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(71) Applicant *(for all designated States except US)*: **THE REGENTS OF CALIFORNIA** [US/US]; Universsity of California, 1111 Franklin Street, 5th Floor, Oakland, CA 94607-5200 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **MINEV, Boris, R.** [US/US]; 4587 Mercurio Street, San Diego, CA 92130 (US).

(74) Agents: **HAILE, Lisa, A.** et al.; DLA Piper Rudnick Gray Cary US LLP, 4365 Executive Drive, Suite 1100, San Diego, CA 92121-2133 (US).

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(54) **Title:** ENHANCING CLASS I ANTIGEN PRESENTATION WITH SYNTHETIC SEQUENCES

(57) Abstract: The invention relates generally to the treatment and prevention of human cancer and viral diseases. More specifically, this invention relates to development of a new generation of vaccines that rely on eliciting cellular immune responses, specifically induction of cytotoxic T lymphocytes (CTL), against cancer cells and virus-infected cells via administration of a vaccine comprising a fusion peptide or a modified peptide. Such a fusion peptide is composed of an insertion signal sequence and a peptide derived from a tumor antigen or a viral antigen, which improves antigen presentation and induces CTL with higher efficiency against cancer cells and virus-infected cells. An exemplary antigen utilized in the invention is HER2/neu. The peptides peptide vaccines of the invention are derived from the antigens PRAME, OFA/iLRP, STEAP and SURVIVIN.

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ENHANCING CLASS I ANTIGEN PRESENTATION WITH SYNTHETIC SEQUENCES

CROSS REFERENCE TO RELATED APPLICATION(S)

[0001] This application claims the benefit of priority under 35 U.S.C. § 119(e) of U.S. Serial No. 60/586,847, filed July 8, 2004, U.S. Serial No.60/586,900, filed July 8, 2004, U.S. Serial No. 60/586,997, filed July 8, 2004, and U.S. Serial No. 60/586,914, filed July 8, 2004, the entire content of which is incorporated herein by reference.

GOVERNMENTAL INTERESTS

[0002] This invention was made in part with government support under Grant Nos. DAMD17-02-1-0028 (2003-180) and DAMD17-00-1-0184 (2003-262) awarded by the U.S. Army Medical Research and Material Command. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates generally to the treatment and prevention of human cancer or viral disease and, more specifically, to development of a new generation of vaccines that rely on eliciting cellular immune responses, specifically induction of cytotoxic T lymphocytes (CTL), against cancer cells and virus-infected cells.

BACKGROUND INFORMATION

[0004] In recent years, peptides derived from tumor-associated antigens (TAA) have been identified for a variety of human cancers. Thus far, however, effective peptide vaccination of patients with cancer has been limited to very few trials. A major obstacle for effective immunotherapy of cancer is that most TAA described to date are expressed in one or a few tumor types, and among patients with these types of tumors, TAA expression is not universal. The relative paucity of responsiveness after conventional peptide vaccination may also be due to the fact that the peptides do not efficiently enter the antigen-presenting cells and do not translocate through the endoplasmic reticulum membrane in order to associate with the MHC molecules. In addition, the immunogenic tumor peptides have short half-life *in vivo*, and their affinity to class I MHC molecules is not optimal.

[0005] Cytotoxic T lymphocytes (CTL) appear to be among the most direct and effective elements of the immune system that are capable of generating anti-tumor immune responses. Tumor cells expressing the appropriate tumor-associated antigens can be effectively recognized and destroyed by these immune effector cells, which may result in dramatic clinical responses. Both the adoptive transfer of tumor-reactive CTL and active immunization designed to elicit CTL responses have been reported to lead to significant therapeutic anti-tumor responses in patients with malignant melanoma. However, these promising approaches and their applicability to other tumor types besides melanoma are more restricted because of the limited number of tumor-associated antigens or epitopes for CTL. Ideal targets for cancer immunotherapy would include antigens that are: (i) highly expressed in the cancer cells; (ii) not expressed in normal tissues; and (iii) able to induce potent cytotoxic immune responses.

[0006] Recently, four class I-restricted PRAME-derived peptides recognized by CTL have been identified. It was shown that CTL clones induced against the four identified epitopes lysed lung carcinoma, melanoma, renal cell carcinoma, and mammary carcinoma cell lines expressing PRAME in a class I-restricted fashion. However, the PRAME-derived peptides have never been modified in any way in order to improve their immunogenicity and efficacy as vaccines.

[0007] The identification of peptide sequences recognized by CTL has led to attempts to directly immunize with synthetic peptides. These approaches have met with mixed success and inconsistent results. Several strategies to improve immunization with peptides have been reported. Successful generation of CTL-responses in mice *in vivo* have been described using peptides formulated with immunostimulating complex (ISCOM), entrapped in liposomes, osmotically loaded into syngeneic splenocytes or coated on their surface. Synthetic viral peptides, covalently linked to a lipophilic compound were also capable of inducing a CTL-response. It was found that immunizations of mice with the minimal peptide $OVA_{257-264}$ were not effective. OVA-ES (OVA with a signal sequence at its carboxy-terminus) was not as effective as ES-OVA (OVA with a signal sequence at its amino-terminus) probably because the signal peptidase requires NH_2-terminal localization of the signal sequence to cleave off the minimal peptide. This possibility has previously been suggested in a study showing that

TAP-deficient T2 cells could be sensitized to lysis by CTL when infected with recombinant vaccinia viruses expressing minimal peptides situated COOH-terminal, but not NH_2-terminal to the signal sequences. A similar strategy later showed that replacing the signal sequence of the influenza virus hemaglutinin molecule H3 with an artificial sequence containing a HLA-A2-restricted T cell epitope results in efficient translocation into the ER and transport to the cell surface. This signal sequence-derived epitope was presented to HLA-A2-restricted T cells. However, the use of synthetic insertion signal sequences to improve the antigen presentation of human tumor antigens has never been attempted before.

[0008] Therefore, there remains a need in the art to discover new peptides useful in vaccination of patients with cancer and viral diseases. Such peptides should lead to a marked improvement in antigen presentation and induction of potent anti-tumor and anti-viral immune responses.

SUMMARY OF THE INVENTION

[0009] The invention relates generally to the treatment and prevention of human cancer and viral diseases. More specifically, the invention relates to development of a new generation of peptides and peptide vaccines for cancer and viral diseases that rely on eliciting cellular immune responses, specifically induction of cytotoxic T lymphocytes (CTL), against cancer cells and virus-infected cells.

[0010] In one aspect, the invention provides new peptides that induce the activity of CTL against cancer cells. In one embodiment, the invention provides non-HLA-A2 peptides and peptide vaccines derived from PRAME. In another embodiment, the invention provides peptides and peptide vaccines derived from OFA/iLRP. In yet another embodiment, the invention provides peptides and peptide vaccines derived from STEAP.

[0011] The peptides of the invention may be modified using any of the approaches described in the invention. In one embodiment, the peptides are operably linked to a signal sequence.

[0012] In another aspect, the invention provides a method of treating or preventing cancer by administering a class I restricted peptide. The cancer may be any type of cancer expressing the antigens PRAME, OFA/iLRP, STEAP, or SURVIVIN. In one embodiment, the cancer is lung cancer. In another embodiment, the cancer is breast cancer. In yet another embodiment, the cancer is prostate cancer. In yet another embodiment, the cancer is a brain tumor.

[0013] In another aspect, the invention provides a vaccine containing one or more fusion peptides for treating or preventing cancer or virus-infected cells. Such fusion peptides are composed of an insertion signal sequence and an antigen-derived peptide, which improves antigen presentation and induces antitumor and antiviral CTL with higher efficiency. The vaccines of the invention are useful for treating or preventing cancer or virus-infected cells as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 sets forth the amino acid sequence of the full length PRAME protein (SEQ ID NO: 1).

[0015] Figure 2 sets forth the nucleic acid and amino acid sequence of the full length OFA/iLRP protein (SEQ ID NO: 70) and (SEQ ID NO: 78).

[0016] Figure 3 sets forth the amino acid sequence of the full length STEAP protein (SEQ ID NO: 95).

[0017] Figure 4 shows the results of loading/pulsing T2 cells with peptide constructs composed of synthetic signal sequences attached to amino-terminus or to carboxy-terminus of HER2/neu$_{48-56}$. T2 cells were loaded (left column) or pulsed (right column) with ES-HER$_{48-56}$ (♦), HER$_{48-56}$ -ES (◇), IS-HER$_{48-56}$ (▼), HER$_{48-56}$-IS (▽) or HER$_{48-56}$ (■). At different periods after loading, T2 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0018] Figure 5 shows the results of loading/pulsing T2 cells with peptide constructs composed of synthetic signal sequences attached to amino-terminus or to carboxy-terminus of HER2/neu$_{369-377}$. T2 cells were loaded (left column) or pulsed (right column)

with ES-$HER_{369-377}$ (♦), $HER_{369-377}$-ES (◇), IS-$HER_{369-377}$ (▼), $HER_{369-377}$-IS (▽) or $HER_{369-377}$ (■). At different periods after loading, T2 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0019] Figure 6 shows the results of loading/pulsing T2 cells with peptide constructs composed of synthetic signal sequences attached to amino-terminus or to carboxy-terminus of HER2/$neu_{654-662}$. T2 cells were loaded (left column) or pulsed (right column) with ES-$HER_{654-662}$ (♦), $HER_{654-662}$-ES (◇), IS-$HER_{654-662}$ (▼), $HER_{654-662}$-IS (▽) or $HER_{654-662}$ (■). At different periods after loading, T2 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0020] Figure 7 shows the results of loading/pulsing T2 cells with peptide constructs composed of synthetic signal sequences attached to amino-terminus or to carboxy-terminus of HER2/$neu_{789-797}$. T2 cells were loaded (left column) or pulsed (right column) with ES-$HER_{789-797}$ (♦), $HER_{789-797}$-ES (◇), IS-$HER_{789-797}$ (▼), $HER_{789-797}$-IS (▽) or $HER_{789-797}$ (■). At different periods after loading, T2 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0021] Figure 8 shows the results of loading/pulsing T2 cells with peptide constructs composed of HER2/neu_{48-56} incorporated into synthetic signal sequences. T2 cells were loaded (left column) or pulsed (right column) with HER_{48-56} -IN-AF (•), HER_{48-56}-IN-ES (▲) or HER_{48-56} (■). At different periods after loading, T2 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0022] Figure 9 shows the results of loading/pulsing T2 cells with peptide constructs composed of HER2/$neu_{369-377}$ incorporated into synthetic signal sequences. T2 cells were loaded (left column) or pulsed (right column) with $HER_{369-377}$-IN-AF (•), $HER_{369-377}$-IN-ES (▲) or $HER_{369-377}$ (■). At different periods after loading, T2 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0023] Figure 10 shows the results of loading/pulsing T2 cells with peptide constructs composed of HER2/$neu_{654-662}$ incorporated into synthetic signal sequences. T2 cells were loaded (left column) or pulsed (right column) with $HER_{654-662}$-IN-AF (•), $HER_{654-662}$-IN-

ES (▲) or $HER_{654\text{-}662}$ (■). At different periods after loading, T2 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0024] Figure 11 shows the results of loading/pulsing T2 cells with peptide constructs composed of $HER2/neu_{789\text{-}797}$ incorporated into synthetic signal sequences. T2 cells were loaded (left column) or pulsed (right column) with $HER_{789\text{-}797}$ -IN-AF (•), $HER_{789\text{-}797}$ -IN-ES (▲) or $HER_{789\text{-}797}$ (■). At different periods after loading, T2 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0025] Figure 12 shows the results of loading/pulsing breast cancer cells MDA-MB-231 with peptide constructs composed of synthetic signal sequences attached to amino-terminus or to carboxy-terminus of $HER2/neu_{369\text{-}377}$. MDA-MB-231 cells were loaded (left column) or pulsed (right column) with ES-$HER2/neu_{369\text{-}377}$ (♦), $HER2/neu_{369\text{-}377}$-ES (◇), IS-$HER2/neu_{369\text{-}377}$ (▼), $HER2/neu_{369\text{-}377}$-IS (▽) or $HER2/neu_{369\text{-}377}$ (■). At different periods after loading, MDA-MB-231 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0026] Figure 13 shows the results of loading/pulsing breast cancer cells MDA-MB-231 with peptide constructs composed of synthetic signal sequences attached to amino-terminus or to carboxy-terminus of $HER2/neu_{654\text{-}662}$. MDA-MB-231 cells were loaded (left column) or pulsed (right column) with ES-$HER2/neu_{654\text{-}662}$ (♦), $HER2/neu_{654\text{-}662}$ -ES (◇), IS-$HER2/neu_{654\text{-}662}$ (▼), $HER2/neu_{654\text{-}662}$ -IS (▽) or $HER2/neu_{654\text{-}662}$ (■). At different periods after loading, MDA-MB-231 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0027] Figure 14 shows the results of loading/pulsing breast cancer cells MDA-MB-231 with peptide constructs composed of $HER2/neu_{369\text{-}377}$ incorporated into synthetic signal sequences. MDA-MB-231 cells were loaded (left column) or pulsed (right column) with $HER_{369\text{-}377}$-IN-AF (•), $HER_{369\text{-}377}$-IN-ES (▲) or $HER_{369\text{-}377}$ (■). At different periods after loading, MDA-MB-231 cells were used as targets in ^{51}Cr-release assays for the CTL.

[0028] Figure 15 shows the results of loading/pulsing breast cancer cells MDA-MB-231 with peptide constructs composed of HER2/neu$_{654-662}$ incorporated into synthetic signal sequences. MDA-MB-231 cells were loaded (left column) or pulsed (right column) with HER$_{654-662}$-IN-AF (•), HER$_{654-662}$-IN-ES (▲) or HER$_{654-662}$ (■). At different periods after loading, MDA-MB-231 cells were used as targets in ^{51}Cr-release assays for the CTL

[0029] Figure 16 is a graph showing loading of dendritic cells with HER2/neu-derived peptides fused to synthetic signal sequences.

[0030] Figure 17 is a graph showing loading of dendritic cells with HER2/neu-derived peptides included within synthetic signal sequences.

[0031] Figure 18 illustrates the path of transport of peptides into a cell and expression of the peptide on the cell surface.

[0032] Figure 19 sets forth the amino acid sequence of the full length SURVIVIN protein (SEQ ID NO: 193).

DETAILED DESCRIPTION OF THE INVENTION

[0033] The present invention relates generally to the treatment and prevention of human cancer and viral diseases and, more specifically, to development of a new generation of vaccines for cancer and viral diseases that rely on eliciting cellular immune responses, specifically induction of antitumor and antiviral cytotoxic T lymphocytes (CTL). CTL can recognize and kill cancer cells and virus-infected cells, but only if they recognize complexes of peptides associated with the major histocompatibility complex (MHC) class I molecules on the cell surface.

[0034] CTL appear to be among the most direct and effective elements of the immune system that are capable of generating anti-tumor immune responses. Tumor cells expressing the appropriate tumor-associated antigens can be effectively recognized and destroyed by these immune effector cells, which may result in dramatic clinical responses in a limited number of patients. The paucity of responsiveness in most patients may be due to the inefficient presentation of the antigens used to immunize patients with cancer.

Consequently, methods to overcome this obstacle should lead to a marked improvement in antigen presentation and induction of potent anti-tumor CTL.

[0035] The recognition of antigens by specific CTL is essential for a successful anti-cancer response. CTL recognize peptides generated from intracellular proteins that are presented by MHC class I molecules on the cell surface. In the cytosol, intracellular proteins are degraded to peptide fragments by multicatalytic protease complexes, the proteasomes. For binding and stabilization of MHC class I molecules these peptides are translocated across the membrane of the endoplasmic reticulum (ER) by the TAP peptide transporter in an ATP-dependent fashion. Following biosynthesis into the ER membrane, MHC class I molecules transiently associate with various helper molecules (chaperones) that facilitate folding and peptide loading. After successful peptide loading MHC class I molecules leave the ER to the cell surface. Once on the cell surface, the peptide is recognized by CTL, which can then kill the cancer cell or virus-infected cell. The present invention, therefore, provides novel peptides, which induce CTL against the cancer or virus on the surface of which the peptides are present, for treatment and prevention of human cancer and virus-infected cells.

[0036] One of the most promising of these new antigens, PRAME, is a member of the cancer/testis family of antigens. PRAME is a particularly attractive antigen because it is widely expressed in many different tumor types, but not in normal tissues, except testis. This antigen is detectable in many lung cancers, as well as in melanoma, renal cell cancer, breast cancer, acute leukemias, and multiple myeloma. Undesirable autoimmune reactivity against the few tissues expressing PRAME at low levels is not to be expected, because expression levels are too low to ensure CTL recognition, as shown *in vitro* with human MAGE-specific CTL and *in vivo* in a murine p53 model. The high immunogenicity of PRAME, and its broad tumor expression make this protein a very promising target for tumor-specific vaccination strategies.

[0037] In one embodiment, the invention provides PRAME-derived peptides for inducing CTL against cancer or virus-infected cells. By "PRAME-derived sequence" is meant an amino acid sequence with: (i) terminal modifications to inhibit proteolytic degradation of the PRAME peptides; (ii) amino-acid substitutions at HLA-A2.1 binding anchor positions to enhance MHC Class I binding affinity of the PRAME peptides; (iii)

amino acid substitutions at NON-anchor positions to enhance the T cell receptor binding affinity for the peptide-MHC complex, or (iv) insertion signal sequences to enhance the immunogenicity of the PRAME peptides.

[0038] Four peptides within the PRAME protein sequence have been utilized in the invention to design optimized synthetic vaccines (Table 1).

Table 1
HLA-A2.1-restricted peptides, identified within the PRAME sequence

PEPTIDES	SEQUENCE	REFERENCE
$PRAME_{100-108}$	VLDGLDVLL (SEQ ID NO: 2)	Kessler, J. (J.Exp.Med. 193: 73-88, 2001)
$PRAME_{142-151}$	SLYSFPEPEA (SEQ ID NO: 3)	Kessler, J. (J.Exp.Med. 193: 73-88, 2001)
$PRAME_{300-309}$	ALYVDSLFFL (SEQ ID NO: 4)	Kessler, J. (J.Exp.Med. 193: 73-88, 2001)
$PRAME_{425-433}$	SLLQHLIGL (SEQ ID NO: 5)	Kessler, J. (J.Exp.Med. 193: 73-88, 2001)

[0039] Another promising target is the oncofetal antigen (OFA/iLRP) identified as a 37-44 kDa immunogenic glycoprotein expressed in all human tumors examined and also in embryos/early fetuses, but not in term fetus, neonate or adult normal tissues. It was found that OFA reappears as an immunogen in early tumor development, which gives all tumor cells the capacity to activate OFA-specific CTL. Recently, it was found that an oncofetal antigen (OFA/iLRP) could induce *in vitro* OFA/iLRP-specific effector and regulatory T lymphocytes in patients with cancer. OFA/iLRP is expressed during early to mid-gestation fetal development and re-expressed as a surface antigen by tumor cells soon after transformation. The antigen is detectable on all types of human and rodent tumors tested, but cannot be detected on normal cells.

[0040] In one embodiment, the invention provides the identification of class I-restricted peptides derived from the widely expressed tumor antigen OFA/iLRP. These natural and modified peptides might be used directly to immunize patients with cancer. Dendritic cells loaded with the OFA/iLRP peptides can also be used to elicit powerful

anti-tumor immune responses. In addition, the OFA/iLRP-specific CTL might be extremely useful for cellular immunotherapy of cancer.

[0041] Recently, another novel gene that is highly expressed in many types of cancer was identified. This gene, named STEAP for six-transmembrane epithelial antigen of the prostate, is found in multiple cancers, including prostate, bladder, colon, ovarian, and Ewing sarcoma. The discovery of immunogenic peptides derived from STEAP is innovative and holds great promise. STEAP may be an ideal target for T-cell-mediated immunotherapy of advanced cancer, as STEAP is highly expressed at all stages of many cancers, including metastases; there is little or no expression of STEAP in normal human tissues; STEAP has cell surface localization and predicted secondary structure; and STEAP is not modulated by hormones, a property that is beneficial when managing hormone-refractory prostate cancer or during anti-androgen therapy for advanced metastatic disease. Protein analysis located STEAP at the cell surface of prostate cancer cells. Its strong expression in many cancers, little or no expression in normal tissues, and cell surface localization suggest that STEAP may be an ideal target for the immunotherapy of cancer.

[0042] Accordingly, in one embodiment, the invention provides immunogenic STEAP-derived peptide sequences that can be used for therapy of a variety of cancers. STEAP-specific CTL were also generated *in vitro* by direct immunization of blood cells from healthy volunteers and from patients with cancer. The STEAP-specific CTL were found to kill STEAP-expressing cancer cells *in vitro*. In addition, the invention demonstrates further enhancement of the immunogenicity of these peptides by specific modifications of their sequence.

[0043] CTL play an important role in eradicating tumor cells and virus-infected cells. Unlike antibodies, which bind foreign proteins in their native form, CTL recognize short fragments of intracellular antigens, 8-10 amino acids in length, complexed with MHC Class I molecules. Cytosolic peptides are transported across the endoplasmic reticulum (ER) membrane with the help of the ATP-dependent transporters associated with antigen processing (TAP). Peptides complexed with Class I molecules in the ER are then transported to the cell surface for recognition by CTL. Studies with cell lines with deficits in antigen processing, (e.g., human T2 and murine RMA-S) have confirmed that

TAP proteins are intimately involved in peptide transport. Alternatively, the translocation of processed proteins from the cytosol across the endoplasmic reticulum (ER) membrane is accomplished by endoplasmic reticulum-insertion signal sequences. As soon as the signal sequence of a growing polypeptide chain has emerged from the ribosome, it is bound by the signal recognition particle (SRP) and the complex is specifically targeted to the ER membrane by an interaction with the membrane bound SRP receptor (Figure 18). An additional targeting pathway is the signal sequence receptor complex, which is a major protein of the eukaryotic ER membrane. While translocation usually occurs during translation, protein precursors have also been shown to be imported into the ER after their synthesis has been completed. After translocation, peptides complexed with class I molecules in the ER are transported to the cell surface for recognition by the CTL.

[0044] The T cell epitopes identified in the invention were utilized to construct fusion peptides with natural or artificial signal sequences. The effectiveness of the following signal sequences were compared in improving the antigen presentation: a) one from early region 3 of the adenovirus type 2, b) one from interferon gamma and c) several artificial sequences, generated according to the structure and the distribution frequency of the amino acids in the natural signal sequences. Since the hydrophobicity of the fusion peptides is higher than that of the minimal peptide, a set of control fusion peptides with signal sequences situated on the carboxy-terminus of the minimal peptides was used. Thus, determination was possible of whether an improved immune response generated with fusion peptides is due only to the higher hydrophobicity of the fusion peptide, or it is related to a better translocation of the minimal peptide through the ER-membrane. Since signal sequences do not contain specific amino acid residues other than a hydrophobic region of about eight residues, it was tested whether replacing this region with the hydrophobic HER2/neu-derived peptides would result in a more efficient presentation of these epitopes. To study the generality of the signal sequence approach similar constructs were designed utilizing several HER2/neu-derived peptides. The amino-acid sequences of the synthetic peptide constructs utilizing the epitopes HER2/neu$_{48-56}$, HER2/neu$_{369-377}$, HER2/neu$_{654-662}$, and HER2/neu$_{789-797}$ are shown in tables 32-35.

[0045] In one embodiment, the invention peptides are administered to a subject as fusion peptides containing a signal sequence. The PRAME-derived, OFA/iLRP derived, STEAP-derived, or SURVIVIN-derived peptide antigen is attached to, or incorporated into a synthetic insertion signal sequence, which can improve the translocation of the peptide antigen into the ER. Such fusion peptides can be used to treat patients with cancer by the following approaches: a) Patients can be immunized with fusion peptides composed of natural or artificial signal sequences and tumor-associated or viral peptide antigens. This way it might be possible to generate specific T-cell responses against the tumor and especially micro-metastases; b) Another way of practicing this invention is to load these peptide constructs into professional antigen-presenting cells and treat patients with these cells. This approach offers the advantage of having the specific antigen presented to the T-cells for a long period of time in the context of appropriate MHC molecules; and c) Patients can be treated with autologous CTL generated *in vitro* with fusion peptide-loaded dendritic cells or other antigen-presenting cells.

[0046] The term “signal sequence,” as used herein, refers to a short amino acid sequence added to an end of an antigenic peptide, or incorporating an antigenic peptide. This modification allows transfer of the antigenic peptide through membranes such as the ER or the cell membrane. The signal sequence is cleaved after the polypeptide has crossed the membrane.

[0047] The term “subject” as used herein refers to any individual or patient to which the subject methods are performed. Generally the subject is human, although as will be appreciated by those in the art, the subject may be an animal. Thus other animals, including mammals such as rodents (including mice, rats, hamsters and guinea pigs), cats, dogs, rabbits, farm animals including cows, horses, goats, sheep, pigs, etc., and primates (including monkeys, chimpanzees, orangutans and gorillas) are included within the definition of subject.

[0048] As used herein, the term “treating” means that the clinical signs and/or the symptoms associated with the cancer or melanoma are lessened as a result of the actions performed. The signs or symptoms to be monitored will be characteristic of a particular cancer or melanoma and will be well known to the skilled clinician, as will the methods for monitoring the signs and conditions. For example, the skilled clinician will know that the

size or rate of growth of a tumor can monitored using a diagnostic imaging method typically used for the particular tumor (e.g., using ultrasound or magnetic resonance image (MRI) to monitor a tumor).

[0049] Immunization with such fusion peptides may be used both for prevention and for treatment of tumors expressing specific tumor antigens. As more specific tumor antigens are revealed, this approach may provide a model for development of more effective vaccines for lung cancer, prostate cancer, melanoma, breast cancer and other tumors. This strategy of immunization may also be useful for eliciting CTL responses against viral diseases. The use of common HLA Class I molecules, such as HLA-A2 may make it possible to immunize a large proportion of patients by this strategy. Moreover, the ability to immunize against a minimal peptide, as opposed to complete proteins, may eliminate cross-reactivity with self-antigens or other highly homologous proteins.

[0050] The term "cancer" as used herein, includes any malignant tumor including, but not limited to, carcinoma and sarcoma. Cancer arises from the uncontrolled and/or abnormal division of cells that then invade and destroy the surrounding tissues. As used herein, "proliferating" and "proliferation" refer to cells undergoing mitosis. As used herein, "metastasis" refers to the distant spread of a malignant tumor from its sight of origin. Cancer cells may metastasize through the bloodstream, through the lymphatic system, across body cavities, or any combination thereof. The term "cancerous cell" as provided herein, includes a cell afflicted by any one of the cancerous conditions provided herein. Thus, the methods of the present invention include treatment of benign overgrowth of melanocytes, glia, brain tumors, prostate cancer, breast cancer, and lung cancer. The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate surrounding tissues, and to give rise to metastases.

[0051] Accordingly, in one embodiment, the invention provides fusion peptides composed of insertion signal sequences and peptides derived from the breast cancer antigen HER2/neu. The fusion peptides improve antigen presentation and induce antitumor CTL with higher efficiency against breast cancer. The addition of a synthetic signal sequence at the NH_2-terminus, but not at the COOH-terminus, of the HER2/neu epitopes greatly enhanced their presentation in T2 target cells, breast cancer cells and dendritic cells. Importantly, peptide constructs, composed of the HER2/neu epitopes

replacing the hydrophobic part of the signal sequences were the most effective. The efficiency of the signal sequences in facilitating the HER2/neu peptide presentation was confirmed also by using cytokine-release assays. The mechanisms involved in the enhancement of antigen presentation by the fusion peptides proved that the effective presentation of the loaded peptide constructs is a result of their efficient loading into the cytosol and not simple binding to the surface HLA molecules.

[0052] By "loading" of the fusion peptides into the cytosol of T2 cells, cancer cells and dendritic cells is meant use of a technology called "osmotic lysis of pinocytic vesicles." T2 cells were exposed to hypertonic medium containing the peptide constructs. Pinocytic vesicles form in this medium, and because of their increased internal osmotic pressure, they break in the cytosol when the cells are placed in hypotonic culture medium. The invention is based on a hypothesis that the signal sequence will translocate the minimal tumor-specific peptide from the cytosol into the ER, improving its presentation to CTL.

[0053] HER2/neu proto-oncogene, expressed in breast cancer and other human cancers, encodes a tyrosine kinase with homology to epidermal growth factor receptor. HER2/neu protein is a receptor-like transmembrane protein comprising a large cysteine-rich extracellular domain that functions in ligand binding, a short transmembrane domain, and a small cytoplasmic domain. HER2/neu is amplified and expressed in many human cancers, largely adenocarcinomas of breast, ovary, colon, and lung. In breast cancer, HER2/neu over-expression is associated with aggressive disease and is an independent predictor of poor prognosis. HER2/neu is considered a possible target for T-cell-mediated immunotherapy for several reasons: (i) the protein is large (1255 amino acids), contains epitopes appropriate for binding to most MHC molecules and thus is potentially recognizable by all individuals; (ii) HER2/neu is greatly over-expressed on malignant cells and thus T-cell therapy may be selective with minimal toxicity; (iii) the oncogenic protein is intimately associated with the malignant phenotype and with the aggressiveness of the malignancy, especially in breast and ovarian carcinomas.

[0054] As shown in the Examples below, peptide signal sequences could improve presentation of the human tumor antigen HER2/neu. Since the transport of antigenic peptides from the cytosol to the endoplasmic reticulum (ER) is a limiting step in the

processing of Class I-restricted antigens, bypassing this step of antigen processing is a clear advantage, resulting in more effective generation of CTL specifically directed against human cancers and viral diseases.

[0055] Signal sequences consist of three regions with specific characteristics shared by both eukaryotes and prokaryotes: (i) basic N-terminal region (n-region, pre-core, 1-3 positively charged residues); (ii) central hydrophobic region (h-region, core, 8-12 hydrophobic residues); and (iii) polar C-terminal region (c-region, post-core, 5-7 residues with higher average polarity than the h-region). The central hydrophobic region is the true hallmark of the signal sequences.

[0056] The primary structure is not critical to signal sequence functions. Comparison to all known signal sequences reveals no regions of strict homology. The cleavage site shows the strongest conservation, probably because it must be recognized by the signal peptidase.

[0057] Accordingly, the present invention provides peptide constructs composed of signal sequences, situated on the amino-terminus or the carboxy-terminus of several HER2/neu-derived peptides. In addition, the invention provides fusion peptides composed of natural or artificial signal sequences and HER2/neu peptides, replacing the hydrophobic part of the signal sequences.

NATURAL SIGNAL SEQUENCES:

E3/19 adenoviral signal sequence: MRYMILGLLALAAVCSA (SEQ ID NO: 68)

Gamma interferon signal sequence: MTNKCLLQIALLLCFSTTALS (SEQ ID NO: 69)

[0058] In performance of the present invention, the hydrophobic region of some signal sequences (natural and artificial) was replaced. Where this was performed, the following was noted: signal sequences do not contain specific amino acid residues other than a hydrophobic region of 8-12 residues; cleavage usually occurs after a small non-polar residue, which is the case with Val in position 9; Ala is the most abundant residue, associated with the cleavage site; the spacer of five Ala residues contributes to the

predicted β-turn, which is found immediately before or after the cleavage site. The β-turn is thought to be important for peptidase access to the cleavage site.

[0059] In one embodiment, protein signal sequences are fused to HLA Class I-restricted minimal peptides for the development of synthetic vaccines against neoplastic and viral diseases. Immunizing with minimal determinant constructs may avoid the possible oncogenic effect of full-length proteins containing ras, p53 or other potential oncogenes. In addition, *in vivo* or *in vitro* immunization with peptide antigens "packaged" in dendritic cells or other antigen-presenting cells opens an exciting opportunity for eliciting powerful CTL-responses.

[0060] In another embodiment, the invention provides vaccines containing one or more fusion peptides as set forth above. The new vaccines can be used in subjects with advanced metastatic cancers, which are normally resistant to the conventional methods for treatment. Other cancers for which the synthetic vaccines are useful include, but are not limited to, melanoma, gliomas (Schwannoma, glioblastoma, astrocytoma), prostate cancer, renal cancer, breast cancer, lung cancer, acute leukemias, and many other cancers expressing known tumor-associated antigens. Dendritic cells loaded with these vaccines can also be used to elicit powerful anti-tumor immune responses in patients with cancer. In addition, fusion-peptide induced CTL might be extremely useful for cellular immunotherapy of cancer. This new approach may also be used to induce potent anti-viral immune responses.

[0061] All of the above-mentioned approaches can be applied using combinations of different tumor-associated or viral peptide antigens. This may allow generation of broader immune responses against the tumor or the virus-infected cell(s).

[0062] In another aspect, the methods of the invention are useful for providing a means for practicing personalized medicine, wherein treatment is tailored to a subject based on the particular characteristics of the cancer cells in the subject. The method can be practiced, for example, by contacting a sample of cells from the subject with at least one test peptide, wherein an increase in CTL in the presence of the test peptide as compared to CTL in the absence of the test peptide identifies the peptide as useful for treating the cancer. The sample of cells examined according to the present method can be

obtained from the subject to be treated, or can be cells of an established cancer cell line of the same type as that of the subject. In one aspect, the established cancer cell line can be one of a panel of such cell lines, wherein the panel can include different cell lines of the same type of cancer and/or different cell lines of different cancers. Such a panel of cell lines can be useful, for example, to practice the present method when only a small number of cancer cells can be obtained from the subject to be treated, thus providing a surrogate sample of the subject's cancer, and also can be useful to include as control samples in practicing the present methods.

[0063] Preferred cell types for use in the invention include, but are not limited to, mammalian cells, including animal (rodents, including mice, rats, hamsters and gerbils), primates, and human cells, particularly cancer cells of all types, including breast, skin, lung, cervix, colorectal, leukemia, brain, etc.

[0064] Once disease is established and a treatment protocol is initiated, the methods of the invention may be repeated on a regular basis to evaluate whether the level of peptide-specific CTL activity in the subject remains elevated as compared to that which is observed in a normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months. Accordingly, the invention is also directed to methods for monitoring a therapeutic regimen for treating a subject having cancer. A comparison of the peptide-specific CTL activity prior to and during therapy indicates the efficacy of the therapy. Therefore, one skilled in the art will be able to recognize and adjust the therapeutic approach as needed.

[0065] All methods may further include the step of bringing the active ingredient(s) into association with a pharmaceutically acceptable carrier, which constitutes one or more accessory ingredients. The term "pharmaceutically acceptable", when used in reference to a carrier, is meant that the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0066] Pharmaceutically acceptable carriers useful for formulating a peptide or synthetic vaccine for administration to a subject are well known in the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic

esters. A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that act, for example, to stabilize or to increase the absorption of the conjugate. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the physico-chemical characteristics of the therapeutic agent and on the route of administration of the composition, which can be, for example, orally or parenterally such as intravenously, and by injection, intubation, or other such method known in the art. The pharmaceutical composition also can contain a second (or more) compound(s) such as a diagnostic reagent, nutritional substance, toxin, or therapeutic agent, for example, a cancer chemotherapeutic agent and/or vitamin(s).

[0067] The peptides and peptide vaccines of the invention can be incorporated within an encapsulating material such as into an oil-in-water emulsion, a microemulsion, a micelle, mixed micelle, a liposome, a microsphere, a polymeric nanoparticle, or other polymer matrix (see, for example, Gregoriadis, *Liposome Technology*, Vol. 1 (CRC Press, Boca Raton, FL 1984); Fraley, et al., *Trends Biochem. Sci.*, 6:77 (1981), each of which is incorporated herein by reference).

[0068] Liposomes, for example, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer. "Stealth" liposomes (see, for example, U.S. Patent Nos. 5,882,679; 5,395,619; and 5,225,212, each of which is incorporated herein by reference) are an example of such encapsulating materials particularly useful for preparing a pharmaceutical composition useful for practicing a method of the invention, and other "masked" liposomes similarly can be used, such liposomes extending the time that the therapeutic agent remain in the circulation. Cationic liposomes, for example, also can be modified with specific receptors or ligands (Morishita et al., *J. Clin. Invest.* 91:2580-2585 (1993), which is incorporated herein by reference). In addition, a polynucleotide agent can be introduced into a cell using, for example, adenovirus-

polylysine DNA complexes (see, for example, Michael et al., *J. Biol. Chem.* 268:6866-6869 (1993), which is incorporated herein by reference).

[0069] Polymeric nanoparticles, on the other hand, are defined as solid particles having a size in the range of 10-1000 nm. Polymeric nanoparticles may allow encapsulation of the peptides or peptide vaccines inside a polymeric matrix, protecting them against enzymatic and hydrolytic degradation. In addition, the nanoparticle-vaccine approach provides the ability to customize various properties of the vaccine materials that may improve their function. Such variable properties include, but are not limited to, particle size, pH sensitivity, surface charge, and hydrophobicity.

[0070] The carriers, in addition to those disclosed above, can include glucose, lactose, mannose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, medium chain length triglycerides, dextrans, and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form. In addition auxiliary, stabilizing, thickening or coloring agents and perfumes can be used, for example a stabilizing dry agent such as triulose (see, for example, U.S. Patent No. 5,314,695, which is incorporated herein by reference).

[0071] The route of administration of a composition containing the peptides of the invention will depend, in part, on the chemical structure of the molecule. As used herein, the terms "administration" or "administering" are defined to include an act of providing a compound or pharmaceutical composition of the invention to a subject in need of treatment. Polypeptides and polynucleotides, for example, are not particularly useful when administered orally because they can be degraded in the digestive tract. However, methods for chemically modifying polynucleotides and polypeptides, for example, to render them less susceptible to degradation by endogenous nucleases or proteases, respectively, or more absorbable through the alimentary tract are well known (see, for example, Blondelle et al., *Trends Anal. Chem.* 14:83-92, 1995; Ecker and Crook, *BioTechnology*, 13:351-360, 1995). For example, a peptide of the invention can be prepared using D-amino acids, or can contain one or more domains based on peptidomimetics, which are organic molecules that mimic the structure of peptide domain; or based on a peptoid such as a vinylogous peptoid. The peptides of the invention can further be administered in a form that releases the peptide at the desired

position in the body (e.g., the stomach), or by injection into a blood vessel such that the peptide circulates to the target cells (e.g., cancer cells).

[0072] Exemplary routes of administration include, but are not limited to, orally or parenterally, such as intravenously, intramuscularly, subcutaneously, intraperitoneally, intrarectally, intracisternally or, if appropriate, by passive or facilitated absorption through the skin using, for example, a skin patch or transdermal iontophoresis, respectively. Furthermore, the pharmaceutical composition can be administered by injection, intubation, orally or topically, the latter of which can be passive, for example, by direct application of an ointment, or active, for example, using a nasal spray or inhalant, in which case one component of the composition is an appropriate propellant. As mentioned above, the pharmaceutical composition also can be administered to the site of a tumor, for example, intravenously or intra-arterially into a blood vessel supplying the tumor.

[0073] The total amount of a peptide or vaccine to be administered in practicing a method of the invention can be administered to a subject as a single dose, either as a bolus or by infusion over a relatively short period of time, or can be administered using a fractionated treatment protocol, in which multiple doses are administered over a prolonged period of time. One skilled in the art would know that the amount of peptide or synthetic vaccine to treat cancer in a subject depends on many factors including the age and general health of the subject as well as the route of administration and the number of treatments to be administered. In view of these factors, the skilled artisan would adjust the particular dose as necessary. In general, the formulation of the pharmaceutical composition and the routes and frequency of administration are determined, initially, using Phase I and Phase II clinical trials.

[0074] In general, a suitable daily dose of a compound or composition of the invention will be that amount of the compound or composition that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. As used herein, the term “therapeutically effective amount” or “effective amount” means the amount of a compound or pharmaceutical composition that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

[0075] Additionally, the compositions and methods of the invention can be used in conjunction with other standard cancer therapies, *e.g.*, surgery, chemotherapy and radiation.

[0076] The following examples are provided to further illustrate the advantages and features of the present invention, but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLE 1

Enhancing the Stability, Immunogenicity, and Antigen Presentation of PRAME-Derived Synthetic Peptides

[0077] Most attempts to treat cancer patients with TAA-derived synthetic peptides have not been successful. The following is therefore further research aimed at enhancing the stability and immunogenicity of the peptides used for vaccination of patients with cancer is essential.

[0078] When biologically active peptides are used clinically in their natural form, their biologic effects are often rapidly lost *in vivo* due to rapid elimination of the active form of the peptide. Since the skin is an enzymatically active organ, in vaccinations that utilize subcutaneous injections, peptides may be degraded by skin peptidases prior to effecting a significant immunological response. Thus, it is critical to design stable peptide formulations for vaccination of patients with cancer. The natural HLA-A2.1 restricted PRAME peptides were modified by N-terminal acetylation and/or C-terminal amidation. Examples of modifications to the native HLA-A2.1 restricted PRAME peptides are shown (Tables 2-5):

Table 2
Terminal modifications of the PRAME-derived peptide PRAME$_{100-108}$

Peptide Name	Peptide Sequence	Modifications	
		N-Terminus	C-Terminus
PRAME$_{100-108}$	^{100}VLDGLDVLL^{108} (SEQ ID NO: 2)	-	-
N- PRAME$_{100-108}$	Ac- VLDGLDVLL (SEQ ID NO: 6)	Acetyl	-
C- PRAME$_{100-108}$	VLDGLDVLL -amide (SEQ ID NO: 7)	-	Amide
Cap- PRAME$_{100-108}$	Ac- VLDGLDVLL -amide (SEQ ID NO: 8)	Acetyl	Amide

Table 3
Terminal modifications of the PRAME-derived peptide PRAME$_{142-151}$

Peptide Name	Peptide Sequence	Modifications	
		N-Terminus	C-Terminus
PRAME$_{142-151}$	^{142} SLYSFPEPEA ^{151} (SEQ ID NO: 3)	-	-
N- PRAME$_{142-151}$	Ac- SLYSFPEPEA (SEQ ID NO: 9)	Acetyl	-
C- PRAME$_{142-151}$	SLYSFPEPEA -amide (SEQ ID NO: 10)	-	Amide
ap- PRAME$_{142-151}$	Ac- SLYSFPEPEA-amide (SEQ ID NO: 11)	Acetyl	Amide

Table 4
Terminal modifications of the PRAME-derived peptide PRAME$_{300-309}$

Peptide Name	Peptide Sequence	Modifications	
		N-Terminus	C-Terminus
PRAME$_{300-309}$	^{300}ALYVDSLFFL^{309} (SEQ ID NO: 4)	-	-
N- PRAME$_{300-309}$	Ac- ALYVDSLFFL (SEQ ID NO: 12)	Acetyl	-
C- PRAME$_{300-309}$	ALYVDSLFFL -amide (SEQ ID NO: 13)	-	Amide
Cap- PRAME$_{300-309}$	Ac- ALYVDSLFFL-amide (SEQ ID NO: 14)	Acetyl	Amide

Table 5
Terminal modifications of the PRAME-derived peptide PRAME$_{425-433}$

Peptide Name	Peptide Sequence	Modifications	
		N-Terminus	C-Terminus
PRAME$_{425-433}$	^{425}SLLQHLIGL^{433} (SEQ ID NO: 5)	-	-
N- PRAME$_{425-433}$	Ac- SLLQHLIGL (SEQ ID NO: 15)	Acetyl	-
C- PRAME$_{425-433}$	SLLQHLIGL -amide (SEQ ID NO: 16)	-	Amide
Cap- PRAME$_{425-433}$	Ac- SLLQHLIGL -amide (SEQ ID NO: 17)	Acetyl	Amide

Amino acid substitutions at HLA-A2.1 binding anchor positions to enhance MHC Class I binding affinity of the PRAME peptides (Fixed Anchor Analogs):

[0079] Upon stimulation with natural peptides, tumor-reactive CTL have been induced *in vitro* from peripheral blood lymphocytes of some patients with cancer. However, tumor-specific CTL could only be induced in a limited number of patients, and numerous re-stimulations were required to generate anti-tumor reactivity. These findings prompted this section of the current invention aimed at enhancing the immunogenicity of peptides derived from PRAME.

[0080] As an example, the following anchor amino acid substitutions to the native HLA-A2.1 restricted PRAME peptides were created (Tables 6-9):

Table 6
Substitutions at the HLA-A2.1 binding anchor positions of the peptide PRAME$_{100-108}$

Peptide Name	Peptide Sequence	Substitutions
PRAME$_{100-108}$	^{100} VLDGLDVLL ^{108} (SEQ ID NO: 2)	--
PRAME$_{100-108}$ -1F	**F**LDGLDVLL (SEQ ID NO: 18)	F for V at position 1
PRAME$_{100-108}$ -3W	VL**W**GLDVLL (SEQ ID NO: 19)	W for D at position 3
PRAME$_{100-108}$ -9V	VLDGLDVL**V** (SEQ ID NO: 20)	V for L at position 9
PRAME$_{100-108}$ -1F/3W/9V	**FLWGLDVLV** (SEQ ID NO: 21)	all of the above

Table 7
Substitutions at the HLA-A2.1 binding anchor positions of the peptide PRAME$_{142-151}$

Peptide Name	Peptide Sequence	Substitutions
PRAME$_{142-151}$	^{142}SLYSFPEPEA^{151} (SEQ ID NO: 3)	--
PRAME$_{142-151}$ – 1F	FLYSFPEPEA (SEQ ID NO: 22)	F for S at position 1
PRAME$_{142-151}$ – 3W	SLWSFPEPEA (SEQ ID NO: 23)	W for Y at position 3
PRAME$_{142-151}$ – 10V	SLYSFPEPEV (SEQ ID NO: 24)	V for A at position 10
PRAME$_{142-151}$ – 1F/3W/10V	FLWSFPEPEV (SEQ ID NO: 25)	all of the above

Table 8
Substitutions at the HLA-A2.1 binding anchor positions of the peptide PRAME$_{300-309}$

Peptide Name	Peptide Sequence	Substitutions
PRAME$_{300-309}$	^{300}ALYVDSLFFL^{309} (SEQ ID NO: 4)	--
PRAME$_{300-309}$ – 3W	ALFVDSLFFL (SEQ ID NO: 26)	F for Y at position 3
PRAME$_{300-309}$ – 10V	ALYVDSLFFV (SEQ ID NO: 27)	V for L at position 10
PRAME$_{300-309}$ –3F/10V	ALFVDSLFFV (SEQ ID NO: 28)	all of the above

Table 9
Substitutions at the HLA-A2.1 binding anchor positions of the peptide PRAME$_{425-433}$

Peptide Name	Peptide Sequence	Substitutions
PRAME$_{425-433}$	^{425}SLLQHLIGL^{433} (SEQ ID NO: 5)	--
PRAME$_{425-433}$ – 1F	FLLQHLIGL (SEQ ID NO: 29)	F for S at position 1
PRAME$_{425-433}$ – 3W	SLWQHLIGL (SEQ ID NO: 30)	W for L at position 3
PRAME$_{425-433}$ – 9V	SLLQHLIGV (SEQ ID NO: 31)	V for L at position 9
PRAME$_{425-433}$ – 1F/3W/9V	FLWQHIIGV (SEQ ID NO: 32)	all of the above

Amino acid substitutions at NON-anchor positions to enhance the T cell receptor binding affinity for the peptide-MHC complex (Heteroclitic Analogs)

[0081] Certain peptide analogs that carry amino acid substitutions at residues other than the main MHC anchors (heteroclitic analogs) have shown a significantly increased potency, and are surprisingly much more antigenic than wild-type peptides. These analogs may provide considerable benefit in vaccine development, as they induce stronger T cell responses than the native epitope, and have been shown to be associated with increased affinity of the epitope/MHC complex for the T cell receptor (TCR) molecule. Important advantages of the heteroclitic analogs related to their clinical application include their ability to break/overcome tolerance by reversing a state of T cell energy and/or recruiting new T cell specificities, and the significantly smaller amounts of heteroclitic analogs that is needed for treatment.

[0082] The scheme that used for selection of the single amino acid substitutions includes rank coefficient scores for PAM250, hydrophobicity, and side chain volume. The Dayhoff PAM250 score (hyper text transfer protocol address prowl.rockefeller.edu/aainfo/pam250.htm) is a commonly used protein alignment scoring matrix which measures the percentage of acceptable point mutations within a defined time frame.

[0083] The following NON-anchor amino acid substitutions were made to the native HLA-A2.1 restricted PRAME peptides (Tables 10-13):

Table 10
Substitutions at NON-anchor positions of the peptide $PRAME_{100-108}$

Peptide Name	Peptide Sequence	Substitutions
$PRAME_{100-108}$	^{100}VLDGLDVLL^{108} (SEQ ID NO: 2)	--
$PRAME_{100-108}$ – 3K	VL**K**GLDVLL (SEQ ID NO: 33)	K for D at position 3
$PRAME_{100-108}$ – 5H	VLDG**H**DVLL (SEQ ID NO: 34)	H for L at position 5
$PRAME_{100-108}$ – 7P	VLDGLD**P**LL (SEQ ID NO: 35)	P for V at position 7

Table 11
Substitutions at NON-anchor positions of the peptide $PRAME_{142-151}$

Peptide Name	Peptide Sequence	Substitutions
$PRAME_{142-51}$	^{142} SLYSFPEPEA ^{151} (SEQ ID NO: 3)	--
$PRAME_{142-151}$ – 3K	SL**K**SFPEPEA (SEQ ID NO: 36)	K for Y at position 3
$PRAME_{142-151}$ – 5H	SLYS**H**PEPEA (SEQ ID NO: 37)	H for F at position 5
$PRAME_{142-151}$ – 7P	SLYSFP**P**PEA (SEQ ID NO: 38)	P for E at position 7

Table 12
Substitutions at NON-anchor positions of the peptide $PRAME_{300-309}$

Peptide Name	Peptide Sequence	Substitutions
$PRAME_{300-309}$	^{300} ALYVDSLFFL ^{309} (SEQ ID NO: 4)	--
$PRAME_{300-309}$ – 3K	AL**K**VDSLFFL (SEQ ID NO: 39)	K for Y at position 3
$PRAME_{300-309}$ – 5H	ALYV**H**SLFFL (SEQ ID NO: 40)	H for D at position 5
$PRAME_{300-309}$ – 7P	ALYVDS**P**FFL (SEQ ID NO: 41)	P for L at position 7

Table 13
Substitutions at NON-anchor positions of the peptide $PRAME_{425-433}$

Peptide Name	Peptide Sequence	Substitutions
$PRAME_{425-433}$	^{425}SLLQHLIGL ^{433} (SEQ ID NO: 5)	--
$PRAME_{425-433}$ – 3K	SL**K**QHLIGL (SEQ ID NO: 42)	K for L at position 3
$PRAME_{425-433}$ – 7P	SLLQHL**P**GL (SEQ ID NO: 43)	P for I at position 7

Enhancing the immunogenicity of the peptides with insertion signal sequences

[0084] The transport of antigenic peptides from the cytosol to the endoplasmic reticulum (ER) is a limiting step in processing and presentation of class I-restricted antigens. Bypassing this step by direct targeting of the antigen to the ER can result in more effective generation of CTL. This could amount to a more potent CTL induction and anti-tumor immunity against cancer. A variety of fusion peptides composed of natural or modified PRAME peptides and endoplasmic reticulum insertion signal sequences were designed. The following signal sequences were utilized to improve the antigen presentation: a) one from early region 3 of the adenovirus type 2 – ES (MRYMILGLLALAAVCSA) (SEQ ID NO: 68), b) one from IFN-beta – IS (MTNKCLLQIALLLCFSTTALS) (SEQ ID NO: 69), and c) several artificial sequences, generated according to the structure and the distribution frequency of the amino acids in the natural signal sequences. Examples of synthetic peptide constructs utilizing the PRAME epitopes are shown (Tables 14-17).

Table 14
Synthetic peptide constructs utilizing the epitope $PRAME_{100-108}$

Designation	Peptide Sequence
1. PRAME - $PRAME_{100-108}$	**VLDGLDVLL** (SEQ ID NO: 2)
2. ES-PRAME	M R Y M I L G L L A L A A V C S A **VLDGLDVLL** (SEQ ID NO: 44)
3. PRAME-ES	**VLDGLDVLL** M R Y M I L G L L A L A A V C S A (SEQ ID NO: 45)
4. IS-PRAME	M T N K C L L Q I A L L L C F S T T A L S **VLDGLDVLL** (SEQ ID NO: 46)
5. PRAME-IS	**VLDGLDVLL** M T N K C L L Q I A L L L C F S T T A L S (SEQ ID NO: 47)
6. PRAME-IN-ES	M R **VLDGLDVLL** A A V C S A (SEQ ID NO: 48)
7. PRAME-IN-AF	M A **VLDGLDVLL** A A A A A G (SEQ ID NO: 49)

Synthetic peptide constructs:

1. Peptide antigen $PRAME_{100-108}$
2. Adenoviral signal sequence ES attached to the amino-terminus of $PRAME_{100-108}$
3. Adenoviral signal sequence ES attached to the carboxy-terminus of $PRAME_{100-108}$
4. Interferon signal sequence IS attached to the amino-terminus of $PRAME_{100-108}$
5. Interferon signal sequence IS attached to the carboxy-terminus of $PRAME_{100-108}$
6. Peptide antigen $PRAME_{100-108}$ replacing the hydrophobic portion of ES
7. Peptide antigen $PRAME_{100-108}$ incorporated into an artificial signal sequence - AF

Table 15
Synthetic peptide constructs utilizing the epitope $PRAME_{142-151}$

Designation	Peptide Sequence
1. PRAME - $PRAME_{142-151}$	**SLYSFPEPEA** (SEQ ID NO: 3)
2. ES-PRAME	M R Y M I L G L L A L A A V C S A **SLYSFPEPEA** (SEQ ID NO: 50)
3. PRAME-ES	**SLYSFPEPEA** M R Y M I L G L L A L A A V C S A (SEQ ID NO: 51)
4. IS-PRAME	M T N K C L L Q I A L L L C F S T T A L S **SLYSFPEPEA** (SEQ ID NO: 52)
5. PRAME-IS	**SLYSFPEPEA** M T N K C L L Q I A L L L C F S T T A L S (SEQ ID NO: 53)
6. PRAME-IN-ES	M R **SLYSFPEPEA** A A V C S A (SEQ ID NO: 54)
7. PRAME-IN-AF	M A **SLYSFPEPEA** A A A A A G (SEQ ID NO: 55)

Synthetic peptide constructs:
1. Peptide antigen $PRAME_{142-151}$
2. Adenoviral signal sequence ES attached to the amino-terminus of $PRAME_{142-151}$
3. Adenoviral signal sequence ES attached to the carboxy-terminus of $PRAME_{142-151}$
4. Interferon signal sequence IS attached to the amino-terminus of $PRAME_{142-151}$
5. Interferon signal sequence IS attached to the carboxy-terminus of $PRAME_{142-151}$
6. Peptide antigen $PRAME_{142-151}$ replacing the hydrophobic portion of ES
7. Peptide antigen $PRAME_{142-151}$ incorporated into an artificial signal sequence - AF

Table 16
Synthetic peptide constructs utilizing the epitope $PRAME_{300-309}$

Designation	Peptide Sequence
1. PRAME - $PRAME_{300-309}$	**ALYVDSLFFL** (SEQ ID NO: 4)
2. ES-PRAME	M R Y M I L G L L A L A A V C S A **ALYVDSLFFL** (SEQ ID NO: 56)
3. PRAME-ES	**ALYVDSLFFL** M R Y M I L G L L A L A A V C S A (SEQ ID NO: 57)
4. IS-PRAME	M T N K C L L Q I A L L L C F S T T A L S **ALYVDSLFFL** (SEQ ID NO: 58)
5. PRAME-IS	**ALYVDSLFFL** M T N K C L L Q I A L L L C F S T T A L S (SEQ ID NO: 59)
6. PRAME-IN-ES	M R **ALYVDSLFFL** A A V C S A (SEQ ID NO: 60)
7. PRAME-IN-AF	M A **ALYVDSLFFL** A A A A A G (SEQ ID NO: 61)

Synthetic peptide constructs:
1. Peptide antigen $PRAME_{300-309}$
2. Adenoviral signal sequence ES attached to the amino-terminus of $PRAME_{300-309}$
3. Adenoviral signal sequence ES attached to the carboxy-terminus of $PRAME_{300-309}$
4. Interferon signal sequence IS attached to the amino-terminus of $PRAME_{300-309}$
5. Interferon signal sequence IS attached to the carboxy-terminus of $PRAME_{300-309}$
6. Peptide antigen $PRAME_{300-309}$ replacing the hydrophobic portion of ES
7. Peptide antigen $PRAME_{300-309}$ incorporated into an artificial signal sequence - AF

Table 17
Synthetic peptide constructs utilizing the epitope $PRAME_{425-433}$

Designation	Peptide Sequence
1. PRAME - $PRAME_{425-433}$	**SLLQHLIGL** (SEQ ID NO: 5)
2. ES-PRAME	M R Y M I L G L L A L A A V C S A **SLLQHLIGL** (SEQ ID NO: 62)
3. PRAME-ES	**SLLQHLIGL** M R Y M I L G L L A L A A V C S A (SEQ ID NO: 63)
4. IS-PRAME	M T N K C L L Q I A L L L C F S T T A L S **SLLQHLIGL** (SEQ ID NO: 64)
5. PRAME-IS	**SLLQHLIGL** M T N K C L L Q I A L L L C F S T T A L S (SEQ ID NO: 65)
6. PRAME-IN-ES	M R **SLLQHLIGL** A A V C S A (SEQ ID NO: 66)
7. PRAME-IN-AF	M A **SLLQHLIGL** A A A A A G (SEQ ID NO: 67)

Synthetic peptide constructs:

1. Peptide antigen $PRAME_{425-433}$
2. Adenoviral signal sequence ES attached to the amino-terminus of $PRAME_{425-433}$
3. Adenoviral signal sequence ES attached to the carboxy-terminus of $PRAME_{425-433}$
4. Interferon signal sequence IS attached to the amino-terminus of $PRAME_{425-433}$
5. Interferon signal sequence IS attached to the carboxy-terminus of $PRAME_{425-433}$
6. Peptide antigen $PRAME_{425-433}$ replacing the hydrophobic portion of ES
7. Peptide antigen $PRAME_{425-433}$ incorporated into an artificial signal sequence - AF

[0085] Since the hydrophobicity of the fusion peptides is higher than that of the minimal peptide, a set of control fusion peptides with signal sequences situated on the carboxy-terminus of the minimal peptides were designed. Since signal sequences do not contain specific amino acid residues other than a hydrophobic region of about eight residues, modified peptides were designed by replacing this region with the hydrophobic PRAME-derived peptides.

EXAMPLE 2
Identification of HLA-A2.1-Restricted Immunogenic Peptides, Derived from the Antigen OFA/iLRP

[0086] OFA/iLRP-derived peptide sequences were identified that are immunogenic and can induce CTL both in healthy volunteers as well as in patients with cancer. The antigen-recognition activity of CTL is intimately linked with recognition of MHC (HLA in humans) molecules. In this invention the focus was on the HLA-A2 allele, which is the most common HLA molecule expressed by the general population in the United States. About 95% of HLA-A2+ individuals express the HLA-A2.1 subtype. For this reason, the identification of immunogenic peptides restricted by the HLA-A2.1 allele would not only

serve as a proof of principle, but would also be applicable to a large portion of the patient population. The following modern methods were utilized for identification of immunogenic peptide sequences:

[0087] Manual step-wise approach to identify peptide sequences based on the known binding motifs for the HLA-A2.1 molecule. The majority of peptides bound to MHC class I molecules have a restricted size of 9±1 amino acids and require free N- and C-terminal ends. In addition to a specific size, different class I molecules appear to require a specific combination of usually two main anchor residues within their peptide ligands. In the case of the human allele HLA-A2.1, these anchor residues have been described as leucine (L) at position 2, and L or valine (V) at the C- terminal end. More recently, it was found that a "canonical" A2.1 motif could be defined as L or M (methionine) at position 2 and L, V, or I (isoleucine) at position 9. Using this approach, several 9 amino acid-long (9^{mer}) peptides have been identified within the OFA/iLRP protein sequence (Table 18):

Table 18
HLA-A2.1-restricted peptides, identified within the OFA/iLRP sequence

ANCHOR POSITION	ANCHOR POSITION	ANCHOR POSITION	ANCHOR POSITION
L at position 2	L at position 2	L at position 2	M at position 2
V at position 9	L at position 9	I at position 9	V, L or I at position 9
7VLQMKEEDV15 (SEQ ID NO: 71)	^{50}NLKRTWEKL^{58} (SEQ ID NO: 72)	^{57}KLLLAARAI^{65} (SEQ ID NO: 73)	NONE
^{58}LLLAARAIV^{66} (SEQ ID NO: 74)	^{146}ALCNTDSPL^{154} (SEQ ID NO: 75)	^{153}PLRYVDIAI^{161} (SEQ ID NO: 76)	

[0088] A combination of three computer algorithms for peptide identification. The predictive algorithm, "BIMAS" ranks potential MHC binders according to the predictive half-time disassociation of peptide/MHC complexes. The second algorithm, "SYFPEITHI" ranks the peptides according to a score that takes into account the presence of primary and secondary MHC-binding anchor residues. The third algorithm, "PAProC", predicts the proteasomal cleavages of the tumor antigens, which is a very important step in the generation of class I-restricted antigenic peptides.

[0089] The amino acid sequence of OFA/iLRP was analyzed using the "BIMAS" and the "SYFPEITHI" predictive algorithms for the existence of 9-amino acid peptides predicted to bind to HLA-A2.1. The focus was on peptides of 9 amino acids because it has been reported that HLA-A2.1 favor binding peptides of this size as compared with peptides of 8 or 10 residues. The analysis resulted in several candidate peptides for HLA-A2.1-restricted CTL epitopes. These epitopes were then analyzed with the third algorithm, "PAProC", to verify the proteasome-mediated generation of the peptides. It was recently found that the COOH terminus of CTL epitopes requires exact cleavage by the proteasome, whereas NH2-terminal extensions of the epitope can be trimmed by putative aminopeptidase activity mainly in the ER, or in the cytosol. Therefore, the focus was on identifying peptides with the highest cleavage strength at the COOH terminus. Using all three algorithms, the search was narrowed to the following four peptides: $OFA/iLRP_{58}$ (LLLAARAIV) (SEQ ID NO: 74), $OFA/iLRP_{7}$ (VLQMKEEDV) (SEQ ID NO: 71), $OFA/iLRP_{57}$ (KLLLAARAI) (SEQ ID NO: 73), and $OFA/iLRP_{146}$ (ALCNTDSPL) (SEQ ID NO: 75). These four natural peptides were used to design synthetic vaccines with modified amino-acid residues to improve their stability, immunogenicity and antigen presentation.

EXAMPLE 3
Enhancing Stability, Immunogenicity, and Antigen Presentation of OFA/iLRP-Derived Synthetic Peptides

[0090] Because most attempts to treat cancer patients with TAA-derived synthetic peptides were not successful, further research aimed at enhancing the stability and immunogenicity of the peptides used for vaccination of patients with cancer is essential. The following methods were utilized.

Terminal modifications to inhibit proteolytic degradation of the OFA/iLRP peptides:

[0091] When biologically active peptides are used clinically in their natural form, their biologic effects are often rapidly lost *in vivo* due to rapid elimination of the active form of the peptide. Since the skin is an enzymatically active organ, in vaccinations that utilize subcutaneous injections, peptides may be degraded by skin peptidases prior to effecting a significant immunological response. Thus, it is critical to design stable peptide

formulations for vaccination of patients with cancer. The natural HLA-A2.1 restricted OFA/iLRP peptides were modified by N-terminal acetylation and/or C-terminal amidation. An example of modifications to the native HLA-A2.1 restricted peptide OFA/iLRP$_{58-66}$ is shown (Table 19):

Table 19
Terminal modifications of the OFA/iLRP-derived peptide OFA/iLRP$_{58-66}$

Peptide Name	Peptide Sequence	Modifications	
		N-terminus	**C-terminus**
OFA/iLRP$_{58-66}$	^{58}LLLAARAIV^{66} (SEQ ID NO: 74)	-	-
N- OFA/iLRP$_{58-66}$	Ac- LLLAARAIV (SEQ ID NO: 77)	**Acetyl**	-
C- OFA/iLRP$_{58-66}$	LLLAARAIV-amide (SEQ ID NO: 79)	-	**Amide**
Cap- OFA/iLRP$_{58-66}$	Ac- LLLAARAIV-amide (SEQ ID NO: 80)	**Acetyl**	**Amide**

Amino acid substitutions at HLA-A2.1 binding anchor positions to enhance MHC Class I binding affinity of the OFA/iLRP peptides (Fixed Anchor Analogs):

[0092] Upon stimulation with natural peptides, tumor-reactive CTL have been induced *in vitro* from peripheral blood lymphocytes of some patients with cancer. However, tumor-specific CTL could only be induced in a limited number of patients, and numerous re-stimulations were required to generate anti-tumor reactivity. These findings prompted this section of the current invention aimed at enhancing the immunogenicity of peptides derived from OFA/iLRP.

[0093] As an example, the following anchor amino acid substitutions were introduced to the native HLA-A2.1 restricted peptide OFA/iLRP$_{57-65}$ (Table 20):

Table 20
Substitutions at the HLA-A2.1 binding anchor positions

Peptide Name	Peptide Sequence	Substitutions
OFA/iLRP$_{57-65}$	^{57}KLLLAARAI^{65} (SEQ ID NO: 73)	--
OFA/iLRP$_{57-65}$– 1F	FLLLAARAI (SEQ ID NO: 81)	F for K at position 1
OFA/iLRP$_{57-65}$ – 3W	KLWLAARAI (SEQ ID NO: 82)	W for L at position 3
OFA/iLRP$_{57-65}$ – 9V	KLLLAARAV (SEQ ID NO: 83)	V for I at position 9
OFA/iLRP$_{57-65}$– 1F/3W/9V	FLWLAARAV (SEQ ID NO: 84)	all of the above

Amino acid substitutions at NON-anchor positions to enhance the T cell receptor binding affinity for the peptide-MHC complex (Heteroclitic Analogs)

[0094] Certain peptide analogs that carry amino acid substitutions at residues other than the main MHC anchors (heteroclitic analogs) have shown a significantly increased potency, and are surprisingly much more antigenic than wild-type peptides. These analogs may provide considerable benefit in vaccine development, as they induce stronger T cell responses than the native epitope, and have been shown to be associated with increased affinity of the epitope/MHC complex for the T cell receptor (TCR) molecule. Important advantages of the heteroclitic analogs related to their clinical application include their ability to break/overcome tolerance by reversing a state of T cell anergy and/or recruiting new T cell specificities, and the significantly smaller amounts of heteroclitic analogs that is needed for treatment.

[0095] The scheme used for selection of the single amino acid substitutions includes rank coefficient scores for PAM250, hydrophobicity, and side chain volume. The Dayhoff PAM250 score (http://prowl.rockefeller.edu/aainfo/ pam250.html) is a commonly used protein alignment scoring matrix which measures the percentage of acceptable point mutations within a defined time frame.

[0096] As an example, the following NON-anchor amino acid substitutions were made to the native HLA-A2.1 restricted peptide OFA/iLRP$_{7-15}$ (Table 21):

Table 21
Substitutions at NON-anchor positions

Peptide Name	Peptide Sequence	Substitutions
OFA/iLRP$_{7-15}$	^{7}VLQMKEEDV^{15} (SEQ ID NO: 71)	--
OFA/iLRP$_{7-15}$ – 3K	VL**K**MKEEDV (SEQ ID NO: 85)	K for Q at position 3
OFA/iLRP$_{7-15}$ – 5H	VLQM**H**EEDV (SEQ ID NO: 86)	H for K at position 5
OFA/iLRP$_{7-15}$ – 7P	VLQMKE**P**DV (SEQ ID NO: 87)	P for E at position 7

Enhancing the immunogenicity of the peptides with insertion signal sequences

[0097] A variety of fusion peptides composed of natural or modified OFA/iLRP peptides and endoplasmic reticulum insertion signal sequences were designed. The following signal sequences were utilized to improve the antigen presentation: a) one from early region 3 of the adenovirus type 2 – ES (MRYMILGLLALAAVCSA) (SEQ ID NO: 68), b) one from IFN-beta – IS (MTNKCLLQIALLLCFSTTALS) (SEQ ID NO: 69), and c) several artificial sequences, generated according to the structure and the distribution frequency of the amino acids in the natural signal sequences. An example of synthetic peptide constructs utilizing the epitope OFA/iLRP$_{58-66}$ is shown (Table 22).

Table 22
Synthetic peptide constructs utilizing the epitope OFA/iLRP$_{58-66}$

Designation	Peptide Sequence
1. OFA/iLRP - OFA/iLRP$_{58-66}$	^{58}**LLLAARAIV**^{66} (SEQ ID NO: 74)
2. ES-OFA/iLRP	M R Y M I L G L L A L A A V C S A **LLLAARAIV** (SEQ ID NO: 89)
3. OFA/iLRP-ES	**LLLAARAIV** M R Y M I L G L L A L A A V C S A (SEQ ID NO: 90)
4. IS-OFA/iLRP	M T N K C L L Q I A L L L C F S T T A L S **LLLAARAIV** (SEQ ID NO: 91)
5. OFA/iLRP-IS	**LLLAARAIV** M T N K C L L Q I A L L L C F S T T A L S (SEQ ID NO: 92)
6. OFA/iLRP-IN-ES	M R **LLLAARAIV** A A V C S A (SEQ ID NO: 93)
7. OFA/iLRP-IN-AF	M A **LLLAARAIV** A A A A A G (SEQ ID NO: 94)

Synthetic peptide constructs:

1. Peptide antigen OFA/iLRP$_{58-66}$
2. Adenoviral signal sequence ES attached to the amino-terminus of OFA/iLRP$_{58-66}$
3. Adenoviral signal sequence ES attached to the carboxy-terminus of OFA/iLRP$_{58-66}$
4. Interferon signal sequence IS attached to the amino-terminus of OFA/iLRP$_{58-66}$
5. Interferon signal sequence IS attached to the carboxy-terminus of OFA/iLRP$_{58-66}$
6. Peptide antigen OFA/iLRP$_{58-66}$ replacing the hydrophobic portion of ES
7. Peptide antigen OFA/iLRP$_{58-66}$ incorporated into an artificial signal sequence - AF

[0098] Since the hydrophobicity of the fusion peptides is higher than that of the minimal peptide, a set of control fusion peptides was designed with signal sequences situated on the carboxy-terminus of the minimal peptides. Since signal sequences do not contain specific amino acid residues other than a hydrophobic region of about eight residues, modified peptides were designed by replacing this region with the hydrophobic OFA/iLRP-derived peptides.

EXAMPLE 4
Identification of HLA-A2.1-Restricted Immunogenic Peptides Derived from the Antigen STEAP

[0099] By the present invention, STEAP-derived peptide sequences are identified that are immunogenic and can induce CTL both in healthy volunteers as well as in patients with cancer. The antigen-recognition activity of CTL is intimately linked with recognition of MHC (HLA in humans) molecules. The invention focuses on the HLA-A2 allele, which is the most common HLA molecule expressed by the general population in the United States. About 95% of HLA-A2+ individuals express the HLA-A2.1 subtype. For this reason, the identification of immunogenic peptides restricted by the HLA-A2.1 allele would not only serve as a proof of principle, but would also be applicable to a large portion of the patient population. The following modern methods were utilized for identification of immunogenic peptide sequences:

[0100] A manual step-wise approach was used to identify peptide sequences based on the known binding motifs for the HLA-A2.1 molecule. The majority of peptides bound to MHC class I molecules have a restricted size of 9±1 amino acids and require free N- and C- terminal ends. In addition to a specific size, different class I molecules appear to require a specific combination of usually two main anchor residues within their peptide ligands. In the case of the human allele HLA-A2.1, these anchor residues have been

described as leucine (L) at position 2, and L or valine (V) at the C- terminal end. More recently, it was found that a "canonical" A2.1 motif could be defined as L or M (methionine) at position 2 and L, V, or I (isoleucine) at position 9. Using this approach several 9 amino acid-long (9^{mer}) peptides have been identified within the STEAP protein sequence (Table 23):

Table 23
HLA-A2.1-restricted peptides, identified within the STEAP sequence

ANCHOR POSITION	ANCHOR POSITION	ANCHOR POSITION	ANCHOR POSITION
L at position 2	L at position 2	L at position 2	M at position 2
V at position 9	L at position 9	I at position 9	V, L or I at position 9
^{86}FLYTLLREV^{94} (SEQ ID NO: 96)	^{83}SLTFLYTLL^{91} (SEQ ID NO: 97)	^{72}HLPIKIAAI^{80} (SEQ ID NO: 98)	^{36}SMLKRPVLL^{44} (SEQ ID NO: 99)
^{165}GLLSFFFAV^{173} (SEQ ID NO: 100)	^{90}LLREVIHPL^{98} (SEQ ID NO: 101)	^{127}ALVYLPGVI^{135} (SEQ ID NO: 102)	^{291}FMIAVFLPI^{299} (SEQ ID NO: 103)
^{192}LLNWAYQQV^{200} (SEQ ID NO: 104)	^{117}VLPMVSITL^{125} (SEQ ID NO: 105)	^{130}YLPGVIAAI^{138} (SEQ ID NO: 106)	
	^{158}MLTRKQFGL^{166} (SEQ ID NO: 107)	^{221}SLGIVGLAI^{229} (SEQ ID NO: 108)	
	^{166}LLSFFFAVL^{174} (SEQ ID NO: 109)	^{263}LLGTIHALI^{271} (SEQ ID NO: 110)	
	^{256}KLGIVSLLL^{264} (SEQ ID NO: 111)	^{309}FLPCLRKKI^{317} (SEQ ID NO: 112)	
	^{262}LLLGTIHAL^{270} (SEQ ID NO: 113)	^{312}CLRKKILKI^{320} (SEQ ID NO: 114)	

[0101] A combination of three computer algorithms was utilized for peptide identification. The predictive algorithm, "BIMAS" ranks potential MHC binders according to the predictive half-time disassociation of peptide/MHC complexes. The second algorithm, "SYFPEITHI" ranks the peptides according to a score that takes into account the presence of primary and secondary MHC-binding anchor residues. The third algorithm, "PAProC", predicts the proteasomal cleavages of the tumor antigens, which is a very important step in the generation of class I-restricted antigenic peptides.

[0102] The amino acid sequence of STEAP was analyzed by using the "BIMAS" and the "SYFPEITHI" predictive algorithms for the existence of 9-amino acid peptides predicted to bind to HLA-A2.1. Peptides of 9 amino acids were the focus because it has

been reported that HLA-A2.1 favor binding peptides of this size as compared with peptides of 8 or 10 residues. The analysis resulted in several candidate peptides for HLA-A2.1-restricted CTL epitopes. These epitopes were then analyzed with the third algorithm, "PAProC", to verify the proteasome-mediated generation of the peptides. It was recently found that the COOH terminus of CTL epitopes requires exact cleavage by the proteasome, whereas NH2-terminal extensions of the epitope can be trimmed by putative aminopeptidase activity mainly in the ER, or in the cytosol. Therefore, the focus was on identifying peptides with the highest cleavage strength at the COOH terminus. Using all three algorithms, the search was narrowed to the following five peptides: ^{130}YLPGVIAAI^{138} (SEQ ID NO: 106), ^{165}GLLSFFFAV^{173} (SEQ ID NO: 100), ^{166}LLSFFFAVL^{174} (SEQ ID NO: 109), ^{192}LLNWAYQQV^{200} (SEQ ID NO: 104) and ^{302}LIFKSILFL^{310} (SEQ ID NO: 115). These five natural peptides were the starting point, however, more peptides were designed with modification of amino-acid residues to improve the stability, immunogenicity and antigen presentation of the peptides.

EXAMPLE 5
Enhancing the Stability, Immunogenicity, and Antigen Presentation of STEAP-Derived Synthetic Peptides

Terminal modifications to inhibit proteolytic degradation of the STEAP peptides:

[0103] When biologically active peptides are used clinically in their natural form, their biologic effects are often rapidly lost *in vivo* due to rapid elimination of the active form of the peptide. Since the skin is an enzymatically active organ, in vaccinations that utilize subcutaneous injections, peptides may be degraded by skin peptidases prior to effecting a significant immunological response. Thus, it is critical to design stable peptide formulations for vaccination of patients with cancer. The natural HLA-A2.1 restricted STEAP peptides were modified by N-terminal acetylation and/or C-terminal amidation. An example of modifications to the native HLA-A2.1 restricted peptide $STEAP_{130-138}$ is shown (Table 24):

Table 24
Terminal modifications of the STEAP-derived peptide $STEAP_{130-138}$

Peptide Name	Peptide Sequence	Modifications	
		N-Terminus	C-Terminus
$STEAP_{130-138}$	^{130}YLPGVIAAI^{138} (SEQ ID NO: 106)	-	-
N-$STEAP_{130-138}$	Ac- YLPGVIAAI (SEQ ID NO: 116)	Acetyl	-
C-$STEAP_{130-138}$	YLPGVIAAI-amide (SEQ ID NO: 117)	-	Amide
Cap- $STEAP_{130-138}$	Ac- YLPGVIAAI-amide (SEQ ID NO: 118)	Acetyl	Amide

Amino acid substitutions at HLA-A2.1 binding anchor positions to enhance MHC Class I binding affinity of the STEAP peptides (Fixed Anchor Analogs):

[0104] Upon stimulation with natural peptides, tumor-reactive CTL have been induced *in vitro* from peripheral blood lymphocytes of some patients with cancer. However, tumor-specific CTL could only be induced in a limited number of patients, and numerous restimulations were required to generate anti-tumor reactivity. These findings prompted this section of the current invention aimed at enhancing the immunogenicity of peptides derived from STEAP.

[0105] As an example, the following anchor amino acid substitutions were made to the native HLA-A2.1 restricted peptide $STEAP_{130-138}$ (Table 25):

Table 25
Substitutions at the HLA-A2.1 binding anchor positions

Peptide Name	**Peptide Sequence**	**Substitutions**
$STEAP_{130-138}$	^{130}YLPGVIAAI^{138} (SEQ ID NO: 106)	--
$STEAP_{130-138}$ – 1F	**F**LPGVIAAI (SEQ ID NO: 119)	F for Y at position 1
$STEAP_{130-138}$ – 3W	YL**W**GVIAAI (SEQ ID NO: 120)	W for P at position 3
$STEAP_{130-138}$ – 9V	YLPGVIAA**V** (SEQ ID NO: 121)	V for I at position 9
$STEAP_{130-138}$ – 1F/3W/9V	**F**L**W**GVIAA**V** (SEQ ID NO: 122)	all of the above

Amino acid substitutions at NON-anchor positions to enhance the T cell receptor binding affinity for the peptide-MHC complex (Heteroclitic Analogs)

[0106] Certain peptide analogs that carry amino acid substitutions at residues other than the main MHC anchors (heteroclitic analogs) have shown a significantly increased potency, and are surprisingly much more antigenic than wild-type peptides. These analogs may provide considerable benefit in vaccine development, as they induce stronger T cell responses than the native epitope, and have been shown to be associated with increased affinity of the epitope/MHC complex for the T cell receptor (TCR) molecule. Important advantages of the heteroclitic analogs related to their clinical application include their ability to break/overcome tolerance by reversing a state of T cell anergy and/or recruiting new T cell specificities, and the significantly smaller amounts of heteroclitic analogs that is needed for treatment.

[0107] The scheme that used for selection of the single amino acid substitutions includes rank coefficient scores for PAM250, hydrophobicity, and side chain volume. The Dayhoff PAM250 score (hyper text transfer protocol address prowl.rockefeller.edu/aainfo/pam250.htm) is a commonly used protein alignment scoring matrix which measures the percentage of acceptable point mutations within a defined time frame.

[0108] As an example, the following NON-anchor amino acid substitutions were made to the native HLA-A2.1 restricted peptide $STEAP_{130-138}$ (Table 26):

Table 26
Substitutions at NON-anchor positions

Peptide Name	Peptide Sequence	Substitutions
$STEAP_{130-138}$	^{130}YLPGVIAAI^{138} (SEQ ID NO: 106)	--
$STEAP_{130-138}$ – 3K	YL**K**GVIAAI (SEQ ID NO: 123)	K for P at position 3
$STEAP_{130-138}$ – 5H	YLPG**H**IAAI (SEQ ID NO: 124)	H for V at position 5
$STEAP_{130-138}$ – 7P	YLPGVI**P**AI (SEQ ID NO: 125)	P for A at position 7

Enhancing the immunogenicity of the peptides with insertion signal sequences

[0109] The transport of antigenic peptides from the cytosol to the endoplasmic reticulum (ER) is a limiting step in processing and presentation of class I-restricted antigens. Bypassing this step by direct targeting of the antigen to the ER can result in more effective generation of CTL. This could amount to a more potent CTL induction and anti-tumor immunity against prostate cancer and breast cancer. A variety of fusion peptides composed of natural or modified STEAP peptides and endoplasmic reticulum insertion signal sequences were designed. The following signal sequences were utilized to improve the antigen presentation: a) one from early region 3 of the adenovirus type 2 – ES (MRYMILGLLALAAVCSA) (SEQ ID NO:68), b) one from IFN-beta – IS (MTNKCLLQIALLLCFSTTALS) (SEQ ID NO: 69), and c) several artificial sequences, generated according to the structure and the distribution frequency of the amino acids in the natural signal sequences. An example of synthetic peptide constructs utilizing the epitope $STEAP_{130\text{-}138}$ is shown (Table 27).

Table 27
Synthetic peptide constructs utilizing the epitope $STEAP_{130\text{-}138}$

Designation	Peptide Sequence
1. STEAP - $STEAP_{130\text{-}138}$	**YLPGVIAAI** (SEQ ID NO: 106)
2. ES-STEAP	M R Y M I L G L L A L A A V C S A **YLPGVIAAI** (SEQ ID NO: 126)
3. STEAP-ES	**YLPGVIAAI** M R Y M I L G L L A L A A V C S A (SEQ ID NO: 127)
4. IS-STEAP	M T N K C L L Q I A L L L C F S T T A L S **YLPGVIAAI** (SEQ ID NO: 128)
5. STEAP-IS	**YLPGVIAAI** M T N K C L L Q I A L L L C F S T T A L S (SEQ ID NO: 129)
6. STEAP-IN-ES	M R **YLPGVIAAI** A A V C S A (SEQ ID NO: 130)
7. STEAP-IN-AF	M A **YLPGVIAAI** A A A A A G (SEQ ID NO: 131)

Synthetic peptide constructs:

1. Peptide antigen $STEAP_{130\text{-}138}$
2. Adenoviral signal sequence ES attached to the amino-terminus of $STEAP_{130\text{-}138}$
3. Adenoviral signal sequence ES attached to the carboxy-terminus of $STEAP_{130\text{-}138}$
4. Interferon signal sequence IS attached to the amino-terminus of $STEAP_{130\text{-}138}$
5. Interferon signal sequence IS attached to the carboxy-terminus of $STEAP_{130\text{-}138}$
6. Peptide antigen $STEAP_{130\text{-}138}$ replacing the hydrophobic portion of ES
7. Peptide antigen $STEAP_{130\text{-}138}$ incorporated into an artificial signal sequence - AF

[0110] Since the hydrophobicity of the fusion peptides is higher than that of the minimal peptide, a set of control fusion peptides were designed with signal sequences situated on the carboxy-terminus of the minimal peptides. Since signal sequences do not contain specific amino acid residues other than a hydrophobic region of about eight residues, modified peptides were designed by replacing this region with the hydrophobic STEAP-derived peptides.

EXAMPLE 6
Induction of Peptide-Specific (CTL) *In Vitro*

[0111] This example tested whether the STEAP-derived natural and modified peptides can induce CTL by *in vitro* immunization of blood cells from healthy donors and from patients with breast cancer with these peptides. Peripheral blood mononuclear cells (PBMC) were isolated from HLA-A2.1+ healthy volunteers and cancer patients by centrifugation on Ficoll-Hypaque gradients. PBMC were then plated in 24-well plates at $5x10^5$ cells/ml/well in RPMI-1640 supplemented with 10% human AB^+ serum, L-glutamine and antibiotics (CM). Autologous PBMC were pulsed with 10 µg/ml STEAP peptide for 3 hours at 37°C. These PBMC (stimulators) were then irradiated at 3000 rads, washed once, and added to the responder cells at responder: stimulator ratios ranging between 1:1 and 1:4. The next day, 12 IU/ml IL-2 and 30 IU/ml IL-7 were added to the cultures. Lymphocytes were then re-stimulated weekly with peptide-pulsed adherent cells as follows: previously frozen autologous PBMC were thawed, washed, re-suspended at $4x10^6$ cells/ml in CM containing 10 µg/ml peptide, and plated in 24-well plates at 1 ml/well. Plates were incubated for 3 hours at 37°C and the non-adherent cells were removed by a gentle wash with PBS. Fresh complete media containing 10 µg/ml peptide were added to the cells, and the plates were incubated again for 3 hours at 37°C. Responder cells were harvested, washed once and added to the peptide-pulsed adherent cells at a concentration of $5x10^5$ cells/ml (2 ml/well) in complete media. IL-2 and IL-7 were added to the cultures on the next day. The activity of these CTL was tested by a LDH-release cytotoxicity assays (Cytotox96 kit, Promega) after at least two rounds of peptide stimulation. K562 cells transfected with HLA-A2.1+ were pulsed with the STEAP peptides, and used as targets.

[0112] Most of the tested STEAP-derived peptides were able to induce peptide-specific CTL. The natural peptides $STEAP_{130-138}$, $STEAP_{166-174}$, and $STEAP_{192-200}$, as well as the modified peptides $STEAP_{130-138-1F}$, $STEAP_{130-138-3W}$, $STEAP_{130-138-9V}$, and $STEAP_{130-138-1F/3W/9V}$ induced potent peptide-specific CTL (Table 28):

Table 28
Specific recognition of peptide-pulsed target cells by STEAP-induced CTL

CTL specific for:	Percent LDH released from[a]:	
	K562-A2	K562-A2 pulsed with peptide[b]
$STEAP_{130-138}$	1	63
$STEAP_{130-138}$ – 1F	4	75
$STEAP_{130-138}$ – 3W	4	63
$STEAP_{130-138}$ – 9V	1	53
$STEAP_{130-138}$ – 1F/3W/9V	1	74
$STEAP_{166-174}$	3	43
$STEAP_{192-200}$	8	56

a. Cytotoxicity was evaluated in a 4-hour LDH-release assay
b. K562-A2 cells were pulsed with corresponding STEAP peptide for 2 hours at 37°C and used as targets

[0113] The peptide $STEAP_{192-200}$ induced peptide-specific CTL in three out of three patients with prostate cancer.

[0114] These findings suggest that most STEAP-derived peptides tested so far are immunogenic, implying that precursor CTL for STEAP are present in the peripheral adult repertoire.

EXAMPLE 7
Testing the Ability of the STEAP-Specific CTL to Recognize and Kill Prostate Cancer Cells in a Class I-Restricted and Antigen-Dependent Fashion

[0115] It was tested whether the STEAP-derived peptides can induce potent CTL capable of recognizing and killing prostate cancer cells *in vitro*. CTL lines selected for their ability to lyse peptide-pulsed target cells were used as effectors in LDH-release cytotoxicity assays against the cancer cell lines. The HLA-A2+ cancer cell line LnCAP was used, with the HLA-A2-negative cancer cell line DU145 as a control (Table 29).

Table 29
Specific recognition of prostate cancer cell lines by CTL reactive against the STEAP-derived peptides

CTL specific for:	Percent LDH released from[a]:	
	LnCap (HLA-A2+)	DU145 (HLA-A2–)
$STEAP_{130-138}$	53	-1
$STEAP_{130-138}$ – 1F	73	-1
$STEAP_{130-138}$ – 3W	69	-2
$STEAP_{130-138}$ – 9V	68	-2
$STEAP_{130-138}$ – 1F/3W/9V	84	1
$STEAP_{166-174}$	65	4
$STEAP_{192-200}$	43	18

a. Cytotoxicity was evaluated in a 4-hour LDH-release assay

[0116] To determine if the lysis of the target cells is HLA-A2 restricted, blocking experiments were performed using the anti-HLA-A2 antibody BB7.2, which was added to the cancer cells prior to the addition of CTL. As an additional control the anti-HLA class II antibody IVA12 was used. With these experiments, it was confirmed that the STEAP-specific CTL can recognize and kill target cells in a class I-restricted fashion (Table 30).

Table 30
Class I-restricted specific recognition of target cells by CTL reactive against the STEAP-derived peptides

CTL specific for:	Percent LDH released from[a]:			
	K562-A2	K562-A2 pulsed[b]	+BB7.2	+IVA12[c]
$STEAP_{130-138}$	1	63	2	37
$STEAP_{130-138}$ -1F	4	75	2	69
$STEAP_{130-138}$ -3W	4	63	5	64
$STEAP_{130-138}$ -9V	1	53	1	47
$STEAP_{130-138}$ - 1F/3W/9V	1	74	1	75
$STEAP_{166-174}$	3	43	11	47
$STEAP_{192-200}$	8	56	7	30

a. Cytotoxicity was evaluated in a 4-hour LDH-release assay
b. K562-A2 cells were pulsed with corresponding STEAP peptide for 2 hours at 37°C and used as targets
c. The blocking antibodies BB7.2 and IVA12 were added to the peptide-pulsed target cells before the 4-hour LDH-release assay

[0117] Collectively, these data indicate that the STEAP-derived peptides are naturally processed in prostate cancer cell lines in a class I-restricted fashion.

EXAMPLE 8
Identification of HLA-A2.1-Restricted Immunogenic Peptides Derived from the Antigen SURVIVIN

[0118] By the present invention, SURVIVIN-derived peptide sequences are identified that are immunogenic and can induce CTL, both in healthy volunteers as well as in patients with cancer. The antigen-recognition activity of CTL is intimately linked with recognition of MHC (HLA in humans) molecules. The invention focuses on the HLA-A2 allele, which is the most common HLA molecule expressed by the general population in the United States. About 95% of HLA-A2+ individuals express the HLA-A2.1 subtype. For this reason, the identification of immunogenic peptides restricted by the HLA-A2.1 allele would not only serve as a proof of principle, but would also be applicable to a large portion of the patient population. The following modern methods were utilized for identification of immunogenic peptide sequences.

[0119] A manual step-wise approach was used to identify peptide sequences based on the known binding motifs for the HLA-A2.1 molecule. The majority of peptides bound to MHC class I molecules have a restricted size of 9±1 amino acids and require free N- and C- terminal ends. In addition to a specific size, different class I molecules appear to require a specific combination of usually two main anchor residues within their peptide ligands. In the case of the human allele HLA-A2.1, these anchor residues have been described as leucine (L) at position 2, and L or valine (V) at the C- terminal end. More recently, it was found that a "canonical" A2.1 motif could be defined as L or M (methionine) at position 2 and L, V, or I (isoleucine) at position 9. Using this approach several 9 amino acid-long (9^{mer}) peptides have been identified within the SURVIVIN protein sequence (Table 31):

Table 31
HLA-A2.1-restricted peptides, identified within the SURVIVIN sequence

HLA-A*0201 nonamers	HLA-A*0201 decamers
^{20}STFKNWPFL^{28} (SEQ ID NO: 160)	^{5}TLPPAWQPFL^{14} (SEQ ID NO: 161)
^{23}KNWPFLEGC^{31} (SEQ ID NO: 162)	^{122}KEFEETAKKV^{131} (SEQ ID NO: 163)
^{96}LTLGEFLKL^{104} (SEQ ID NO: 164)	^{95}ELTLGEFLKL^{104} (SEQ ID NO: 165)
^{6}LPPAWQPFL^{14} (SEQ ID NO: 166)	^{19}ISTFKNWPFL^{28} (SEQ ID NO: 167)
^{33}CTPERMAEA^{41} (SEQ ID NO: 168)	^{48}TENEPDLAQC^{57} (SEQ ID NO: 169)
^{46}CPTENEPDL^{54} (SEQ ID NO: 170)	^{93}FEELTLGEFL^{102} (SEQ ID NO: 171)
^{130}KVRRAIEQL^{138} (SEQ ID NO: 172)	^{87}LSVKKQFEEL^{96} (SEQ ID NO: 173)
^{37}RMAEAGFIH^{45} (SEQ ID NO: 174)	^{129}KKVRRAIEQL^{138} (SEQ ID NO: 175)
^{88}SVKKQFEEL^{96} (SEQ ID NO: 176)	^{13}FLKDHRISTF^{22} (SEQ ID NO: 177)
	^{32}ACTPERMAEA^{41} (SEQ ID NO: 178)

[0120] A combination of three computer algorithms was utilized for peptide identification. The predictive algorithm, "BIMAS" ranks potential MHC binders according to the predictive half-time disassociation of peptide/MHC complexes. The second algorithm, "SYFPEITHI" ranks the peptides according to a score that takes into account the presence of primary and secondary MHC-binding anchor residues. The third algorithm, "PAProC", predicts the proteasomal cleavages of the tumor antigens, which is a very important step in the generation of class I-restricted antigenic peptides.

[0121] The amino acid sequence of SURVIVIN was analyzed by using the "BIMAS" and the "SYFPEITHI" predictive algorithms for the existence of 9-amino acid peptides predicted to bind to HLA-A2.1. Peptides of 9 amino acids were the focus because it has been reported that HLA-A2.1 favor binding peptides of this size as compared with peptides of 8 or 10 residues. The analysis resulted in several candidate peptides for HLA-A2.1-restricted CTL epitopes. These epitopes were then analyzed with the third algorithm, "PAProC", to verify the proteasome-mediated generation of the peptides. It was recently found that the COOH terminus of CTL epitopes requires exact cleavage by

the proteasome, whereas NH2-terminal extensions of the epitope can be trimmed by putative aminopeptidase activity mainly in the ER, or in the cytosol. Therefore, the focus was on identifying peptides with the highest cleavage strength at the COOH terminus.

EXAMPLE 9
Enhancing the Stability, Immunogenicity, and Antigen Presentation of SURVIVIN -Derived Synthetic Peptides

Terminal modifications to inhibit proteolytic degradation of the SURVIVIN peptides:

[0122] When biologically active peptides are used clinically in their natural form, their biologic effects are often rapidly lost *in vivo* due to rapid elimination of the active form of the peptide. Since the skin is an enzymatically active organ, in vaccinations that utilize subcutaneous injections, peptides may be degraded by skin peptidases prior to effecting a significant immunological response. Thus, it is critical to design stable peptide formulations for vaccination of patients with cancer. The natural HLA-A2.1 restricted SURVIVIN peptides were modified by N-terminal acetylation and/or C-terminal amidation. An example of modifications to the native HLA-A2.1 restricted peptide **survivin$_{20-28}$** is shown (Table 32):

Table 32
Terminal modifications of the survivin-derived peptide survivin$_{20-28}$

Peptide Name	Peptide Sequence	Modifications	
		N-Terminus	C-Terminus
survivin$_{20-28}$	^{20}STFKNWPFL^{28} (SEQ ID NO: 160)	-	-
N- survivin$_{20-28}$	Ac-STFKNWPFL (SEQ ID NO: 179)	Acetyl	-
C- survivin$_{20-28}$	STFKNWPFL-amide (SEQ ID NO: 180)	-	Amide
Cap- survivin$_{20-28}$	Ac-STFKNWPFL-amide (SEQ ID NO: 181)	Acetyl	Amide

Amino acid substitutions at HLA-A2.1 binding anchor positions to enhance MHC Class I binding affinity of the SURVIVIN peptides (Fixed Anchor Analogs):

[0123] Upon stimulation with natural peptides, tumor-reactive CTL have been induced *in vitro* from peripheral blood lymphocytes of some patients with cancer. However, tumor-specific CTL could only be induced in a limited number of patients, and numerous restimulations were required to generate anti-tumor reactivity. These findings prompted this section of the current invention aimed at enhancing the immunogenicity of peptides derived from SURVIVIN.

[0124] As an example, the following anchor amino acid substitutions were made to the native HLA-A2.1 restricted peptide **survivin$_{20-28}$** (Table 33):

Table 33
Substitutions at the HLA-A*0201 binding anchor positions

Peptide Name	Peptide Sequence	Substitutions
survivin$_{20-28}$	^{20}STFKNWPFL^{28} (SEQ ID NO: 160)	-
survivin$_{20-28}$-2L	S<u>L</u>FKNWPFL (SEQ ID NO: 182)	L for T at P2
survivin$_{20-28}$-1A	<u>A</u>TFKNWPFL (SEQ ID NO: 183)	A for S at P1
survivin$_{20-28}$-2L/1A	<u>AL</u>FKNWPFL (SEQ ID NO: 184)	both substitutions

Amino acid substitutions at NON-anchor positions to enhance the T cell receptor binding affinity for the peptide-MHC complex (Heteroclitic Analogs)

[0125] Certain peptide analogs that carry amino acid substitutions at residues other than the main MHC anchors (heteroclitic analogs) have shown a significantly increased potency, and are surprisingly much more antigenic than wild-type peptides. These analogs may provide considerable benefit in vaccine development, as they induce stronger T cell responses than the native epitope, and have been shown to be associated with increased affinity of the epitope/MHC complex for the T cell receptor (TCR) molecule. Important advantages of the heteroclitic analogs related to their clinical application include their ability to break/overcome tolerance by reversing a state of T cell anergy and/or recruiting new T cell specificities, and the significantly smaller amounts of heteroclitic analogs that is needed for treatment.

[0126] The scheme that used for selection of the single amino acid substitutions includes rank coefficient scores for PAM250, hydrophobicity, and side chain volume. The Dayhoff PAM250 score (hyper text transfer protocol address prowl.rockefeller.edu/aainfo/pam250.htm) is a commonly used protein alignment scoring matrix which measures the percentage of acceptable point mutations within a defined time frame.

[0127] As an example, the following NON-anchor amino acid substitutions were made to the native HLA-A2.1 restricted peptide survivin$_{20-28}$ (Table 34):

Table 34
Substitutions at NON-anchor positions

Peptide Name	Peptide Sequence	Substitutions
survivin$_{20-28}$	^{20}STFKNWPFL^{28} (SEQ ID NO: 160)	-
survivin$_{20-28}$ -3K	ST**<u>K</u>**KNWPFL (SEQ ID NO: 185)	K for F at P3
survivin$_{20-28}$ -5H	STFK**<u>H</u>**WPFL (SEQ ID NO: 186)	H for N at P5

Enhancing the immunogenicity of the peptides with insertion signal sequences

[0128] The transport of antigenic peptides from the cytosol to the endoplasmic reticulum (ER) is a limiting step in processing and presentation of class I-restricted antigens. Bypassing this step by direct targeting of the antigen to the ER can result in more effective generation of CTL. This could amount to a more potent CTL induction and anti-tumor immunity against prostate cancer and breast cancer. A variety of fusion peptides composed of natural or modified STEAP peptides and endoplasmic reticulum insertion signal sequences were designed. The following signal sequences were utilized to improve the antigen presentation: a) one from early region 3 of the adenovirus type 2 – ES (MRYMILGLLALAAVCSA) (SEQ ID NO:68), b) one from IFN-beta – IS (MTNKCLLQIALLLCFSTTALS) (SEQ ID NO: 69), and c) several artificial sequences, generated according to the structure and the distribution frequency of the amino acids in the natural signal sequences. An example of synthetic peptide constructs utilizing the epitope survivin$_{20-28}$ is shown (Table 35):

Table 35
Synthetic peptide constructs utilizing the epitope survivin$_{20-28}$

DESIGNATION	PEPTIDE SEQUENCE
survivin$_{20-28}$	**STFKNWPFL** (SEQ ID NO: 160)
ES-survivin$_{20-28}$	M R Y M I L G L L A L A A V C S A **STFKNWPFL** (SEQ ID NO: 187)
survivin$_{20-28}$-ES	**STFKNWPFL** M R Y M I L G L L A L A A V C S A (SEQ ID NO: 188)
IS-survivin$_{20-28}$	M T N K C L L Q I A L L L C F S T T A L S **STFKNWPFL** (SEQ ID NO: 189)
survivin$_{20-28}$-IS	**STFKNWPFL** M T N K C L L Q I A L L L C F S T T A L S (SEQ ID NO: 190)
survivin$_{20-28}$-IN-ES	M R **STFKNWPFL** A A V C S A (SEQ ID NO: 191)
survivin$_{20-28}$-IN-AF	M A **STFKNWPFL** A A A A A G (SEQ ID NO: 192)

Synthetic peptide constructs:

1. Peptide antigen survivin
2. Adenoviral signal sequence ES attached to the amino-terminus of survivin$_{20-28}$
3. Adenoviral signal sequence ES attached to the carboxy-terminus of survivin$_{20-28}$
4. Interferon signal sequence IS attached to the amino-terminus of survivin$_{20-28}$
5. Interferon signal sequence IS attached to the carboxy-terminus of survivin$_{20-28}$
6. Peptide antigen survivin$_{20-28}$ replacing the hydrophobic portion of ES
7. Peptide antigen survivin$_{20-28}$ incorporated into an artificial signal sequence – AF

[0129] Since the hydrophobicity of the fusion peptides is higher than that of the minimal peptide, a set of control fusion peptides were designed with signal sequences situated on the carboxy-terminus of the minimal peptides. Since signal sequences do not contain specific amino acid residues other than a hydrophobic region of about eight residues, modified peptides were designed by replacing this region with the hydrophobic SURVIVIN-derived peptides.

EXAMPLE 10
Testing the Effectiveness of the Fusion Peptides with T2 Cells

[0130] To probe class I presentation of cells loaded with the fusion peptides and their counterpart minimal peptides CTL recognizing the HER2/neu-derived peptides were generated. *In vitro* peripheral blood mononuclear cells (PBMCs) were immunized from healthy donors with these peptides in the presence of interleukin 2 and interleukin 7 using the following technique:

[0131] PBMCs were separated by centrifugation on Ficoll-Hypaque gradients and plated in 24-well plates at 5 x 10^5 cells/ml per well in RPMI medium 1640 supplemented with 10% human AB^+ serum, L-glutamine, and antibiotics. Autologous PBMC (stimulators) were pulsed with the HER2/neu synthetic peptides (10 μg/ml) for 3 h at 37°C. Cells were then irradiated at 3,000 rads, washed once, and added to the responder cells at a responder to stimulator ratio ranging between 1:1 and 1:4. The next day, 12 units/ml IL-2 (Chiron) and 30 units/ml IL-7 (R & D Systems) were added to the cultures. Lymphocytes were re-stimulated weekly with peptide-pulsed autologous adherent cells as follows: First, autologous PBMC were incubated with HER2/neu peptide (10 μg/ml) for 3 h at 37°C. Nonadherent cells were then removed by a gentle wash and the adherent cells were incubated with fresh medium containing the HER2/neu peptide (10 μg/ml) for an additional 3 h at 37°C. Second, responder cells from a previous stimulation cycle were harvested, washed, and added to the peptide-pulsed adherent cells at a concentration of 5 x 10^5 cells/ml (2 ml/well) in medium without peptide. Recombinant IL-2 and IL-7 were added to the cultures the next day.

[0132] The induction of CTL in human PBMC was monitored in a conventional ^{51}Cr-labeling release assay. Briefly, peptide-pulsed TAP^{-}/HLA-$A2.1^{+}$ human T2 cells were incubated with 10 μg of HER2/neu peptides or the MART-1 control peptide for 90 min during labeling with ^{51}Cr. After washing, the target cells were added to serially diluted effectors in 96-well microplates. After a 6-h incubation at 37°C, supernatants were harvested and counted in a gamma counter. Results are expressed as the percentage of specific lysis and determined as follows: [(experimental cpm--spontaneous cpm)/(maximum cpm--spontaneous cpm)] x 100. (Table 36).

[0133] Peptide-loaded or pulsed T2 cells were tested for their ability to present HER2/neu peptides at different periods of time after loading or pulsing. T2 cells loaded with most of the constructs composed of signal sequence at the amino-terminus of HER2/neu peptides were recognized by CTL up to eight days after loading (Figures 4-6, left column). In contrast, constructs with carboxy-terminal position of the signal sequence were not efficient, even when ^{51}Cr-release assays were performed immediately after loading. This recognition was not due to surface binding of these constructs since pulsing of T2 cells with any of the constructs was not efficient (Figures 4-7, right

column). Loading or pulsing with the minimal HER2/neu peptides resulted in a significant recognition and lysis of T2 cells for only one day after loading or pulsing, followed by a rapid decrease of recognition on day 3 and complete lack of recognition on days 5 and 8 after loading or pulsing. This finding suggests that the recognition of T2 cells resulted from simple binding of the HER2/neu peptides to surface HLA molecules, from which it rapidly dissociated. T2 cells loaded with the constructs composed of signal sequence at the amino-terminus of the peptide HER2/neu$_{789-797}$ were recognized by CTL up to three days after loading (Figure 7, left column).

Table 36
HER2/neu-derived HLA-A2 restricted peptides

PEPTIDES	SEQUENCE	LOCATION	REFERENCE
HER2/neu$_{48-56}$	HLYQGCQVV (SEQ ID NO: 132)	EXTRACELLULAR	Disb, M.(Canccr Res. 54:1071-6,1994)
HER2/neu$_{369-377}$	KIFGSLAFL (SEQ ID NO: 133)	EXTRACELLULAR	Fisk, B.(J.Exp.Med. 181:2109-17, 1995)
HER2/neu$_{654-662}$	IISAVVGIL (SEQ ID NO: 134)	TRANSMEMBRANE	Peoples,G.(P.N.A.S 92:432-6, 1995)
HER2/neu$_{789-797}$	CLTSTVQLV (SEQ ID NO: 135)	INTRACELLULAR	Disis,M.(CancerRes. 54:1071-6,1994)

Table 37
Synthetic peptide constructs with HER2/neu$_{48-56}$

DESIGNATION	PEPTIDE SEQUENCE
HER - HER2/neu$_{48-56}$	**HLYQGCQVV** (SEQ ID NO: 132)
ES-HER$_{48-56}$	MRYMILGLLALAAVCSA **HLYQGCQVV** (SEQ ID NO: 136)
HER$_{48-56}$-ES	**HLYQGCQVV** MRYMILGLLALAAVCSA (SEQ ID NO: 137)
IS- HER$_{48-56}$	MTNKCLLQIALLLCFSTTALS **HLYQGCQVV** (SEQ ID NO:138)
HER$_{48-56}$ - IS	**HLYQGCQVV** MTNKCLLQIALLLCFSTTALS (SEQ ID NO: 139)
HER$_{48-56}$-IN-ES	MR **HLYQGCQVV** AAVCSA (SEQ ID NO: 140)
HER$_{48-56}$-IN-AF	MA **HLYQGCQVV** AAAAAG (SEQ ID NO: 141)

Synthetic peptide constructs;
1. Peptide antigen HER2/neu$_{48-56}$
2. Adenoviral signal sequence ES attached to the atnino-terminus of HER2/neu$_{48-56}$
3. Adenoviral signal sequence ES attached to the carboxy-terminus of HER2/neu$_{48-56}$
4. Interferon signal sequence IS attached to the amino-terminus of HER2/neu$_{48-56}$

5. Interferon signal sequence IS attached to the carboxy-terminus of $HER2/neu_{48-56}$
6. Peptide antigen $HER2/neu_{48-56}$ replacing the hydrophobic portion of ES
7. Peptide antigen $HER2/neu_{48-56}$ incorporated into an artificial signal sequence - AF

Table 38
Synthetic peptide constructs with $HER2/neu_{369-377}$

DESIGNATION	PEPTIDE SEQUENCE
HER - $HER2/neu_{369-377}$	**KIFGSLAFL** (SEQ ID NO: 133)
ES- $HER2_{369-377}$	MRYMILGLLALAAVCSA **KIFGSLAFL** (SEQ ID NO: 142)
$HER2_{369-377}$-ES	**KIFGSLAFL** MRYMILGLLALAAVCSA (SEQ ID NO: 143)
IS- $HER2_{369-377}$	MTNKCLLQIALLLCFSTTALS **KIFGSLAFL** (SEQ ID NO: 144)
$HER2_{369-377}$-IS	**KIFGSLAFL** MTNKCLLQIALLLCFSTTALS (SEQ ID NO: 145)
$HER2_{369-377}$-IN-ES	M R **KIFGSLAFL** A A V C S A (SEQ ID NO: 146)
$HER2_{369-377}$-IN-AF	M A **KIFGSLAFL** A A A A A G (SEQ ID NO: 147)

Table 39
Synthetic peptide constructs with $HER2/neu_{654-622}$

DESIGNATION	PEPTIDE SEQUENCE
HER - $HER2/neu_{654-622}$	**IISAVVGIL** (SEQ ID NO: 134)
ES - $HER_{654-622}$	MRYMILGLLALAAVCSA **IISAVVGIL** (SEQ ID NO: 148)
$HER_{654-622}$-ES	**IISAVVGIL** MRYMILGLLALAAVCSA (SEQ ID NO: 149)
IS-$HER_{654-622}$	MTNKCLLQIALLLCFSTTALS **IISAVVGIL** (SEQ ID NO: 150)
$HER_{654-622}$-IS	**IISAVVGIL** MTNKCLLQIALLLCFSTTALS (SEQ ID NO: 151)
$HER_{654-622}$-IN-ES	M R **IISAVVGIL** A A V C S A (SEQ ID NO: 152)
$HER_{654-622}$-IN-AF	M A **IISAVVGIL** A A A A A G (SEQ ID NO: 153)

Table 40
Synthetic peptide constructs with HER2/ neu$_{789-797}$

DESIGNATION	PEPTIDE SEQUENCE
HER-HER2/neu$_{789-797}$	**CLTSTVQLV** (SEQ ID NO: 135)
ES-HER$_{789-797}$	MRYMILGLLALAAVCSA **CLTSTVQLV** (SEQ ID NO: 154)
HER$_{789-797}$-ES	**CLTSTVQLV** MRYMILGLLALAAVCSA (SEQ ID NO: 155)
IS- HER$_{789-797}$	MTNKCLLQIALLLCFSTTALS **CLTSTVQLV** (SEQ ID NO: 156)
HER$_{789-797}$-IS	**CLTSTVQLV** MTNKCLLQIALLLCFSTTA (SEQ ID NO: 157)
HER$_{789-797}$-IN-ES	M R **CLTSTVQLV** A A V C S A (SEQ ID NO: 158)
HER$_{789-797}$-IN-AF	M A **CLTSTVQLV** A A A A A G (SEQ ID NO: 159)

Table 41
^{51}Cr-release assay using T2 cells pulsed with HER2/neu-derived peptides as targets for CTL

E:T ratio	50:1	25:1	12:1	6:1	3.1	1.5:1
T2	1	2	1	0	1	0
T2 pulsed with HER2/neu48-56	88	53	41	33	19	8
T2 pulsed with HER2/neu$_{369-377}$	94	66	57	42	23	12
T2 pulsed with HER2/neu$_{654-662}$	91	71	59	38	26	13
T2 pulsed with HER2/neu$_{789-797}$	83	62	52	31	22	9

EXAMPLE 11
Signal Sequences Containing HER2/neu Peptides

[0134] Since signal sequences do not contain specific amino acid residues other than a hydrophobic region of about eight residues, it was tested whether replacing this region with the hydrophobic HER2/neu peptides would result in a more efficient presentation of these epitopes (Figures 8-11). It was found that one of the two constructs of this type (HER-IN-AF) was the most efficient in facilitating the HER2/neu peptide presentation. Eight days after loading with the construct HER$_{369-377}$-IN-AF, T2 cells were still lysed with more than 60% specific ^{51}Cr-release (Figure 9). The constructs HER$_{48-56}$-IN-AF and

$HER_{654-662}$-IN-AF were also effective (Figures 8 and 10). The second construct of this type (HER-IN-ES), although not as effective as HER-IN-AF, was able to facilitate the recognition of T2 cells (Figures 8-10). Pulsing of T2 cells with these constructs did not resulted in efficient presentation. Again, as in the first group of experiments, loading or pulsing with the minimal HER2/neu peptides resulted in recognition of T2 cells for only a short period of time.

[0135] In interferon gamma-release assays, 10^5 HER2/neu-specific CTL were co-incubated with 10^5 peptide-loaded T2 cells for 20 hours at 37°C. The concentrations of human interferon gamma in co-cultured supernatants were then determined by ELISA. The results of the ELISA experiments are shown in table 37 A-D. These findings are in parallel with the ^{51}Cr-release experiments, and confirm that the most efficient constructs in facilitating the HER2/neu peptide presentation are the constructs of the type HER-IN-AF. As in the ^{51}Cr-release experiments, the constructs with the peptides $HER_{369-377}$ and $HER_{654-662}$ were the most efficient, while the constructs with the peptide $HER_{789-797}$ were the least efficient, especially on days 5 and 8 after peptide loading.

Table 42

Release of IFNγ bv CTL after incubation with non-loaded or peptide-loaded T2 cells

A. Day 1 after peptide loading

CTL elicited with:	**Stimulators in ELISA assays: T2 cells loaded with:[a]**							
	---	HER	ES-HER	HER-ES	IS-HER	HER-IS	HER-IN-ES	HER-IN-AF
$HER2/neu_{48-56}$	210[b]	2280	2894	418	3268	288	3368 2227	3488 3288
$HER2/neu_{369-377}$	186	3120	3368	172	2120	212	2929 246	3321 296
$HER2/neu_{654-662}$	121	2827	2667	144	2590	111		
$HER2/neu_{789-797}$	234	2924	1824	58	1717	69		

B. Day 3 after peptide loading

CTL elicited with:	Stimulators in ELISA assavs: T2 cells loaded with:[a]							
	—	HER	ES-HER	HER-ES	IS-HER	HER-IS	HER-IN-ES	HER-IN-AF
HER2/neu$_{48-56}$	129[b]	488	1876	218	1264	148	1349 2029	1229
HER2/neu$_{369-377}$	143	127	2377	142	1818	112	2773 312	2401
HER2/neu$_{654-662}$	111	429	2518	124	1990	99 315		2981 327
HER2/neu$_{789-797}$	215	317	526	78	78			

C. Day 5 after peptide loading

CTL elicited with:	Stimulators in ELISA assays: T2 cells loaded with: [a]							
	---	HER	ES-HER	HER-ES	IS-HER	HER-IS	HER-IN-ES	HER-IN-AF
HER2/neu$_{48-56}$	181[b]	134	953	99	943	155	988	1010 1663
HER2/neu$_{369-377}$	111	211	1073	121	1323	117	1773 1699	1892
HER2/neu$_{654-662}$	97	121	1245	137	1557	121	121	178
HER2/neu$_{789-797}$	136	116	168	69	125	87		

D. Day 8 after peptide loading

CTL elicited with:	**Stimulators in ELISA assays: T2 cells loaded with:** [a]							
	---	HER	ES-HER	HER-ES	IS-HER	HER-IS	HER-IN-ES	HER-IN-AF
HER2/neu$_{48-56}$	116-	177	589	101	614	121	228	718 690
HER2/neu$_{369-377}$	93	89	592	118	545	103	878 671	881
HER2/neu$_{654-662}$	115	167	581	83 98	615	76 104	107	117
HER2/neu$_{789-797}$	88	91	110			59		

[a]CTL were coincubated with stimulator cells (non-loaded or peptide-loaded T2 cells) for 20 h. The concentration of IFNγ in coculture supernatants was then determined by ELISA.

[b]IFNγ (pg/ml) - mean numbers of IFNγ release in triplicate wells with 10^5 CTL/well.

EXAMPLE 12
Testing the Effectiveness of the Fusion Peptides with Breast Cancer Cells

[0136] It was tested whether the most effective signal sequence constructs, already selected in the experiments with the TAP-deficient T2 cells, can also improve HER2/neu antigen presentation in human breast cancer cells.

[0137] In this series of studies the HLA-A2+ human breast cancer cell line MCF-7 expressing high levels of HER2/neu and the cell line MDA-MB-231 expressing only

basal levels of HER2/neu were used. $HER2/neu_{369-377}$-specific CTL and $HER2/neu_{654-662}$-specific CTL failed to recognize the breast cancer cell line MDA-MB-231, although the same effectors specifically recognized T2 cells pulsed with $HER2/neu_{369-377}$, $HER2/neu_{654-662}$ and the cell line MCF7 expressing HER2/neu (Table 38). Thus, it was concluded that MDA-MB-231 cells do not express $HER2/neu_{369-377}$ and $HER2/neu_{654-662}$, and that this cell line was appropriate for our peptide-loading experiments.

[0138] ^{51}Cr-release assays were used to test to see if the low HER2/neu-expressing breast cancer cells MDA-MB-231 can be recognized more efficiently by the HER2/neu-specific CTL after loading with the fusion peptides. Determination was also made by ELISA to see if the peptide-loaded breast cancer cells can induce release of interferon gamma by the HER2/neu-specific CTL.

[0139] The lysis of the tumor cells by the HER2/neu-specific CTL was monitored in a conventional ^{51}Cr-labeling release assay. Briefly, peptide-loaded tumor cells were added to serially diluted effectors in 96-well microplates. After a 6-h incubation at 37°C, supernatants were harvested and counted in a gamma counter. Results are expressed as the percentage of specific lysis and determined as follows: [(experimental cpm--spontaneous cpm)/(maximum cpm--spontaneous cpm)] x 100.

[0140] Peptide-loaded breast cancer cells MDA-MB-231 were tested for their ability to present HER2/neu peptides at different periods of time after loading or pulsing. Tumor cells loaded with the constructs composed of signal sequence at the amino-terminus of the peptides were recognized by CTL up to eight days after loading (Figures 12-13, left column). In contrast, constructs with carboxy-terminal position of the signal sequence were not efficient, even when ^{51}Cr-release assays were performed immediately after loading. This recognition was not due to surface binding of these constructs since pulsing of the tumor cells with any of the constructs was not efficient (Figures 12-13, right column). Loading or pulsing with the minimal HER2/neu peptides resulted in a significant recognition and lysis of the tumor cells for only one day after loading or pulsing, followed by a rapid decrease of recognition on day 3 and complete lack of recognition on days 5 and 8 after loading or pulsing. This finding suggests that the recognition of the tumor cells resulted from simple binding of the HER2/neu peptides to surface HLA molecules, from which it rapidly dissociated.

[0141] An experiment was also performed to test whether replacing the hydrophobic region of the signal sequences with the HER2/neu peptides would result in a more efficient presentation of these epitopes (Figures 14-15). The construct HER-IN-AF was found to be the most efficient in facilitating the HER2/neu peptide presentation. Eight days after loading with the construct $HER_{369-377}$-IN-AF, the tumor cells were still lysed (Figure 14). The construct $HER_{654-662}$-IN-AF was also effective (Figure 15). The second construct of this type (HER-IN-ES), although not as effective as HER-IN-AF, was able to facilitate the recognition of the tumor cells (Figures 14-15). Pulsing of the tumor cells with these constructs did not resulted in efficient presentation. Loading or pulsing with the minimal HER2/neu peptides resulted in recognition of the tumor cells for only a short period of time.

[0142] In interferon gamma release assays, 10^5 HER2/neu-specific CTL were co-incubated with 10^5 peptide-loaded tumor cells for 20 hours at 37°C. The concentration of human interferon gamma in co-cultured supernatants was then determined by ELISA. The results of the ELISA experiments are shown in Table 39 A-D. These findings are in parallel with the ^{51}Cr-release experiments, and confirm that the most efficient constructs in facilitating the HER2/neu peptide presentation are the constructs of the type HER-IN-AF.

Table 43
Lack of recognition of breast cancer cell line MDA-MB-231 by CTL reactive against $HER2/neu_{369-377}$ and $HER2/neu_{654-622}$

			Percent ^{51}Cr Released From[a]:		
Effectors	**E:T**	**T2**	**T2-pulsed[b]**	**MCF7**	**MDA-MB-231**
$CTL_{369-377}$	40:1	2 1	94 73	68	2
	20:1			33	1
$CTL_{654.662}$	40:1	1 2	71	58	2 31
	20:1		57	0	

a) Cytotoxicity was evaluated in a 6-hour ^{51}Cr-release assay.
b) T2 cells were pulsed with $HER2/neu_{369-377}$, or $HER2/neu_{654-622}$ at 1 µg/ml for 2 hours at 37°C, labeled with ^{51}Cr and used as targets.

Table 44
Release of IFNγ by CTL after incubation with non-loaded or peptide-loaded breast cancer cells MDA-MB-231

A. Day 1 after peptide loading

CTL elicited with:	Stimulators in ELISA assays: T2 cells loaded with: [a]							
	---	HER	ES-HER	HER-ES	IS-HER	HER-IS	HER-IN-ES	HER-IN-AF
HER2/neu$_{369-377}$	177[b]	2820	3688	182	2126	224	2627	3381
HER2/neu$_{654-622}$	153	2826	2767	188	2678	144	2727	3321

B. Day 3 after **peptide loading**

CTL elicited with:	Stimulators in ELISA assays: T2 cells loaded with: [a]							
	---	HER	ES-HER	HER-ES	IS-HER	HER-IS	HER-IN-ES	HER-IN-AF
HER2/neu$_{369-377}$	138[b]	141	2417	187	1777	131	2187	2347
HER2/neu$_{654-622}$	121	438	2622	138	1974	102	2666	2994

C. Day 5 after peptide loading

CTL elicited with:	Stimulators in ELISA assays: T2 cells loaded with: [a]							
	---	HER	ES-HER	HER-ES	IS-HER	HER-IS	HER-IN-ES	HER-IN-AF
HER2/neu$_{369-377}$	122[b]	274	1278	137	1444	128	1778	1897
HER2/neu$_{654-622}$	102	131	1445	135	1604	132	1708	1933

D. Day 8 after peptide loading

CTL elicited with:	Stimulators in ELISA assays: T2 cells loaded with: [a]							
	---	HER	ES-HER	HER-ES	IS-HER	HER-IS	HER-IN-ES	HER-IN-AF
HER2/neu$_{369-377}$	102[b]	99	577	122	587	113	687	889
HER2/neu$_{654-622}$	125	147	578	93	628	86	667	899

[a]CTL were coincubated with stimulator cells (non-loaded or peptide-loaded MDA-MB-231 cells) for 20 h. The concentration of IFNg in coculture supernatants was then determined by ELISA. [b]IFNg (pg/ml) - mean numbers of IFNg release in triplicate wells with 10^5 CTL/well.

EXAMPLE 13
Identification of the Mechanisms Involved in the Enhancement of Antigen Presentation by the Fusion Peptides

[0143] The goal of this set of experiments was to prove that the effective presentation of the loaded peptide constructs is a result of their efficient loading into the cytosol and not simple binding to the surface HLA molecules. The role of TAP in class I presentation in human cancer cells was also tested, along with a test of the efficiency of different signal peptides in cancer cells with different levels of TAP expression.

EXAMPLE 14
Probing the Mechanisms of Peptide Loading

[0144] To distinguish between loading of the peptides into the cytosol and simple binding of these peptides to the surface MHC molecules several approaches were used. First, β_2-microglobulin was removed from the surface of peptide-loaded tumor cells by acid stripping. It was found that acid-stripping solution with pH=3.5 was most efficient in decreasing the specific recognition of peptide-loaded cells. Second, pronase was used for complete enzymatic digestion of HLA molecules on the cell surface after loading in order to be able to detect the appearance of new internally formed HLA-peptide complexes on the cell surface, but not pulsing of the cells. Third, Brefeldin A (BFA), a metabolite of the fungus *Eupenicillium brefeldianum*, was used which specifically blocks protein transport from the ER to Golgi apparatus.

[0145] It was found that Brefeldin A specifically blocks the recognition of the peptide-loaded tumor cells by the HER2/neu-specific CTL. In contrast, the acid stripping and the treatment with pronase was not able to block antigen recognition for more than 24 hours (Table 40). These experiments confirmed that the antigenic peptides were introduced into the cytosol of the cells, resulting in a prolonged and more efficient antigen presentation.

Table 45
Mechanisms of peptide loading: Recognition of breast cancer cells MDA-MB-231 by CTL reactive against HER2/neu$_{369\text{-}377}$ and HER2/neu$_{654\text{-}622}$

		Percent ^{51}Cr Released From MDA-MB-231 cells treated with[a]:			
Effectors	**E:T**	**acid**	**pronase**	**brefeldin**	**non-treated**
CTL$_{369\text{-}377}$	40:1	62 41	59	18 7	62 39
	20:1		33		
CTL$_{654\text{-}662}$	40:1	61 28	61 37	13 6	58 30
	20:1				

a) Cytotoxicity was evaluated in a 6-hour ^{51}Cr-release assay 3 days after peptide loading

EXAMPLE 15
Inducing a Functional Blockade of TAP by ICP47

[0146] Another aspect in these studies was to determine the mechanisms of enhancement of the antigen presentation by the fusion peptides in human tumor cell lines. Therefore, a new test system was developed utilizing the Herpes Simplex virus (HSV) protein ICP47. ICP47 is a cytoplasmic protein, which interferes with antigen presentation by physically associating with TAP within the cell and inhibiting peptide transport across the ER-membrane. By transfecting the ICP47 gene into several cancer cell lines a novel system for screening different fusion peptides for TAP-**in**dependent translocation of peptide antigens through the ER-membrane was generated.

[0147] The breast cancer cell line MCF7 was transfected with ICP47, and observed permanent block of the function of TAP, and therefore lack of recognition of these cells by the CTL, which normally recognize and kill them. To select the sequences most effective in translocation of antigenic peptides across the ER-membrane of the breast cancer cells, the ICP47-transfected cells were loaded with several fusion peptides with different signal sequences. The expression of these antigens was detected by ^{51}Cr-release assays. It was found that only the most efficient peptide constructs - HER$_{369\text{-}377}$-IN-AF and HER$_{654\text{-}662}$-IN-AF - were able to restore the antigen presentation in the ICP47-transfected breast cancer cells (Table 41). This confirms that the signal sequence approach is very effective in improving antigen presentation, even in tumor cells with deficiency of antigen processing/presentation.

Table 46
Mechanisms of peptide loading: Recognition of ICP47-transfected cells MCF7 by CTL reactive against HER2/neu$_{369-377}$ and HER2/neu$_{654-622}$

Effectors	E:T	Percent ^{51}Cr Released From MCF7 cells loaded with[a]:					
		MCF7	MCF7-ICP47	ES-HER	IS-HER	HER-IN-ES	HER-IN-AF
CTL$_{369-377}$	40:1	62	3	1 2	2 3	4	58
	20:1	29	3			3	24
CTL$_{654-662}$	40:1	74 31	4 2	3	5	2	66
	20:1			2	3	2	22

a) Cytotoxicity was evaluated in a 6-hour ^{51}Cr-release assay 3 days after peptide loading

EXAMPLE 16
Loading of Dendritic Cells with the Fusion Peptides

[0148] Human dendritic cells (DC) derived from healthy donors were utilized. The nonamer HER2/neu peptides were introduced alone, fused to, or included within, synthetic signal sequences into the cytosol of DC with a technology called "osmotic lysis of pinocytic vesicles." With a standard ^{51}Cr-release assay, the ability of HER2/neu-specific tumor infiltrating lymphocytes (TIL) to recognize peptide-loaded DC at various intervals after loading was tested. Significant lysis of DC loaded with a peptide construct composed of a signal sequence fused to the amino-terminus was observed, but not the carboxy-terminus of HER2/neu peptide (Figure 16). Of all constructs tested, DC loaded with the HER2/neu peptide included within an artificial signal sequence were recognized most efficiently, for at least 6 days after loading (Figure 17). DC loaded with the minimal peptide were only marginally recognized. In all of our experiments, non-loaded DC were not recognized by the HER2/neu specific CTL. These studies suggest that with signal sequences combined with minimal antigenic peptides, it may be possible to enhance antigen-presentation and stimulation of cytotoxic T lymphocytes. This approach may facilitate the development of synthetic peptide vaccines for human cancer.

EXAMPLE 17
Nanoparticle-Based Synthetic Vaccines for Cancer and Infectious Diseases

[0149] A major obstacle affecting the activity of peptides that function intracellularly is the cytoplasmic delivery. Biomolecules usually enter cells via fluid-phase or receptor-mediated endocytosis, and are initially localized in the endosomal compartment. A high percentage of these biomolecules are subsequently sent to lysosomes, resulting in high levels of protein degradation and thus limiting antigen delivery. Accordingly the design and synthesis of specialized carriers that can enhance the intracellular delivery of biotherapeutics, in particular to overcome the important barrier of lysosomal trafficking, is important for vaccine development.

[0150] A new strategy will be implemented for the design and synthesis of polymeric nanoparticles that enhance the cytoplasmic delivery of the peptide vaccines into the antigen-presenting cells by disrupting the endosomal membrane at the acidic pH of the endosome. These acid-sensitive nanoparticles will be designed to disrupt endosomes and deliver protein antigens into the cytoplasm of antigen-presenting cells (APC) for class I antigen presentation. The nanoparticles will be chemically stable at pH 7.4, but will degrade into linear polymer chains and small molecules under mildly acidic conditions.

[0151] It is hypothesized that tumor/pathogen antigen-derived peptide vaccines encapsulated in acid-sensitive nanoparticles will induce potent and specific CTL responses against cancer and infectious diseases. This approach may provide a potential avenue for vaccine development using the cancer-associated antigens and pathogen-associated antigens described herein.

[0152] The development of nanoparticle-based vaccines is innovative and holds great promise. Like the biological systems, these nanoparticles combine targeting elements that direct cellular uptake, together with the sensing of pH changes within the endosome to activate membrane destabilization and cytosolic delivery. The intrinsic modular design of these nanoparticle-based vaccines makes it possible to customize the targeting and membrane destabilizing activities for a wide range of biotherapeutics and vaccine applications. These vaccines might be used directly to immunize patients with cancer or infectious diseases. In addition, they may be used to generate and expand *in vitro* CTL for adoptive transfer therapies.

EXAMPLE 18
Survivin-Based Synthetic Vaccines for Immunotherapy of Brain Tumors

[0153] Gliomas are among the most common tumors of the central nervous system (CNS). Even with conventional treatments, including surgery, radiation, and chemotherapy, the median survival time for patients with gliomas, is only one year. As these tumors are incurable, the aim of the current conventional treatments is to improve the neurological deficits and to increase survival while maintaining the best possible quality of life. It is has recently been discovered that with Gliomas, there is a significant trafficking of activated T cells through the CNS, and that T cells primed by tumor cells in the periphery can recirculate and reach the brain to mediate their anti-tumor effects.

[0154] A newly described inhibitor of apoptosis, survivin, has been found to induce *in vitro* survivin-specific effector T lymphocytes in healthy donors, as well as in patients with cancer. Most importantly, spontaneous T cell reactivity against survivin in patients with leukemia, melanoma and breast cancer has been observed. The over-expression of survivin in most gliomas and many other human tumors suggests a general role of apoptosis inhibition during tumor progression. Survivin may be an ideal target for the immunotherapy of gliomas because of its strong expression in most gliomas, little or no expression in adult tissues, and its essential role for the survival of the tumor cells.

[0155] The development strategy will be to (i) identify and obtain class I-restricted immunogenic survivin-derived peptides, (ii) generate *in vitro* survivin-specific CTL lines and clones from healthy volunteers and from patients with glioma, (iii) test the ability of the survivin-specific CTL to kill glioma tumor cells *in vitro* in a class I-restricted and survivin-dependent fashion, and (iv) enhance the stability and immunogenicity of the survivin-derived synthetic vaccines. Several survivin peptides have already been observed to expand precursor CTL in PBMC of healthy individuals and induce MHC class I-restricted, peptide-specific CTL responses. Therefore, it is hypothesized that survivin-derived peptides may be used for vaccination of HLA-A2.1 positive cancer patients.

[0156] The identification of immunogenic peptides derived from survivin, a widely expressed tumor antigen, is innovative and holds great promise. Identification of immunogenic survivin peptides will allow for the development of synthetic vaccines for patients with glioma. Furthermore, immunogenic survivin peptides will be used to generate and expand *in vitro* CTL for adoptive transfer therapies, or for dendritic cell-based immunotherapy.

[0157] Although the invention has been described with reference to the above example, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

WHAT IS CLAIMED IS:

1. An isolated class I restricted peptide, wherein the peptide is:
   a) a non-HLA-A2 peptide derived from PRAME (SEQ ID NO: 1);
   b) derived from OFA/iLP (SEQ ID NO: 70);
   c) derived from STEAP (SEQ ID NO: 95); or
   d) derived from SURVIVIN (SEQ ID NO: 193).

2. The peptide of claim 1, wherein the peptide is selected from any one of SEQ ID NOs: 2 to 5.

3. The peptide of claim 1, wherein the peptide is modified by using any of the approaches described in this invention.

4. The peptide of claim 3, wherein the peptide is selected from any one of SEQ ID NOs: 6 to 67.

5. The peptide of claim 1, wherein the peptide is selected from any one of SEQ ID NOs: 71 to 76.

6. The peptide of claim 3, wherein the peptide is selected from any one of SEQ ID NOs: 77 to 94.

7. The peptide of claim 1, wherein the peptide is selected from any one of SEQ ID NOs: 96 to 115.

8. The peptide of claim 3, wherein the peptide is selected from any one of SEQ ID NOs: 116 to 131.

9. The peptide of any one of claims 1-8, wherein a signal sequence is operably linked to the peptide.

10. A method of treating or preventing cancer in a subject comprising administering to the subject a class I restricted peptide of claim 1.

11. The method of claim 10, wherein the cancer is any type of cancer expressing the antigens PRAME, OFA/iLP, STEAP, or SURVIVIN.

12. The method of claim 11, wherein the cancer is lung cancer.

13. The method of claim 11, wherein the cancer is breast cancer.

14. The method of claim 11, wherein the cancer is prostate cancer.

15. The method of claim 11, wherein the cancer is a brain tumor.

16. A vaccine comprising a fusion peptide, wherein the fusion peptide comprises a signal sequence and an antigen-derived peptide, wherein the antigen-derived peptide is derived from an antigen expressed on the surface of a cancer cell or a virus-infected cell.

17. The vaccine of claim 16, wherein the antigen-derived peptide is selected from any one of SEQ ID NOs: 132-159.

18. The vaccine of claim 16, wherein the antigen-derived peptide consists of the peptide according to claims 1-8.

19. The vaccine of claim 16, wherein the cell is a cancer cell and is a cancerous prostate cell, cancerous breast cell or cancerous lung cell.

20. A method of treating or preventing cancer in a subject comprising administration of a vaccine of any of claims 16-19.

21. The method of claim 20, wherein the cancer is prostate cancer, breast cancer or lung cancer.

22. A method of treating or preventing a viral disease in a subject comprising administration of a vaccine of any of claims 16-19.

Figure 1

Amino acid sequence of PRAME

1	MERRRLWGSI QSRYISMSVW TSPRRLVELA GQSLLKDEAL AIAALELLPR ELFPPLFMAA
61	FDGRHSQTLK AMVQAWPFTC LPLGVLMKGQ HLHLETFKAV LDGLDVLLAQ EVRPRRWKLQ
121	VLDLRKNSHQ DFWTVWSGNR ASLYSFPEPE AAQPMTKKRK VDGLSTEAEQ PFIPVEVLVD
181	LFLKEGACDE LFSYLIEKVK RKKNVLRLCC KKLKIFAMPM QDIKMILKMV QLDSIEDLEV
241	TCTWKLPTLA KFSPYLGQMI NLRRLLLSHI HASSYISPEK EEQYIAQFTS QFLSLQCLQA
301	LYVDSLFFLR GRLDQLLRHV MNPLETLSIT NCRLSEGDVM HLSQSPSVSQ LSVLSLSGVM
361	LTDVSPEPLQ ALLERASATL QDLVFDECGI TDDQLLALLP SLSHCSQLTT LSFYGNSISI
421	SALQSLLQHL IGLSNLTHVL YPVPLESYED IHGTLHLERL AYLHARLREL LCELGRPSMV
481	WLSANPCPHC GDRTFYDPEP ILCPCFMPN

Figure 2

Nucleic acid sequence of OFA/iLRP

1 gtcgacccac gcgtccgcta cccggggacg ggtccatacg gcgttgttct tgattcccat
61 cgtaacttaa agggaaactt acacaatgtc cggagccctt gacgtcctgc agatgaagga
121 ggaggatgtc ctcaaattcc ttgctgcggg aacccactta ggtggcacca accttgactt
181 tcagatggag cagtacatct acaaaaggaa aagtgacggt atctacatca taaacctgaa
241 gaggacctgg gagaagctgt tgctcgcagc tcgagctatt gttgccatcg agaatcctgc
301 tgacgtcagc gtcatctcct ccaggaacac tggccagcga gctgtgctga agtttgctgc
361 tgccacagga gccactccga tcgctggccg cttcacacct gggaccttca ctaaccagat
421 ccaagcagcc ttcagggagc cacggcttct agtggtgacc gatcccaggg ctgaccatca
481 gccactcaca gaggcctctt atgtcaacct gcccaccatt gctctgtgta acacagattc
541 tcccctgcgc tatgtggaca ttgccatccc atgcaacaac aagggagctc actcagtggg
601 tctgatgtgg tggatgctgg ccagggaagt actccgcatg cgaggtacta tctcccgtga
661 gcacccctgg gaggtcatgc ctgatcttta cttctacaga gacccagagg agattgagaa
721 ggaggagcag gctgctgctg agaaggctgt gaccaaggag gaattccagg gtgaatggac
781 cgcaccagct cctgagttca ctgctgctca gcctgaggtg gccgactggt ctgagggtgt
841 gcaggttccc tctgtgccca tccagcagtt ccccacggaa gactggagtg cacagccagc
901 cactgaggat tggtcagcag ctcccacagc gcaggccact gagtgggttg gagccaccac
961 tgagtggtcc tgagctgctg tgcaggtgcc tgagcaaagg gaaaaagat ggaaggaaaa
1021 taaagttgct aaaagctgaa aaaaaaaaa aaaaaaa

Amino acid sequence of OFA/iLRP

MSGALDVLQMKEEDVLKFLAAGTHLGGTNLDFQMEQYIYKRKSDGIYIINLKRTWEKLLLAARAIVAIENPA
DVSVISSRNTGQRAVLKFAAATGATPIAGRFTPGTFTNQIQAAFREPRLLVVTDPRADHQPLTEASYVNLP
TIALCNTDSPLRYVDIAIPCNNKGAHSVGLMWWMLAREVLRMRGTISREHPWEVMPDLYFYRDPEEIEKE
EQAAAEKAVTKEEFQGEWTAPAPEFTAAQPEVADWSEGVQVPSVPIQQFPTEDWSAQPATEDWSAAPT
AQATEWVGATTEWS

Figure 3

Amino acid sequence of STEAP

1 MESRKDITNQ EELWKMKPRR NLEEDDYLHK DTGETSMLKR PVLLHLHQTA HADEFDCPSE
61 LQHTQELFPQ WHLPIKIAAI IASLTFLYTL LREVIHPLAT SHQQYFYKIP ILVINKVLPM
121 VSITLLALVY LPGVIAAIVQ LHNGTKYKKF PHWLDKWMLT RKQFGLLSFF FAVLHAIYSL
181 SYPMRRSYRY KLLNWAYQQV QQNKEDAWIE HDVWRMEIYV SLGIVGLAIL ALLAVTSIPS
241 VSDSLTWREF HYIQSKLGIV SLLLGTIHAL IFAWNKWIDI KQFVWYTPPT FMIAVFLPIV
301 VLIFKSILFL PCLRKKILKI RHGWEDVTKI NKTEICSQL

Figure 4

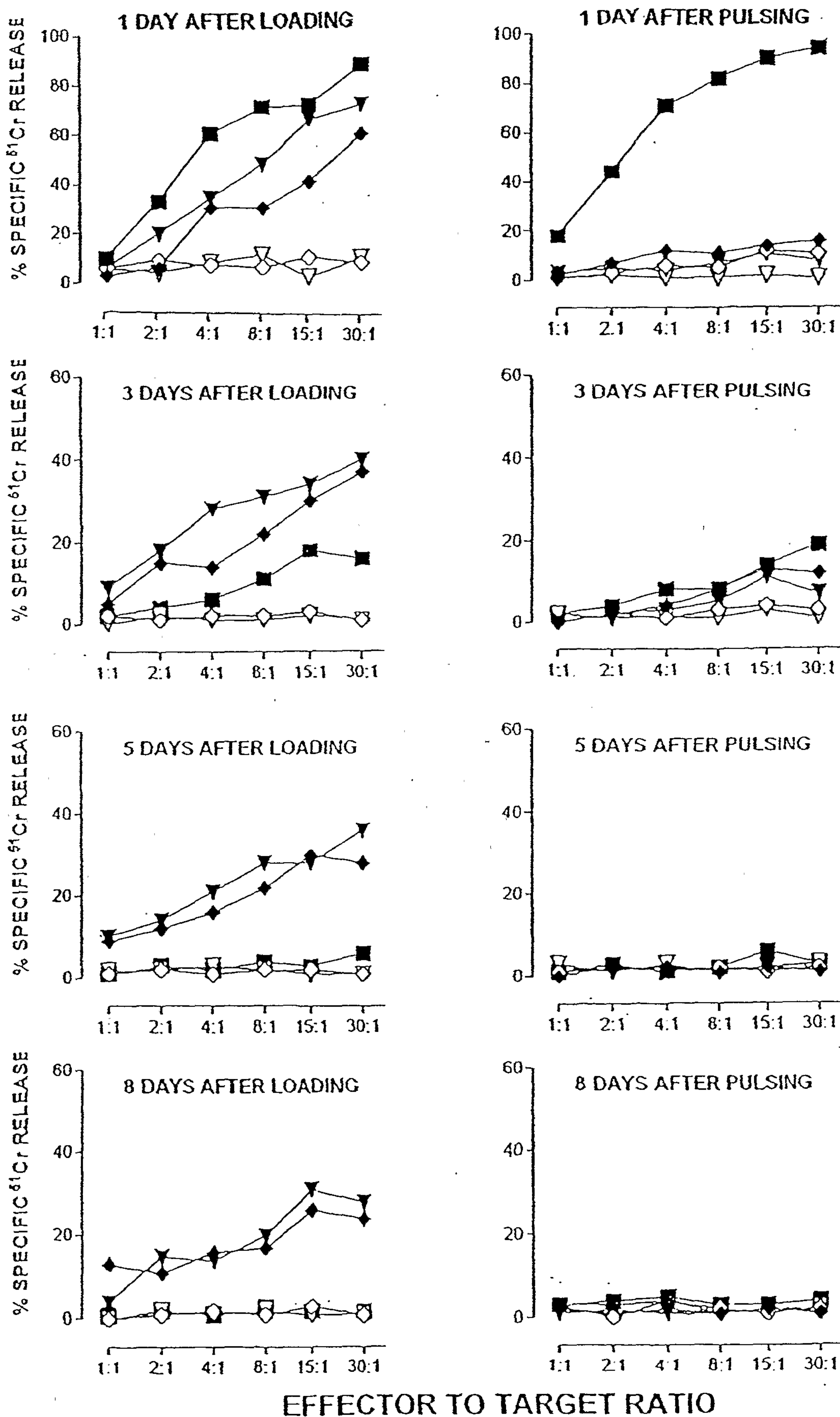

Figure 5

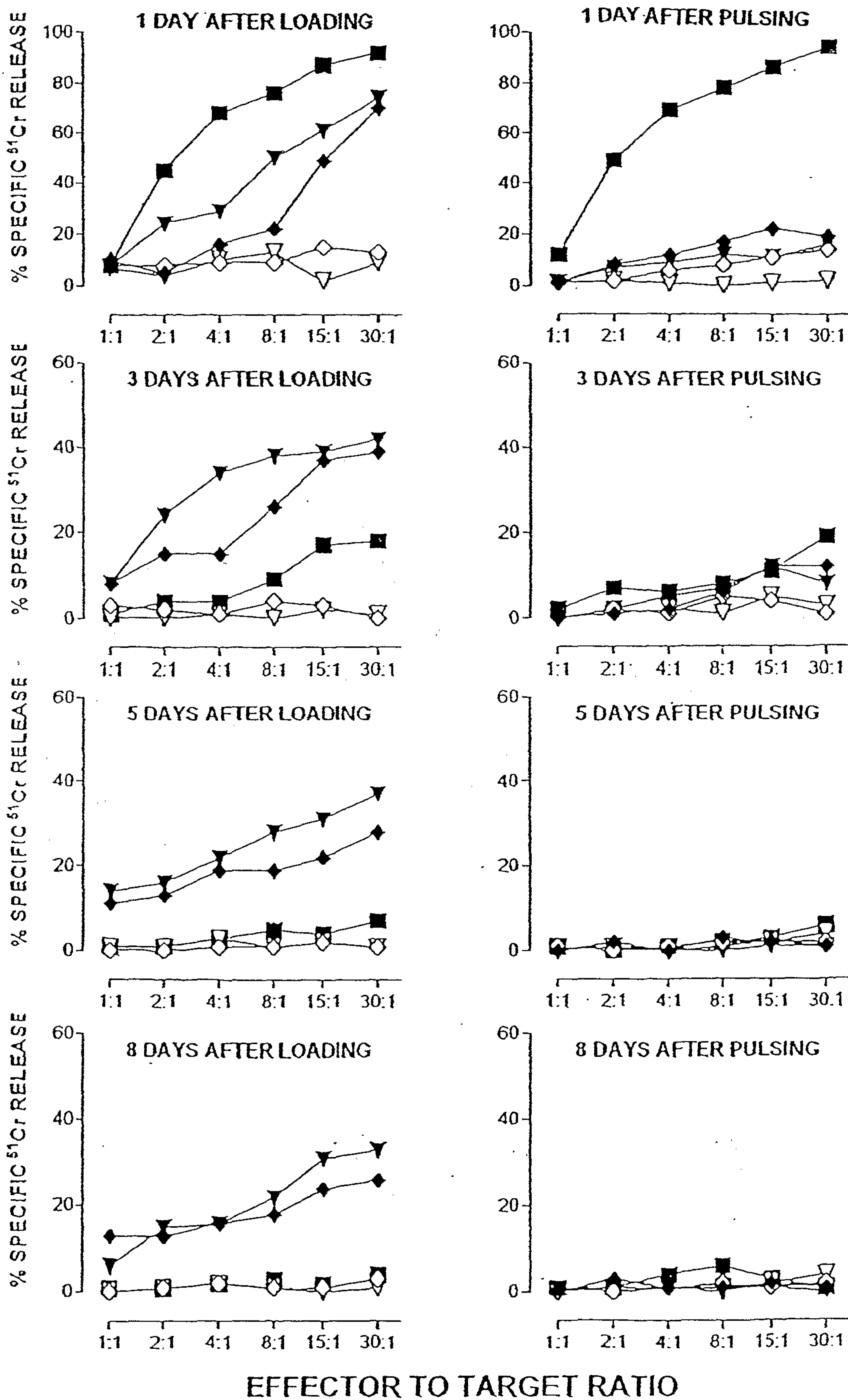

Figure 6

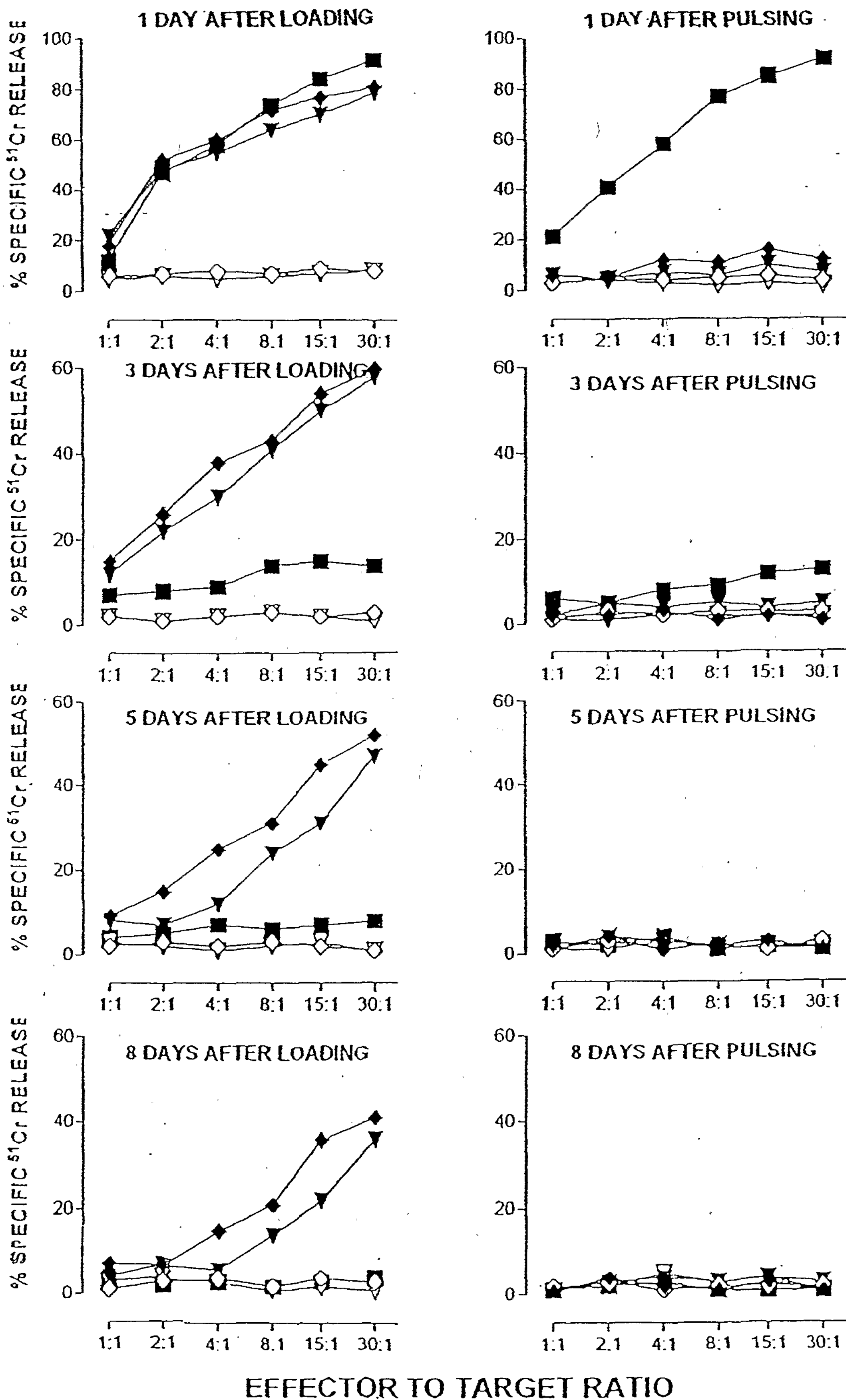

Figure 7

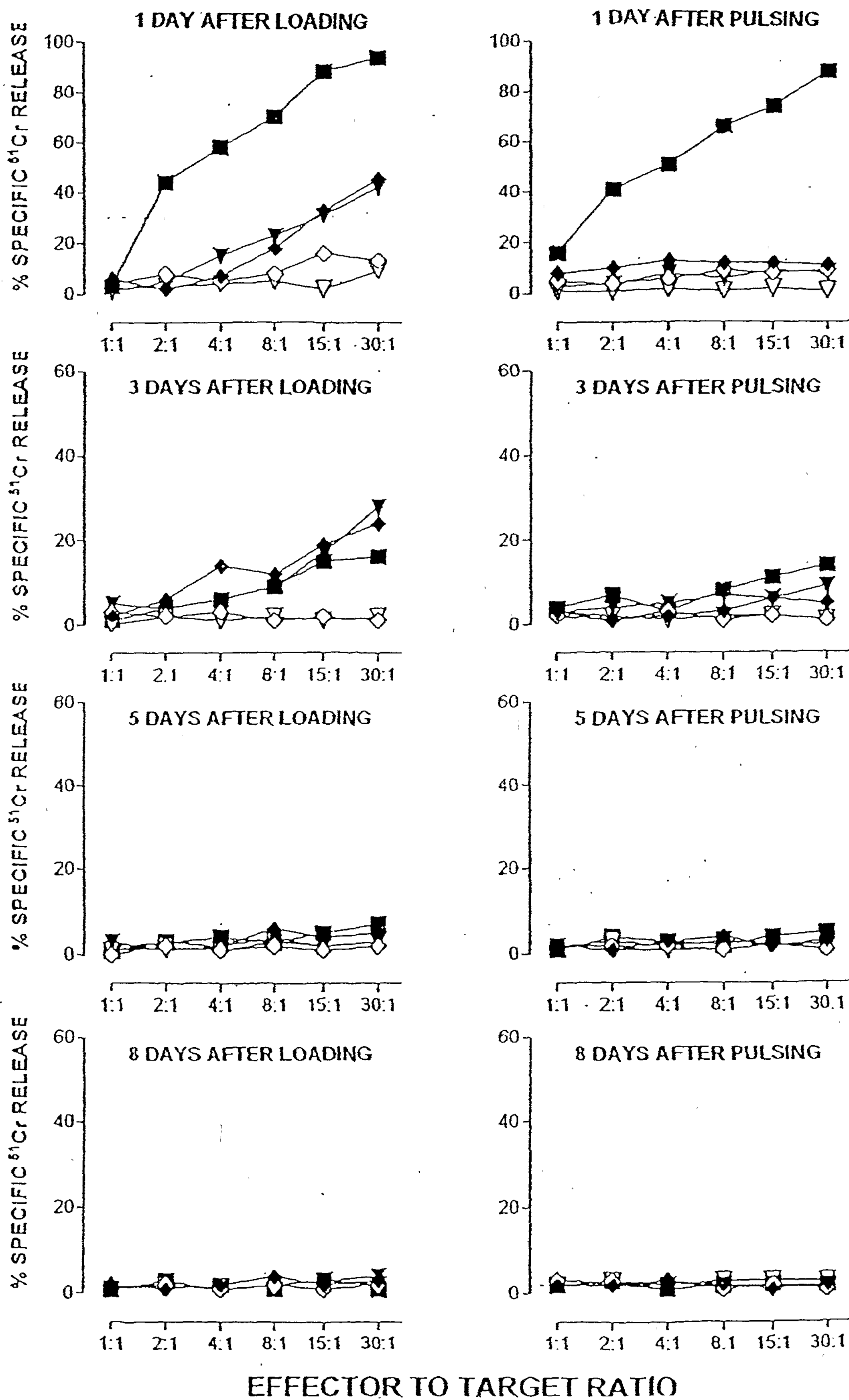

Figure 8

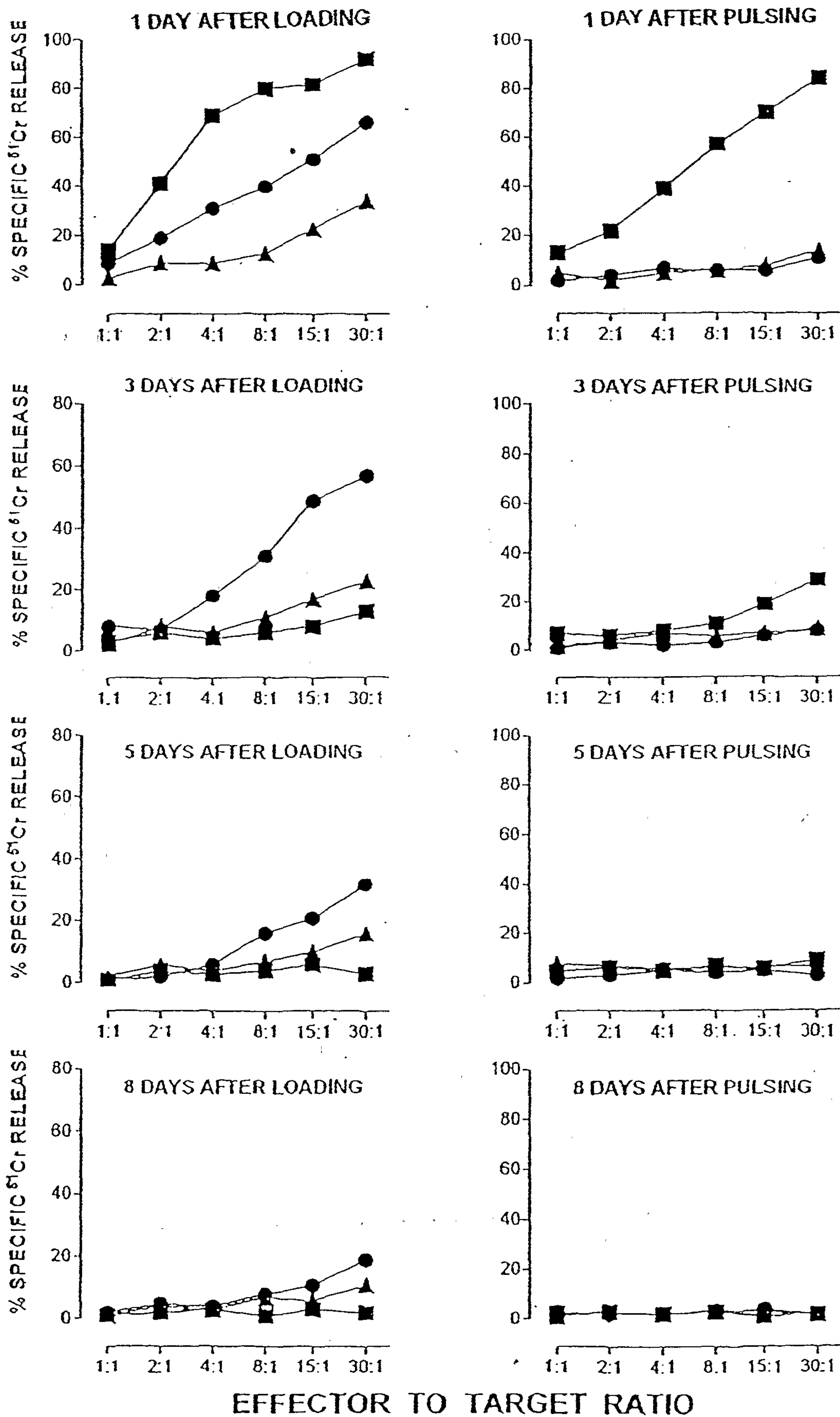

Figure 9

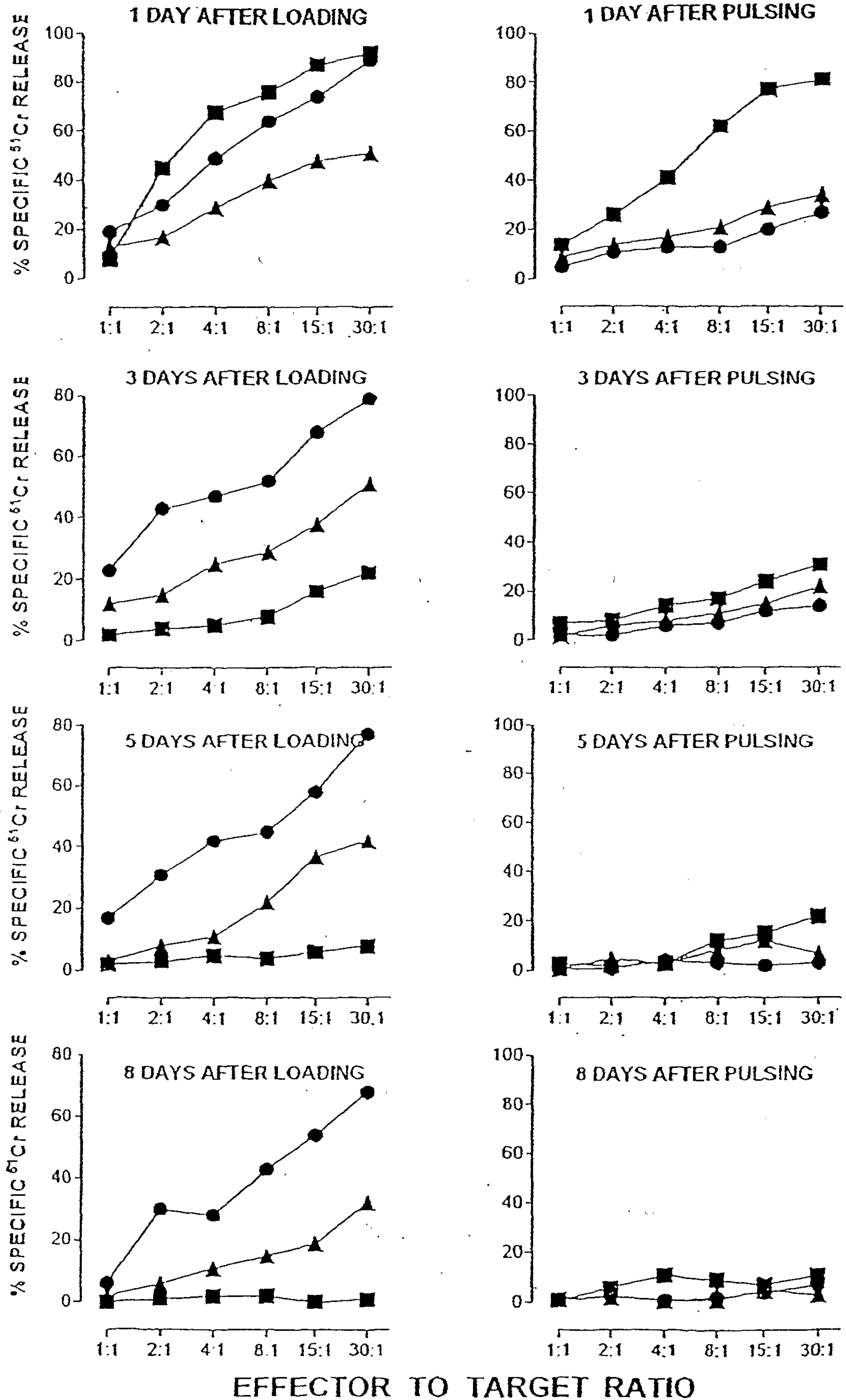

Figure 10

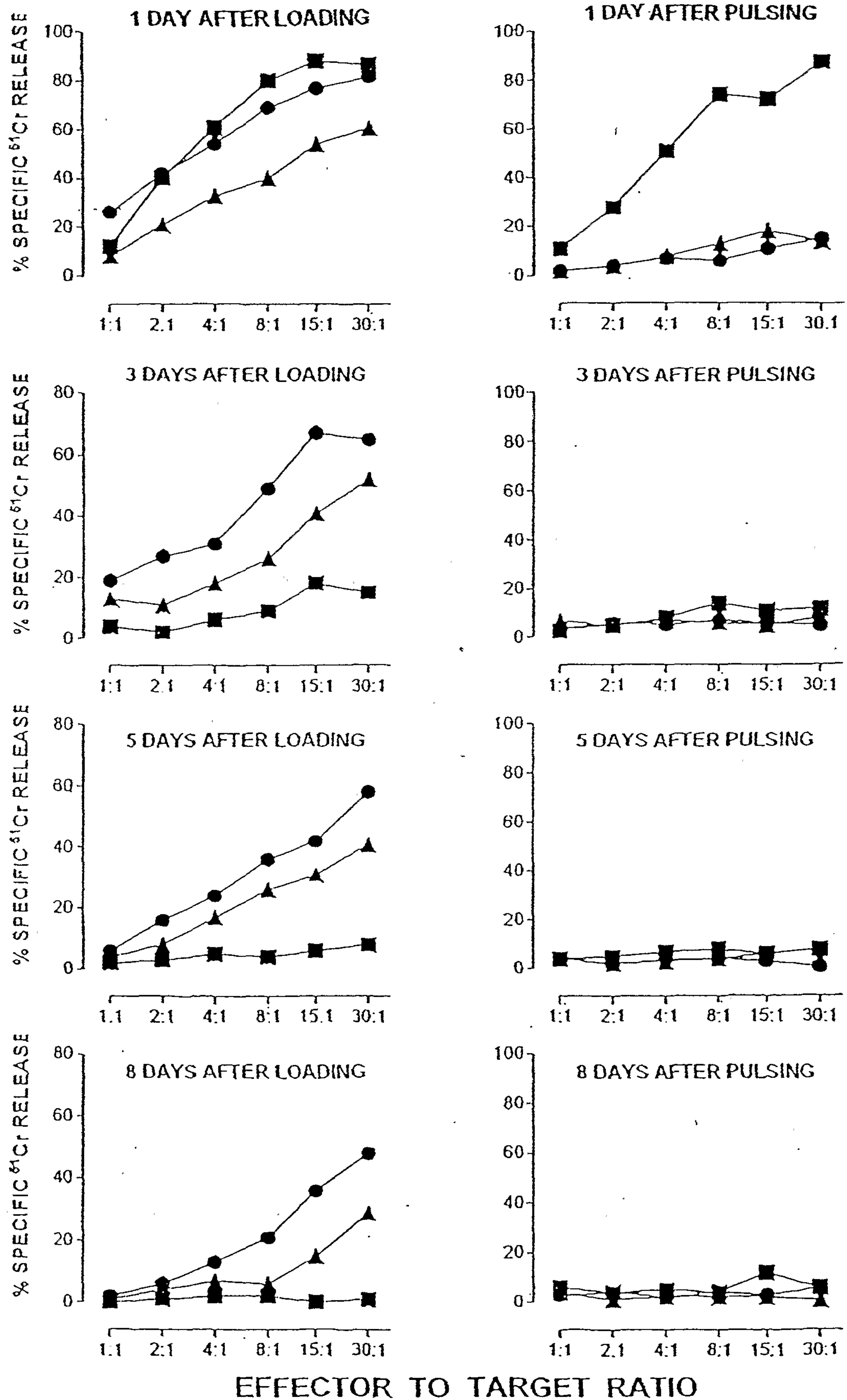

Figure 11

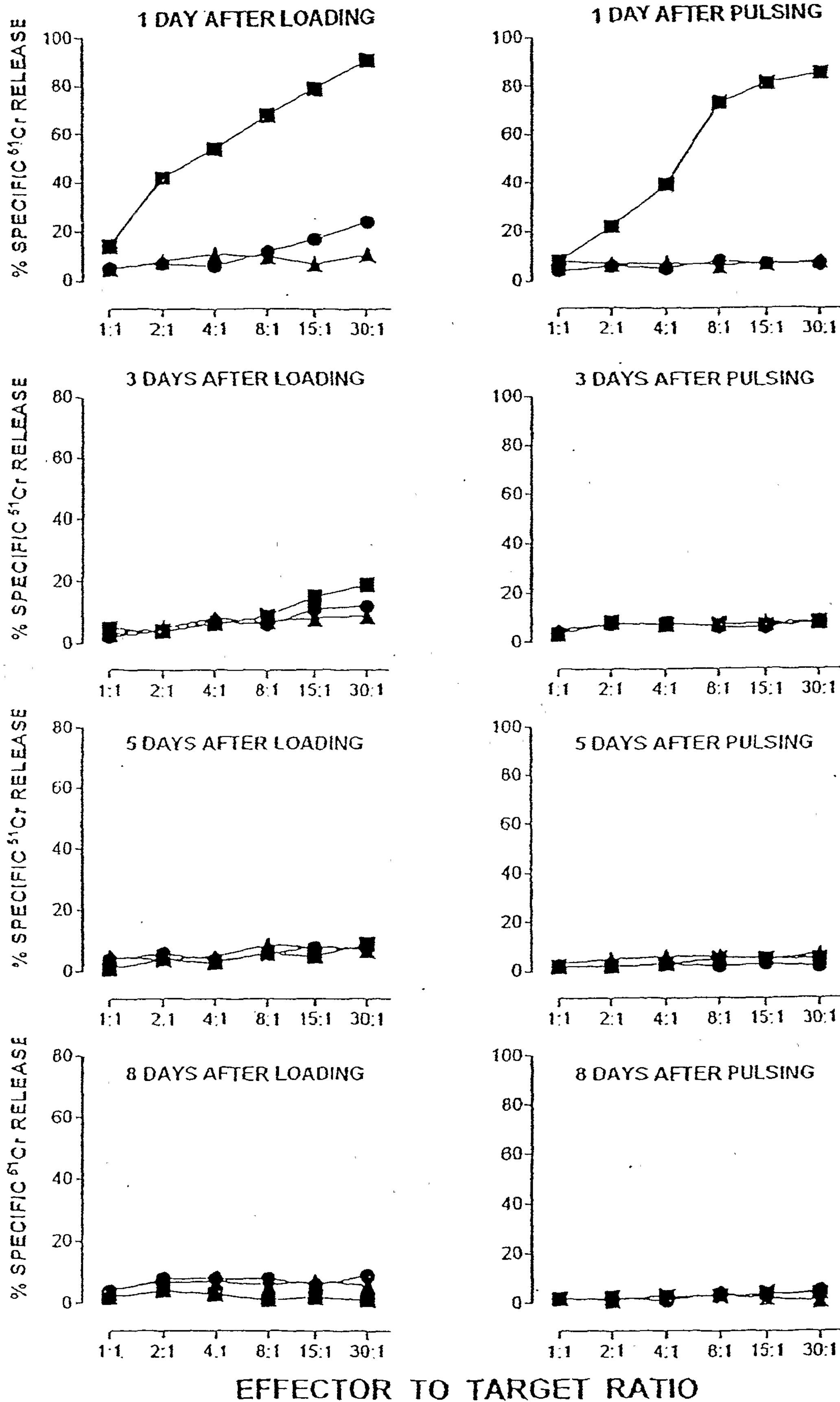

Figure 12

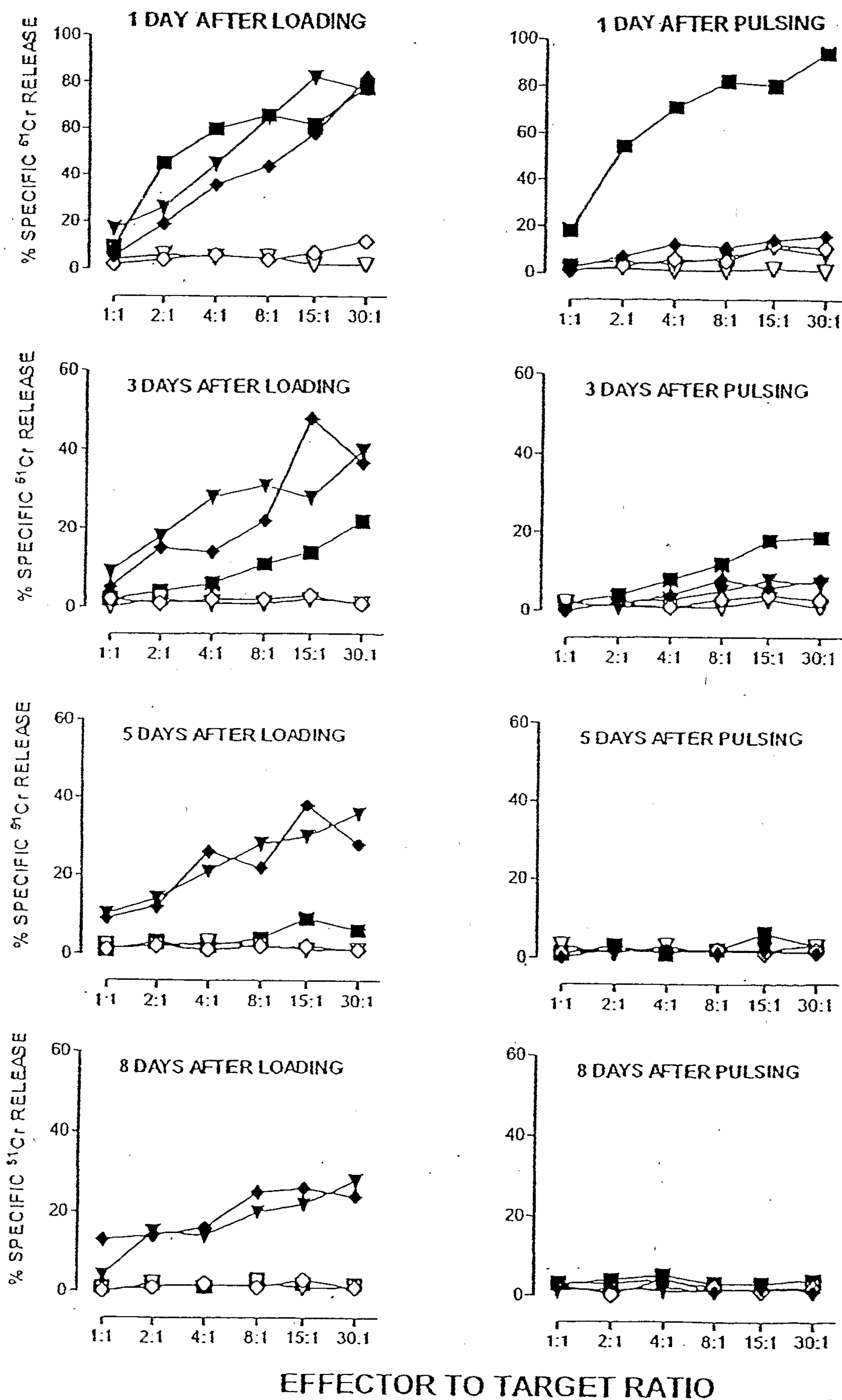

Figure 13

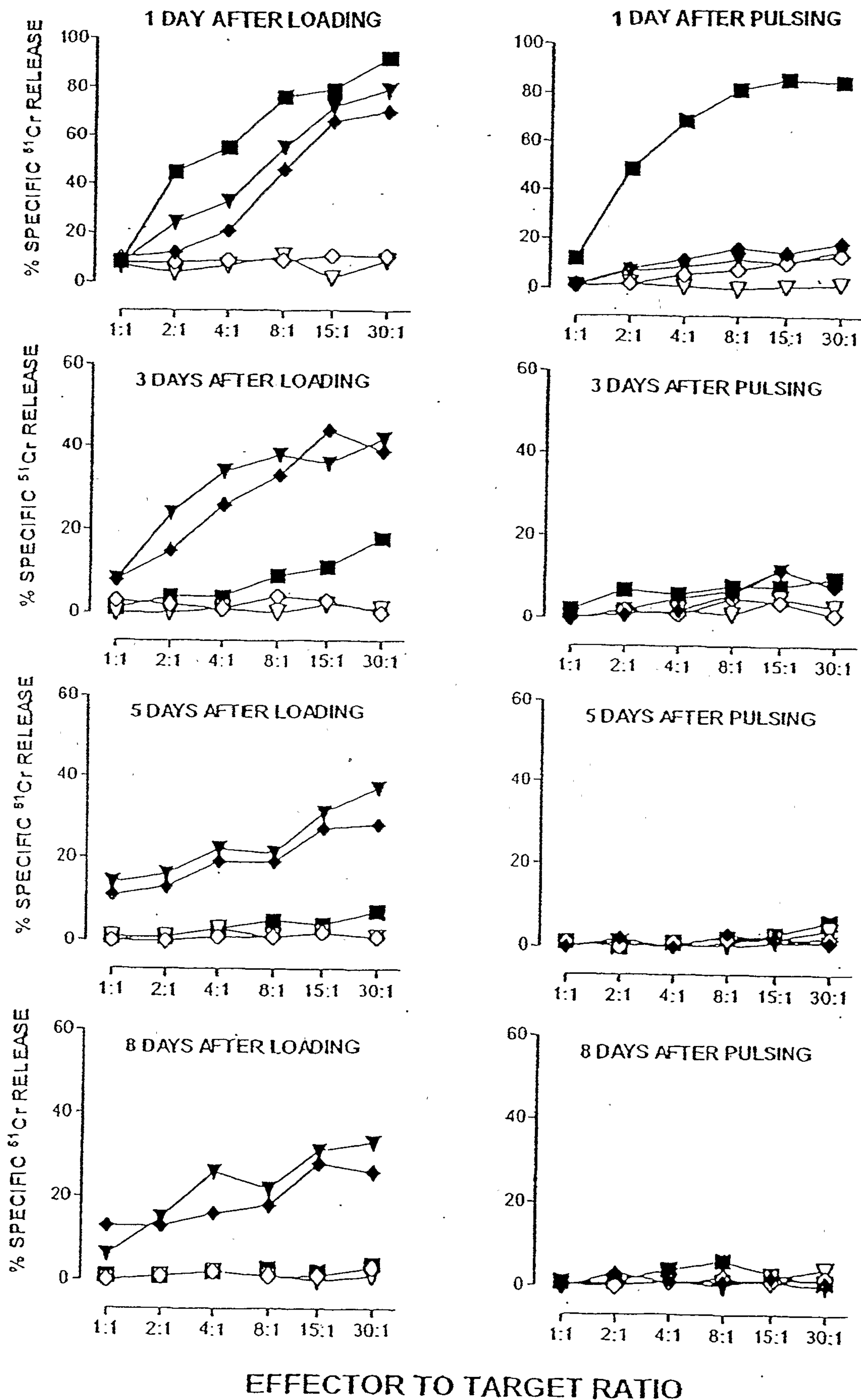

Figure 14

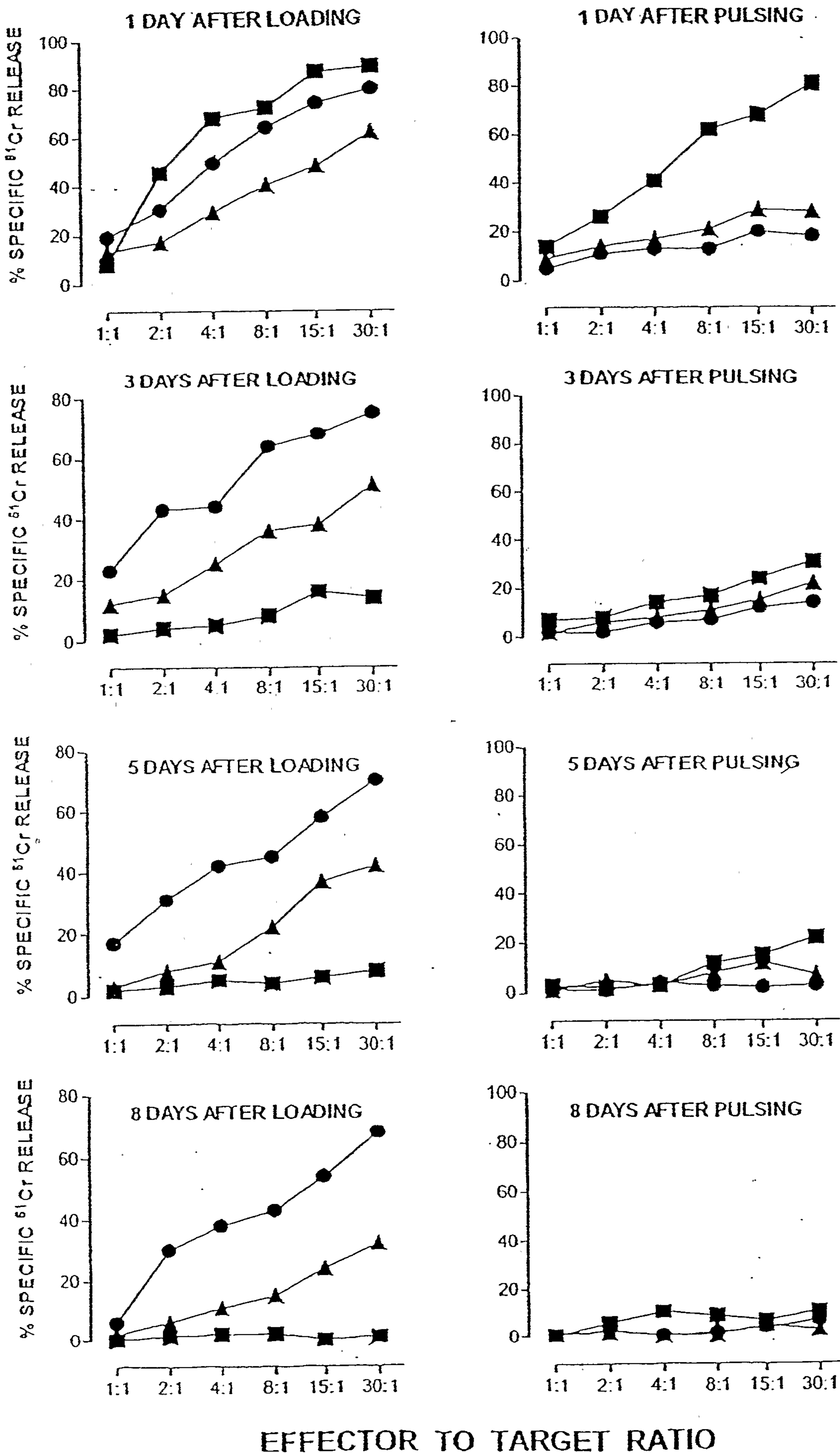

Figure 15

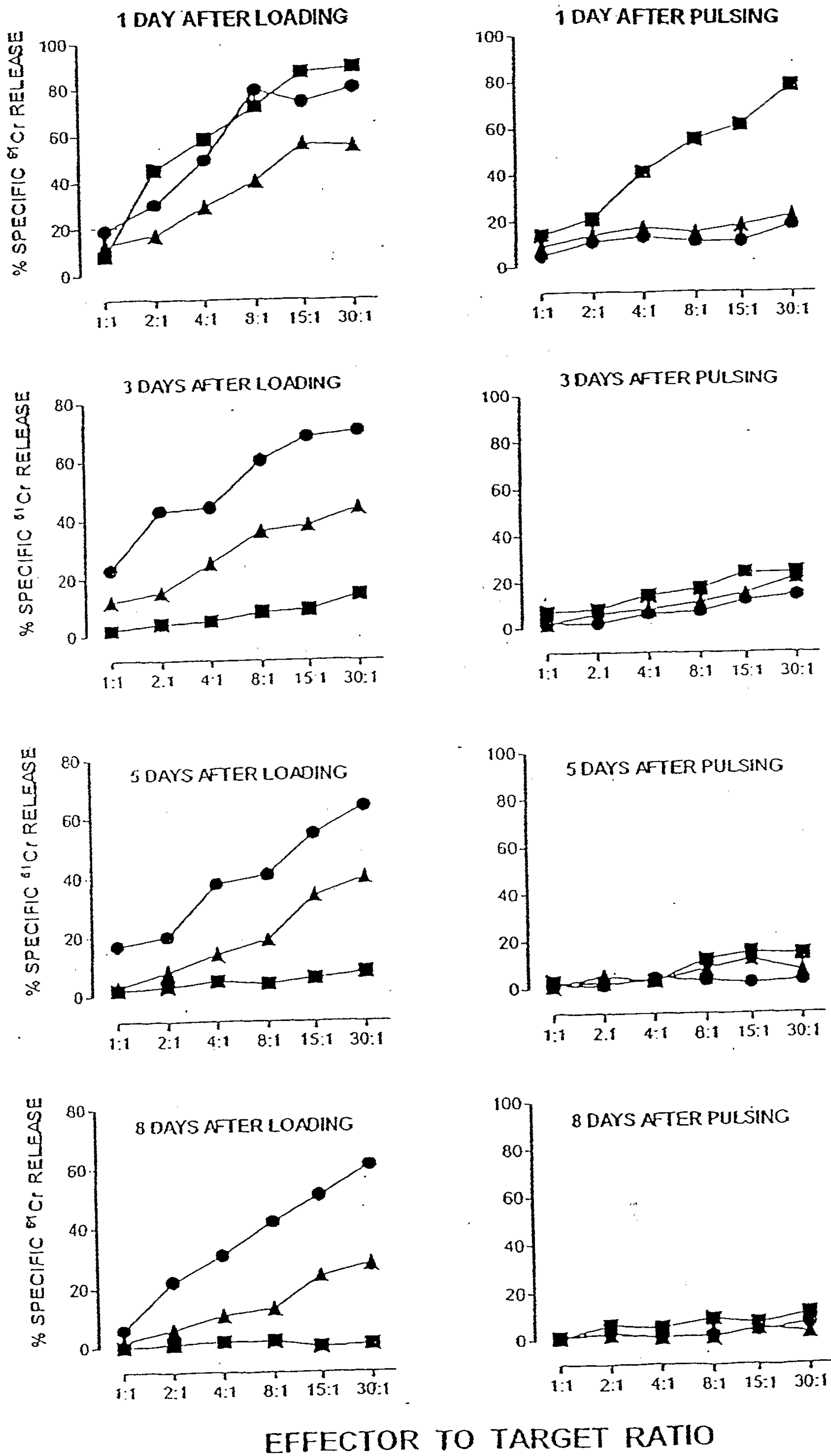

Figure 16

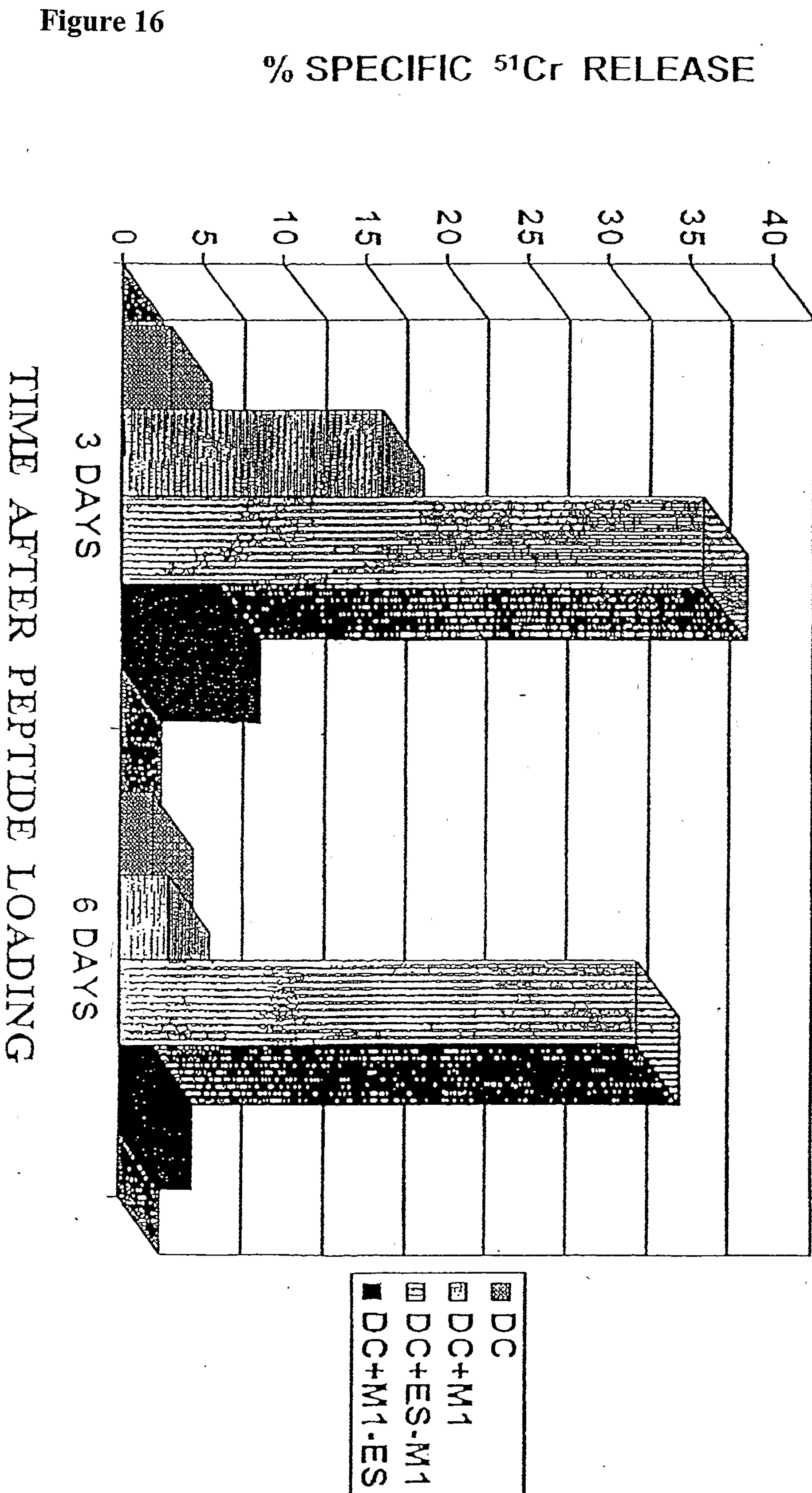

Figure 17

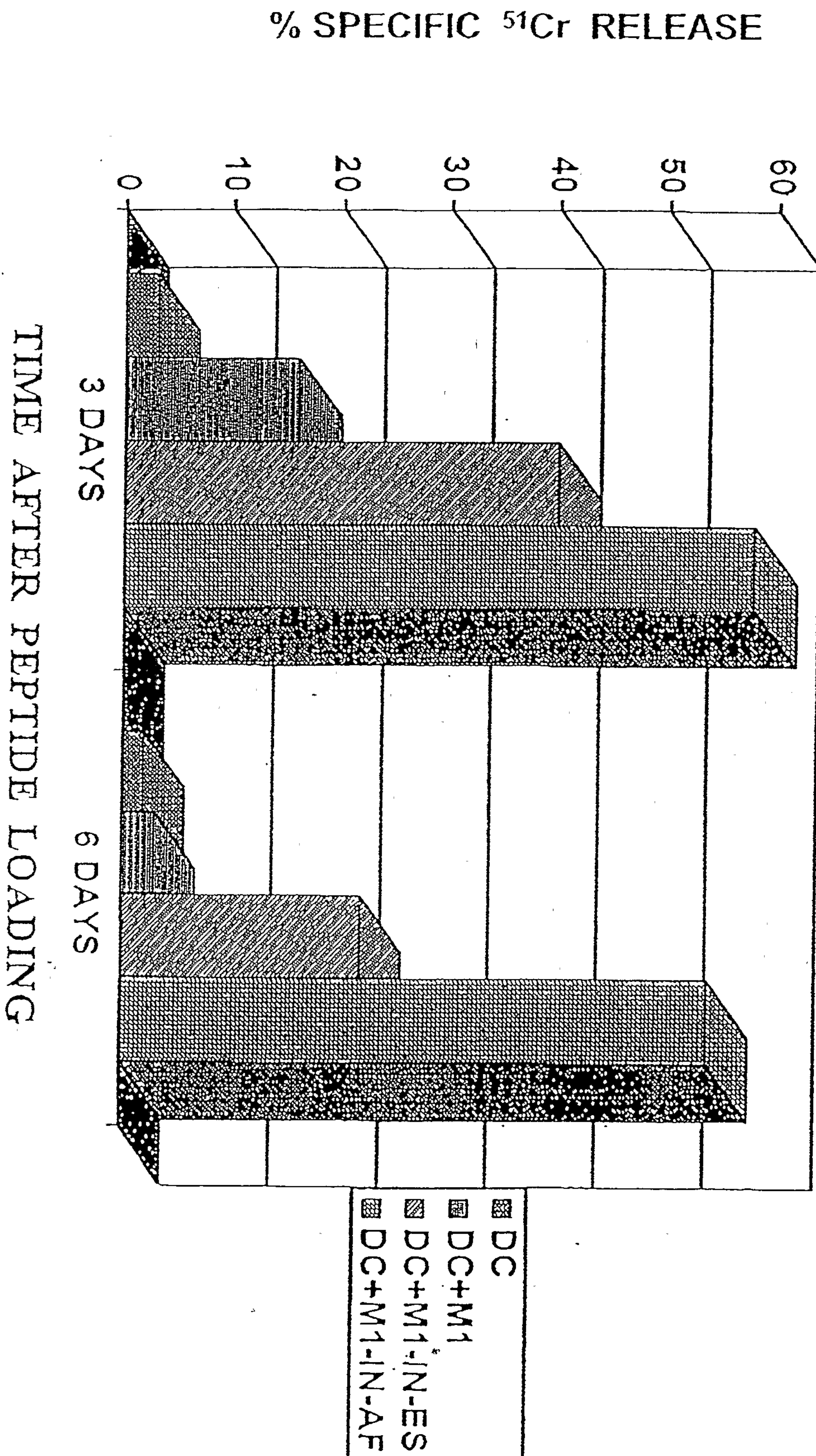

Figure 18

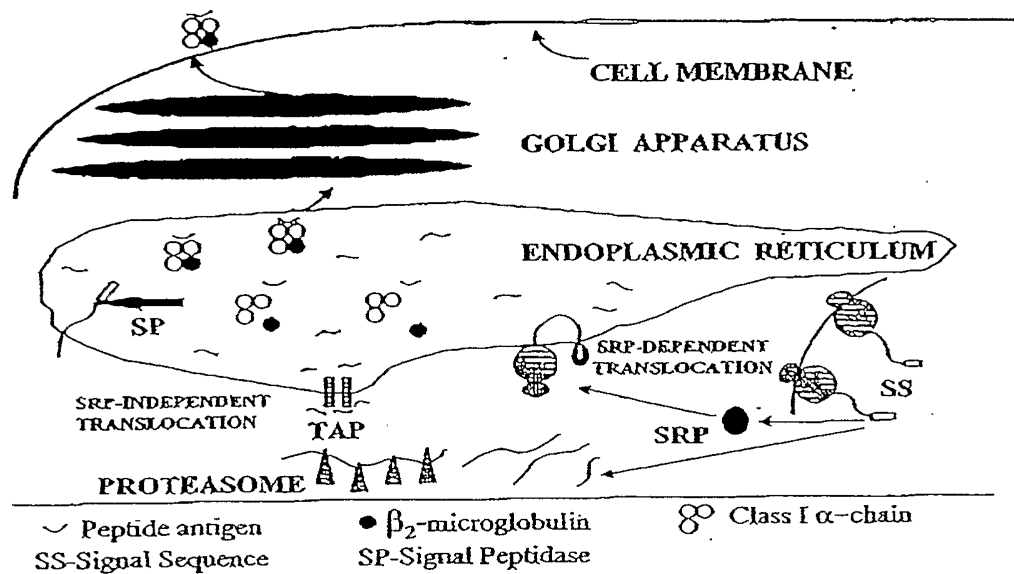

Figure 19

Amino acid sequence of SURVIVIN

MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCACTPERMAEAGFIHCPTENEPDLA
QCFFCFKELEGWEPDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDRERAK
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SEQUENCE LISTING

<110> THE REGENTS OF THE UNIVERSITY OF CALIFORNIA
MINEV, Boris R.

<120> ENHANCING CLASS I ANTIGEN PRESENTATION WITH SYNTHETIC SEQUENCES

<130> UCSD1640-1WO

<150> US 60/586,997
<151> 2004-07-08

<150> US 60/586,914
<151> 2004-07-08

<150> US 60/586,900
<151> 2004-07-08

<150> US 60/586,847
<151> 2004-07-08

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<170> PatentIn version 3.3

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Met Ser Val Trp Thr Ser Pro Arg Arg Leu Val Glu Leu Ala Gly Gln
20 25 30

Ser Leu Leu Lys Asp Glu Ala Leu Ala Ile Ala Ala Leu Glu Leu Leu
35 40 45

Pro Arg Glu Leu Phe Pro Pro Leu Phe Met Ala Ala Phe Asp Gly Arg
50 55 60

His Ser Gln Thr Leu Lys Ala Met Val Gln Ala Trp Pro Phe Thr Cys
65 70 75 80

Leu Pro Leu Gly Val Leu Met Lys Gly Gln His Leu His Leu Glu Thr
85 90 95

Phe Lys Ala Val Leu Asp Gly Leu Asp Val Leu Leu Ala Gln Glu Val
100 105 110

Arg Pro Arg Arg Trp Lys Leu Gln Val Leu Asp Leu Arg Lys Asn Ser

115 120 125

His Gln Asp Phe Trp Thr Val Trp Ser Gly Asn Arg Ala Ser Leu Tyr
130 135 140

Ser Phe Pro Glu Pro Glu Ala Ala Gln Pro Met Thr Lys Lys Arg Lys
145 150 155 160

Val Asp Gly Leu Ser Thr Glu Ala Glu Gln Pro Phe Ile Pro Val Glu
165 170 175

Val Leu Val Asp Leu Phe Leu Lys Glu Gly Ala Cys Asp Glu Leu Phe
180 185 190

Ser Tyr Leu Ile Glu Lys Val Lys Arg Lys Lys Asn Val Leu Arg Leu
195 200 205

Cys Cys Lys Lys Leu Lys Ile Phe Ala Met Pro Met Gln Asp Ile Lys
210 215 220

Met Ile Leu Lys Met Val Gln Leu Asp Ser Ile Glu Asp Leu Glu Val
225 230 235 240

Thr Cys Thr Trp Lys Leu Pro Thr Leu Ala Lys Phe Ser Pro Tyr Leu
245 250 255

Gly Gln Met Ile Asn Leu Arg Arg Leu Leu Leu Ser His Ile His Ala
260 265 270

Ser Ser Tyr Ile Ser Pro Glu Lys Glu Glu Gln Tyr Ile Ala Gln Phe
275 280 285

Thr Ser Gln Phe Leu Ser Leu Gln Cys Leu Gln Ala Leu Tyr Val Asp
290 295 300

Ser Leu Phe Phe Leu Arg Gly Arg Leu Asp Gln Leu Leu Arg His Val
305 310 315 320

Met Asn Pro Leu Glu Thr Leu Ser Ile Thr Asn Cys Arg Leu Ser Glu
325 330 335

Gly Asp Val Met His Leu Ser Gln Ser Pro Ser Val Ser Gln Leu Ser
340 345 350

Val Leu Ser Leu Ser Gly Val Met Leu Thr Asp Val Ser Pro Glu Pro
355 360 365

Leu Gln Ala Leu Leu Glu Arg Ala Ser Ala Thr Leu Gln Asp Leu Val
370 375 380

Phe Asp Glu Cys Gly Ile Thr Asp Asp Gln Leu Leu Ala Leu Leu Pro
385 390 395 400

Ser Leu Ser His Cys Ser Gln Leu Thr Thr Leu Ser Phe Tyr Gly Asn
405 410 415

Ser Ile Ser Ile Ser Ala Leu Gln Ser Leu Leu Gln His Leu Ile Gly
420 425 430

Leu Ser Asn Leu Thr His Val Leu Tyr Pro Val Pro Leu Glu Ser Tyr
435 440 445

Glu Asp Ile His Gly Thr Leu His Leu Glu Arg Leu Ala Tyr Leu His
450 455 460

Ala Arg Leu Arg Glu Leu Leu Cys Glu Leu Gly Arg Pro Ser Met Val
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Trp Leu Ser Ala Asn Pro Cys Pro His Cys Gly Asp Arg Thr Phe Tyr
485 490 495

Asp Pro Glu Pro Ile Leu Cys Pro Cys Phe Met Pro Asn
500 505

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Val Leu Asp Gly Leu Asp Val Leu Leu
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Phe Leu Trp Gly Leu Asp Val Leu Val
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Val Leu Lys Gly Leu Asp Val Leu Leu
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Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
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Ala Val Leu Asp Gly Leu Asp Val Leu Leu
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Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
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Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
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Thr Thr Ala Leu Ser Val Leu Asp Gly Leu Asp Val Leu Leu
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Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
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Ala Ser Leu Tyr Ser Phe Pro Glu Pro Glu Ala
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Gly Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
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Thr Thr Ala Leu Ser Ser Leu Tyr Ser Phe Pro Glu Pro Glu Ala
20 25 30

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Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
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Met Arg Ser Leu Tyr Ser Phe Pro Glu Pro Glu Ala Ala Ala Val Cys
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Ser Ala

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Ala Gly

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Ala Ala Leu Tyr Val Asp Ser Leu Phe Phe Leu
20 25

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Gly Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
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Thr Thr Ala Leu Ser Ala Leu Tyr Val Asp Ser Leu Phe Phe Leu
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Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
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Ser Ala

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Met Ala Ala Leu Tyr Val Asp Ser Leu Phe Phe Leu Ala Ala Ala Ala
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Ala Gly

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Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
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Ala Ser Leu Leu Gln His Leu Ile Gly Leu
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Ser Leu Leu Gln His Leu Ile Gly Leu Met Arg Tyr Met Ile Leu Gly
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Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
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Thr Thr Ala Leu Ser Ser Leu Leu Gln His Leu Ile Gly Leu
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Ser Leu Leu Gln His Leu Ile Gly Leu Met Thr Asn Lys Cys Leu Leu
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Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
20 25 30

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Ala

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Met Ala Ser Leu Leu Gln His Leu Ile Gly Leu Ala Ala Ala Ala Ala
1 5 10 15

Gly

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Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
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Ala

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Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
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Thr Thr Ala Leu Ser
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Lys Phe Leu Ala Ala Gly Thr His Leu Gly Gly Thr Asn Leu Asp Phe
20 25 30

Gln Met Glu Gln Tyr Ile Tyr Lys Arg Lys Ser Asp Gly Ile Tyr Ile
35 40 45

Ile Asn Leu Lys Arg Thr Trp Glu Lys Leu Leu Leu Ala Ala Arg Ala
50 55 60

Ile Val Ala Ile Glu Asn Pro Ala Asp Val Ser Val Ile Ser Ser Arg
65 70 75 80

Asn Thr Gly Gln Arg Ala Val Leu Lys Phe Ala Ala Ala Thr Gly Ala
85 90 95

Thr Pro Ile Ala Gly Arg Phe Thr Pro Gly Thr Phe Thr Asn Gln Ile
100 105 110

Gln Ala Ala Phe Arg Glu Pro Arg Leu Leu Val Val Thr Asp Pro Arg
115 120 125

Ala Asp His Gln Pro Leu Thr Glu Ala Ser Tyr Val Asn Leu Pro Thr
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Ile Ala Leu Cys Asn Thr Asp Ser Pro Leu Arg Tyr Val Asp Ile Ala
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Ile Pro Cys Asn Asn Lys Gly Ala His Ser Val Gly Leu Met Trp Trp
165 170 175

Met Leu Ala Arg Glu Val Leu Arg Met Arg Gly Thr Ile Ser Arg Glu
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His Pro Trp Glu Val Met Pro Asp Leu Tyr Phe Tyr Arg Asp Pro Glu
195 200 205

Glu Ile Glu Lys Glu Glu Gln Ala Ala Ala Glu Lys Ala Val Thr Lys
210 215 220

Glu Glu Phe Gln Gly Glu Trp Thr Ala Pro Ala Pro Glu Phe Thr Ala
225 230 235 240

Ala Gln Pro Glu Val Ala Asp Trp Ser Glu Gly Val Gln Val Pro Ser
245 250 255

Val Pro Ile Gln Gln Phe Pro Thr Glu Asp Trp Ser Ala Gln Pro Ala
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Gly Ala Thr Thr Glu Trp Ser
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Val Leu Gln Met Lys Glu Glu Asp Val
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Asn Leu Lys Arg Thr Trp Glu Lys Leu
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Lys Leu Leu Leu Ala Ala Arg Ala Ile
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Leu Leu Leu Ala Ala Arg Ala Ile Val
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Ala Leu Cys Asn Thr Asp Ser Pro Leu
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Pro Leu Arg Tyr Val Asp Ile Ala Ile
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ggaggatgtc ctcaaattcc ttgctgcggg aacccactta ggtggcacca accttgactt 180

tcagatggag cagtacatct acaaaaggaa aagtgacggt atctacatca taaacctgaa 240

gaggacctgg gagaagctgt tgctcgcagc tcgagctatt gttgccatcg agaatcctgc 300

tgacgtcagc gtcatctcct ccaggaacac tggccagcga gctgtgctga agtttgctgc 360

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ccaagcagcc ttcagggagc cacggcttct agtggtgacc gatcccaggg ctgaccatca 480

gccactcaca gaggcctctt atgtcaacct gcccaccatt gctctgtgta acacagattc 540

tcccctgcgc tatgtggaca ttgccatccc atgcaacaac aagggagctc actcagtggg 600

tctgatgtgg tggatgctgg ccagggaagt actccgcatg cgaggtacta tctcccgtga 660

gcacccctgg gaggtcatgc ctgatcttta cttctacaga gacccagagg agattgagaa 720

ggaggagcag gctgctgctg agaaggctgt gaccaaggag gaattccagg gtgaatggac 780

cgcaccagct cctgagttca ctgctgctca gcctgaggtg gccgactggt ctgagggtgt 840

gcaggttccc tctgtgccca tccagcagtt ccccacggaa gactggagtg cacagccagc 900

cactgaggat tggtcagcag ctcccacagc gcaggccact gagtgggttg gagccaccac 960

tgagtggtcc tgagctgctg tgcaggtgcc tgagcaaagg gaaaaaagat ggaaggaaaa 1020

taaagttgct aaaagctgaa aaaaaaaaaa aaaaaaa 1057

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<220>
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<223> AMIDATION

<400> 79

Leu Leu Leu Ala Ala Arg Ala Ile Val

1 5

<210> 80
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<220>
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<223> ACETYLATION

<220>
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<222> (9)..(9)
<223> AMIDATION

<400> 80

Leu Leu Leu Ala Ala Arg Ala Ile Val
1 5

<210> 81
<211> 9
<212> PRT
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<220>
<223> Synthetic construct

<400> 81

Phe Leu Leu Leu Ala Ala Arg Ala Ile
1 5

<210> 82
<211> 9
<212> PRT
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<220>
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<400> 82

Lys Leu Trp Leu Ala Ala Arg Ala Ile
1 5

<210> 83
<211> 9
<212> PRT
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<220>
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<400> 83

Lys Leu Leu Leu Ala Ala Arg Ala Val
1 5

<210> 84
<211> 9
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<213> Artificial sequence

<220>
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<400> 84

Phe Leu Trp Leu Ala Ala Arg Ala Val
1 5

<210> 85
<211> 9
<212> PRT
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<220>
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<400> 85

Val Leu Lys Met Lys Glu Glu Asp Val
1 5

<210> 86
<211> 9
<212> PRT
<213> Artificial sequence

<220>
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<400> 86

Val Leu Gln Met His Glu Glu Asp Val
1 5

<210> 87
<211> 9
<212> PRT
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<220>
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<400> 87

Val Leu Gln Met Lys Glu Pro Asp Val
1 5

<210> 88
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 88

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala Leu Leu Leu Ala Ala Arg Ala Ile Val
20 25

<210> 89
<211> 26
<212> PRT
<213> Artificial sequence

<220>
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<400> 89

Leu Leu Leu Ala Ala Arg Ala Ile Val Met Arg Tyr Met Ile Leu Gly
1 5 10 15

Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
20 25

<210> 90
<211> 30
<212> PRT
<213> Artificial sequence

<220>
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<400> 90

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Leu Leu Leu Ala Ala Arg Ala Ile Val
20 25 30

<210> 91
<211> 30
<212> PRT

<213> Artificial sequence

<220>
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<400> 91

Leu Leu Leu Ala Ala Arg Ala Ile Val Met Thr Asn Lys Cys Leu Leu
1 5 10 15

Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
20 25 30

<210> 92
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 92

Met Arg Leu Leu Leu Ala Ala Arg Ala Ile Val Ala Ala Val Cys Ser
1 5 10 15

Ala

<210> 93
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 93

Met Ala Leu Leu Leu Ala Ala Arg Ala Ile Val Ala Ala Ala Ala Ala
1 5 10 15

Gly

<210> 94
<211> 339
<212> PRT
<213> Homo sapiens

<400> 94

Met Glu Ser Arg Lys Asp Ile Thr Asn Gln Glu Glu Leu Trp Lys Met
1 5 10 15

Lys Pro Arg Arg Asn Leu Glu Glu Asp Asp Tyr Leu His Lys Asp Thr
20 25 30

Gly Glu Thr Ser Met Leu Lys Arg Pro Val Leu Leu His Leu His Gln
35 40 45

Thr Ala His Ala Asp Glu Phe Asp Cys Pro Ser Glu Leu Gln His Thr
50 55 60

Gln Glu Leu Phe Pro Gln Trp His Leu Pro Ile Lys Ile Ala Ala Ile
65 70 75 80

Ile Ala Ser Leu Thr Phe Leu Tyr Thr Leu Leu Arg Glu Val Ile His
85 90 95

Pro Leu Ala Thr Ser His Gln Gln Tyr Phe Tyr Lys Ile Pro Ile Leu
100 105 110

Val Ile Asn Lys Val Leu Pro Met Val Ser Ile Thr Leu Leu Ala Leu
115 120 125

Val Tyr Leu Pro Gly Val Ile Ala Ala Ile Val Gln Leu His Asn Gly
130 135 140

Thr Lys Tyr Lys Lys Phe Pro His Trp Leu Asp Lys Trp Met Leu Thr
145 150 155 160

Arg Lys Gln Phe Gly Leu Leu Ser Phe Phe Phe Ala Val Leu His Ala
165 170 175

Ile Tyr Ser Leu Ser Tyr Pro Met Arg Arg Ser Tyr Arg Tyr Lys Leu
180 185 190

Leu Asn Trp Ala Tyr Gln Gln Val Gln Gln Asn Lys Glu Asp Ala Trp
195 200 205

Ile Glu His Asp Val Trp Arg Met Glu Ile Tyr Val Ser Leu Gly Ile
210 215 220

Val Gly Leu Ala Ile Leu Ala Leu Leu Ala Val Thr Ser Ile Pro Ser
225 230 235 240

Val Ser Asp Ser Leu Thr Trp Arg Glu Phe His Tyr Ile Gln Ser Lys
245 250 255

Leu Gly Ile Val Ser Leu Leu Leu Gly Thr Ile His Ala Leu Ile Phe
260 265 270

Ala Trp Asn Lys Trp Ile Asp Ile Lys Gln Phe Val Trp Tyr Thr Pro
275 280 285

Pro Thr Phe Met Ile Ala Val Phe Leu Pro Ile Val Val Leu Ile Phe
290 295 300

Lys Ser Ile Leu Phe Leu Pro Cys Leu Arg Lys Lys Ile Leu Lys Ile
305 310 315 320

Arg His Gly Trp Glu Asp Val Thr Lys Ile Asn Lys Thr Glu Ile Cys
325 330 335

Ser Gln Leu

<210> 95
<211> 9
<212> PRT
<213> Homo sapiens

<400> 95

Phe Leu Tyr Thr Leu Leu Arg Glu Val
1 5

<210> 96
<211> 9
<212> PRT
<213> Homo sapiens

<400> 96

Ser Leu Thr Phe Leu Tyr Thr Leu Leu
1 5

<210> 97
<211> 9
<212> PRT
<213> Homo sapiens

<400> 97

His Leu Pro Ile Lys Ile Ala Ala Ile
1 5

<210> 98
<211> 9
<212> PRT
<213> Homo sapiens

<400> 98

Ser Met Leu Lys Arg Pro Val Leu Leu
1 5

<210> 99
<211> 9
<212> PRT
<213> Homo sapiens

<400> 99

Gly Leu Leu Ser Phe Phe Phe Ala Val
1 5

<210> 100
<211> 9
<212> PRT
<213> Homo sapiens

<400> 100

Leu Leu Arg Glu Val Ile His Pro Leu
1 5

<210> 101
<211> 9
<212> PRT
<213> Homo sapiens

<400> 101

Ala Leu Val Tyr Leu Pro Gly Val Ile
1 5

<210> 102
<211> 9
<212> PRT
<213> Homo sapiens

<400> 102

Phe Met Ile Ala Val Phe Leu Pro Ile
1 5

<210> 103
<211> 9
<212> PRT
<213> Homo sapiens

<400> 103

Leu Leu Asn Trp Ala Tyr Gln Gln Val
1 5

<210> 104
<211> 9
<212> PRT
<213> Homo sapiens

<400> 104

Val Leu Pro Met Val Ser Ile Thr Leu
1 5

<210> 105
<211> 9
<212> PRT
<213> Homo sapiens

<400> 105

Tyr Leu Pro Gly Val Ile Ala Ala Ile
1 5

<210> 106
<211> 9
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<213> Homo sapiens

<400> 106

Met Leu Thr Arg Lys Gln Phe Gly Leu
1 5

<210> 107
<211> 9
<212> PRT
<213> Homo sapiens

<400> 107

Ser Leu Gly Ile Val Gly Leu Ala Ile
1 5

<210> 108
<211> 9
<212> PRT
<213> Homo sapiens

<400> 108

Leu Leu Ser Phe Phe Phe Ala Val Leu
1 5

<210> 109
<211> 9
<212> PRT
<213> Homo sapiens

<400> 109

Leu Leu Gly Thr Ile His Ala Leu Ile
1 5

<210> 110
<211> 9
<212> PRT
<213> Homo sapiens

<400> 110

Lys Leu Gly Ile Val Ser Leu Leu Leu
1 5

<210> 111
<211> 9
<212> PRT
<213> Homo sapiens

<400> 111

Phe Leu Pro Cys Leu Arg Lys Lys Ile
1 5

<210> 112
<211> 9
<212> PRT
<213> Homo sapiens

<400> 112

Leu Leu Leu Gly Thr Ile His Ala Leu
1 5

<210> 113
<211> 9
<212> PRT
<213> Homo sapiens

<400> 113

Cys Leu Arg Lys Lys Ile Leu Lys Ile
1 5

<210> 114
<211> 9
<212> PRT
<213> Homo sapiens

<400> 114

Leu Ile Phe Lys Ser Ile Leu Phe Leu
1 5

<210> 115
<211> 9
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<220>
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<220>
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<222> (1)..(1)
<223> ACETYLATION

<400> 115

Tyr Leu Pro Gly Val Ile Ala Ala Ile
1 5

<210> 116
<211> 9
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<213> Artificial sequence

<220>
<223> Synthetic construct

<220>
<221> MOD_RES
<222> (9)..(9)
<223> AMIDATION

<400> 116

Tyr Leu Pro Gly Val Ile Ala Ala Ile
1 5

<210> 117
<211> 9
<212> PRT
<213> Artificial sequence

<220>
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<220>
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<222> (1)..(1)
<223> ACETYLATION

<220>
<221> MOD_RES
<222> (9)..(9)
<223> AMIDATION

<400> 117

Tyr Leu Pro Gly Val Ile Ala Ala Ile
1   5

<210> 118
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 118

Phe Leu Pro Gly Val Ile Ala Ala Ile
1   5

<210> 119
<211> 9
<212> PRT
<213> Artificial sequence

<220>
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<400> 119

Tyr Leu Trp Gly Val Ile Ala Ala Ile
1   5

<210> 120
<211> 9
<212> PRT
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<220>
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<400> 120

Tyr Leu Pro Gly Val Ile Ala Ala Val
1   5

<210> 121
<211> 9
<212> PRT
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<220>
<223> Synthetic construct

<400> 121

Phe Leu Trp Gly Val Ile Ala Ala Val
1   5

<210> 122

<211> 9
<212> PRT
<213> Artificial sequence

<220>
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<400> 122

Tyr Leu Lys Gly Val Ile Ala Ala Ile
1 5

<210> 123
<211> 9
<212> PRT
<213> Artificial sequence

<220>
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<400> 123

Tyr Leu Pro Gly His Ile Ala Ala Ile
1 5

<210> 124
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 124

Tyr Leu Pro Gly Val Ile Pro Ala Ile
1 5

<210> 125
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 125

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala Tyr Leu Pro Gly Val Ile Ala Ala Ile
20 25

<210> 126
<211> 26

<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 126

Tyr Leu Pro Gly Val Ile Ala Ala Ile Met Arg Tyr Met Ile Leu Gly
1 5 10 15

Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
20 25

<210> 127
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 127

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Tyr Leu Pro Gly Val Ile Ala Ala Ile
20 25 30

<210> 128
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 128

Tyr Leu Pro Gly Val Ile Ala Ala Ile Met Thr Asn Lys Cys Leu Leu
1 5 10 15

Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
20 25 30

<210> 129
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 129

Met Arg Tyr Leu Pro Gly Val Ile Ala Ala Ile Ala Ala Val Cys Ser
1 5 10 15

Ala

<210> 130
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 130

Met Ala Tyr Leu Pro Gly Val Ile Ala Ala Ile Ala Ala Ala Ala Ala
1 5 10 15

Gly

<210> 131
<211> 9
<212> PRT
<213> Homo sapiens

<400> 131

His Leu Tyr Gln Gly Cys Gln Val Val
1 5

<210> 132
<211> 9
<212> PRT
<213> Homo sapiens

<400> 132

Lys Ile Phe Gly Ser Leu Ala Phe Leu
1 5

<210> 133
<211> 9
<212> PRT
<213> Homo sapiens

<400> 133

Ile Ile Ser Ala Val Val Gly Ile Leu
1 5

<210> 134

<211> 9
<212> PRT
<213> Homo sapiens

<400> 134

Cys Leu Thr Ser Thr Val Gln Leu Val
1 5

<210> 135
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 135

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala His Leu Tyr Gln Gly Cys Gln Val Val
20 25

<210> 136
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 136

His Leu Tyr Gln Gly Cys Gln Val Val Met Arg Tyr Met Ile Leu Gly
1 5 10 15

Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
20 25

<210> 137
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 137

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser His Leu Tyr Gln Gly Cys Gln Val Val

20 25 30

<210> 138
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 138

His Leu Tyr Gln Gly Cys Gln Val Val Met Thr Asn Lys Cys Leu Leu
1 5 10 15

Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
20 25 30

<210> 139
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 139

Met Arg His Leu Tyr Gln Gly Cys Gln Val Val Ala Ala Val Cys Ser
1 5 10 15

Ala

<210> 140
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 140

Met Ala His Leu Tyr Gln Gly Cys Gln Val Val Ala Ala Ala Ala Ala
1 5 10 15

Gly

<210> 141
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 141

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala Lys Ile Phe Gly Ser Leu Ala Phe Leu
20 25

<210> 142
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 142

Lys Ile Phe Gly Ser Leu Ala Phe Leu Met Arg Tyr Met Ile Leu Gly
1 5 10 15

Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
20 25

<210> 143
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 143

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Lys Ile Phe Gly Ser Leu Ala Phe Leu
20 25 30

<210> 144
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 144

Lys Ile Phe Gly Ser Leu Ala Phe Leu Met Thr Asn Lys Cys Leu Leu

1 5 10 15

Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
20 25 30

<210> 145
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 145

Met Arg Lys Ile Phe Gly Ser Leu Ala Phe Leu Ala Ala Val Cys Ser
1 5 10 15

Ala

<210> 146
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 146

Met Ala Lys Ile Phe Gly Ser Leu Ala Phe Leu Ala Ala Ala Ala Ala
1 5 10 15

Gly

<210> 147
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 147

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala Ile Ile Ser Ala Val Val Gly Ile Leu
20 25

<210> 148
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 148

Ile Ile Ser Ala Val Val Gly Ile Leu Met Arg Tyr Met Ile Leu Gly
1 5 10 15

Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
20 25

<210> 149
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 149

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Ile Ile Ser Ala Val Val Gly Ile Leu
20 25 30

<210> 150
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 150

Ile Ile Ser Ala Val Val Gly Ile Leu Met Thr Asn Lys Cys Leu Leu
1 5 10 15

Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
20 25 30

<210> 151
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 151

Met Arg Ile Ile Ser Ala Val Val Gly Ile Leu Ala Ala Val Cys Ser
1 5 10 15

Ala

<210> 152
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 152

Met Ala Ile Ile Ser Ala Val Val Gly Ile Leu Ala Ala Ala Ala Ala
1 5 10 15

Gly

<210> 153
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 153

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala Cys Leu Thr Ser Thr Val Gln Leu Val
20 25

<210> 154
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 154

Cys Leu Thr Ser Thr Val Gln Leu Val Met Arg Tyr Met Ile Leu Gly
1 5 10 15

Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
20 25

<210> 155
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 155

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Cys Leu Thr Ser Thr Val Gln Leu Val
20 25 30

<210> 156
<211> 28
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 156

Cys Leu Thr Ser Thr Val Gln Leu Val Met Thr Asn Lys Cys Leu Leu
1 5 10 15

Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala
20 25

<210> 157
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 157

Met Arg Cys Leu Thr Ser Thr Val Gln Leu Val Ala Ala Val Cys Ser
1 5 10 15

Ala

<210> 158
<211> 17
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 158

Met Ala Cys Leu Thr Ser Thr Val Gln Leu Val Ala Ala Ala Ala Ala
1 5 10 15

Gly

<210> 159
<211> 9
<212> PRT
<213> Homo sapiens

<400> 159

Ser Thr Phe Lys Asn Trp Pro Phe Leu
1 5

<210> 160
<211> 10
<212> PRT
<213> Homo sapiens

<400> 160

Thr Leu Pro Pro Ala Trp Gln Pro Phe Leu
1 5 10

<210> 161
<211> 9
<212> PRT
<213> Homo sapiens

<400> 161

Lys Asn Trp Pro Phe Leu Glu Gly Cys
1 5

<210> 162
<211> 10
<212> PRT
<213> Homo sapiens

<400> 162

Lys Glu Phe Glu Glu Thr Ala Lys Lys Val
1 5 10

<210> 163
<211> 9

<212> PRT
<213> Homo sapiens

<400> 163

Leu Thr Leu Gly Glu Phe Leu Lys Leu
1 5

<210> 164
<211> 10
<212> PRT
<213> Homo sapiens

<400> 164

Glu Leu Thr Leu Gly Glu Phe Leu Lys Leu
1 5 10

<210> 165
<211> 9
<212> PRT
<213> Homo sapiens

<400> 165

Leu Pro Pro Ala Trp Gln Pro Phe Leu
1 5

<210> 166
<211> 10
<212> PRT
<213> Homo sapiens

<400> 166

Ile Ser Thr Phe Lys Asn Trp Pro Phe Leu
1 5 10

<210> 167
<211> 9
<212> PRT
<213> Homo sapiens

<400> 167

Cys Thr Pro Glu Arg Met Ala Glu Ala
1 5

<210> 168
<211> 10
<212> PRT
<213> Homo sapiens

<400> 168

Thr Glu Asn Glu Pro Asp Leu Ala Gln Cys

1 5 10

<210> 169
<211> 9
<212> PRT
<213> Homo sapiens

<400> 169

Cys Pro Thr Glu Asn Glu Pro Asp Leu
1 5

<210> 170
<211> 10
<212> PRT
<213> Homo sapiens

<400> 170

Phe Glu Glu Leu Thr Leu Gly Glu Phe Leu
1 5 10

<210> 171
<211> 9
<212> PRT
<213> Homo sapiens

<400> 171

Lys Val Arg Arg Ala Ile Glu Gln Leu
1 5

<210> 172
<211> 10
<212> PRT
<213> Homo sapiens

<400> 172

Leu Ser Val Lys Lys Gln Phe Glu Glu Leu
1 5 10

<210> 173
<211> 9
<212> PRT
<213> Homo sapiens

<400> 173

Arg Met Ala Glu Ala Gly Phe Ile His
1 5

<210> 174
<211> 10
<212> PRT

<213> Homo sapiens

<400> 174

Lys Lys Val Arg Arg Ala Ile Glu Gln Leu
1 5 10

<210> 175
<211> 9
<212> PRT
<213> Homo sapiens

<400> 175

Ser Val Lys Lys Gln Phe Glu Glu Leu
1 5

<210> 176
<211> 10
<212> PRT
<213> Homo sapiens

<400> 176

Phe Leu Lys Asp His Arg Ile Ser Thr Phe
1 5 10

<210> 177
<211> 10
<212> PRT
<213> Homo sapiens

<400> 177

Ala Cys Thr Pro Glu Arg Met Ala Glu Ala
1 5 10

<210> 178
<211> 9
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<213> Artificial sequence

<220>
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<220>
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<400> 178

Ser Thr Phe Lys Asn Trp Pro Phe Leu
1 5

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Ser Thr Phe Lys Asn Trp Pro Phe Leu
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Ser Thr Phe Lys Asn Trp Pro Phe Leu
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<400> 181

Ser Leu Phe Lys Asn Trp Pro Phe Leu
1   5

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<400> 182

Ala Thr Phe Lys Asn Trp Pro Phe Leu
1 5

<210> 183
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<400> 183

Ala Leu Phe Lys Asn Trp Pro Phe Leu
1 5

<210> 184
<211> 9
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<400> 184

Ser Thr Lys Lys Asn Trp Pro Phe Leu
1 5

<210> 185
<211> 9
<212> PRT
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<220>
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<400> 185

Ser Thr Phe Lys His Trp Pro Phe Leu
1 5

<210> 186
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 186

Met Arg Tyr Met Ile Leu Gly Leu Leu Ala Leu Ala Ala Val Cys Ser
1 5 10 15

Ala Ser Thr Phe Lys Asn Trp Pro Phe Leu
20 25

<210> 187
<211> 26
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 187

Ser Thr Phe Lys Asn Trp Pro Phe Leu Met Arg Tyr Met Ile Leu Gly
1 5 10 15

Leu Leu Ala Leu Ala Ala Val Cys Ser Ala
20 25

<210> 188
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 188

Met Thr Asn Lys Cys Leu Leu Gln Ile Ala Leu Leu Leu Cys Phe Ser
1 5 10 15

Thr Thr Ala Leu Ser Ser Thr Phe Lys Asn Trp Pro Phe Leu
20 25 30

<210> 189
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 189

Ser Thr Phe Lys Asn Trp Pro Phe Leu Met Thr Asn Lys Cys Leu Leu
1 5 10 15

Gln Ile Ala Leu Leu Leu Cys Phe Ser Thr Thr Ala Leu Ser
20 25 30

<210> 190
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 190

Met Arg Ser Thr Phe Lys Asn Trp Pro Phe Leu Ala Ala Val Cys Ser
1 5 10 15

Ala

<210> 191
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic construct

<400> 191

Met Ala Ser Thr Phe Lys Asn Trp Pro Phe Leu Ala Ala Ala Ala Ala
1 5 10 15

Gly

<210> 192
<211> 142
<212> PRT
<213> Homo sapiens

<400> 192

Met Gly Ala Pro Thr Leu Pro Pro Ala Trp Gln Pro Phe Leu Lys Asp
1 5 10 15

His Arg Ile Ser Thr Phe Lys Asn Trp Pro Phe Leu Glu Gly Cys Ala
20 25 30

Cys Thr Pro Glu Arg Met Ala Glu Ala Gly Phe Ile His Cys Pro Thr
35 40 45

Glu Asn Glu Pro Asp Leu Ala Gln Cys Phe Phe Cys Phe Lys Glu Leu
50 55 60

Glu Gly Trp Glu Pro Asp Asp Asp Pro Ile Glu Glu His Lys Lys His
65 70 75 80

Ser Ser Gly Cys Ala Phe Leu Ser Val Lys Lys Gln Phe Glu Glu Leu
85 90 95

Thr Leu Gly Glu Phe Leu Lys Leu Asp Arg Glu Arg Ala Lys Asn Lys
100 105 110

Ile Ala Lys Glu Thr Asn Asn Lys Lys Lys Glu Phe Glu Glu Thr Ala
115 120 125

Lys Lys Val Arg Arg Ala Ile Glu Gln Leu Ala Ala Met Asp
130 135 140