-class I H-2K. S9L is also presented by MHC HLA A2, whereas, V8L.is not presented by MHC HLA A2. The TRP2 and p53 peptides were stored as a 1 mg/ml stock solution in DMSO. Further dilutions for vaccine manufacture were made using PBS.

All formulations of the vaccines, except those containing coupled mK9M, contained PADRE (25 μg/dose) and CpG ODN 1826 (50 μg/dose). Coupled mK9M contained PADRE as part of its structure, therefore, addition of free PADRE was unnecessary.

The amino acid sequence of the irrelevant peptide used in ELISPOT determinations was KIMCNSSCM (Dalton Chemical Laboratories Inc.).

Adjuvants

CpG ODN (Synthetic ODN 1826 with CpG motifs underlined 5'-TCCATGACGTTCCTGACGTT-3', 50 μg/dose) (SEQ ID

NO; 12) was obtained from Coley Pharmaceutical (Wellesley, MA). Lipopetide (Pam3Cys-SKKKK, (100 μg/dose) was obtained from EMC Microcollections, Germany.

Treatments

Liposomes were prepared as follows; lecithin and cholesterol in a ratio of 10:1 (0.2 g lecithin and 0.02 g cholesterol/dose) were dissolved in chloroform/methanol (1:1; v/v) and the solution filter-sterilized using a PTFE 0.2 μm filter. Chloroform and methanol were removed under reduced pressure using a rotary evaporator and traces of the solvents were further removed from the resulting thin lipid layer in vacuo. For liposome encapsulation, fusion peptides with CpG were dissolved in sterile PBS and the resulting solution added to the thin lipid layer with mixing to form liposomes. The resulting suspension of liposomes was emulsified in FIA (Sigma, St Louis, MO) by adding the liposome/PBS suspension to FIA to form a water-in-oil emulsion (PBS:FIA; 1:1,v/v; 100 μl/dose). In some experiments, Montanide ISA51 (Seppic, France) was used in place of FIA as the oil carrier.

Pathogen-free C57BL/6 female mice, 6-8 weeks of age, were obtained from Charles River Laboratories (Wilmington, MA) and were housed under filter top conditions with water and food ad libitum. Institutional animal care and use guidelines were followed for all experiments. Mice were treated with compositions of the invention by subcutaneous injection at the base of the tail. Unless stated otherwise, all treatments were single administration and all treatment groups contained 10 mice. Control mice were injected subcutaneously with PBS or a fusion peptide (a selected CTL epitope fused to PADRE), R9F peptide, CpG ODN

(or Pam3c), fusion peptide with CpG in PBS (100 μl) or liposome encapsulated fusion peptide, R9F, CpG (or Pam3c) in a water-in-oil emulsion (PBS/FIA; 1:1,v/v, 100 μl/dose).

Tumor implantation

C3 cells used in tumor implantation were grown to 95 % confluency and harvested with 0.05 % trypsin. To establish tumors in mice, mice were injected with 0.5 x 10^6 C3 cells s. c. in the left flank. Tumor sizes were determined every 4-5 days using the following formula: longest measurement x (shortest measurement)^2 divided by 2.

Mice (HLA A2) were challenged with TC1/A2 tumor cells (1 X 10^5 cells/mouse) implanted subcutaneously in the left flank. Tumor size was measured every 5 days and is reported as tumor size in individual mice and as percent tumor bearing mice.

B16-F10 cells (10 x 10^3 cells/mouse) were implanted subcutaneously in the left flank of pathogen-free C57BL/6 mice that were 6-8 weeks of age at time of challenge. Tumor size was measured every 2-5 days and the results reported as percent tumor bearing mice.

Cytotoxicity assays

CTL assays, ELISPOT and intracellular staining for interferon (IFN)-γ showed the therapeutic response was specific for the selected E7 peptide since an irrelevant peptide did not elicit CTL activity or IFN-γ production above background. These studies indicate that increases in activated treatment-specific cytotoxic T-cells in splenocytes from mice given the therapeutic treatment correlate with tumor size reduction. Details of the procedures used are described below.

Lymphoblast generation and in-vitro stimulation (IVS)

To examine the acute and memory CTL response, splenocytes from treated mice were analyzed 7, 14 or 130 days post-immunization respectively, unless stated otherwise. Where stated, the cytotoxicity assay was performed upon one round of IVS. Briefly, three days before in vitro stimulation, naïve C57BL/6 mice were sacrificed by CO_2 asphyxiation and spleens were harvested and disassociated. Splenocytes were washed and counted in RPMI-10 where RPMI is supplemented with 10 % heat-inactivated fetal calf serum (Sigma, St Louis, MO), 50 mM 2-mercaptoethanol (Gibco), 100 U/ml penicillin and 100 □g/ml streptomycin (Gibco). Splenocytes (10^6 cells/ml) were cultured with lipopolysaccharide (25 μg/ml) and dextran sulphate (7 μg/ml) treated lymphoblasts.

Syngeneic lymphoblasts were irradiated (by 4000 rad using a ^{137}Sc source for 15 minutes) and loaded with the R9F peptide (100 μM). Peptide-loaded LPS activated lymphoblasts (3 × 10^6cells/ml) were used to stimulate splenocytes of immunized mice in a ratio of 3:1 where effector cells were adjusted to 3 ×10^6cells/ml, and T-stim (BD Biosciences, Mississauga, ON) was added to wells to obtain a final concentration of 20 %. Cells were incubated at 37°C /5 % CO_2 for 6 days.

JAM assay

EL-4 cells were labeled with 5 μCi/ml [Methyl-^{3}H] thymidine (Amersham Pharmacia, Erlangen, Germany). The cells were incubated at 37°C /5 % CO_2 for 24 hours then loaded with R9F or irrelevant peptides (10 μg/ml) for one hour. Suspensions of labelled target cells were then harvested, washed twice in RPMI-10, and seeded in 96-well U-bottom

plates at a density of 2 x 10^3cells/well. The effector cells were added by serial dilution starting at a concentration of 2 x 10^5 effector cells/well. The plates were incubated for 4 hours at 37°C /5 % CO_2. The cells were aspirated onto fiberglass filters and tritium counted using a Packard TopCount scintillation counter. The percent DNA fragmentation was calculated using the following formula: % DNA fragmentation = (S - E)/E x 100, where S is retained DNA (counts) in the absence of treatment (spontaneous) and E is retained DNA (counts) in the presence of effector cells.

Ex vivo analysis of antigen-specific T cells by ELISPOT

Activated antigen-specific CTLs in splenocytes harvested from treated C57BL/6 mice were detected using a BD ELISPOT (BD Bioscience, San Diego, CA). Briefly, on day 7 post-treatment a 96-well nitrocellulose plate was coated with the capture antibody, a purified anti-mouse IFN-γ antibody, and incubated overnight at 4°C. The antibody was discarded and the plate was blocked for 2 hours then the blocking solution was removed. Splenocytes were each added to their respective wells at an initial concentration of 1 million cells/well in a final volume of 100μl followed by serial dilutions in subsequent wells of a row. The following stimulators and controls were added to 100μl of media to obtain their desired final concentration. Either, C3 cells (5 x 10^5 cells/ml), the R9F peptide (10μg/ml), the irrelevant peptide (10μg/ml), or no peptides were added to the wells. PMA (5ng/ml, Sigma), ionomycin (500ng/ml, Sigma), served as positive controls and the irrelevant peptide and media alone served as negative controls. The plate was incubated overnight at 37°C / 5% CO_2 after which the detection antibody, a biotinylated anti-mouse IFN-γ antibody, was added for 2 hours at room temperature.

Following the incubation period, the detection antibody was discarded and the enzyme conjugate (Streptavidin-HRP) was added for 1 hour and lastly the plate was stained with an AEC substrate solution for 20 minutes. The plate was washed and left to air dry overnight for visualization of spots using a magnifying lens.

Intracellular cytokine staining (ICS)

Splenocytes were retrieved from spleens of tumor-free mice as previously described, washed twice with RPMI-10 (500 x g, 5 minutes) and resuspended in RMPI-10 (10 x 10^6 cells/ml). Splenocytes (1 x 10^6 cells/well) were added to wells of a 96-well flat bottom plate and incubated with R9F or an irrelevant peptide at a final concentration of 3 μg/ml in duplicate columns for each peptide. In experiments that used EL-4 cells to demonstrate the protective function of IFNγ-producing $CD8^+$ CTLs, EL-4 cells (1 x 10^5 cells/well) loaded with either R9F or the irrelevant peptide were incubated for 6 hours at 37°C / 5% CO_2 before cytotoxicity measurements.

Intracellular cytokine staining was performed as described in the Cytofix™/ Cytoperm™ kit instruction manual (BD Biosciences, Mississauga, ON). In brief, after addition of stimulants, GolgiStop was added to each well and the plates were incubated (37°C / 5% CO_2) for 4 hours. Cells were washed with staining buffer then incubated (20 minutes at 4°C, in the dark) with anti-CD8 serum, washed again with staining buffer followed by incubation with anti-IFN-γ (30 minutes at 4°C in the dark). This was followed by washes with perm/wash buffer after which cells were resuspended with

perm/wash buffer and transferred to FACS tubes (BD Falcon). Staining was assessed by FACSCalibur (BD Biosciences, San Jose, CA), and data were analysed using CellQuest software.

REFERENCES

Allegra, C.J. and R.W. Childs, Cytotoxins and cancer immunotherapy: The dance of the macabre. J. National Cancer Institute, 97:1396-1397, 2005.

Antonia, S.J. et al., Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. Clinical Cancer Research, 12:878-887, 2006.

Bellone, M. et al., Relevance of the tumor antigen in the validation of three vaccination strategies for melanoma, *Journal of Immunology*, 165(5):2651-6, 2000.

Bronte, V. et al., Genetic Vaccination with "Self" Tyrosinase-related Protein 2 Causes Melanoma Eradication but not Vitiligo, *Cancer Research*, 60:253-258, January 15, 2000.

Celis E. *et al.*, Recognition of hepatitis B surface antigen by human T lymphocytes. Proliferative and cytotoxic responses to a major antigenic determinant defined by synthetic peptides, *Journal of Immunology*, 140:1808-1815, 1988.

Chakraborty, M. *et al.*, External beam radiation of tumors alters phenotype of tumor cells to render them susceptible to vaccine-mediated T-cell killing. Cancer Research, 64:4328-4337, 2004.

Chong P. *et al.*, Identification of T- and B-cell epitopes of the S2 and S3 subunits of pertussis toxin by use of synthetic peptides, *Infection and Immunity*, 60:4640-4647, 1992.

Correale, P. *et al*, Fluorouracil-based chemotherapy enhances the antitumor activity of a thymidylate synthase-directed

polyepitopic peptide vaccine. Journal of the National Cancer Institute 97:1437-1445, 2005.

DeLeo, A.B., p53-based immunotherapy of cancer, *Critical Reviews in Immunololgy, 18:29,* 1998.

Demotz S. *et al.*, Delineation of several DR-restricted tetanus toxin T cell epitopes, *Journal of Immunology,* 142:394-402, 1989.

Diethelm-Okita, B.M. *et al.*, Universal epitopes for human $CD4^+$ cells on tetanus and diphtheria toxins. *Journal of Infectious Diseases,* 181:1001-1009, 2000.

Dudley, M.E. et al., Adoptive transfer of cloned melanoma-reactive T lymphocytes for the treatment of patients with metastatic melanoma, *Journal of Immunotherapy,* 24(4): 363-73 2001.

Fernando, G.J. et al., Vaccine-induced Th1-type responses are dominant over Th2-type responses in the short term whereas pre-existing Th2 responses are dominant in the longer term. *Scandinavian Journal of Immunology,* 47(5): 459-65, 1998.

Frezard, F., Liposomes: From biophysics to the design of peptide vaccines. *Brazilian Journal Medical Biology Research,* 32:181-189, 1999.

Gregoriadis, G., Immunological adjuvants: A role for liposomes, *Immunology Today,* 11:89-97, 1990.

Gulley, J.L. *et al.*, Combining a recombinant cancer vaccine with standard definitiveradiotherapy in patients with localized protate cancer. Clinical Cancer Research, 11: 3353-3362, 2005.

Knutson, K.L., et al., Immunization of cancer patients with a HER-2/neu, HLA-A2 peptide, p369-377, results in short-lived peptide-specific immunity." *Clinical Cancer Research*, 8(5): 1014-8, 2002.

Pilon-Thomas, S. et al., Immunostimulatory Effects of CpG-ODN Upon Dendritic Cell-Based Immunotherapy in a Murine Melanoma Model, *Journal Immunotherapy*, 29(4), July/August 2006.

Vierboom, M.P.M. et al., p53: a target for T-cell mediated immunotherapy, *Peptide-Based Cancer Vaccines*, W. M. Kast, Ed. Landes Bioscience, Georgetown, p.40, 2000.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

It must be noted that as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

CLAIMS:

1. A composition comprising:

a carrier comprising a continuous phase of a hydrophobic substance;

liposomes; and

at least one antigen capable of inducing a cytotoxic T lymphocyte (CTL) response.

2. The composition of claim 1 further comprising at least one T helper epitope.

3. The composition of claim 2, wherein the T helper epitope is a separate molecule from the at least one antigen.

4. The composition of claim 2, wherein the antigen comprises the T helper epitope.

5. The composition of any one of claims 1 to 4, wherein the antigen is a polypeptide.

6. The composition of any one of claims 1 to 5, wherein the antigen comprises a CTL epitope, or a combination of CTL epitopes.

7. The composition of claim 6, wherein the CTL epitope is derived from a virus.

8. The composition of claim 7, wherein CTL epitope is derived from human papilloma virus.

9. The composition of claim 8, wherein the CTL epitope is an epitope of an E6 or E7 protein of human papilloma virus (HPV).

10. The composition of claim 9, wherein the E6 epitope comprises the peptide sequence TIHDIILECV (T10V; SEQ ID NO: 5).

11. The composition of claim 9, wherein the E7 epitope comprises a peptide sequence selected from the group consisting of: RAHYNIVTF (R9F; SEQ ID NO: 1), YMLDLQPETT (Y10T; SEQ ID NO: 2), LLMGTLGIV (L9V; SEQ ID NO: 3), and TLGIVCPI (T81; SEQ ID NO: 4).

12. The composition of claim 9, wherein the CTL epitope is a mixture of CTL epitopes of E6 and E7 proteins of HPV.

13. The composition of claim 12, wherein the mixture of epitopes comprises Y10T (SEQ ID NO: 2), L9V (SEQ ID NO: 3), T81 (SEQ ID NO: 4) and T10V (SEQ ID NO: 5) peptide sequences.

14. The composition of claim 13, wherein said epitopes are joined together to form one peptide (AB2) (SEQ ID NO: 14).

15. The composition of claim 6, comprising a plurality of CTL epitopes linked to form a single polypeptide.

16. The composition of claim 6, wherein the CTL epitope is derived from a tumor-associated protein.

17. The composition of claim 16 wherein the tumor associated protein is p53.

18. The composition of claim 16, wherein the tumor-associated protein is tyrosinase-related protein-2 (TRP-2).

19. The composition of claim 6, wherein the antigen comprises a mixture of a p53 epitope and a TRP-2 epitope.

20. The composition of claim 17 or 19, wherein the p53 epitope comprises the peptide sequence KYICNSSCM (mK9M) (SEQ ID NO: 8).

21. The composition of claim 18 or 1, wherein the TRP-2 epitope comprises the peptide sequence SVYDFFVWL (SEQ ID NO: 7).

22. The composition of claim 18 or 19, wherein the TRP-2 epitope comprises the peptide sequence VYDFFVWL (SEQ ID NO: 6).

23. The composition of any one of claims 1 to 22, wherein the T helper epitope is a universal T helper epitope.

24. The composition of claim 23, wherein the T helper epitope is PADRE (pan-DR epitope) (SEQ ID NO: 10).

25. The composition of any one of claims 1 to 22, wherein the T helper epitope is F21E (SEQ ID NO: 11).

26. The composition of any one of claims 1 to 25, wherein the T helper epitope is fused to the at least one antigen.

27. The composition of claim 26, wherein the T helper epitope is PADRE and at least one antigen is a CTL epitope.

28. The composition of any one of claims 1 to 27, wherein the composition is capable of increasing IFN-γ producing cells.

29. The composition of any one of claims 1 to 27, further comprising an adjuvant.

30. The composition of claim 29, wherein the adjuvant is a CpG oligodeoxynucleotide (CpG ODN) (SEQ ID NO: 12).

31. The composition of claim 29, wherein the adjuvant is a lipopeptide.

32. The composition of claims 31, wherein the lipopeptide is Pam3Cys-SKKKK.

33. The composition of any one of claims 1 to 32 for the treatment of cancer.

34. The composition of claim 33, wherein the cancer is selected from the group consisting of: cervical, vulvar, melanoma, breast, lung, ovarian, multiple myeloma, B cell lymphoma, hepatoma, sarcoma, bladder, prostate cancer, thyroid, H/N tumors, colon, rectal, renal, pancreatic, gastric, adenocarcinoma, T cell leukemia, lymphosarcoma, uterine, esophageal, non-Hodgkin's lymphomas, endometrial, and RCC tumors.

35. The composition of any one of claims 1 to 34 for reducing tumor size.

36. A method for treating cancer or inhibiting or preventing the growth or proliferation of cancer cells in a subject comprising administering the composition of any one of claims 1 to 35.

37. The method of claim 36 wherein the cancer is selected from the group consisting of: cervical, vulvar, melanoma, breast, lung, ovarian, multiple myeloma, B cell lymphoma, hepatoma, sarcoma, bladder, prostate cancer, thyroid, H/N tumors, colon, rectal, renal, pancreatic, gastric, adenocarcinoma, T cell leukemia, lymphosarcoma,

uterine, esophageal, non-Hodgkin's lymphomas, endometrial, and RCC tumors.

38. The method of claim 36 or 37, further comprising the use of a topical composition comprising 1-isobutyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine at the site of administration of said composition.

39. A kit useful for treating cancer in a subject comprising a composition of any one of claims 1 to 31, and instructions for its use thereof.

40. The method of any one of claims 36 to 38, wherein the subject is a mammal.

41. The method of claims 40, wherein the mammal is a human.

42. The composition of any one of claims 1 to 35, wherein the liposome comprises a phospholipid.

43. The composition of claim 42, wherein the phospholipid has at least one head group selected from phosphoglycerol, phosphoethanolamine, phosphoserine, phosphocholine and phosphoinositol.

44. The composition of any one of claims 1 to 35, wherein the liposome comprises unesterified cholesterol.

45. The composition of any one of claims 1 to 35, wherein the liposome comprises lipids in phospholipon 90 G.

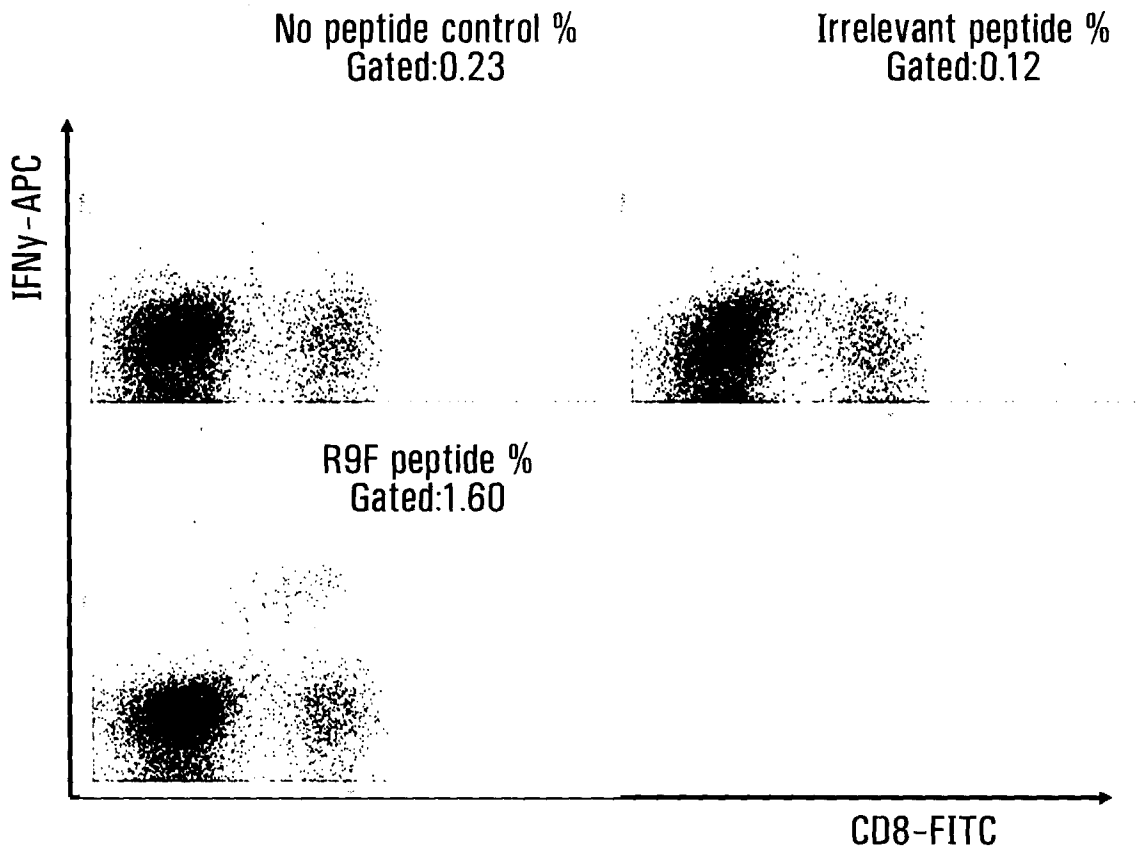
44. The composition of any one of claims 1 to 35, wherein the liposome is obtained from archaebacterial bacteria.

45. The composition of any one of claims 1 to 35, wherein the liposome is obtained from natural lipids, synthetic lipids, sphingolipids, ether lipids, sterols, cardiolipin, cationic lipids and lipids modified with poly (ethylene glycol) and other polymers.

46. The composition of claim 45 wherein the synthetic lipids includes fatty acid constituents selected from lauroyl, myristoyl, palmitoyl, stearoyl, arachidoyl, oleoyl, linoleoyl, and erucoyl, or combinations thereof.

SMART & BIGGAR
OTTAWA, CANADA

PATENT AGENTS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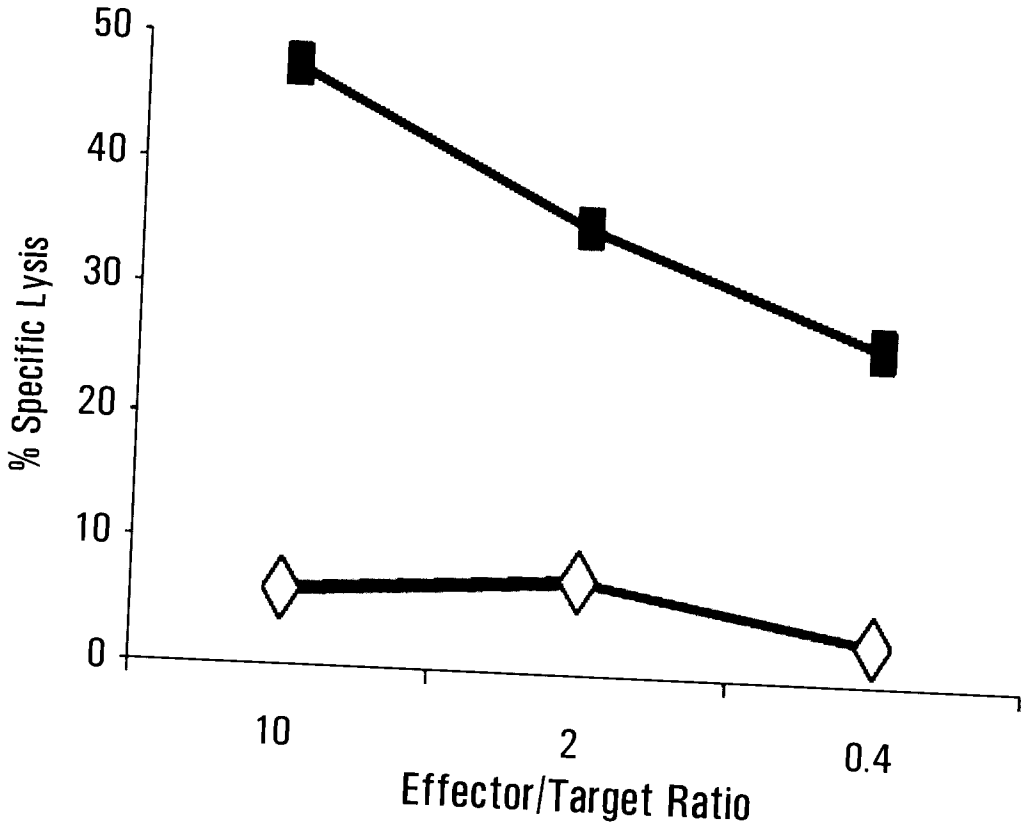
Irrelevant peptide %
Gated:0.12
IFNγ-APC
R9F peptide %
Gated:1.60
CD8-FITC

FIG. 1



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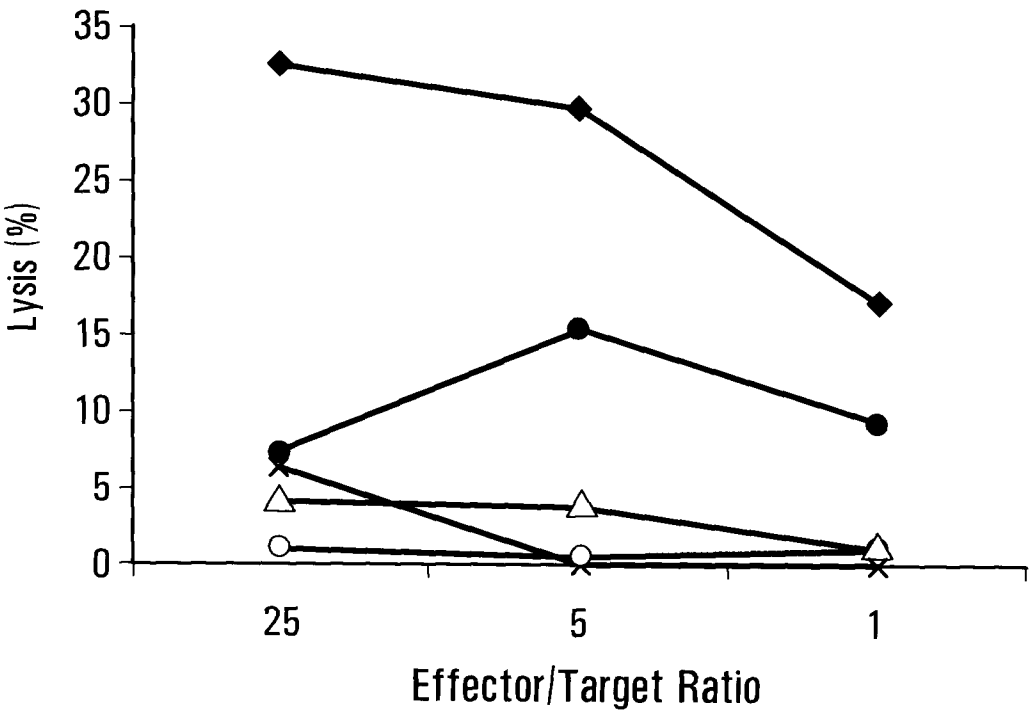

FIG. 3

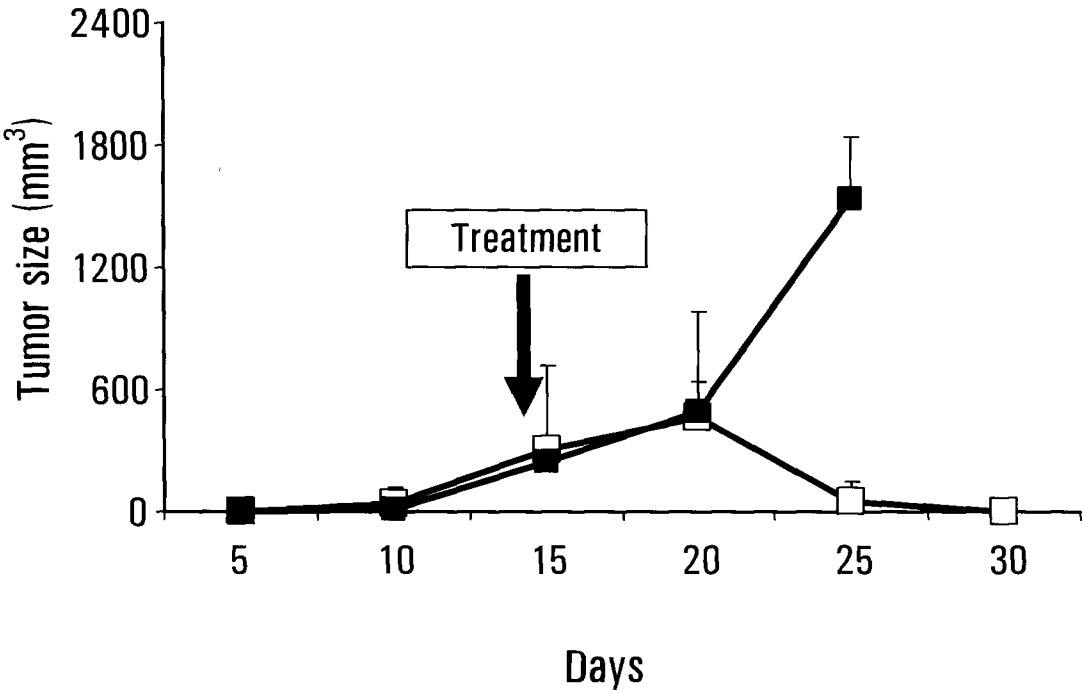
2400
1800
1200
600
0
Tumor size (mm3)
Treatment
5
10
15
20
25
30
Days

FIG. 4

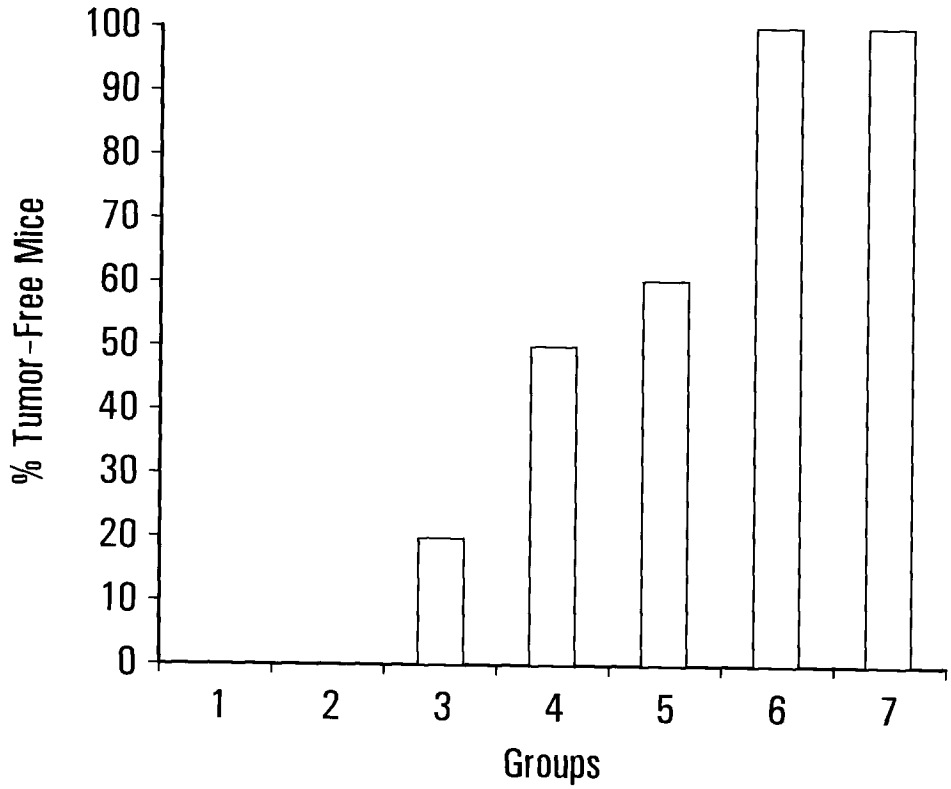

FIG. 5

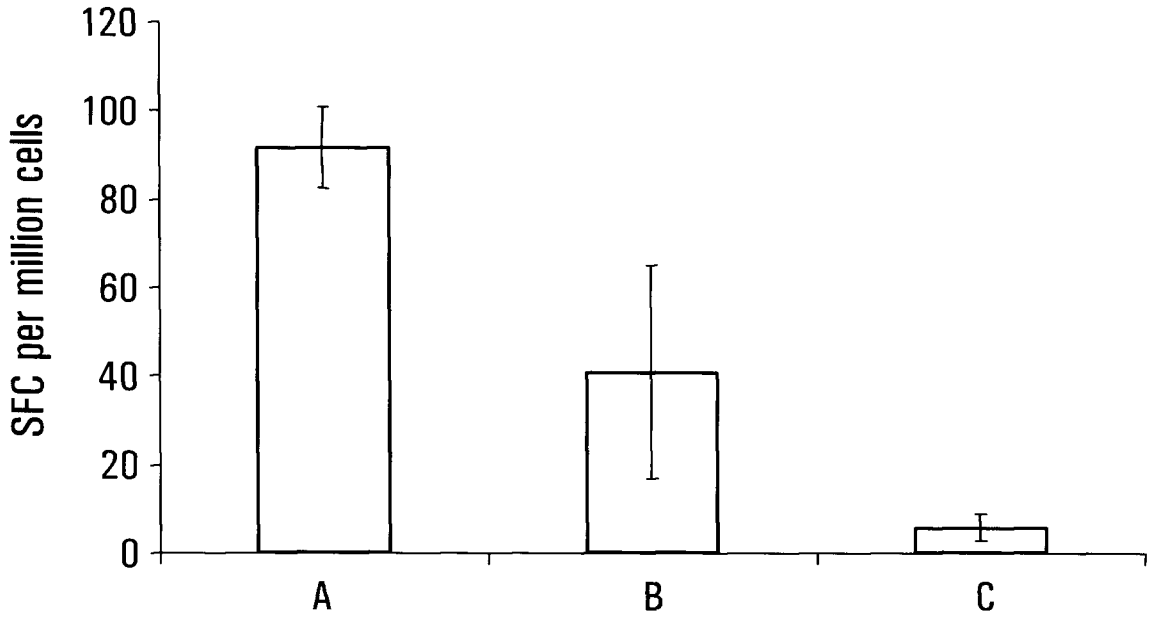

FIG. 6

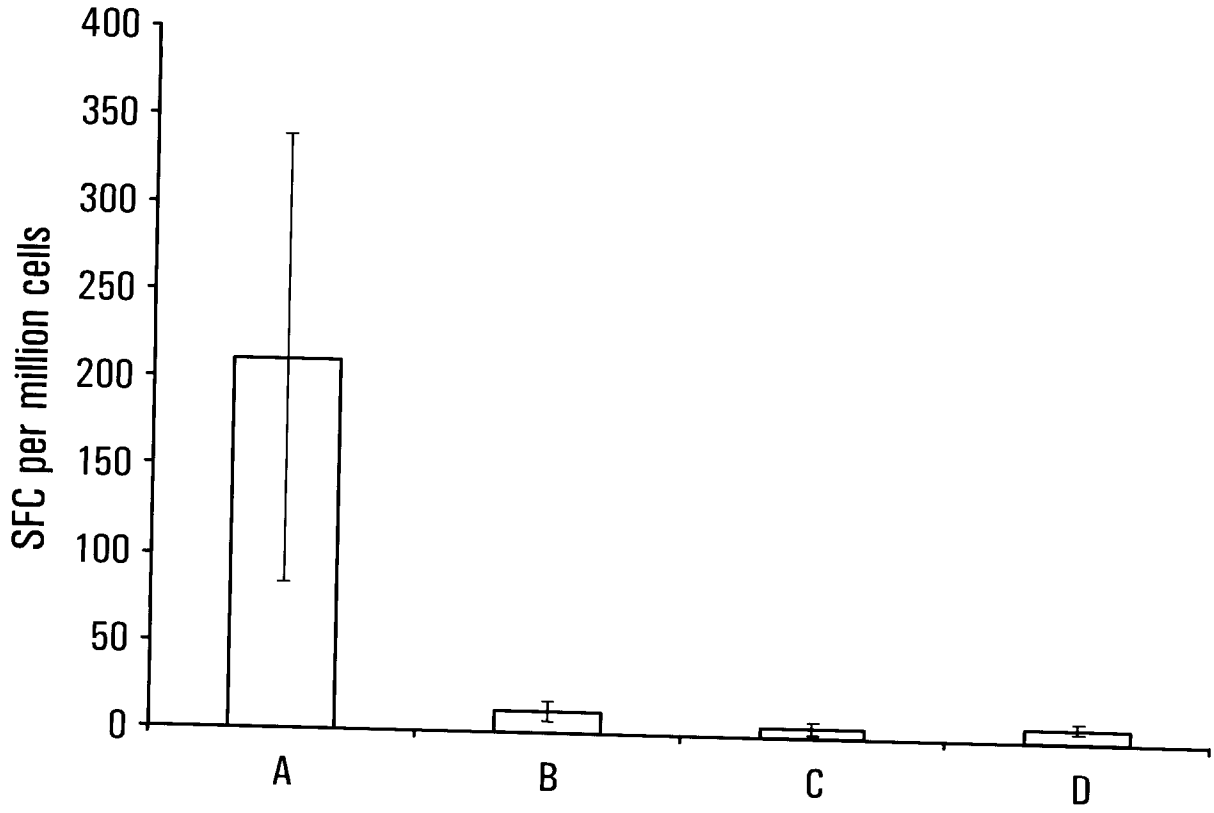

FIG. 7

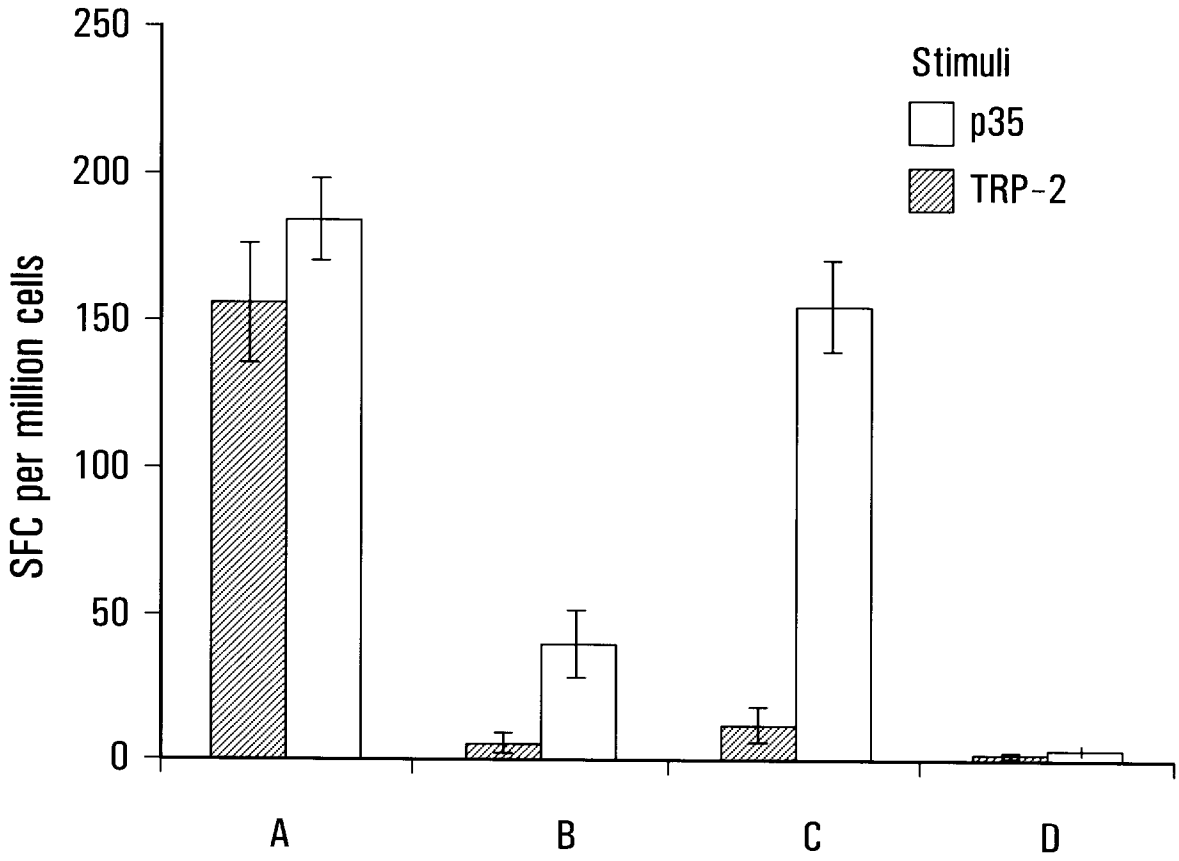
250
200
150
100
50
0
SFC per million cells
Stimuli
p35
TRP-2
A
B
C
D

FIG. 8

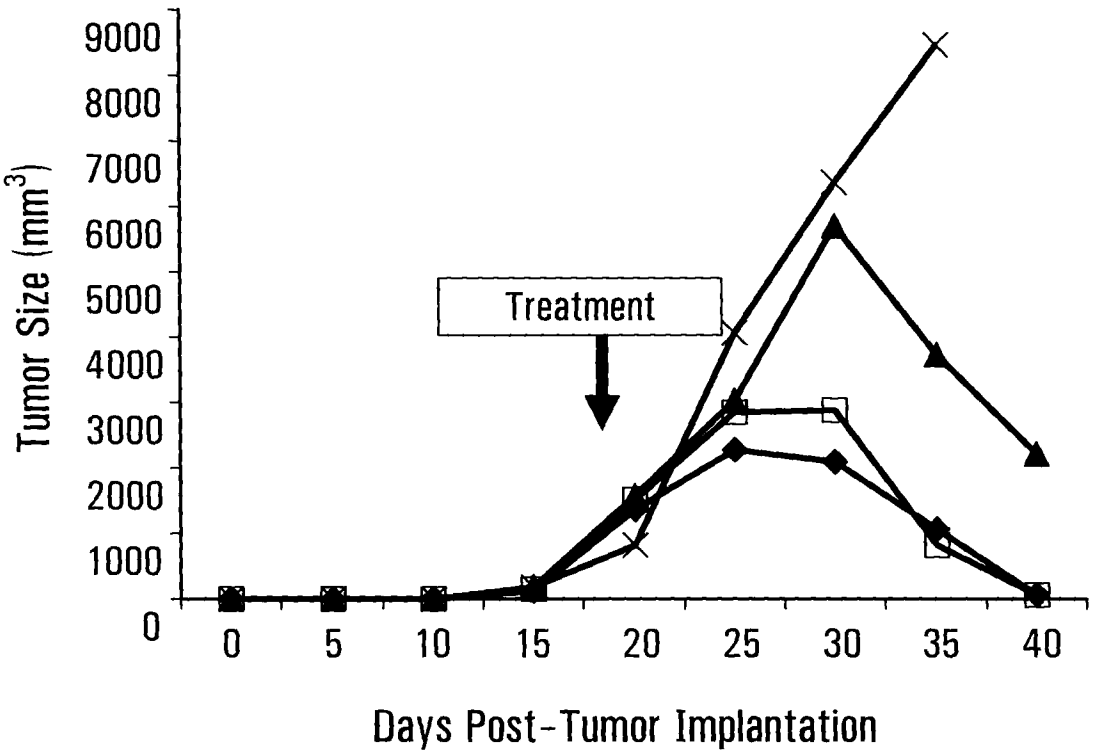
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Tumor Size (mm3)
Treatment
0
5
10
15
20
25
30
35
40
Days Post-Tumor Implantation

FIG. 9

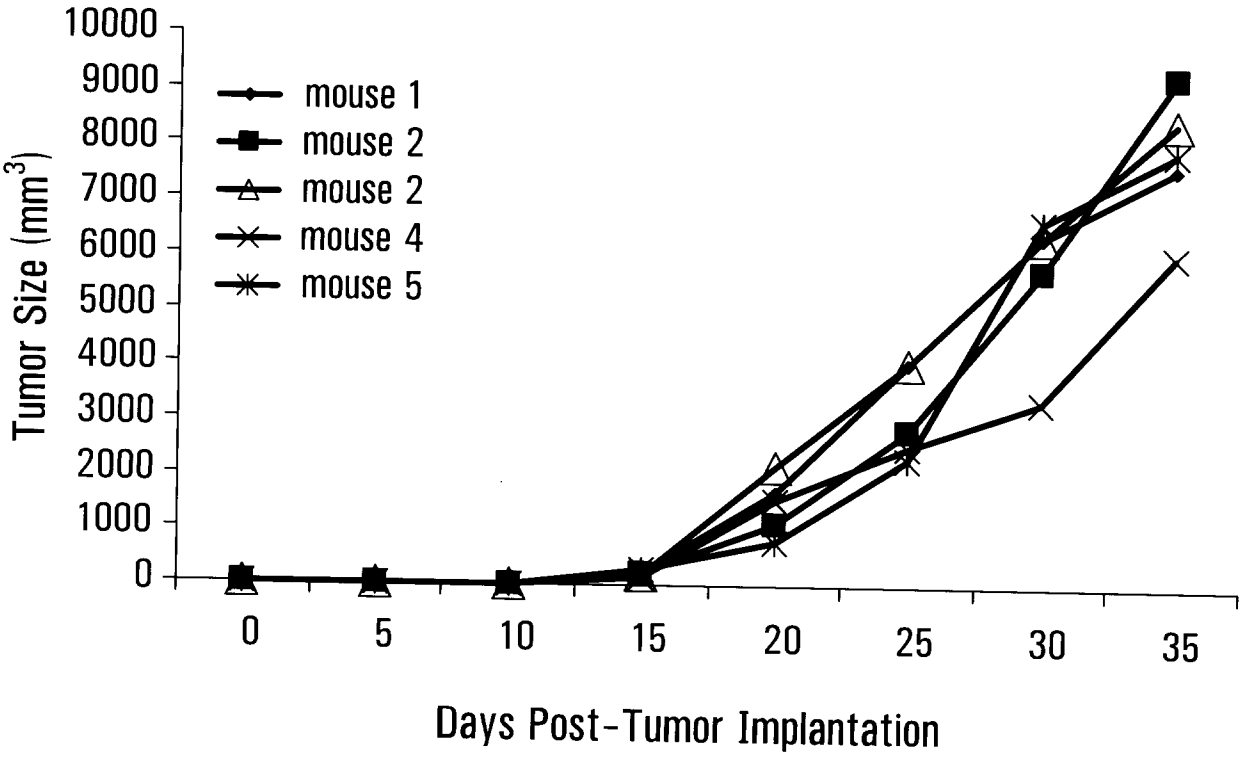
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Tumor Size (mm3)
mouse 1
mouse 2
mouse 2
mouse 4
mouse 5
0
5
10
15
20
25
30
35
Days Post-Tumor Implantation

FIG. 10

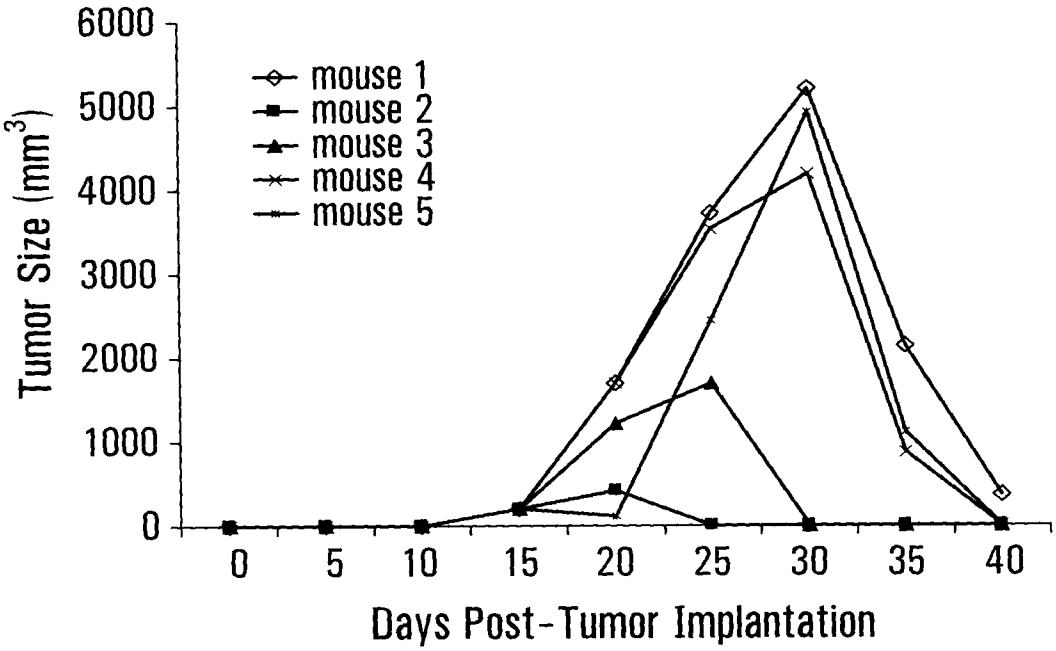
6000
5000
4000
3000
2000
1000
0
Tumor Size (mm3)
mouse 1
mouse 2
mouse 3
mouse 4
mouse 5
0
5
10
15
20
25
30
35
40
Days Post-Tumor Implantation

FIG. 11

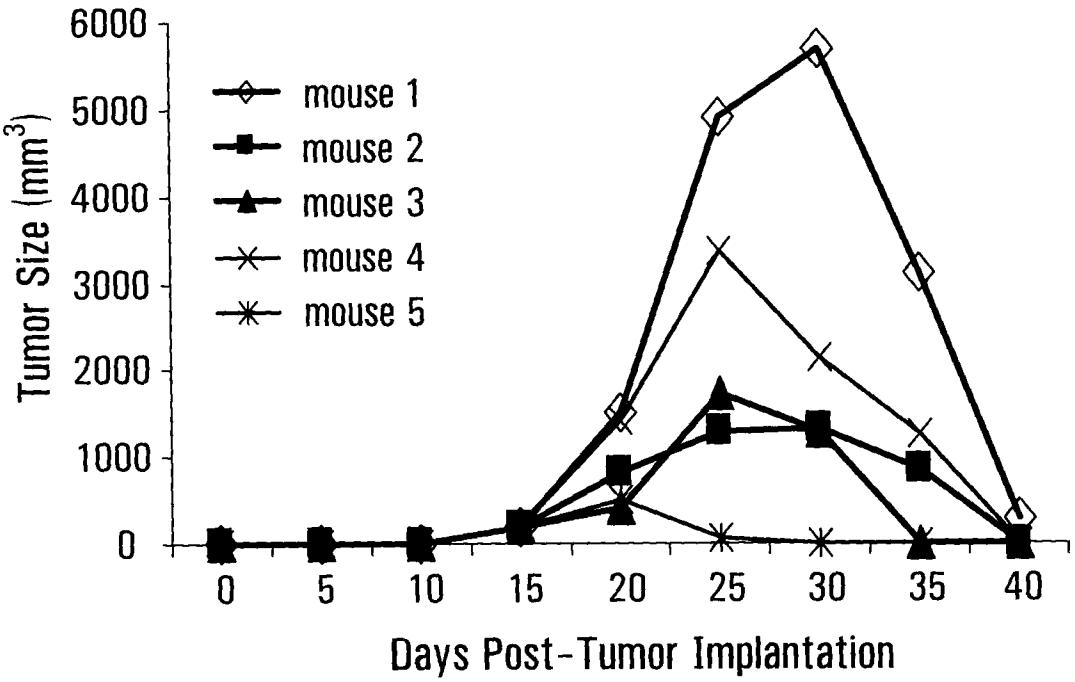
6000
5000
4000
3000
2000
1000
0
Tumor Size (mm3)
mouse 1
mouse 2
mouse 3
mouse 4
mouse 5
0
5
10
15
20
25
30
35
40
Days Post-Tumor Implantation

FIG. 12

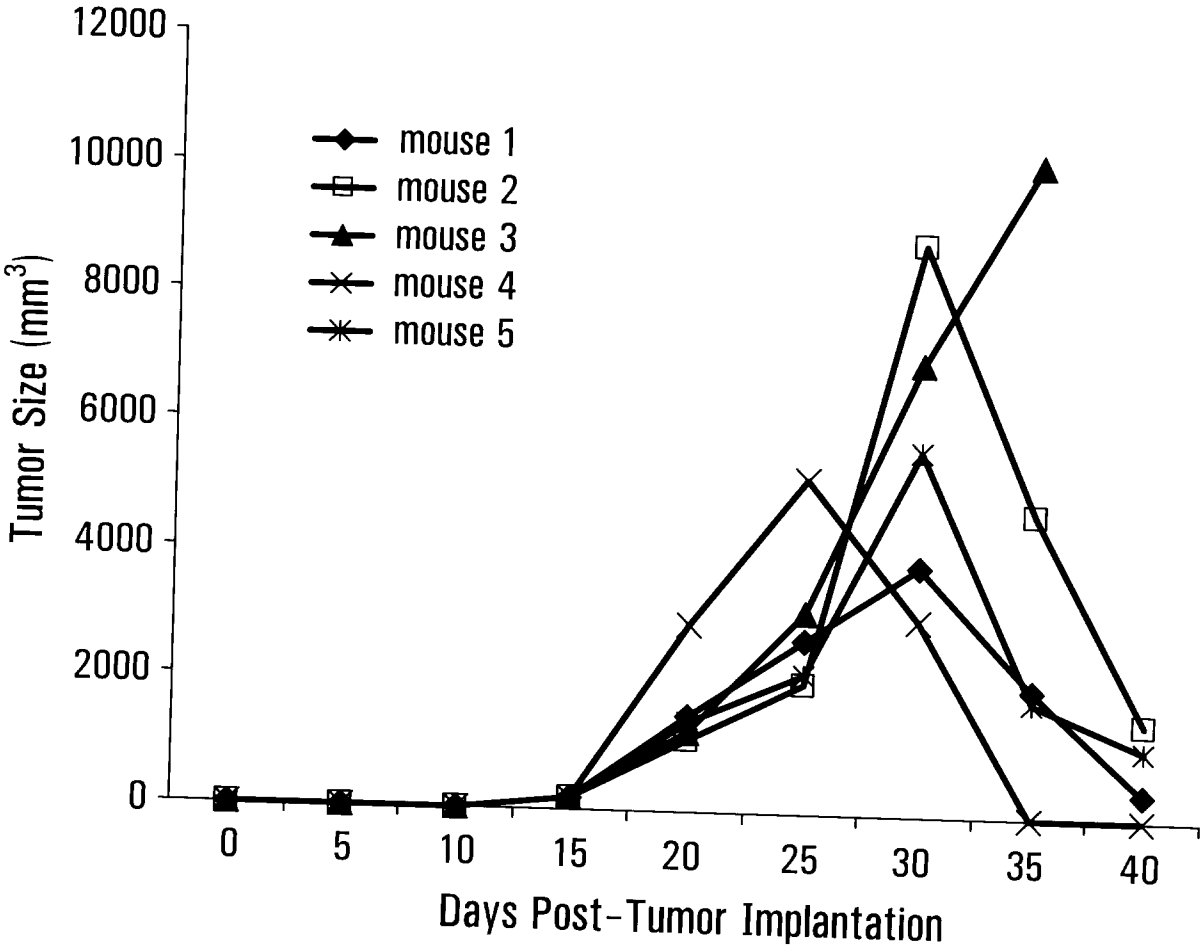
12000
10000
8000
6000
4000
2000
0
Tumor Size (mm3)
mouse 1
mouse 2
mouse 3
mouse 4
mouse 5
0
5
10
15
20
25
30
35
40
Days Post-Tumor Implantation

FIG. 13

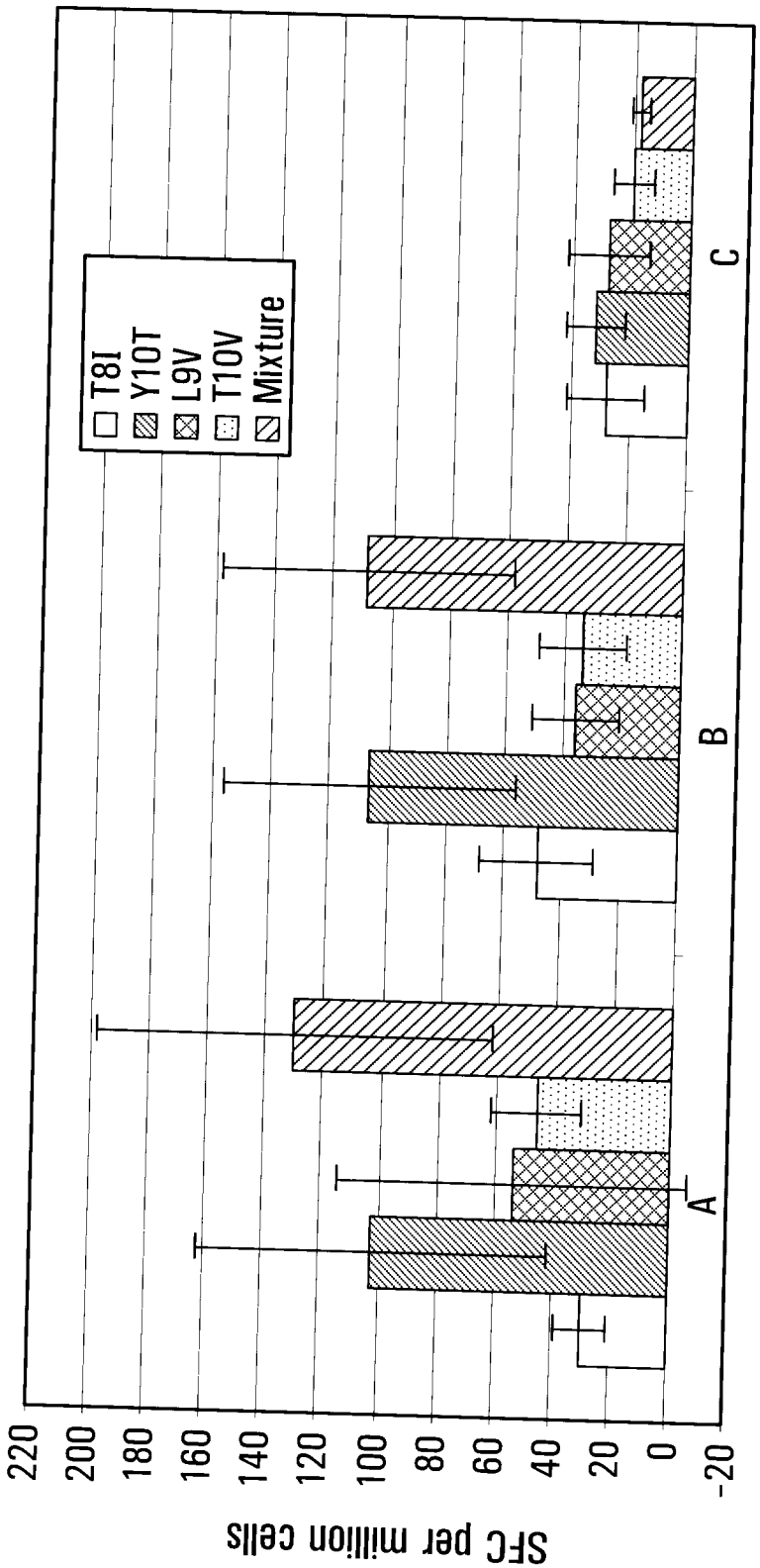
SFC per million cells
220
200
180
160
140
120
100
80
60
40
20
0
-20
T8I
Y10T
L9V
T10V
Mixture
A
B
C
Treatment Group

FIG. 14

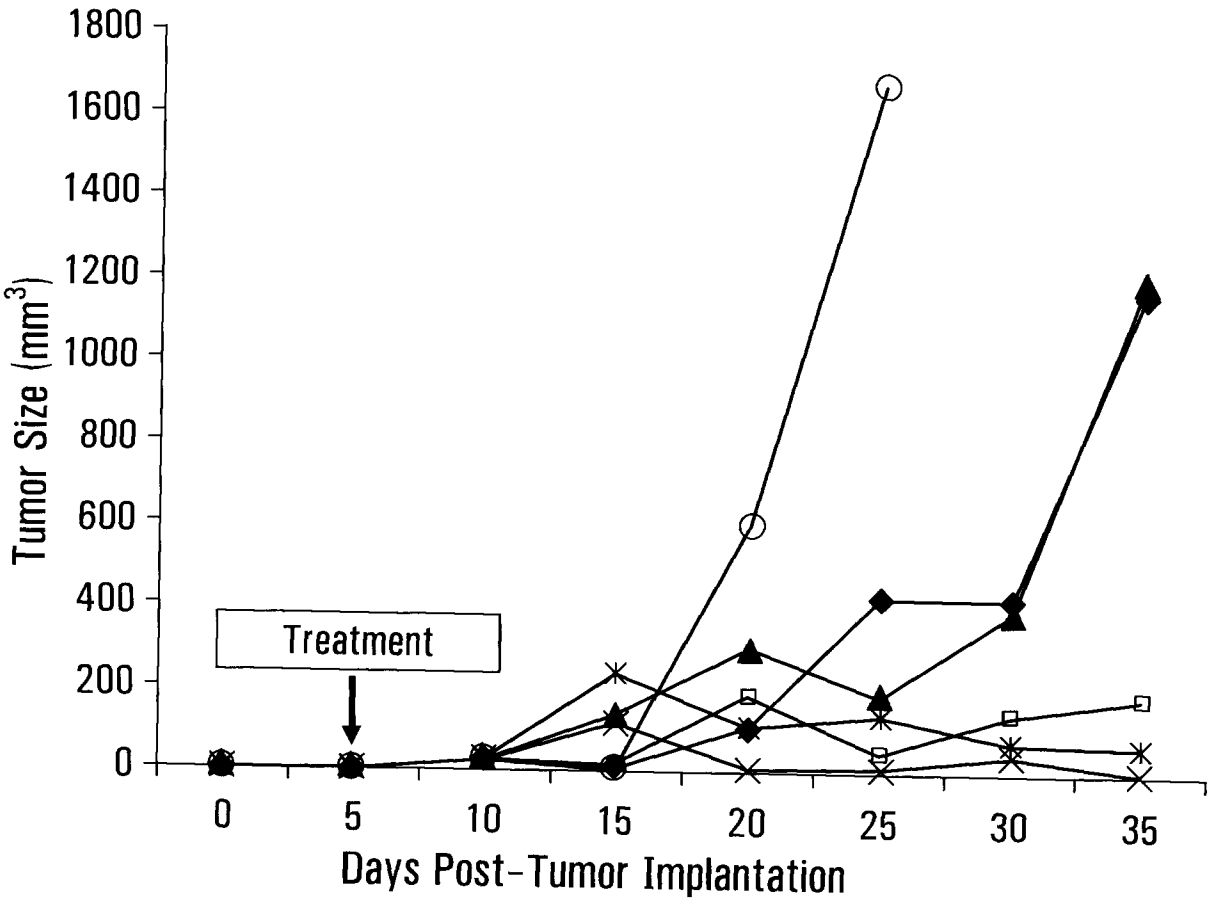
1800
1600
1400
1200
1000
800
600
400
200
0
Tumor Size (mm3)
Treatment
0
5
10
15
20
25
30
35
Days Post-Tumor Implantation

FIG. 15

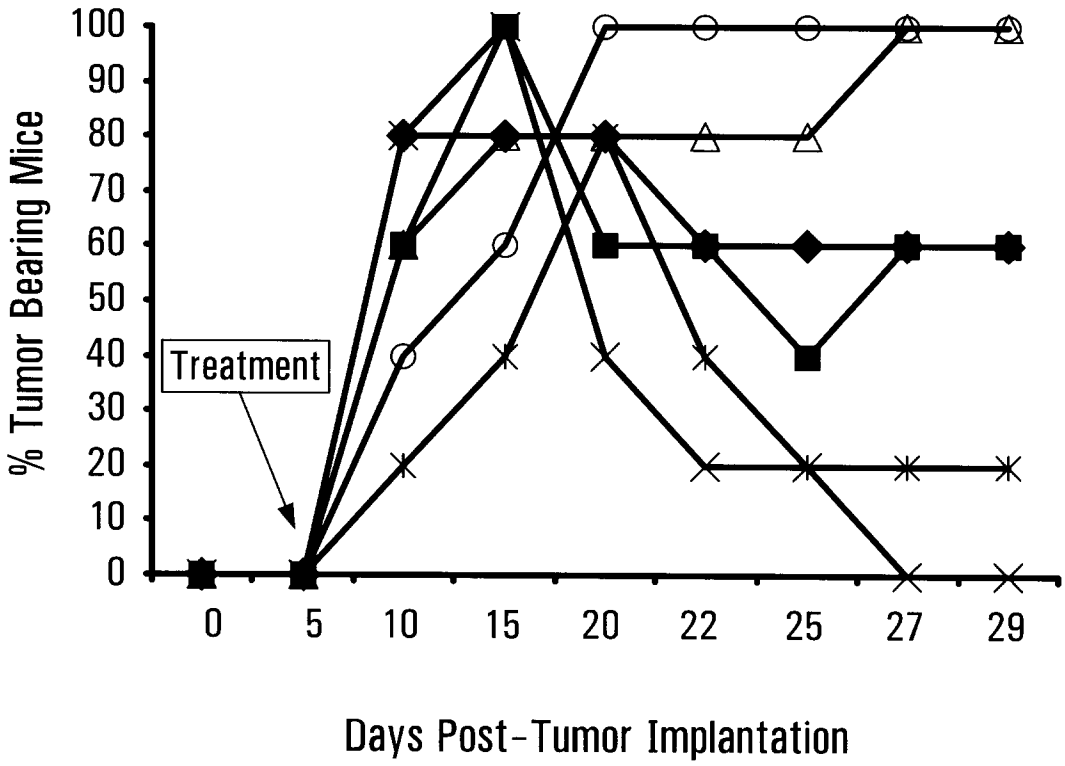
% Tumor Bearing Mice
100
90
80
70
60
50
40
30
20
10
0
Treatment
0
5
10
15
20
22
25
27
29
Days Post-Tumor Implantation

FIG. 16

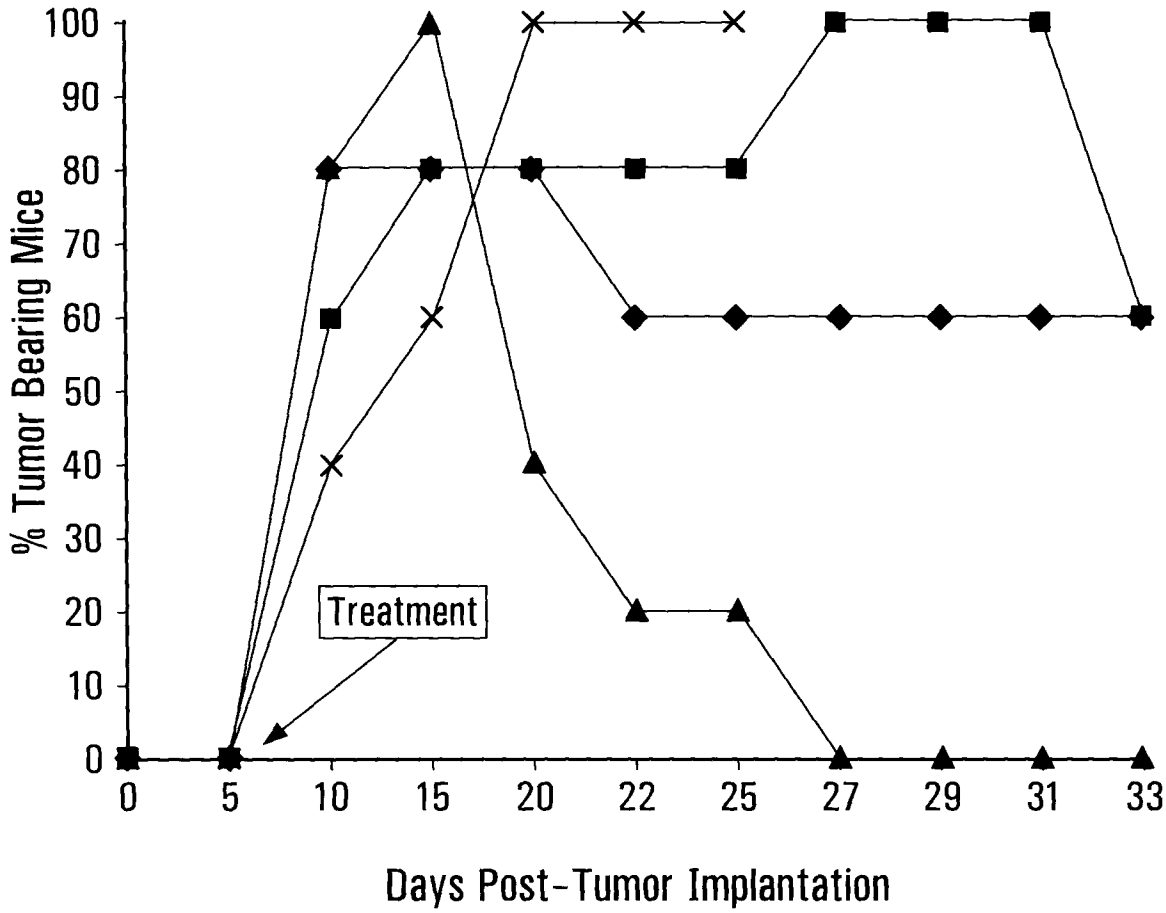
100
90
80
70
60
50
40
30
20
10
0
% Tumor Bearing Mice
Treatment
0
5
10
15
20
22
25
27
29
31
33
Days Post-Tumor Implantation

FIG. 17

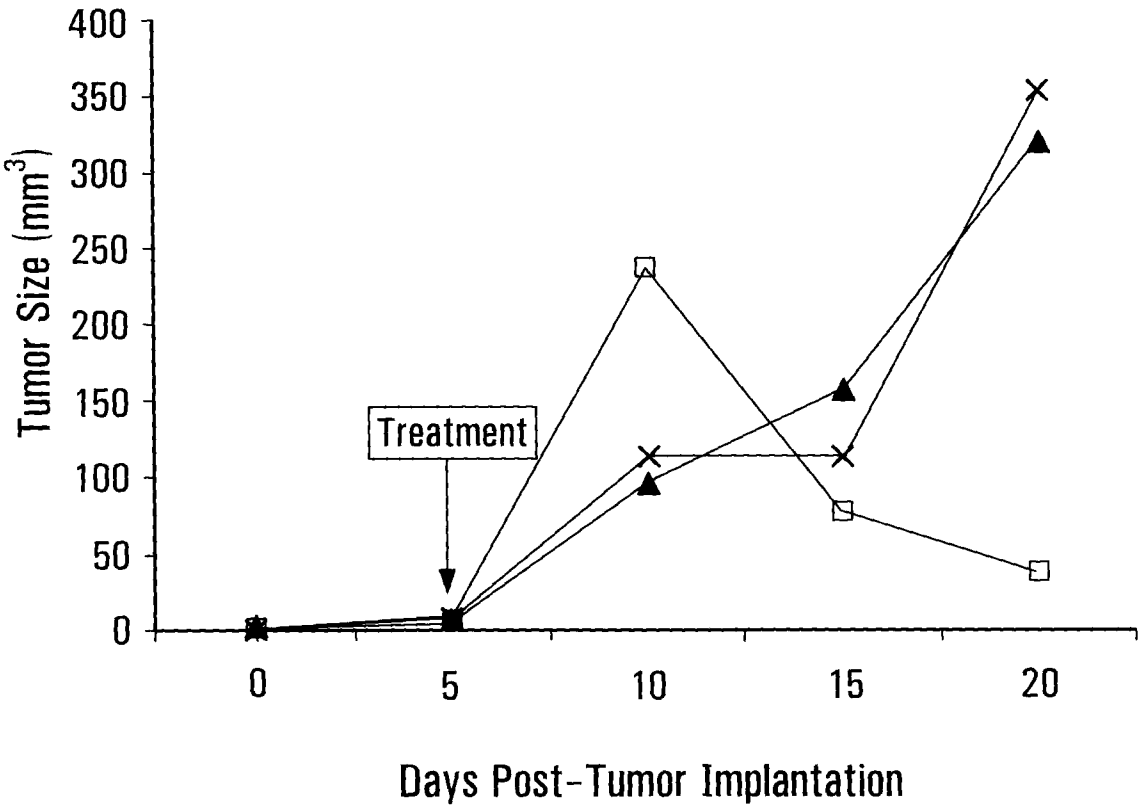
400
350
300
250
200
150
100
50
0
Tumor Size (mm3)
Treatment
0
5
10
15
20
Days Post-Tumor Implantation

FIG. 18

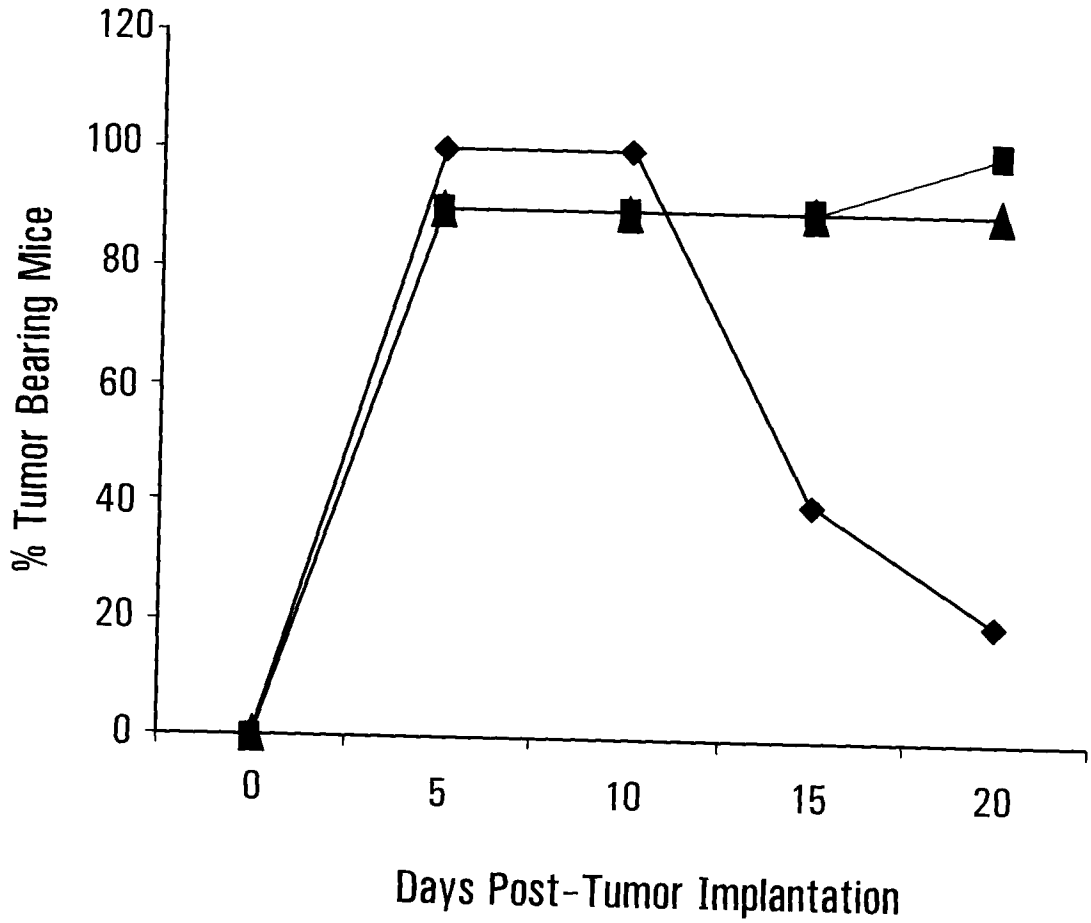
120
100
80
60
40
20
0
% Tumor Bearing Mice
0
5
10
15
20
Days Post-Tumor Implantation

FIG. 19

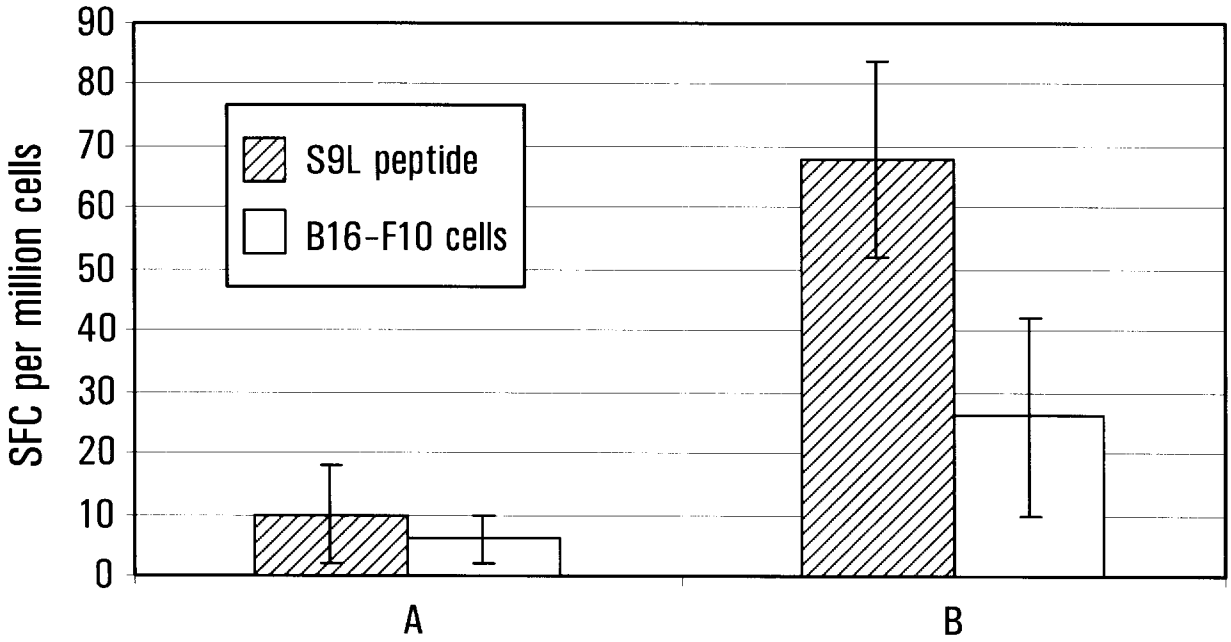
90
80
70
60
50
40
30
20
10
0
SFC per million cells
S9L peptide
B16-F10 cells
A
B

FIG. 20

SEQUENCE LISTING

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<120> Use of liposomes in a carrier comprising a continious hydrophobic phase as a vehicle for cancer treatment

<130> 78961-49

<150> CA 2,523,032
<151> 2005-10-07

<150> CA 2,533,705
<151> 2006-01-13

<150> CA 2,542,212
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<170> PatentIn version 3.3

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1 5

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<211> 10
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1 5 10

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<212> PRT
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001640

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 39/00*** (2006.01) , ***A61K 31/4745*** (2006.01) , ***A61K 38/00*** (2006.01) , ***A61K 39/12*** (2006.01) , ***A61K 9/127*** (2006.01) , ***A61P 35/00*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***A61K 39/00*** (2006.01) , ***A61K 31/4745*** (2006.01) , ***A61K 38/00*** (2006.01) , ***A61K 39/12*** (2006.01) , ***A61K 9/127*** (2006.01) , ***A61P 35/00*** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used):
CANADIAN PATENT DATABASE, DELPHION, USPTO, ESPACENET, STN / BIOSIS, PUBMED; Keywords: cytotoxic T lymphocyte response, CTL epitope, human papilloma virus, HPV, tarosinase related protein 2, TRP-2, P53 epitope, tumour associated protein, vaccine, cancer, antigen, liposome, phospholipid, mineral oil, ISA51, T helper epitope, carrier, adjuvant, hydrophobic, continuous phase, IFN-γ, CpG ODN 1826, Pam3Cys-SKKKK, Pam3Cys-CSK4, topical, imiquimod, 1-isobutyl-1H-imidazo [4,5-c]quinolin-4-amine, 1H-imidazo [4, 5-c] quinolin-4-amine, 1-(2-methylpropyl), aldara, R 837, S 26308; GENOMEQUEST, GenBank; Sequences: SEQ ID NOs 1-5 and 10-14 .

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant	Relevant to claim No.
X	MUDERHWA, J.M. et al. Oil-in-water liposomal emulsions: characterization and potential use in vaccine delivery. J. Pharm. Sci. December 1999, Vol.88, No.12, pages 1332-1339, ISSN 0022-3549. See the abstract; and the Materials and Methods.	1, 5, 6, 16, 28, 29, 33-37, 39-44(first), 44(second), 45(first), 45(second) and 46
Y		11, 25, 30-32 and 38
X	RICHARDS, R.L. et al. Liposome-stabilized oil-in-water emulsions as adjuvants: increased emulsion stability promotes induction of cytotoxic T lymphocytes against an HIV envelope antigen. Immunol. Cell Biol. October 2004, Vol.82, No.5, pages 531-538, ISSN 0818-9641. See the abstract; pages 531 and 532, bridging	1-7, 23, 26, 28, 29, 42-44(first), 44(second), 45(first), 45(second) and 46
Y	paragraph; and the Materials and Methods.	11, 25 and 30-32

[X] Further documents are listed in the continuation of Box

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 January 2007 (09-01-2007)	29 January 2007 (29-01-2007)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Qianfa Chen 819- 994-1374

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant	Relevant to claim No.
X	WO 2004/052917 A2 (KEOGH, E.A. et al.) 24 June 2004. See page 6, paragraphs 0017-0019; page 12, paragraph 0041; page 34, paragraphs 0095-0097; page 35 paragraph 0103; page 57, paragraph 0149, page 64, paragraphs 0171 and 0172, and page 69, paragraph 0184.	1-9, 12, 15, 16, 23, 24, 26-29, 33-37, 39-44(first), 44(second), 45(first), 45(second) and 46
Y		11, 25, 30-32 and 38
Y	WO 92/10513 A1 (TINDLE, R. et al.) 25 June 1992. See the abstract; and page 22, lines 2-10.	11
Y	WO 2005/089164 A2 (CHESNUT, R. et al.) 29 September 2005. See the abstract; page 11, line 1 to page 45, line 5; and Table 36, 37, and 48.	11
Y	US 6,183,746 B1 (URBAN, R.G. et al.) 6 February, 2001 See the abstract; column 1, line 48 to column 3, line 45; and Table I.	11
Y	WO 95/31480 A1 (HOUSTON, M.E. et al.) 23 November 1995. See the abstract; page 33, lines 9-29; claim 7; and SEQ ID NO: 14.	25
Y	CHEN, Y.F. et al. Cytotoxic-T-lymphocyte human papillomavirus type 16 E5 peptide with CpG-oligodeoxynucleotide can eliminate tumour growth in C57BL/6 mice. J. Virol. February 2004, Vol.78, No.3, pages 1333-1343, ISSN 0022-538X. See the abstract; and Materials and Methods.	30
Y	TAKEUCHI, O. et al. Cutting edge: role of toll-like receptor 1 in mediating immune response to microbial lipoproteins. The Journal of Immunology, 2002, Vol.169, pages 10-14, ISSN 0022-1767. See the entire document.	31 and 32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001640

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2006/001640

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

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

1. [X] Claim Nos. : 36-38, 40 and 41
because they relate to subject matter not required to be searched by this Authority, namely :

Claims 36-38, 40 and 41 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search under Rule 39.1 (iv) of the PCT. Regardless, this Authority has carried out a search based on the alleged effect(s) or purpose(s)/use(s) of the product defined in claims 36-38, 40 and 41.

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

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

This International Search Authority found multiple inventions (groups) in this international application
See the extra sheet.

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Claims 1-6(partial, or P), 7-14, 15(P), 16 (P) and 23-46(P)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[X] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant	Relevant to claim No.
Y	WO 2005/019435 A2 (MOLLDREM, J. and BARRETT, J.A.) 3 March 2005. See the abstract; page 4, line 31 to page 7, line 18; and claim 5.	38
A	HARADA, M. et al. Vaccination of cytotoxic T lymphocyte-directed peptides elicited and spread humoral and Th1-type immune responses to prostate-specific antigen protein in a prostate cancer patient. J. Immunother. July-August 2005, Vol.28, No.4, pages 368-375, ISSN 1524-9557. See the abstract.	1-44(first), 44(second), 45(first), 45(second) and 46
A	CHIKH, G. and SHUTZE-REDELMEIER, M.P. Liposomal delivery of CTL epitopes to dendritic cells. Biosci Rep. April 2002, Vol.22, No.2, page 339-353, ISSN 0144-8463. See the entire document.	1-44(first), 44(second), 45(first), 45(second) and 46

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2004052917 A2	24-06-2004	AU2003296330 A1	30-06-2004
		CA2511775 A1	24-06-2004
		EP1583548 A2	12-10-2005
		US2006094649 A1	04-05-2006
		WO2004052917 A3	23-06-2005
		WO2004052917 A9	30-09-2004
WO9210513 A1	25-06-1992	AT189818T T	15-03-2000
		AU660954B B2	13-07-1995
		AU9070991 A	08-07-1992
		CA2097916 A1	13-06-1992
		DE69131992D D1	23-03-2000
		DE69131992T T2	29-06-2000
		EP0561885 A1	29-09-1993
		JP6503559T T	21-04-1994
		KR100252371B B1	01-05-2000
		US6183745 B1	06-02-2001
WO2005089164 A2	29-09-2005	AU2005222776 A1	29-09-2005
		CA2552508 A1	29-09-2005
		EP1732598 A2	20-12-2006
		WO2005089164 A3	01-12-2005
US6183746 B1	06-02-2001	US6582704 B2	24-06-2003
		US2001006639 A1	09-07-2001
		US7097843 B2	29-08-2006
		US2004229809 A1	18-11-2004
WO9531480 A1	23-11-1995	AT196297T T	15-09-2000
		AU708472B B2	05-08-1999
		AU719469B B2	11-05-2000
		AU2441895 A	05-12-1995
		AU2805995 A	05-01-1996
		AU4753996 A	31-07-1996
		AU6118196 A	15-01-1997
		CA2190494 A1	23-11-1995
		CA2224086 A1	03-01-1997
		CA2361012 A1	03-01-1997
		CA2372569 A1	23-11-1995
		DE69518838D D1	19-10-2000
		DE69518838T T2	03-05-2001
		EP0759941 A1	05-03-1997
		EP0802991 A1	29-10-1997
		EP0833836 A1	08-04-1998
		JP10504018T T	14-04-1998
		JP10512228T T	24-11-1998
		JP11507648T T	06-07-1999
		JP2006257095 A	28-09-2006
		US5549748 A	27-08-1996
		US5738996 A	14-04-1998
		US5824483 A	20-10-1998
		WO9534575 A1	21-12-1995
		WO9621756 A1	18-07-1996
		WO9700267 A1	03-01-1997

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2005019435 A2	03-03-2005	AU2004267506 A1	03-03-2005
		CA2536654 A1	03-03-2005
		EP1660636 A2	31-05-2006
		EP1670899 A2	21-06-2006
		WO2005035714 A2	21-04-2005
		WO2005019435 A3	29-06-2006
		WO2005019435 A8	27-04-2006

This international Search Authority found multiple inventions (groups) in this international application as follows:

Group 1: Claims 1-6 (partial, or P), 7-14, 15 (P), 16 (P) and 23-46 (P)

Group 1 is directed to a composition or kit comprising a cytotoxic T-lymphocyte (CTL) epitope derived from human papilloma virus, and the use thereof.

Group 2: Claims 1-6 (P), 15 (P), 16 (P), 17, 19 (P), 20, and 23-46 (P)

Group 2 is directed to a composition or kit comprising a CTL epitope derived from P53, and the use thereof.

Group 3: Claims 1-6 (P), 15 (P), 16 (P), 18, 19 (P), 21, 22, and 23-46 (P)

Group 3 is directed to a composition or kit comprising a CTL epitope derived fromTRP2, and the use thereof.

In the response dated December 22, 2006 to an invitation under Section 36 to pay additional fees dated November 23, 2006 in view of D3 (KEOGH, E.A. et al.), applicant argued that "The pending claims require the combination of a carrier comprising a continuous phase of a hydrophobic substance; liposomes; and at least one antigen capable of inducing a cytotoxic T lymphocyte (CTL) response". Applicant further argued that "The cited reference KEOGH et al. (WO 2004/052917) does not in any way disclose this specific combination". Applicant's comments have been carefully considered but have not been persuasive. Having regard to applicants' arguments, the unity objection is maintained in view of newly cited document D1 (MUDERHWA, J.M. et al. J. Pharm. Sci. December 1999, Vol.88, No.12, pages 1332-1339) or D2 (RICHARDS, R.L. et al. Cell Biol. October 2004, Vol.82, No.5, pages 531-538).

D1 describes a vaccine composition comprising an oil-in-water liposomal emulsion (e.g., a carrier comprising a continuous phase of a hydrophobic substance, and liposomes), and encapsulated prostate-specific antigen (PSA). The PSA antigen is inherently capable of inducing a CTL response in view of D4 (HARADA, M. et al. J. Immunother. July-August 2005, Vol.28, No.4, pages 368-375) which describes that a PSA-derived peptide, was able to elicit and spread humoral and Th1-type immune response to the PSA protein in a prostate cancer patient. D2 describes a vaccine composition comprising liposome-stabilized oil-in-water emulsions (e.g., a carrier comprising a continuous phase of a hydrophobic substance, and liposomes), and encapsulated oligomeric gp140 (ogp 140) derived from the HIV-1 envelope protein as the antigen. The ogp140 antigen is capable of inducing a cytotoxic T lymphocyte (CTL) response. Therefore, D1 or D2 separately discloses the specific combination as argued in applicant's response of December 22, 2006. As such, the requirements of the unity of invention are not fulfilled, in view of D1 and D2, in that there is no technical relationship among the three groups of inventions as they do not involve one or more of the same or corresponding special technical features. The expression "special technical features" means those features which define a contribution which each of the claimed inventions considered as a whole makes over the prior art.