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(54) Title: ADENOVIRAL VECTORS COMPRISING A NUCLEIC ACID ENCODING A MHC CLASS I MOLECULE AND THE USE OF SUCH VECTORS IN CANCER THERAPY.

(57) Abstract: The present invention provides compositions and methods for facilitating expression of genetic material encoding an immunogenic molecule on the surface of a target cell. In particular, the present invention relates to a method for facilitating expression of genetic material encoding a major histocompatibility (MHC) protein on the surface of cancer cells in order to induce an immune response against such cells. Even more particularly, the subject invention provides viral vectors encoding MHC molecules foreign to a host for use in facilitating expression of such molecules on the surface of cancer cells in order to induce an immune response against the cells.

Adenoviral vectors comprising a nucleic acid encoding a MHC Class I molecule and the use of such vectors in cancer therapy

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention provides compositions and methods for facilitating expression of genetic material encoding an immunogenic molecule on the surface of a target cell. In particular, the present invention relates to a method for facilitating expression of genetic material encoding a major histocompatibility (MHC) protein on the surface of cancer cells in order to induce an immune response against such cells. Even more particularly, the subject invention provides viral vectors encoding MHC molecules foreign to a host for use in facilitating expression of such molecules on the surface of cancer cells in order to induce an immune response against the cells.

DESCRIPTION OF THE PRIOR ART

Bibliographic details of references in the subject specification are also listed at the end of the specification.

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in any country.

The primary function of the MHC molecules is to bind and "present" antigenic peptides on the surfaces of cells for recognition (binding) by the antigen-specific T cell receptors (TCRs) of lymphocytes. Differential structural properties of MHC Class I and Class II molecules account for their respective roles in activating different populations of T lymphocytes. MHC Class I molecules are expressed on all nucleated somatic cells except neurons, consistent with the protective function of the cytotoxic lymphocytes (CTLs) which continuously survey cell surfaces and kill cells harboring foreign peptides. MHC

Class I molecules bind peptide fragments that have been derived from proteolytically degraded proteins endogenously synthesized in the cell (small peptides are transported to the endoplasmic reticulum where they associate with nascent MHC I molecules before being routed through the Golgi apparatus and displayed on the surface for recognition by T cells). MHC Class II molecules are restricted to antigen presenting cells (APC) consistent with the functions of helper (Th) lymphocytes which are found locally wherever these cells encounter macrophages, dendritic cells or B cells that have internalized and processed antigens produced by pathogenic mechanisms. Thus, while MHC Class I molecules provide identity of self information to the immune system, MHC Class II molecules provide defence against foreign microbiologicals.

The two major types of MHC protein molecules- Class I and Class II- that span the membrane of almost every cell in an organism are encoded by several genes, all clustered in the same region on chromosome 6. Each gene has an unusual number of alleles (alternate forms of a gene). As a result, it is very rare for two individuals to have the same set of MHC molecules, which are collectively called a tissue type.

The MHC Class I membrane spanning molecule is composed of two proteins: the spanning protein which is approximately 350 amino acids in length, with approximately 70 amino acids at the carboxylic end which comprises the transmembrane and cytoplasmic portion, with the remaining 270 amino acids divided into 3 globular domains, alpha-1, alpha-2 and alpha-3 (closest to membrane). The second portion of the molecule is a globular protein, beta-2 microglobulin which associates with the alpha-3 prome domain and is necessary for MHC stability.

In humans, the MHC I and II genes are located at separate but nearby loci on the 6^{th} chromosome. The Class I locus contains three smaller loci of genes for 3 Class 1 genes, A, B and C. Every human processes at least one version of the A, B and C Class I molecules. Since each individual inherits one strand of DNA from each parent most people have 2 distinct variants of each of A, B and C for a total of 6 distinct MHC I genes. The Class I gene codes only for the alpha (α) component of the protein of the Class I molecule. The

beta-2 component of the Class I molecule is located elsewhere in the genome and is non-variable. The complex is also called the human leukocyte antigen (HLA) system. The prevalence of different HLA types vary widely in different populations.

MHC molecules are important components of the immune response. They allow cells that have been invaded by an infectious organism to be detected by cells of the immune system called T lymphocytes, or T cells. The MHC molecules do this by presenting fragments of proteins (peptides) belonging to the invader on the cell's surface. The T cell recognizes the foreign peptide attached to the MHC molecule and binds to it, stimulating the T cell to either destroy or cure the infected cell. In uninfected healthy cells the MHC molecule presents peptides from its own cell (self peptides), to which T cells do not normally react. However, if the immune mechanism malfunctions and T cells react against self peptides, an autoimmune disease arises.

MHC molecules were initially defined as antigens that stimulate an organism's immunologic response to transplanted organs and tissues. In the 1940s, skin graft experiments showed that graft rejection was an immune reaction mounted by the host organism against foreign tissue. This response was elicited because the host recognized the MHC molecules on cells of the graft tissue as foreign antigens and attacked them.

As indicated above, one of the critical functions of MHC Class I antigens, present on the surface of nearly every cell in the body, is to constantly present for inspection by the immune surveillance system, peptides that are representative of the molecular activity in the cell. The evolution of such a system has enabled the host to recognise and attack cells that display unfamiliar peptides through either viral infection or aberrant behaviour such as that of tumorigenesis. When a tumor escapes from the immune system, it generally involves the loss, downregulation or alteration of MHC Class I profiles that enable a tumorigenic cell to avoid cytotoxic T cell (CTL) lysis even if immunogenic peptides are being present on the cell surface.

Advances in adenovirus (Ad) vector biology have allowed bypassing of the native coxsackie and adenovirus receptor (CAR) to allow CAR independent tropism such that an Ad vector can be targeted *via* the use of adapter molecules to virtually any receptor type on the cell surface.

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In accordance with the present invention, MHC Class I- and Ad-based technologies are proposed to be combined to engineer target cells, such as cancer cells, which express an MHC Class I molecule on a cancer cell which is foreign to the host. This in turn facilitates the induction of an immune response against the target cell.

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SUMMARY OF THE INVENTION

Throughout the specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not to the exclusion of any other element or integer or group of elements or integers.

The present invention provides an Ad-delivered construct containing a gene encoding a foreign MHC Class I molecule whose expression is under the control of a tumor specific promoter. Administration of the construct to a subject results in infection of tumorigenic and non-tumorigenic cells. However, transcription of the foreign MHC Class I molecule only occurs when the cell is expressing tumor related proteins. Cancer cells expressing foreign MHC Class I molecules on their surface become a target of the immune surveillance system including CTLs.

As the specificity of foreign MHC Class I is pivotal to the therapeutic efficacy as well as avoidance of foreign MHC Class I on non-tumorigenic cells tissue specific promoters form part of the construct. Further specificity is provided by using conditionally replicating or adapter-passed targeting Ad vectors. Temporal activation control is provided by an inducible promoter.

Accordingly, the present invention provides an Ad-derived vector or a functional equivalent thereof comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression, the MHC Class I molecule is present on the surface of the cancer cell wherein the MHC Class I molecule is foreign relative to a subject carrying said cancer cell.

The present invention further contemplates a method of cancer therapy in a subject said method comprising administering to said subject an Ad-derived vector or a functional equivalent thereof comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression, the MHC Class I

molecule is present on the surface of the cancer cell wherein the MHC Class I molecule is foreign relative to a subject carrying said cancer cell thereby permitting an immune response to be generated against said cancer cell.

The present invention also provides a therapeutic composition comprising an Ad-derived vector or a functional equivalent thereof comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression the MHC Class I molecule is present on the surface of the cancer cell wherein the MHC Class I molecule is foreign relative to a subject carrying said cancer cell, said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

The present invention provides viral vectors and in particular Ad-derived vectors or their functional equivalents which are capable of infecting cells and facilitating expression of nucleic acid molecules carried by the vector while not inducing substantial cytopathic effects on the infected cell. The vectors carry a nucleic acid molecule comprising a sequence of nucleotides encoding an MHC Class I molecule capable of inducing an immune response in a particular subject. The nucleic acid molecule is operably linked to a cancer-specific promoter or a promoter encoding a protein which is specific for the cancer cell.

Accordingly, one aspect of the present invention provides an Ad-derived vector or a functional equivalent thereof comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression the MHC Class I molecule is present on the surface of the cancer cell wherein the MHC Class I molecule is foreign relative to a subject carrying said cancer cell.

If additional specificity is required, the cancer-specific promoter may also be developmentally regulated or tissue-specific. In addition, conditionally replicating or adapter-based targeting Ad vectors may be employed.

The terms "cancer" and "tumor" may be used interchangeably throughout the subject specification.

Examples of cancers contemplated herein include, but are not limited to, ABL1 protooncogene, AIDS related cancers, acoustic neuroma, acute lymphocytic leukaemia, acute myeloid leukaemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous t-cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, haematological malignancies, hairy cell leukaemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, intraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, Langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukaemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(nsclc), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer,

osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (sclc), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia or Wilms' tumor.

DETAILED DESCRIPTION OF THE INVENTION

Prior to describing the present invention in detail, it is to be understood that unless otherwise indicated, the subject invention is not limited to specific formulation components, manufacturing methods, dosage regimens, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to a "vector" includes a single vector, as well as two or more vector; reference to a "cell" includes a single cell, as well as two or more cells; and so forth.

The vector, and in particular the Ad vector, comprise in a preferred embodiment, the MHC coding sequences, a cancer-specific promoter region operating in kind to the MHC coding sequence and a 3' regulatory region.

In a preferred aspect, reference to "MHC", includes reference to the isoforms HLA-A, HLA-B, HLA-C, HLA-DQ, HLA-DP, HLA-DM, HLA-DR or subtypes thereof.

The MHC Class I molecule is generally a polypeptide. As used herein, the term "polypeptide" is used in its conventional meaning, i.e., as a sequence of amino acids. The recombinant MHC Class I polypeptides of the present invention, therefore, should be understood to also encompass peptides, oligopeptides and proteins. The protein may be glycosylated or unglycosylated. Reference hereinafter to a "protein" includes a protein comprising a sequence of amino acids as well as a protein associated with other molecules such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins.

The MHC Class I molecules of the invention are immunogenic, i.e., they are able to stimulate T cells and/or B-cells from a subject to which the MHC molecule is foreign. Screening for immunogenic activity can be performed using techniques well known to the skilled artisan. For example, such screens can be performed using methods such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA, 1988. In one illustrative example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, I^{125} labeled Protein A.

The present invention extends to an "immunogenic portion," or "epitope" of the MHC Class I molecule which is a fragment of the MHC molecule of the subject invention that itself is immunologically reactive (i.e., specifically binds) with the B-cells and/or T cell surface antigen receptors that recognize the polypeptide. Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared using well-known techniques.

In order to express a recombinant MHC Class I molecule or immunogenic portion thereof, the nucleotide sequences encoding the MHC molecule is inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Preferably, the expression vector is an Ad-derived vector. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include

in vitro recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook, J. *et al.* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. *et al.* (1989) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York. N.Y.

The "control elements" may also be referred to as "regulatory sequences". These sequences present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. In mammals, cancer-specific promoters are generally preferred.

Examples of cancer-specific promoters include those associated with genes encoding carcinoembryonic antigen, alpha-fetoprotein, cyclooxygenase-2, leukocyte plastin, leukoprotease inhibitor, mucin-like glycoprotein and melanoma antigen Family A2

In mammalian host cells, a number of viral-based expression systems are generally available. Where an Ad-virus is used as an expression vector, sequences encoding the MHC molecule of interest is ligated into an Ad transcription/translation complex consisting of the cancer-specific promoter. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan and Shenk. *Proc. Natl. Acad. Sci.* 81:3655-3659, 1984). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding the MHC molecule of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate

expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, *et al. Results Probl. Cell Differ.* 20:125-162, 1994).

Candidate compounds of the present invention are also referred to herein as "lead" compounds. In the present invention, the nucleic acid molecules in the Ad-vectors encodes an immunogenic MHC Class I cell surface molecule. As indicated above, an immunogenic cell surface molecule can be an MHC molecule is preferably an MHC Class I molecule. In a preferred aspect, the molecules are HLA-A, HLA-B, HLA-C, HLA-DQ, HLA-DR, HLA-DP and/or HLA-DM.

The nucleic acid molecule generally comprises a translation initiation codon such as 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine. It is also known in the art that eukaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the subject invention, "start codon" and "translation initiation codon" refer to the codon or codons that are used *in vivo* to initiate translation of an mRNA transcribed from a gene encoding an MHC Class I cell surface molecule, regardless of the sequence(s) of such codons. A translation termination codon (or "stop codon") of the MHC Class I coding

sequence may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively).

The terms "start codon region" and "translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

The open reading frame (ORF) or "coding region", is used to define the art to refer to the region between the translation initiation codon and the translation termination codon of the MHC Class I-coding sequence.

The MHC Class I-coding sequence may also comprise a 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene), and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene).

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns", which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. the present invention extends to both genomic MHC Class I-coding sequences as well as cDNA encoding the spliced mRNA sequence.

It is also known in the art that alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as "variants". More specifically, "pre-mRNA variants" are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequence.

Upon excision of one or more exon or intron regions, or portions thereof during splicing, pre-mRNA variants produce smaller "mRNA variants". Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as "alternative splice variants". If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

It is also known in the art that variants can be produced through the use of alternative signals to start or stop transcription and that pre-mRNAs and mRNAs can possess more that one start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as "alternative start variants" of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as "alternative stop variants" of that pre-mRNA or mRNA. One specific type of alternative stop variant is the "polyA variant" in which the multiple transcripts produced result from the alternative selection of one of the "polyA stop signals" by the transcription machinery, thereby producing transcripts that terminate at unique polyA sites. Within the context of the invention, the types of variants described herein are also preferred target nucleic acids.

T cells are considered to be specific for the MHC molecule of the present invention if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the molecule or expressing a gene encoding the molecule. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen *et al.*, *Cancer Res.* 54:1065-1070, 1994.

Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (e.g., by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a tumor polypeptide (100 ng/ml -100 μg/ml, preferably 200 ng/ml - 25 μg/ml) for 3-7 days will typically result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (e.g., TNF or IFN-.gamma.) is indicative of T cell activation (see Coligan *et al., Current Protocols in Immunology*, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to a foreign MHC molecule may be $CD4^+$ and/or $DC8^+$. Foreign MHC specific T cells may be expanded using standard techniques.

For therapeutic purposes, $CD4^+$ or $CD8^+$ T cells that proliferate in response to a specific foreign MHC molecule can be expanded in number either in vitro or *in vivo*. Proliferation of such T cells in vitro may be accomplished in a variety of ways. For example, the T cells can be re-exposed to the polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a tumor polypeptide. Alternatively, one or more T cells that proliferate in the presence of the MHC molecule can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

The present invention contemplates the use of therapeutic agents to treat subjects undergoing carrying cancer cells. Subjects treated using the compositions and compounds of the present invention include any animal who may benefit from such treatment. These include, without limitation, humans, marmosets, orangutans and gorillas, livestock animals (e.g. cows, sheep, pigs, horses, donkeys), laboratory test animals (e.g. mice, rats, guinea pigs, hamsters, rabbits), companion animals (e.g. cats, dogs) and captured wild animals

(e.g. rodents, foxes, deer, kangaroos. A particularly preferred host is a human, primate or livestock animal. A most preferred host is a human.

As one non-limiting example, expression patterns within cancer cells or tissues treated with one or more of the Ad vector-derived compounds are compared to control cell or tissues not treated or treated with an Ad vector not encoding an MHC Class I molecule and the cells tested for immunogenicity.

Initially, the cells need to be tested for MHC Class I gene expression. Examples of methods of gene expression analysis include DNA arrays or microarrays (Brazma and Vilo, *FEBS Lett. 480:* 17-24, 2000; Celis *et al.*, *FEBS Lett. 480:* 2-16, 2000), SAGE (serial analysis of gene expression)(Madden *et al.*, *Drug Discov. Today 5:* 415-425, 2000), READS (restriction enzyme amplification of digested cDNAs) (Prashar and Weissman, *Methods Enzymol.303:* 258-272, 1999), TOGA (total gene expression analysis) (Sutcliffe *et al.*, *Proc. Natl. Acad. Sci. USA 97:* 1976-1981, 2000), protein arrays and proteomics (Celis *et al.* 2000, *supra*; Jungblut *et al.*, *Electrophoresis 20:* 2100-2110, 1999), expressed sequence tag (EST) sequencing (Celis *et al.*, 2000, *supra*; Larsson *et al.*, *J. Biotechnol.80:* 143-157, 2000), subtractive RNA fingerprinting (SuRF) (Fuchs *et al.*, *Anal. Biochem. 286:* 91-98, 2000; Larson *et al.*, *Cytometry 41:* 203-208, 2000), subtractive cloning, differential display (DD) (Jurecic and Belmont, *Curr. Opin. Microbiol. 3:* 316-321, 2000), comparative genomic hybridization (Carulli *et al.*, *J. Cell Biochem. Suppl.31:* 286-296, 1998), FISH (fluorescent *in situ* hybridization) techniques (Going and Gusterson, *Eur. J. Cancer, 35:* 1895-1904, 1999) and mass spectrometry methods (To, *Comb. Chem. High Throughput Screen, 3:* 235-241, 2000).

For therapeutics, an animal, preferably a human, requires induction of an immune response specifically against cancer cells. As such, the compounds and compositions of the present invention can be used to facilitate expression of MHC Class I molecules on the surface of cancer cells wherein the MHC molecule is foreign to the host carrying the cancer cells. In one embodiment, the expression of the immunogenic cell surface molecule gene is treated by administering the Ad-derived vectors in accordance with this invention, generally *in*

vivo. For example, in one non-limiting embodiment, the methods comprise the step of administering to the animal in need of treatment, a therapeutically effective amount of an Ad-derived vector or a functional equivalent thereof comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression the MHC Class I molecule is present on the surface of the cancer cell wherein the MHC Class I molecule is foreign relative to a subject carrying said cancer cell.

Accordingly, the present invention provides a method of cancer therapy said method comprising administering to a subject an Ad-derived vector or a functional equivalent thereof comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression the MHC Class I molecule is present on the surface of the cancer cell wherein the MHC Class I molecule is foreign relative to a subject carrying said cancer cell thereby permitting an immune response to be generated against said cancer..

Another aspect of the present invention contemplates a therapeutic composition comprising an Ad-derived vector or a functional equivalent thereof comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression the MHC Class I molecule is present on the surface of the cancer cell wherein the MHC Class I molecule is foreign relative to a subject carrying said cancer cell, said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of a compound to a suitable pharmaceutically acceptable diluent, carrier or other delivery mechanism including means of introduction of the Ad-derived vector which allows the insertion of exogenous nucleic acid into a cell. Use of the compounds and methods of the invention may also be useful prophylactically. The compounds or compositions of the present invention may be administered in conjunction with other cancer therapies. These therapies, in conjunction with the compositions and compounds of the present invention, can be administered in lower doses and for longer

periods of time, thereby decreasing or preventing the usual side effects associated with cancer therapies.

The present invention includes, therefore, pharmaceutical compositions and formulations which include Ad-derived vectors encoding MHC Class I molecule for use in *in vitro* culture or *in vivo*. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oral doses may also be possible if the Ad vector is suitably protected. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be

formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Patent Number: 6,287,860, which is incorporated herein in its entirety.

Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such

specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Patent Number: 6,287,860, which is incorporated herein in its entirety.

The pharmaceutical formulations and compositions of the present invention may also include surfactants. The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are further described in U.S. Patent Number: 6,287,860.

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability cells to viruses. Penetration enhancers may be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. Penetration enhancers and their uses are further described in U.S. Patent Number: 6,287,860.

One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Certain embodiments of the invention provide pharmaceutical compositions containing one or more Ad-derived vectors and one or more other chemotherapeutic agents. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin,

esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES).

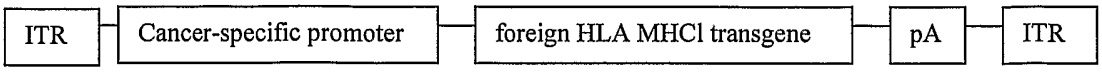
The formulation of therapeutic compositions and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 ug to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the Ad-derived vectors in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years or greater. Examples of effective amounts include 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44,

45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 and 100g/kg body weight.

The present invention is further described by the following non-limiting example.

EXAMPLE 1

Generalized Ad construct



The cancer-specific promoter may be selected based upon the specific application, i.e. host, cell type, etc. Other necessary viral genes may be provided in trans and carried by a helper virus.

EXAMPLE 2

Treatment for ovarian cancer

A serotype 3 conditionally replicating Ad is selectively uptaken by ovarian and other cancer cells and is used as the vector to deliver a construct encoding foreign MHC Class I molecule under the control of the leukocyte plasmin promoter (LP-P), also overexpressed in ovarian cancer.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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CLAIMS:

1. An adenovirus (Ad) vector comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression of the MHC Class I molecule is foreign relative to a subject carrying said cancer cell.

2. The Ad vector of Claim 1 wherein the MHC comprises isoforms selected from HLA-A, HLA-B, HLA-C, HLA-DQ, HLA-DP, HLA-DM and HLA-DR.

3. The Ad vector of Claim 1 or 2 wherein the MHC Class I molecule is a polypeptide or a fragment thereof comprising an immunogenic epitope.

4. The Ad vector of Claim 1 wherein the cancer-specific promoter is selected from promoters associated with genes encoding carcinoembryonic antigen, alpha-fetoprotein, cyclooxygenase-2, leukocyte plastin, leukoprotease inhibitor, mucin-like glycoprotein or melanoma antigen Family A2.

5. The Ad vector of Claim 1 wherein the vector is capable of expression in a mammalian cell.

6. The Ad vector of Claim 5 wherein the mammalian cell is a human cell.

7. The Ad vector of Claim 1 wherein the cancer is selected from ABL1 protooncogene, AIDS related cancers, acoustic neuroma, acute lymphocytic leukaemia, acute myeloid leukaemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal

cancers, cutaneous t-cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, haematological malignancies, hairy cell leukaemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, intraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, Langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukaemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(nsclc), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (sclc), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia or Wilms' tumor.

8. A method of cancer therapy in a subject, said method comprising administering to said subject an Ad vector comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer specific promoter such that upon expression, the MHC Class I molecule is present of the surface of the cancer cell wherein the MHC Class I molecule is foreign relative to a subject carrying said cancer cell thereby permitting an immune response to be generated against said cancer cell.

9. The method of Claim 8 wherein the MHC comprises isoforms selected from HLA-A, HLA-B, HLA-C, HLA-DQ, HLA-DP, HLA-DM and HLA-DR.

10. The method of Claim 8 or 9 wherein the MHC Class I molecule is a polypeptide or a fragment thereof comprising an immunogenic epitope.

11. The method of Claim 8 wherein the cancer-specific promoter is selected from promoters associated with genes encoding carcinoembryonic antigen, alpha-fetoprotein, cyclooxygenase-2, leukocyte plastin, leukoprotease inhibitor, mucin-like glycoprotein or melanoma antigen Family A2.

12. The method of Claim 8 wherein the subject is a human.

13. The method of Claim 8 wherein the claim is selected from ABL1 protooncogene, AIDS related cancers, acoustic neuroma, acute lymphocytic leukaemia, acute myeloid leukaemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous t-cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal

cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, haematological malignancies, hairy cell leukaemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, intraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, Langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukaemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(nsclc), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (sclc), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia or Wilms' tumor.

14. Use of an Ad vector comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression of the MHC Class I molecule is foreign relative to a subject carrying said cancer cell.

15. Use of Claim 14 wherein the MHC comprises isoforms selected from HLA-A, HLA-B, HLA-C, HLA-DQ, HLA-DP, HLA-DM and HLA-DR.

16. Use of Claim 14 or 15 wherein the MHC Class I molecule is a polypeptide or a fragment thereof comprising an immunogenic epitope.

17. Use of Claim 14 wherein the cancer-specific promoter is selected from promoters associated with genes encoding carcinoembryonic antigen, alpha-fetoprotein, cyclooxygenase-2, leukocyte plastin, leukoprotease inhibitor, mucin-like glycoprotein or melanoma antigen Family A2.

18. Use of Claim 14 wherein the subject is a human.

19. Use of Claim 14 in the treatment of a cancer selected from ABL1 protooncogene, AIDS related cancers, acoustic neuroma, acute lymphocytic leukaemia, acute myeloid leukaemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous t-cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, haematological malignancies, hairy cell leukaemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human

papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, intraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, Langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukaemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders, nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(nsclc), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (sclc), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia or Wilms' tumor.

20. A therapeutic composition comprising an Ad-derived vector or a functional equivalent thereof comprising a nucleic acid molecule encoding an MHC Class I molecule operably linked to a cancer-specific promoter such that upon expression the MHC Class I molecule is present on the surface of the cancer cell wherein the MHC Class I molecule is foreign relative to a subject carrying said cancer cell, said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

21. The composition of Claim 20 wherein the MHC Class I molecule is a polypeptide or a fragment thereof comprising an immunogenic epitope.

22. The composition of Claim 20 or 21 wherein the cancer-specific promoter is is selected from promoters associated with genes encoding carcinoembryonic antigen, alpha-fetoprotein, cyclooxygenase-2, leukocyte plastin, leukoprotease inhibitor, mucin-like glycoprotein or melanoma antigen Family A2.

23. The composition of Claim 20 when used in cancer therapy against ABL1 protooncogene, AIDS related cancers, acoustic neuroma, acute lymphocytic leukaemia, acute myeloid leukaemia, adenocystic carcinoma, adrenocortical cancer, agnogenic myeloid metaplasia, alopecia, alveolar soft-part sarcoma, anal cancer, angiosarcoma, aplastic anaemia, astrocytoma, ataxia-telangiectasia, basal cell carcinoma (skin), bladder cancer, bone cancers, bowel cancer, brain stem glioma, brain and CNS tumors, breast cancer, CNS tumors, carcinoid tumors, cervical cancer, childhood brain tumors, childhood cancer, childhood leukaemia, childhood soft tissue sarcoma, chondrosarcoma, choriocarcinoma, chronic lymphocytic leukaemia, chronic myeloid leukaemia, colorectal cancers, cutaneous t-cell lymphoma, dermatofibrosarcoma-protuberans, desmoplastic-small-round-cell-tumor, ductal carcinoma, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extra-hepatic bile duct cancer, eye cancer, eye: melanoma, retinoblastoma, fallopian tube cancer, fanconi anaemia, fibrosarcoma, gall bladder cancer, gastric cancer, gastrointestinal cancers, gastrointestinal-carcinoid-tumor, genitourinary cancers, germ cell tumors, gestational-trophoblastic-disease, glioma, gynaecological cancers, haematological malignancies, hairy cell leukaemia, head and neck cancer, hepatocellular cancer, hereditary breast cancer, histiocytosis, Hodgkin's disease, human papillomavirus, hydatidiform mole, hypercalcemia, hypopharynx cancer, intraocular melanoma, islet cell cancer, Kaposi's sarcoma, kidney cancer, Langerhan's-cell-histiocytosis, laryngeal cancer, leiomyosarcoma, leukaemia, li-fraumeni syndrome, lip cancer, liposarcoma, liver cancer, lung cancer, lymphedema, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, male breast cancer, malignant-rhabdoid-tumor-of-kidney, medulloblastoma, melanoma, Merkel cell cancer, mesothelioma, metastatic cancer, mouth cancer, multiple endocrine neoplasia, mycosis fungoides, myelodysplastic syndromes, myeloma, myeloproliferative disorders,

nasal cancer, nasopharyngeal cancer, nephroblastoma, neuroblastoma, neurofibromatosis, nijmegen breakage syndrome, non-melanoma skin cancer, non-small-cell-lung-cancer-(nsclc), ocular cancers, oesophageal cancer, oral cavity cancer, oropharynx cancer, osteosarcoma, ostomy ovarian cancer, pancreas cancer, paranasal cancer, parathyroid cancer, parotid gland cancer, penile cancer, peripheral-neuroectodermal-tumors, pituitary cancer, polycythemia vera, prostate cancer, rare-cancers-and-associated-disorders, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, Rothmund-Thomson syndrome, salivary gland cancer, sarcoma, schwannoma, Sezary syndrome, skin cancer, small cell lung cancer (sclc), small intestine cancer, soft tissue sarcoma, spinal cord tumors, squamous-cell-carcinoma-(skin), stomach cancer, synovial sarcoma, testicular cancer, thymus cancer, thyroid cancer, transitional-cell-cancer-(bladder), transitional-cell-cancer-(renal-pelvis-/-ureter), trophoblastic cancer, urethral cancer, urinary system cancer, uroplakins, uterine sarcoma, uterus cancer, vaginal cancer, vulva cancer, Waldenstrom's-macroglobulinemia or Wilms' tumor.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/000145

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C12N 15/861 (2006.01) ***A61K 48/00*** (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)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS, Medline, Caplus, Biotechabs: tumor specific, tumour specific, cancer specific, promoter, MHC, HLA, major histocompatibility, human leukocyte antigen, carcinoembryonic antigen, alpha-fetoprotein, cyclooxygenase, leukocyte plastin, leukoprotease inhibitor, mucin like glycoprotein, melanoma antigen, adenovir?, adv

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 1991/02805 A2 (VIAGENE INC) 7 March 1991 See in particular page 44, line 15 to page 46 line 30	1-23
A	WO 2003/051402 A1 (UAB RESEARCH FOUNDATION) 26 June 2003 See the whole document	1-23

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

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"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
22 March 2006	29 MAR 2006
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**LEXIE PRESS** Telephone No : (02) 6283 2677

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/000145

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROTS MG et al (2003) 'Targeted cancer gene therapy: the flexibility of adenoviral gene therapy vectors' Journal of Controlled Release. Vol 87: 159-165 See the whole document	1-23
A	REIN DT et al (2005) 'A fiber-modified, secretory leukoprotease inhibitor promoter-based conditionally replicating adenovirus for treatment of ovarian cancer' Clinical Cancer Research. Vol 11: 1327-1335 See the whole document	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2006/000145

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member					
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	CH	684094	CN	1038306	DK	226890
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX