(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 October 2007 (25.10.2007)

PCT

(10) International Publication Number
WO 2007/120603 A2

(51) International Patent Classification:
C07K 14/74 (2006.01)

(21) International Application Number:
PCT/US2007/008747

(22) International Filing Date: 10 April 2007 (10.04.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/790,525 10 April 2006 (10.04.2006) US

(71) Applicant *(for all designated States except US)*: **SLOAN KETTERING INSTITUTE FOR CANCER RESEARCH** [US/US]; 1275 York Avenue, 460 East 63rd Street, New York, NY 10021 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SCHEINBERG, David, A.** [US/US]; 415 East 68th; 19th Floor, New York, NY 10025 (US). **MAY, Rena** [US/US]; 87-10 204th Street, Apt B23, Hollis, NY 11423 (US).

(74) Agent: **COHEN, Mark, S.**; Pearl Cohen Zedek Latzer, LLP, 1500 Broadway, 12th Floor, New York, NY 10036 (US).

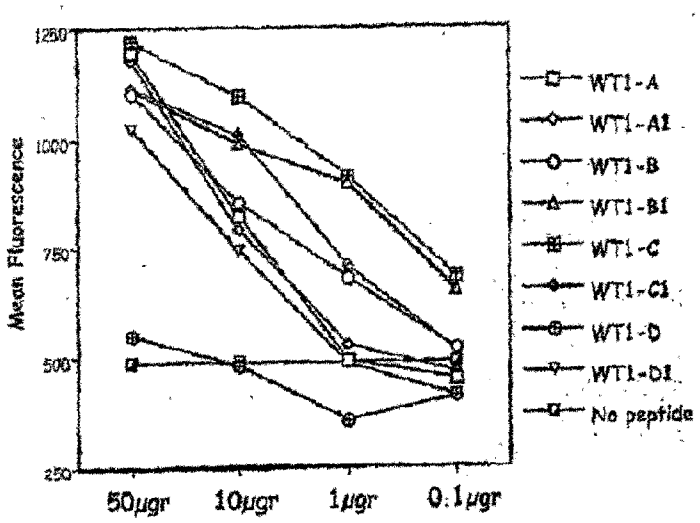
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** IMMUNOGENIC BCR-ABL PEPTIDES AND METHODS OF USE THEREOF



(57) **Abstract:** This invention provides peptides, immunogenic compositions and vaccines comprising same, and methods of treating, reducing the incidence of, and inducing immune responses to a bcr-abl- expressing cancer, comprising same.

WO 2007/120603 A2

IMMUNOGENIC BCR-ABL PEPTIDES AND METHODS OF USE THEREOF

FIELD OF INVENTION

[001] This invention provides peptides, immunogenic compositions and vaccines comprising same, and methods of treating, reducing the incidence of, and inducing immune responses to a bcr-abl-expressing cancer, comprising same.

BACKGROUND OF THE INVENTION

[002] Leukemias, including chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML) and acute lymphocytic leukemia (ALL) are pluripotent stem cell disorders, which may be characterized by the presence of the Philadelphia chromosome (Ph). Because of the unique features, these cancers present a unique opportunity to develop therapeutic strategies using vaccination against a truly tumor specific antigen that is also the oncogenic protein required for neoplasia.

[003] The chimeric fusion proteins are potential antigens for two reasons. The proteins are uniquely expressed in the leukemic cells in which the junctional regions contain a sequence of amino acids that is not expressed on any normal protein. In addition, as a result of the codon split on the fused message, a new amino acid (lysine in b3a2) and a conserved one (glutamic acid in b2a2) are present at the exact fusion point in each of the proteins. Therefore, the unique amino acid sequences encompassing the b3a2 and b2a2 breakpoint region can be considered truly tumor specific antigens. Despite the intracellular location of these proteins, short peptides produced by cellular processing of the products of the fusion proteins can be presented on the cell surface within the cleft of human leukocyte antigen (HLA) molecules, and in this form, may be recognized by T cells.

[004] Tumor specific, bcr-abl derived multivalent vaccine can be safely administered to patients with chronic phase CML; the vaccine reliably elicits a bcr-abl peptide specific CD4 immune response, as measured by DTH in vivo, $CD4^+$ T cell proliferation ex vivo and gamma interferon secretion in a ELISPOT assay. CD8 responses in A0201 patients, however, were undetectable, and only weak responses in HLA A0301 patients using a sensitive gamma interferon ELISPOT assay were found. For stimulation of responses the strength of CD8 responses depends upon the binding affinity of the target peptide to class I MHC molecules, the peptide-HLA complex stability, and the avidity of the T cell receptor binding for the peptide complex. Killing of native CML cells also requires adequate processing and presentation of the natural antigen. Therefore the lack of reproducible CD8 responses

may reflect the biochemistry of the class I peptide-HLA interaction, which resulted in their weak immunogenicity to cytotoxic CD8 cells.

[005] Thus, there remains a need to design peptides that are more immunogenic and that produce a robust CTL response. Ideally, such peptides should generate an immune response that not only recognizes the immunizing epitopes, but also that cross reacts with the original native peptides, producing a heteroclitic response, which as yet, is lacking.

SUMMARY OF THE INVENTION

[006] This invention provides peptides, immunogenic compositions and vaccines comprising same, and methods of treating, reducing the incidence of, and inducing immune responses to a bcr-abl-expressing cancer, comprising same.

[007] In one embodiment, the present invention provides an isolated, mutated bcr-abl peptide, comprising: (a) a binding motif of a human leukocyte antigen (HLA) Class II molecule; and (b) a binding motif of an HLA class I molecule, having a point mutation in one or more anchor residues of the binding motif of an HLA class I molecule. In another embodiment, the bcr-abl peptide is 11-30 amino acids in length.

[008] In another embodiment, the present invention provides a method of treating a subject with a bcr-abl-expressing cancer, the method comprising administering to the subject a peptide of the present invention, thereby treating a subject with a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of treating a subject with a bcr-abl-expressing cancer, the method comprising administering to the subject a vaccine of the present invention, thereby treating a subject with a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of treating a subject with a bcr-abl-expressing cancer, the method comprising administering to the subject an immunogenic composition of the present invention, thereby treating a subject with a bcr-abl-expressing cancer.

[009] In another embodiment, the present invention provides a method of suppressing or halting the progression of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject a peptide of the present invention, thereby suppressing or halting the progression of a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of suppressing or halting the progression of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject a vaccine of the present invention, thereby suppressing or halting the

progression of a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of suppressing or halting the progression of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject an immunogenic composition of the present invention, thereby suppressing or halting the progression of a bcr-abl-expressing cancer

[0010] In another embodiment, the present invention provides a method of inducing formation and proliferation of bcr-abl-specific CTL, the method comprising contacting a lymphocyte population with a peptide of the present invention, thereby inducing formation and proliferation of bcr-abl-specific CTL. In another embodiment, the cell is a cell of a bcr-abl-expressing leukemia.

[0011] In another embodiment, the present invention provides a method of inducing formation and proliferation of (a) a $CD8^+$ lymphocyte specific for a bcr-abl protein; and (b) a $CD4^+$ lymphocyte specific for the bcr-abl protein, the method comprising contacting a lymphocyte population with a peptide of the present invention, thereby inducing formation and proliferation of (a) a $CD8^+$ lymphocyte specific for a bcr-abl protein; and (b) a $CD4^+$ lymphocyte specific for the bcr-abl protein.

BRIEF DESCRIPTION OF THE FIGURES

[0012] Figure 1: T2 stabilization assay of native and synthetic WT-1 peptides to HLA A0201 cells (A) and HLA A0301 cells (B-E). Fluorescence index is ratio between median fluorescence with peptide tested: median fluorescence with no peptide. X axis: concentration per well of the peptide tested.

[0013] Figure 2: $CD8^+/CD3^+$ gamma interferon (IFN) ELISPOT (A) and cytotoxicity (B) from healthy HLA A0201 donors against T2 cells pulsed with the following peptides: 1st bar in each series: no peptide; 2nd bar: same peptide used for stimulation; 3rd bar: corresponding native peptide; 4th bar: negative control peptide. X axis: peptides used for stimulations. Experiments were performed in triplicate and confirmed 3-5 times.

[0014] Figure 3: $CD8^+$ (A) and $CD3^+$ (B-D) gamma IFN ELISPOT from healthy HLA A0201 donors using the indicated peptides- assignment of bars in each series is the same as for Figure 2. Each subfigure in B-D represents a separate repetition of the experiment].

[0015] Figure 4: Cytotoxicity assays using $CD8^+$ T cells stimulated with synthetic WT-1 A1 peptides from a HLA A0201 donor against HLA-matched CML blasts presenting native peptide sequences. A. Bar graphs of results. 1st bar in each series: SKLY-16 ($WT1^-$); 2nd bar: BV173 ($WT1^+$); 3rd bar: LAMA81 ($WT1^+$); 4th bar: CMLA (additional negative control). B. Killing curves. Squares: SKLY-16. Diamonds: 697 cells. G3, F4, C5, and G5 are T-cell clones generated from a healthy HLA-A0201

donor after multiple stimulations in vitro. Y axis: percentage of cytotoxicity. X axis: T cell: target cell ratio.

[0016] Figure 5, part 1. Gamma interferon ELISPOT after stimulation with WT1 peptides of $CD3^+$ T cells from healthy donors with different HLA-DRB1 types. Part 2. $CD3^+$ T cells (A: HLA-DRB1*1001/1501; B: HLA-DRB1*0701/1202; C: HLA-DRB1*0301/901; D: HLA-DRB1*0407/1302) were stimulated twice with peptide WT1DR 328 or WT1DR 423. Stimulated T cells were challenged in an IFN-gamma ELISPOT assay with the following: Grey Bars: unchallenged control; Black Bars: $CD14^+$ cells pulsed with stimulating peptide (either WT1DR 328 or WT1DR 423); White Bars: $CD14^+$ cells pulsed with irrelevant $CD4^+$ peptide epitope (RAS); Hatched Bars: unpulsed $CD14^+$ cells. * - $p < 0.05$ compared to controls. Y axis: number of spots per $1x10^5$ $CD3^+$ T cells. X axis: peptide used for T cell stimulations.

[0017] Figure 6. Peptides with buried heteroclitic epitopes are processed, presented, and recognized by human T cells. A. $CD3^+$ T cells from an HLA A0201/301 DRB1*1301/1302 healthy donor were stimulated with autologous DCs previously incubated with 697 tumor lysates, then challenged in an IFN-gamma ELISPOT assay with autologous DCs previously incubated with either 697 tumor lysate, individual WT1 peptides, control peptides or unpulsed DCs (X axis). B. $CD3^+$ T cells from an HLA A0201/101 DRB1*0301/1601 healthy donor were stimulated with autologous DCs previously incubated with tumor lysates from either JMN (Black Bars), or MeWo (White Bars). T cells were challenged in an IFN-gamma ELISPOT assay with autologous DCs previously incubated with JMN or MeWo tumor lysates, individual WT1DR peptides, or control class II peptide (X axis). Hatched bars: background level of spots from autologous DCs incubated in the absence of T cells. * - $P < 0.05$ compared to control peptides. Y axis: number of spots per $1x10^5$ CD3+ cells.

[0018] Figure 7A-B. A. $CD3^+$ gamma interferon ELISPOT with peptides WT1DR 122 and WT1DR 122A1. $CD3^+$ T cells from healthy donors with different HLA-DRB1 types (A: HLA-DRB1*1401; B: HLA-DRB1*0104/1104) were stimulated twice with either peptide WT1DR 122 or WT1DR 122A1, then challenged in an IFN-gamma ELISPOT assay with the following: $CD14^+$ cells pulsed with peptide WT1DR 122 (Grey Bars); $CD14^+$ cells pulsed with peptide WT1DR 122A1 (Black Bars); $CD14^+$ cells pulsed with irrelevant CD4 peptide epitope (White Bars; RAS); unpulsed $CD14^+$ cells (Hatched Bars). * - $p < 0.05$ compared to controls. Y axis: number of spots per $1x10^5$ $CD3^+$ T cells. X axis: peptide used for stimulations.

[0019] Figure 7C. WT1DR peptide 122 and 122A1 stimulate $CD8^+$ T cell responses. Left panel: $CD3^+$ T cells from an HLA-A0201/DRB1*1401 donor were stimulated twice with WT1DR 122, then

challenged in an IFN-gamma ELISPOT assay with autologous $CD14^+$ cells. Right panel: $CD3^+$ T cells from an HLA-A0201/DRB1*1501 donor were stimulated twice with WT1DR 122A1, then challenged in an IFN-gamma ELISPOT assay with control melanoma cell line MeWo (A0201/DRB1*15XX, $WT1^-$). * - $p < 0.05$ compared to no peptide controls. Y axis represents the number of spots per $1x10^5$ $CD3^+$ cells. X axis shows the different test peptides used in the ELISPOT.

[0020] Figure 8. CD3+ gamma interferon ELISPOT against Mesothelioma cell lines. Left panel: Total PBMCs from an HLA-A0201 donor were stimulated twice with the different WT1DR peptides, then T cells were challenged in an IFN-gamma ELISPOT assay with the following: Mesothelioma H-Meso1A cell line (Black Bars; WT1+, A0201+); control melanoma MeWo cell line (WT1-, A0201+; Grey Bars). * - $p \leq 0.01$ compared to MeWo controls. Y axis: number of spots per $2x10^5$ PBMCs. X axis: peptide used for T cell stimulation. Right panel: $CD3^+$ T cells from an HLA-A0201/DRB1*1501 donor were stimulated twice with WT1DR 122A1, then T cells were challenged in an IFN-gamma ELISPOT assay with the following target cells: JMN, an A0201/DRB1*1505 WT1 positive mesothelioma cell line or MeWo, an A0201/DRB1*15XX WT1 negative melanoma cell line. Target cells were either pulsed with WT1DR 122A1 (Black Bars) or not pulsed (Grey Bars). * $p < 0.05$ compared to the unpulsed Mewo target cell. Y axis: number of spots per $1x10^5$ $CD3^+$ T cells. X axis: cell lines used as target cells.

[0021] Figure 9, left panel. $CD3^+$ T cells from an HLA-A0201/DRB1*0101/15XX donor were stimulated twice with WT1DR 122A1, then $CD8^+$ T cells were isolated by negative selection and used as effector cells in a ^{51}Cr release cytotoxicity assay. $CD8^+$ T cells were incubated with radiolabeled target cells (pulsed or unpulsed 697 ($A0201^+$, $WT1^+$) or SKLY16 (A0201+, WT1-) at 3 different E:T ratios: Grey bars 100:1; Black bars 30:1; White bars 10:1. Y axis: percentage of cytotoxicity. X axis: target cell conditions. * - $p<0.05$ compared to SKLY16 controls at the same E:T ratio. Right panel: CD8+ T cells isolated as described for the left panel were incubated with radiolabeled JMN (solid line A0201+ WT1+) or MeWo (dashed line A0201+, WT1-) target cells at 4 different E:T ratios: Y axis: percentage of cytotoxicity. X axis: E:T ratios. P was <0.001 compared to MeWo controls.

[0022] Figure 10: T2 stabilization assay using peptides derived from b3a2 translocation (left panel) and b2a2 translocations (right panel). Peptide sequences are delineated in Table 5. The fluorescence index is the value obtained for the ratio between median fluorescence obtained with the indicated peptide divided by background fluorescence. The X-axis represents different peptide concentrations. "n" denotes native sequences from b3a2. p210Cn, p210Dn, CMLA2, and CMLA3 are native b3a2 sequences; b2a2A is the native sequence for b2a2.

[0023] Figure 11: gamma interferon (IFN) production detected by ELISPOT of $CD8^+$ T cells from a healthy HLA A0201 donor following two *in vitro* stimulations with the peptides p210 C and F. After stimulation, $CD8^+$ cells were challenged with the following: T2 (APC), or T2 pulsed with tested peptide (p210C or p210F), corresponding native peptide, or negative control peptide, as indicated.

[0024] Figure 12: secretion of gamma IFN detected by ELISPOT of $CD8^+$ T cells from an HLA A0201, chronic phase CML patient following 2 *in vitro* stimulations with p210C. T cells were challenged with the following: media, APC T2, or T2 pulsed with p210C, corresponding native peptide, or negative control peptide. Empty bars: CD8+ cells plus media. Dot bars: $CD8^+$ plus APC T2. Diagonal bars: $CD8^+$ plus T2 pulsed with p210C. Black bars: $CD8^+$ plus T2 pulsed with corresponding native peptide p210Cn. Grey bars: $CD8^+$ plus T2 pulsed with irrelevant control peptide.

[0025] Figure 13: production of gamma IFN detected by ELISPOT of $CD3^+$ cells of two healthy HLA A0201 donors after two *in vitro* stimulations. T cells were challenged with the following: media, APC T2, or T2 pulsed with test peptide (b2a2 A3, A4 or A5); corresponding native peptide, or negative control peptide. Dot bars: CD8+ plus APC T2. diagonal bars: CD8+ plus T2 pulsed with tested peptide (b2a2 A3, A4 or A5). black bars: CD8+ plus T2 pulsed with native peptide (cross reactivity). grey bars: CD8+ plus T2 pulsed with irrelevant control peptide.

[0026] Figure 14: cytotoxicity assay with T cells isolated from a healthy HLA A0201 donor following 3 *in vitro* stimulations with p210F. Target cells used were T2 cell lines pulsed with the indicated peptides. The Y-axis reflects the percent cytotoxicity, and the X-axis reflects the varied T cell/target ratio. Open squares: T2 with no peptide. Open diamonds: T2 pulsed with p210F. Open circles: T2 pulsed with CMLA2. Open triangles: T2 pulsed with irrelevant control peptide.

[0027] Figure 15: results of two cytotoxicity assays with T cells isolated from a healthy HLA A0201 donor following five *in vitro* stimulations with b2a2 A3 peptide. Target cells used were T2 cell line pulsed with the indicated peptides. Y-axis reflects the percent cytotoxicity, and the X-axis reflects the different T cell/target ratio. Open squares: T2 with no peptide. Open diamonds: T2 pulsed with b2a2 A3 peptide. Open circles: T2 pulsed with negative control peptide.

DETAILED DESCRIPTION OF THE INVENTION

[0028] This invention provides peptides, immunogenic compositions and vaccines comprising same, and methods of treating, reducing the incidence of, and inducing immune responses to a bcr-abl-expressing cancer, comprising same.

[0029] In one embodiment, the present invention provides an isolated, mutated bcr-abl peptide, comprising: (a) a binding motif of a human leukocyte antigen (HLA) Class II molecule; and (b) a binding motif of an HLA class I molecule, having a point mutation in one or more anchor residues of the binding motif of an HLA class I molecule. In another embodiment, the bcr-abl peptide is 11-30 amino acids in length.

[0030] In another embodiment, the bcr-abl peptide is 11-30 amino acids in length. In another embodiment, the bcr-abl peptide is 11 or more amino acids in length.

[0031] In another embodiment, a bcr-abl peptide of the present invention is 16-22 amino acids (AA) in length. In another embodiment, the length is 16-22 AA. In another embodiment, the length is 19 AA. In another embodiment, the bcr-abl peptide is 15-23 AA in length. In another embodiment, the length is 15-24 AA. In another embodiment, the length is 15-25 AA. In another embodiment, the length is 15-26 AA. In another embodiment, the length is 15-27 AA. In another embodiment, the length is 15-28 AA. In another embodiment, the length is 14-30 AA. In another embodiment, the length is 14-29 AA. In another embodiment, the length is 14-28 AA. In another embodiment, the length is 14-26 AA. In another embodiment, the length is 14-24 AA. In another embodiment, the length is 14-22 AA. In another embodiment, the length is 14-20 AA. In another embodiment, the length is 16-30 AA. In another embodiment, the length is 16-28 AA. In another embodiment, the length is 16-26 AA. In another embodiment, the length is 16-24 AA. In another embodiment, the length is 16-22 AA. In another embodiment, the length is 18-30 AA. In another embodiment, the length is 18-28 AA. In another embodiment, the length is 18-26 AA. In another embodiment, the length is 18-24 AA. In another embodiment, the length is 18-22 AA. In another embodiment, the length is 18-20 AA. In another embodiment, the length is 20-30 AA. In another embodiment, the length is 20-28 AA. In another embodiment, the length is 20-26 AA. In another embodiment, the length is 20-24 AA. In another embodiment, the length is 22-30 AA. In another embodiment, the length is 22-28 AA. In another embodiment, the length is 22-26 AA. In another embodiment, the length is 24-30 AA. In another embodiment, the length is 24-28 AA. In another embodiment, the length is 24-26 AA.

[0032] In another embodiment, a bcr-abl peptide of methods and compositions of the present invention is longer than the minimum length for binding to an HLA class II molecule, which is, in another embodiment, about 12 AA. In another embodiment, increasing the length of the HLA class II-binding bcr-abl peptide enables binding to more than one HLA class II molecule. In another embodiment, increasing the length enables binding to an HLA class II molecule whose binding motif

is not known. In another embodiment, increasing the length enables binding to an HLA class I molecule. In one embodiment, the binding motif of the HLA class I molecule is known. In another embodiment, the binding motif of the HLA class I molecule is not known. Each possibility represents a separate embodiment of the present invention.

[0033] Each of the above peptide lengths represents a separate embodiment of the present invention.

[0034] The "point mutation," in another embodiment, indicates that the fragment is mutated with respect to the native sequence of the protein, thus creating the HLA class I molecule binding motif. In another embodiment, the "point mutation" strengthens the binding capacity of an HLA class I molecule binding motif present in the native sequence. Each possibility represents a separate embodiment of the present invention.

[0035] In another embodiment, the point mutation is in 1-3 anchor residues of the HLA class I molecule binding motif. In another embodiment, the point mutation is in 1 anchor residue of the HLA class I molecule binding motif. In another embodiment, the point mutation is in 2 anchor residues of the HLA class I molecule binding motif. In another embodiment, the point mutation is in 1-2 anchor residues of the HLA class I molecule binding motif. In another embodiment, the point mutation is in 2-3 anchor residues of the HLA class I molecule binding motif. In another embodiment, the point mutation is in 1-4 anchor residues of the HLA class I molecule binding motif. Each possibility represents a separate embodiment of the present invention.

[0036] "Peptide," in another embodiment of methods and compositions of the present invention, refers to a compound of two or more subunit AA connected by peptide bonds. In another embodiment, the term is used hereinbelow to refer to peptides (e.g. peptides of the present invention). In another embodiment, the peptide comprises an AA analogue. In another embodiment, the AA analogue is one of those enumerated below. In another embodiment, the peptide is a peptidomimetic. The subunits are, in another embodiment, linked by peptide bonds. In another embodiment, the subunit is linked by another type of bond, e.g. ester, ether, etc. In another embodiment, a peptide of the present invention is one of the types of peptidomimetics enumerated below. Each possibility represents a separate embodiment of the present invention.

[0037] HLA molecules, known in another embodiment as major histocompatibility complex (MHC) molecules, bind peptides and present them to immune cells. Thus, in another embodiment, the immunogenicity of a peptide is partially determined by its affinity for HLA molecules. HLA class I molecules interact with CD8 molecules, which are generally present on cytotoxic T lymphocytes

(CTL). HLA class II molecules interact with CD4 molecules, which are generally present on helper T lymphocytes.

[0038] In another embodiment, a peptide of the present invention is immunogenic. In one embodiment, the term "immunogenic" refers to an ability to stimulate, elicit or participate in an immune response. In one embodiment, the immune response elicited is a cell-mediated immune response. In another embodiment, the immune response is a combination of cell-mediated and humoral responses.

[0039] In another embodiment, T cells that bind to the MHC molecule-peptide complex become activated and induced to proliferate and lyse cells expressing a protein comprising the peptide. T cells are typically initially activated by "professional" antigen presenting cells ("APC"; e.g. dendritic cells, monocytes, and macrophages), which present costimulatory molecules that encourage T cell activation as opposed to anergy or apoptosis. In another embodiment, the response is heteroclitic, as described herein, such that the CTL lyses a cell expressing a protein which has an AA sequence homologous to a peptide of this invention, or a different peptide than that used to first stimulate the T cell.

[0040] In another embodiment, an encounter of a T cell with a peptide of this invention induces its differentiation into an effector and/or memory T cell. Subsequent encounters between the effector or memory T cell and the same peptide, or, in another embodiment, with a related peptide of this invention, leads to a faster and more intense immune response. Such responses are gauged, in one embodiment, by measuring the degree of proliferation of the T cell population exposed to the peptide. In another embodiment, such responses are gauged by any of the methods enumerated hereinbelow.

[0041] In one embodiment, as described herein, the subject is exposed to a peptide, or a composition/cell population comprising a peptide of this invention, which differs from the native protein expressed, wherein subsequently a host immune response cross-reactive with the native protein/antigen develops.

[0042] In another embodiment, peptides, vaccines, and compositions of this invention stimulate an immune response that results in lysis of a tumor cell.

[0043] In another embodiment, the HLA class I molecule binding motif of a peptide of the present invention is contained within the HLA class II molecule binding motif of the peptide. In another embodiment, the HLA class I molecule binding motif overlaps with the HLA class II molecule binding motif. In another embodiment, the HLA class I molecule binding motif does not overlap with the HLA

class II molecule binding motif. Each possibility represents a separate embodiment of the present invention.

[0044] The HLA class II molecule whose binding motif is contained in a peptide of the present invention is, in another embodiment, an HLA-DR molecule. In another embodiment, the HLA class II molecule is an HLA-DP molecule. In another embodiment, the HLA class II molecule is an HLA-DQ molecule.

[0045] In another embodiment, the HLA class II molecule is an HLA-DRB molecule. In another embodiment, the HLA class II molecule is DRB101. In another embodiment, the HLA class II molecule is DRB301. In another embodiment, the HLA class II molecule is DRB401. In another embodiment, the HLA class II molecule is DRB701. In another embodiment, the HLA class II molecule is DRB1101. In another embodiment, the HLA class II molecule is DRB1501. In another embodiment, the HLA class II molecule is any other HLA-DRB molecule known in the art. In another embodiment, the HLA class II molecule is an HLA-DRA molecule. In another embodiment, the HLA class II molecule is an HLA-DQA1 molecule. In another embodiment, the HLA class II molecule is an HLA-DQB1 molecule. In another embodiment, the HLA class II molecule is an HLA-DPA1 molecule. In another embodiment, the HLA class II molecule is an HLA-DPB1 molecule. In another embodiment, the HLA class II molecule is an HLA-DMA molecule. In another embodiment, the HLA class II molecule is an HLA-DMB molecule. In another embodiment, the HLA class II molecule is an HLA-DOA molecule. In another embodiment, the HLA class II molecule is an HLA-DOB molecule. In another embodiment, the HLA class II molecule is any other HLA class II-molecule known in the art.

[0046] In another embodiment, a peptide of the present invention binds to 2 distinct HLA class II molecules. In another embodiment, the peptide binds to three distinct HLA class II molecules. In another embodiment, the peptide binds to four distinct HLA class II molecules. In another embodiment, the peptide binds to five distinct HLA class II molecules. In another embodiment, the peptide binds to six distinct HLA class II molecules. In another embodiment, the peptide binds to more than six distinct HLA class II molecules.

[0047] In another embodiment, the HLA class II molecules that are bound by a peptide of the present invention are encoded by two or more distinct alleles at a given HLA class II locus. In another embodiment, the HLA class II molecules are encoded by three distinct alleles at a locus. In another embodiment, the HLA class II molecules are encoded by four distinct alleles at a locus. In another embodiment, the HLA class II molecules are encoded by five distinct alleles at a locus. In another

embodiment, the HLA class II molecules are encoded by six distinct alleles at a locus. In another embodiment, the HLA class II molecules are encoded by more than six distinct alleles at a locus.

[0048] In another embodiment, the HLA class II molecules bound by the peptide are encoded by HLA class II genes at two distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at 2 or more distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at 3 distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at 3 or more distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at 4 distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at 4 or more distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at 5 distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at 5 or more distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at 6 distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at 6 or more distinct loci. In another embodiment, the HLA class II molecules are encoded by HLA class II genes at more than 6 distinct loci. Each possibility represents a separate embodiment of the present invention.

[0049] In another embodiment, a peptide of the present invention binds to 2 distinct HLA-DRB molecules. In another embodiment, the peptide binds to three distinct HLA-DRB molecules. In another embodiment, the peptide binds to four distinct HLA-DRB molecules. In another embodiment, the peptide binds to five distinct HLA-DRB molecules. In another embodiment, the peptide binds to six distinct HLA-DRB molecules. In another embodiment, the peptide binds to more than six distinct HLA-DRB molecules.

[0050] In another embodiment, a peptide of the present invention binds to HLA-DRB molecules that are encoded by 2 distinct HLA-DRB alleles. In another embodiment, the HLA-DRB molecules are encoded by three distinct HLA-DRB alleles. In another embodiment, the HLA-DRB molecules are encoded by four distinct HLA-DRB alleles. In another embodiment, the HLA-DRB molecules are encoded by five distinct HLA-DRB alleles. In another embodiment, the HLA-DRB molecules are encoded by six distinct HLA-DRB alleles. In another embodiment, the HLA-DRB molecules are encoded by more than six distinct HLA-DRB alleles. Each possibility represents a separate embodiment of the present invention.

[0051] In another embodiment, a peptide of the present invention binds to HLA-DRB molecules that are encoded by 2 distinct HLA-DRB alleles selected from DRB 101, DRB 301, DRB 401, DRB 701, DRB 1101, and DRB 1501. In another embodiment, the peptide binds to HLA-DRB molecules

encoded by 3 distinct HLA-DRB alleles selected from DRB 101, DRB 301, DRB 401, DRB 701, DRB 1101, and DRB 1501. In another embodiment, the peptide binds to HLA-DRB molecules encoded by 4 distinct HLA-DRB alleles selected from DRB 101, DRB 301, DRB 401, DRB 701, DRB 1101, and DRB 1501. In another embodiment, the peptide binds to HLA-DRB molecules encoded by 5 distinct HLA-DRB alleles selected from DRB 101, DRB 301, DRB 401, DRB 701, DRB 1101, and DRB 1501. In another embodiment, the peptide binds to HLA-DRB molecules encoded by each of the following HLA-DRB alleles: DRB 101, DRB 301, DRB 401, DRB 701, DRB 1101, and DRB 1501. Each possibility represents a separate embodiment of the present invention.

[0052] Each of the above HLA class II molecule, types, classes, and combinations thereof represents a separate embodiment of the present invention.

[0053] The HLA class I molecule whose binding motif is contained in a peptide of the present invention is, in another embodiment, an HLA-A molecule. In another embodiment, the HLA class I molecule is an HLA-B molecule. In another embodiment, the HLA class I molecule is an HLA-C molecule. In another embodiment, the HLA class I molecule is an HLA-A0201 molecule. In another embodiment, the molecule is HLA A1. In another embodiment, the HLA class I molecule is HLA A2. In another embodiment, the HLA class I molecule is HLA A2.1. In another embodiment, the HLA class I molecule is HLA A3. In another embodiment, the HLA class I molecule is HLA A3.2. In another embodiment, the HLA class I molecule is HLA A11. In another embodiment, the HLA class I molecule is HLA A24. In another embodiment, the HLA class I molecule is HLA B7. In another embodiment, the HLA class I molecule is HLA B27. In another embodiment, the HLA class I molecule is HLA B8. Each possibility represents a separate embodiment of the present invention.

[0054] In another embodiment, a peptide of methods and compositions of the present invention binds to a superfamily of HLA class I molecules. In another embodiment, the superfamily is the A2 superfamily. In another embodiment, the superfamily is the A3 superfamily. In another embodiment, the superfamily is the A24 superfamily. In another embodiment, the superfamily is the B7 superfamily. In another embodiment, the superfamily is the B27 superfamily. In another embodiment, the superfamily is the B44 superfamily. In another embodiment, the superfamily is the C1 superfamily. In another embodiment, the superfamily is the C4 superfamily. In another embodiment, the superfamily is any other superfamily known in the art. Each possibility represents a separate embodiment of the present invention.

[0055] In another embodiment, an HLA class I molecule binding motif of a peptide of the present invention exhibits an increased affinity for the HLA class I molecule. In another embodiment, the

point mutation increases the affinity of the mutated peptide for the HLA class I molecule. In another embodiment, the increase in affinity is relative to the affinity (for the same HLA class I molecule) of the unmutated protein fragment wherefrom the mutated peptide was derived. Each possibility represents a separate embodiment of the present invention.

[0056] In another embodiment, a peptide of the present invention retains ability to bind multiple HLA class II molecules, as exhibited by the isolated unmutated protein fragment wherefrom the peptide of the present invention was derived.

[0057] The bcr-abl protein of methods and compositions of the present invention can be any bcr-abl protein known in the art. In other embodiments, the bcr-abl protein comprises one of the sequences set forth a GenBank sequence entry having one of the following Accession Numbers: X02596, NM_004327, X02596, U07000, Y00661, X06418, NM_005157, NM_007313, U07563, M15025, BAB62851, AAL05889, AAL99544, CAA10377, CAA10376, AAD04633, M14752, M14753, AAA35592, AAA35594, AAA87612, AAA88013, 1314255A, AAF61858, AAA35596, AAF89176, AAD04633. In other embodiments, the bcr-abl protein has one of the sequences set forth in a GenBank sequence entry having one of the following Accession Numbers: X02596, NM_004327, X02596, U07000, Y00661, X06418, NM_005157, NM_007313, U07563, M15025, BAB62851, AAL05889, AAL99544, CAA10377, CAA10376, AAD04633, M14752, M14753, AAA35592, AAA35594, AAA87612, AAA88013, 1314255A, AAF61858, AAA35596, AAF89176, AAD04633. In another embodiment, the bcr-abl protein has any other bcr-abl sequence known in the art.

[0058] In another embodiment, a peptide of methods and compositions of the present invention is derived from a fragment of a bcr-abl protein. In another embodiment, the process of derivation comprises introduction of the point mutation in the anchor residues of the HLA class I molecule binding motif. In another embodiment, the process of derivation consists of introduction of the point mutation in the anchor residues of the HLA class I molecule binding motif. In another embodiment, a peptide of the present invention differs from the corresponding fragment of a antigenic protein only by the point mutation in the HLA class I molecule binding motif anchor residue. In another embodiment, an HLA class I molecule binding motif of a peptide of the present invention differs from the corresponding wild-type protein sequence only by the point mutation in the anchor residue. Each possibility represents a separate embodiment of the present invention.

[0059] In another embodiment, the process of derivation of a peptide of the present invention further comprises one or more modifications of an amino acid (AA) to an AA analogue. In another embodiment, the process of derivation further comprises a modification of one or more peptide bond

connecting two or more of the AA. In another embodiment, the AA analogue or peptide bond modification is one of the AA analogues or peptide bond modifications enumerated below. Each possibility represents a separate embodiment of the present invention.

[0060] The unmutated fragment of a bcr-abl protein wherefrom a peptide of the present invention is derived (the "counterpart" in the wild-type sequence), in another embodiment, has the sequence IVHSATGFKQSSKALQRPVASDFEP (SEQ ID No: 62) and VHSIPLTINKEEALQRPVASDFE (SEQ ID No: 63). In another embodiment, the unmutated bcr-abl fragment is any other bcr-abl fragment that contains an HLA class II molecule binding motif. In another embodiment, the unmutated bcr-abl fragment is any other bcr-abl fragment that contains an HLA-DR molecule binding motif. In another embodiment, the unmutated bcr-abl fragment contains multiple HLA-DR molecule binding motifs. In another embodiment, the unmutated bcr-abl fragment is any other bcr-abl fragment that contains an HLA-DRB molecule binding motif. In another embodiment, the unmutated bcr-abl fragment contains multiple HLA-DRB molecule binding motifs. Methods for designing and deriving peptides of the present invention are described, for example, in Example 5. Each possibility represents a separate embodiment of the present invention.

[0061] In another embodiment, a peptide of the present invention retains the ability to bind an HLA class II molecule, as exhibited by the unmutated protein fragment wherefrom the peptide was derived. In another embodiment, a peptide of the present invention retains ability to bind multiple HLA class II molecules, as exhibited by the unmutated protein fragment. Each possibility represents a separate embodiment of the present invention.

[0062] The HLA class I molecule binding motif contained in a peptide of the present invention, in another embodiment, has the sequence YLKALQRPV (SEQ ID No: 45). In another embodiment, the HLA class I molecule binding motif has the sequence KQSSKALQV (SEQ ID No: 47). In another embodiment, the HLA class I molecule binding motif has the sequence KLSSKALQV (SEQ ID No: 48). In another embodiment, the HLA class I molecule binding motif has the sequence KLLQRPVAV (SEQ ID No: 50). In another embodiment, the HLA class I molecule binding motif has the sequence TLFKQSSKV (SEQ ID No: 52). In another embodiment, the HLA class I molecule binding motif has the sequence YLFKQSSKV (SEQ ID No: 53). In another embodiment, the HLA class I molecule binding motif has the sequence LLINKEEAL (SEQ ID No: 55). In another embodiment, the HLA class I molecule binding motif has the sequence LTINKVEAL (SEQ ID No: 56). In another embodiment, the HLA class I molecule binding motif has the sequence YLINKEEAL (SEQ ID No: 57). In another embodiment, the HLA class I molecule binding motif has the sequence YLINKEEAV

(SEQ ID No: 58). In another embodiment, the HLA class I molecule binding motif has the sequence YLINKVEAL (SEQ ID No: 59). In another embodiment, the HLA class I molecule binding motif is any other HLA class I motif known in the art. In another embodiment, the motif is any other HLA-A motif known in the art. Each possibility represents a separate embodiment of the present invention.

[0063] In another embodiment, a peptide of methods and compositions of the present invention binds with high affinity to the HLA class I molecule whose binding motif is contained therein. For example, peptides WT1-A1, B1, and C1 exhibited stable binding to HLA-A0201 (Example 1). In other embodiments, the HLA class I molecule is any HLA class I molecule enumerated herein. In another embodiment, the peptide binds to the HLA class I molecule with medium affinity. In another embodiment, the peptide binds to the HLA class I molecule with significant affinity. In another embodiment, the peptide binds to the HLA class I molecule with measurable affinity. In another embodiment, the peptide exhibits stable binding to the HLA class I molecule. Each possibility represents a separate embodiment of the present invention.

[0064] In another embodiment, a peptide of methods and compositions of the present invention binds with high affinity to the HLA class II molecule whose binding motif is contained therein. In other embodiments, the HLA class II molecule is any HLA class II molecule enumerated herein. In another embodiment, the peptide binds with high affinity to more than 1 HLA class II molecules. (give example) In another embodiment, the peptide binds to the HLA class II molecule with medium affinity. In another embodiment, the peptide binds with medium affinity to more than 1 HLA class II molecules. In another embodiment, the peptide binds to the HLA class II molecule with significant affinity. In another embodiment, the peptide binds with significant affinity to more than 1 HLA class II molecules. In another embodiment, the peptide binds to the HLA class II molecule with measurable affinity. In another embodiment, the peptide binds with measurable affinity to more than 1 HLA class II molecules. In another embodiment, the peptide exhibits stable binding to the HLA class II molecule. In another embodiment, the peptide exhibits stable binding to more than 1 HLA class II molecules. Each possibility represents a separate embodiment of the present invention.

[0065] In another embodiment, a peptide of methods and compositions of the present invention binds to both an HLA class I molecule and an HLA class II molecule with significant affinity. In another embodiment, the peptide binds to both an HLA class I molecule and an HLA class II molecule with high affinity. In another embodiment, the peptide binds to both an HLA class I molecule and an HLA class II molecule with medium affinity. In another embodiment, the peptide binds to both an HLA

class I molecule and an HLA class II molecule with measurable affinity. Each possibility represents a separate embodiment of the present invention.

[0066] "Fragment," in another embodiment, refers to a peptide of 11 or more AA in length. In another embodiment, a peptide fragment of the present invention is 16 or more AA long. In another embodiment, the fragment is 12 or more AA long. In another embodiment, the fragment is 13 or more AA. In another embodiment, the fragment is 14 or more AA. In another embodiment, the fragment is 15 or more AA. In another embodiment, the fragment is 17 or more AA. In another embodiment, the fragment is 18 or more AA. Each possibility represents a separate embodiment of the present invention.

[0067] In another embodiment, the present invention provides a composition comprising an isolated peptide of the invention in combination with at least one additional bcr-abl peptide. In certain embodiments, a composition comprising at least two different isolated peptides of the present invention is provided. In certain embodiments, a composition comprising at least three or at least four different isolated peptides of the present invention is provided. Each possibility represents a separate embodiment of the present invention. In certain embodiments, the composition of the present invention is a vaccine.

[0068] The additional bcr-abl peptide, in another embodiment, has the sequence IVHSATGFKQSSKALQRPVASDFEP; SEQ ID No: 62) are utilized. In another embodiment, the additional bcr-abl peptide comprises the sequence IVHSATGFKQSSKALQRPVASDFEP. In another embodiment, the additional bcr-abl peptide has the sequence VHSIPLTINKEEALQRPVASDFE (SEQ ID No: 63). In another embodiment, the additional bcr-abl peptide comprises the sequence VHSIPLTINKEEALQRPVASDFE. In another embodiment, the additional bcr-abl peptide has a sequence selected from the sequences set forth in SEQ ID No: 44-59. In another embodiment, the additional bcr-abl peptide is a heteroclitic peptide having a sequence selected from SEQ ID No: 44-59. In another embodiment, the additional bcr-abl peptide is a wild-type peptide having a sequence selected from SEQ ID No: 44-59. In another embodiment, the additional bcr-abl peptide is another heteroclitic bcr-abl peptide. In another embodiment, the additional bcr-abl peptide is another wild-type bcr-abl peptide. In another embodiment, the additional bcr-abl peptide is any other bcr-abl peptide known in the art. Each possibility represents a separate embodiment of the present invention.

[0069] In another embodiment, the additional bcr-abl peptide has a length of 8-22 AA. In another embodiment, the additional bcr-abl peptide has a length of 8-30 AA. In another embodiment, the additional bcr-abl peptide has a length of 11-30 AA. In another embodiment, the length is 16-22 AA.

In another embodiment, the length is 19 AA. In another embodiment, the peptide is 15-23 AA in length. In another embodiment, the length is 15-24 AA. In another embodiment, the length is 15-25 AA. In another embodiment, the length is 15-26 AA. In another embodiment, the length is 15-27 AA. In another embodiment, the length is 15-28 AA. In another embodiment, the length is 14-30 AA. In another embodiment, the length is 14-29 AA. In another embodiment, the length is 14-28 AA. In another embodiment, the length is 14-26 AA. In another embodiment, the length is 14-24 AA. In another embodiment, the length is 14-22 AA. In another embodiment, the length is 14-20 AA. In another embodiment, the length is 16-30 AA. In another embodiment, the length is 16-28 AA. In another embodiment, the length is 16-26 AA. In another embodiment, the length is 16-24 AA. In another embodiment, the length is 16-22 AA. In another embodiment, the length is 18-30 AA. In another embodiment, the length is 18-28 AA. In another embodiment, the length is 18-26 AA. In another embodiment, the length is 18-24 AA. In another embodiment, the length is 18-22 AA. In another embodiment, the length is 18-20 AA. In another embodiment, the length is 20-30 AA. In another embodiment, the length is 20-28 AA. In another embodiment, the length is 20-26 AA. In another embodiment, the length is 20-24 AA. In another embodiment, the length is 22-30 AA. In another embodiment, the length is 22-28 AA. In another embodiment, the length is 22-26 AA. In another embodiment, the length is 24-30 AA. In another embodiment, the length is 24-28 AA. In another embodiment, the length is 24-26 AA.

[0070] In another embodiment, the additional bcr-abl peptide has any other length. Each possibility represents a separate embodiment of the present invention.

[0071] In one embodiment, "affinity" refers to the concentration of peptide necessary for inhibiting binding of a standard peptide to the indicated MHC molecule by fifty percent. In one embodiment, "high affinity" refers to an affinity is such that a concentration of about 100 nanomolar (nM) or less of the peptide is required for inhibition of binding of a standard peptide. In another embodiment, the binding affinity is 80 nM. In another embodiment, the binding affinity is 60 nM. In another embodiment, the binding affinity is 40 nM. In another embodiment, the binding affinity is 30 nM. In another embodiment, the binding affinity is 20 nM. In another embodiment, the binding affinity is 15 nM. In another embodiment, the binding affinity is 10 nM. In another embodiment, the binding affinity is 8 nM. In another embodiment, the binding affinity is 6 nM. In another embodiment, the binding affinity is 4 nM. In another embodiment, the binding affinity is 3 nM. In another embodiment, the binding affinity is 2 nM. In another embodiment, the binding affinity is 1.5 nM. In another embodiment, the binding affinity is 1 nM. In another embodiment, the binding affinity is 0.8 nM. In another embodiment, the binding affinity is 0.6 nM. In another embodiment, the binding affinity is 0.5

nM. In another embodiment, the binding affinity is 0.4 nM. In another embodiment, the binding affinity is 0.3 nM. In another embodiment, the binding affinity is less than 0.3 nM.

[0072] In another embodiment, "high affinity" refers to a binding affinity of 0.5-100 nM. In another embodiment, the binding affinity is 1-100 nM. In another embodiment, the binding affinity is 1.5-200 nM. In another embodiment, the binding affinity is 2-100 nM. In another embodiment, the binding affinity is 3-100 nM. In another embodiment, the binding affinity is 4-100 nM. In another embodiment, the binding affinity is 6-100 nM. In another embodiment, the binding affinity is 10-100 nM. In another embodiment, the binding affinity is 30-100 nM. In another embodiment, the binding affinity is 3-80 nM. In another embodiment, the binding affinity is 4-60 nM. In another embodiment, the binding affinity is 5-50 nM. In another embodiment, the binding affinity is 6-50 nM. In another embodiment, the binding affinity is 8-50 nM. In another embodiment, the binding affinity is 10-50 nM. In another embodiment, the binding affinity is 20-50 nM. In another embodiment, the binding affinity is 6-40 nM. In another embodiment, the binding affinity is 8-30 nM. In another embodiment, the binding affinity is 10-25 nM. In another embodiment, the binding affinity is 15-25 nM. Each affinity and range of affinities represents a separate embodiment of the present invention.

[0073] In another embodiment, "medium affinity" refers to a binding affinity of 100-500 nM. In another embodiment, the binding affinity is 100-300 nM. In another embodiment, the binding affinity is 100-200 nM. In another embodiment, the binding affinity is 50-100 nM. In another embodiment, the binding affinity is 50-80 nM. In another embodiment, the binding affinity is 50-60 nM. Each affinity and range of affinities represents a separate embodiment of the present invention.

[0074] "Significant affinity" refers, in another embodiment, to sufficient affinity to mediate recognition of a target cell by a T cell carrying a T cell receptor (TCR) that recognizes the MHC molecule-peptide complex. In another embodiment, the term refers to sufficient affinity to mediate recognition of a cancer cell by a T cell carrying a TCR that recognizes the MHC molecule-peptide complex. In another embodiment, the term refers to sufficient affinity to mediate activation of a naive T cell by a dendritic cell presenting the peptide. In another embodiment, the term refers to sufficient affinity to mediate activation of a naive T cell by an APC presenting the peptide. In another embodiment, the term refers to sufficient affinity to mediate re-activation of a memory T cell by a dendritic cell presenting the peptide. In another embodiment, the term refers to sufficient affinity to mediate re-activation of a memory T cell by an APC presenting the peptide. In another embodiment, the term refers to sufficient affinity to mediate re-activation of a memory T cell by a somatic cell presenting the peptide. Each possibility represents a separate embodiment of the present invention.

[0075] "Measurable affinity" refers, in another embodiment, to sufficient affinity to be measurable by an immunological assay. In another embodiment, the immunological assay is any assay enumerated herein. Each possibility represents a separate embodiment of the present invention.

[0076] In another embodiment, a peptide of methods and compositions of the present invention binds to a superfamily of HLA molecules. Superfamilies of HLA molecules share very similar or identical binding motifs. In one embodiment, the superfamily is a HLA class I superfamily. In one embodiment, the superfamily is a HLA class II superfamily. Each possibility represents a separate embodiment of the present invention.

[0077] In another embodiment, a peptide of methods and compositions of the present invention is heteroclitic. "Heteroclitic" refers, in one embodiment, to a peptide that generates an immune response that recognizes the original peptide from which the heteroclitic peptide was derived (e.g. the peptide not containing the anchor residue mutations). In one embodiment, "original peptide" refers to a fragment of bcr-abl. For example, a peptide termed "WT1 122A1," having the sequence SGQAYMFPNAPYLPSCLES (SEQ ID No: 41), was generated from the wild-type WT1 peptide SGQARMFPNAPYLPSCLES (SEQ ID No: 39) by mutation of residue 5 to arginine (Example 6). The mutation introduced the $CD8^+$ heteroclitic WT1A1 peptide YMFPNAPYL (SEQ ID No: 6) into the WT 1 peptide. In another embodiment, "heteroclitic" refers to a peptide that generates an immune response that recognizes the original peptide from which the heteroclitic peptide was derived, wherein the immune response generated by vaccination with the heteroclitic peptide is greater than the immune response generated by vaccination with the original peptide. In another embodiment, a "heteroclitic" immune response refers to an immune response that recognizes the original peptide from which the improved peptide was derived (e.g. the peptide not containing the anchor residue mutations). In another embodiment, a "heteroclitic" immune response refers to an immune response that recognizes the original peptide from which the heteroclitic peptide was derived, wherein the immune response generated by vaccination with the heteroclitic peptide is greater than the immune response generated by vaccination with the original peptide. Each possibility represents a separate embodiment of the present invention.

[0078] In one embodiment, a heteroclitic peptide of the present invention induces an immune response that is increased at least 2-fold relative to the peptide from which the heteroclitic peptide was derived ("native peptide"). In another embodiment, the increase is 3-fold relative to the native peptide. In another embodiment, the increase is 5-fold relative to the native peptide. In another embodiment, the increase is 7-fold relative to the native peptide. In another embodiment, the increase is 10-fold relative

to the native peptide. In another embodiment, the increase is 15-fold relative to the native peptide. In another embodiment, the increase is 20-fold relative to the native peptide. In another embodiment, the increase is 30-fold relative to the native peptide. In another embodiment, the increase is 50-fold relative to the native peptide. In another embodiment, the increase is 100-fold relative to the native peptide. In another embodiment, the increase is 150-fold relative to the native peptide. In another embodiment, the increase is 200-fold relative to the native peptide. In another embodiment, the increase is 300-fold relative to the native peptide. In another embodiment, the increase is 500-fold relative to the native peptide. In another embodiment, the increase is 1000-fold relative to the native peptide. In another embodiment, the increase is more than 1000-fold relative to the native peptide. Each possibility represents a separate embodiment of the present invention.

[0079] "Anchor motifs" or "anchor residues" refers, in one embodiment, to one or a set of preferred residues at particular positions in an HLA-binding sequence. For example, residues at positions 1, 2, 3, 6, and 9 are used as anchor residues in the Examples herein. In one embodiment, the HLA-binding sequence is an HLA class II-binding sequence. In another embodiment, the HLA-binding sequence is an HLA class I-binding sequence. In another embodiment, the positions corresponding to the anchor motifs are those that play a significant role in binding the HLA molecule. In one embodiment, the anchor residue is a primary anchor motif. In another embodiment, the anchor residue is a secondary anchor motif. Each possibility represents a separate embodiment of the present invention.

[0080] In another embodiment, "anchor residues" are residues in positions 1, 3, 6, and 9 of the HLA class I binding motif. In another embodiment, the term refers to positions 1, 2, 6, and 9 of the HLA class I binding motif. In another embodiment, the term refers to positions 1, 6, and 9 of the HLA class I binding motif. In another embodiment, the term refers to positions 1, 2, and 9 of the HLA class I binding motif. In another embodiment, the term refers to positions 1, 3, and 9 of the HLA class I binding motif. In another embodiment, the term refers to positions 2 and 9 of the HLA class I binding motif. In another embodiment, the term refers to positions 6 and 9 of the HLA class I binding motif. Each possibility represents a separate embodiment of the present invention.

[0081] Methods for identifying MHC class II epitopes are well known in the art. In one embodiment, the MHC class II epitope is predicted using TEPITOPE (Meister GE, Roberts CG et al, Vaccine 1995 13: 581-91). In another embodiment, the MHC class II epitope is identified using EpiMatrix (De Groot AS, Jesdale BM et al, AIDS Res Hum Retroviruses 1997 13: 529-31). In another embodiment, the MHC class II epitope is identified using the Predict Method (Yu K, Petrovsky N et al, Mol Med. 2002 8: 137-48). In another embodiment, the MHC class II epitope is identified using the SYFPEITHI

epitope prediction algorithm (Examples). SYFPEITHI is a database comprising more than 4500 peptide sequences known to bind class I and class II MHC molecules. SYFPEITHI provides a score based on the presence of certain amino acids in certain positions along the MHC-binding groove. Ideal amino acid anchors are valued at 10 points, unusual anchors are worth 6–8 points, auxiliary anchors are worth 4–6 points, preferred residues are worth 1–4 points; negative amino acid effect on the binding score between –1 and –3. The maximum score for HLA-A*0201 is 36.

[0082] In another embodiment, the MHC class II epitope is identified using Rankpep. Rankpep uses position specific scoring matrices (PSSMs) or profiles from sets of aligned peptides known to bind to a given MHC molecule as the predictor of MHC-peptide binding. Rankpep includes information on the score of the peptide and the % optimum or percentile score of the predicted peptide relative to that of a consensus sequence that yields the maximum score, with the selected profile. Rankpep includes a selection of 102 and 80 PSSMs for the prediction of peptide binding to MHC I and MHC II molecules, respectively. Several PSSMs for the prediction of peptide binders of different sizes are usually available for each MHC I molecule.

[0083] In another embodiment, the MHC class II epitope is identified using SVMHC (Donnes P, Elofsson A. Prediction of MHC class I binding peptides, using SVMHC. BMC Bioinformatics. 2002 Sep 11;3:25). In another embodiment, the MHC class II epitope is identified using any other method known in the art. The above methods are utilized, in another embodiment, to identify MHC class II binding will be perturbed by introduction of an MHC class I anchor residue mutation into the sequence. Each possibility represents a separate embodiment of the present invention.

[0084] Methods for identifying MHC class I epitopes are well known in the art. In one embodiment, the MHC class I epitope is predicted using BIMAS software (Example 1). In another embodiment, the MHC class I epitope is identified using SYFPEITHI. In another embodiment, the MHC class I epitope is identified using SVMHC (Donnes P, Elofsson A. Prediction of MHC class I binding peptides, using SVMHC. BMC Bioinformatics. 2002 Sep 11;3:25). In another embodiment, the MHC class I epitope is identified using NetMHC-2.0 (Sensitive quantitative predictions of peptide-MHC binding by a 'Query by Committee' artificial neural network approach. Buus S, Lauemoller SL, Worning P, Kesmir C, Frimurer T, Corbet S, Fomsgaard A, Hilden J,Holm A, Brunak S. Tissue Antigens., 62:378-84, 2003). In another embodiment, the MHC class I epitope is identified using any other method known in the art. The above methods are utilized, in another embodiment, to identify MHC class I epitopes that can be created by introduction of an anchor residue mutation into the bcr-abl sequence. Each possibility represents a separate embodiment of the present invention.

[0085] In another embodiment, the mutation that enhances MHC binding is in the residue at position 1 of the HLA class I binding motif. In one embodiment, the residue is changed to tyrosine. In another embodiment, the residue is changed to glycine. In another embodiment, the residue is changed to threonine. In another embodiment, the residue is changed to phenylalanine. In another embodiment, the residue is changed to any other residue known in the art. In another embodiment, a substitution in position 1 (e.g. to tyrosine) stabilizes the binding of the position 2 anchor residue.

[0086] In another embodiment, the mutation is in position 2 of the HLA class I binding motif. In one embodiment, the residue is changed to leucine. In another embodiment, the residue is changed to valine. In another embodiment, the residue is changed to isoleucine. In another embodiment, the residue is changed to methionine. In another embodiment, the residue is changed to any other residue known in the art.

[0087] In another embodiment, the mutation is in position 6 of the HLA class I binding motif. In one embodiment, the residue is changed to valine. In another embodiment, the residue is changed to cysteine. In another embodiment, the residue is changed to glutamine. In another embodiment, the residue is changed to histidine. In another embodiment, the residue is changed to any other residue known in the art.

[0088] In another embodiment, the mutation is in position 9 of the HLA class I binding motif. In another embodiment, the mutation changes the residue at the C-terminal position thereof. In one embodiment, the residue is changed to valine. In another embodiment, the residue is changed to threonine. In another embodiment, the residue is changed to isoleucine. In another embodiment, the residue is changed to leucine. In another embodiment, the residue is changed to alanine. In another embodiment, the residue is changed to cysteine. In another embodiment, the residue is changed to any other residue known in the art.

[0089] In another embodiment, the point mutation is in a primary anchor residue. In another embodiment, the HLA class I primary anchor residues are positions 2 and 9. In another embodiment, the point mutation is in a secondary anchor residue. In another embodiment, the HLA class I secondary anchor residues are positions 1, 3, 6, and 7. In another embodiment, the point mutation is in a position selected from positions 4, 5, and 8. Each possibility represents a separate embodiment of the present invention.

[0090] In another embodiment, the point mutation is in one or more residues in positions selected from positions 1, 3, 6, and 9 of the HLA class I binding motif. In another embodiment, the point

mutation is in one or more residues in positions selected from positions 1, 2, 6, and 9 of the HLA class I binding motif. In another embodiment, the point mutation is in one or more residues in positions selected from positions 1, 6, and 9 of the HLA class I binding motif. In another embodiment, the point mutation is in one or more residues in positions selected from positions 1, 2, and 9 of the HLA class I binding motif. In another embodiment, the point mutation is in one or more residues in positions selected from positions 1, 3, and 9 of the HLA class I binding motif. In another embodiment, the point mutation is in one or more residues in positions selected from positions 2 and 9 of the HLA class I binding motif. In another embodiment, the point mutation is in one or more residues in positions selected from positions 6 and 9 of the HLA class I binding motif. Each possibility represents a separate embodiment of the present invention.

[0091] In another embodiment, the mutation is in the 4 position of the HLA class I binding motif. In another embodiment, the mutation is in the 5 position of the HLA class I binding motif. In another embodiment, the mutation is in the 7 position of the HLA class I binding motif. In another embodiment, the mutation is in the 8 position of the HLA class I binding motif. Each possibility represents a separate embodiment of the present invention.

[0092] Each of the above anchor residues and substitutions represents a separate embodiment of the present invention.

[0093] As provided herein, peptides of the present invention exhibited significant ability to stimulate both $CD4^+$ and $CD8^+$ T cells (Examples). Moreover, the peptides exhibited enhanced immuno-stimulating activity, relative to the native peptides from which they were derived.

[0094] In another embodiment, the HLA class II binding site in a peptide of the present invention is created or improved by mutation of an HLA class II motif anchor residue. In another embodiment, the anchor residue that is modified is in the P1 position (e.g. a position corresponding to F263 of the CII(259-273) peptide). In another embodiment, the anchor residue is in the P2 position (e.g. a position corresponding to K264 of the CII(259-273) peptide). In another embodiment, the anchor residue is in the P6 position. In another embodiment, the anchor residue is in the P9 position. In another embodiment, the anchor residue is selected from the P1, P2, P6, and P9 positions.

[0095] In another embodiment, the HLA class II motif anchor residue is the P3 position. In another embodiment, the HLA class II motif anchor residue is the P4 position. In another embodiment, the HLA class II motif anchor residue is the P5 position. In another embodiment, the HLA class II motif anchor residue is the P6 position. In another embodiment, the HLA class II motif anchor residue is the

P8 position. In another embodiment, the HLA class II motif anchor residue is the P10 position. In another embodiment, the HLA class II motif anchor residue is the P11 position. In another embodiment, the HLA class II motif anchor residue is the P12 position. In another embodiment, the HLA class II motif anchor residue is the P13 position. In another embodiment, the anchor residue is any other anchor residue of an HLA class II molecule that is known in the art. In another embodiment, residues other than P1, P2, P6, and P9 serve as secondary anchor residues; therefore, mutating them can improve HLA class II binding. Each possibility represents a separate embodiment of the present invention.

[0096] In another embodiment, a peptide of the present invention is homologous to a peptide enumerated in the Examples. The terms "homology," "homologous," etc, when in reference to any protein or peptide, refer, in one embodiment, to a percentage of amino acid residues in the candidate sequence that are identical with the residues of a corresponding native polypeptide, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. Methods and computer programs for the alignment are well known in the art.

[0097] In another embodiment, the term "homology," when in reference to any nucleic acid sequence similarly indicates a percentage of nucleotides in a candidate sequence that are identical with the nucleotides of a corresponding native nucleic acid sequence.

[0098] Homology is, in one embodiment, determined by computer algorithm for sequence alignment, by methods well described in the art. In other embodiments, computer algorithm analysis of nucleic acid sequence homology includes the utilization of any number of software packages available, such as, for example, the BLAST, DOMAIN, BEAUTY (BLAST Enhanced Alignment Utility), GENPEPT and TREMBL packages.

[0099] In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 70%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 72%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 75%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 78%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 80%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 82%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 83%.

In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 85%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 87%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 88%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 90%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 92%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 93%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 95%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 96%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 97%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 98%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of greater than 99%. In another embodiment, "homology" refers to identity to a sequence of a peptide of the present invention of 100%. Each possibility represents a separate embodiment of the present invention.

[00100] In another embodiment, homology is determined is via determination of candidate sequence hybridization, methods of which are well described in the art (See, for example, "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., Eds. (1985); Sambrook et al., 2001, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y). In another embodiments, methods of hybridization are carried out under moderate to stringent conditions, to the complement of a DNA encoding a native caspase peptide. Hybridization conditions being, for example, overnight incubation at 42 °C in a solution comprising: 10-20 % formamide, 5 X SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7. 6), 5 X Denhardt's solution, 10 % dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA.

[00101] Each of the above homologues and variants of peptides enumerated in the Examples represents a separate embodiment of the present invention.

[00102] In another embodiment, the present invention provides a composition comprising a peptide of this invention. In another embodiment, the composition further comprises a pharmaceutically acceptable carrier. In another embodiment, the composition further comprises an adjuvant. In another

embodiment, the composition comprises two or more peptides of the present invention. In another embodiment, the composition further comprises any of the additives, compounds, or excipients set forth hereinbelow. In one embodiment, the adjuvant is QS21, Freund's complete or incomplete adjuvant, aluminum phosphate, aluminum hydroxide, BCG or alum. In other embodiments, the carrier is any carrier enumerated herein. In other embodiments, the adjuvant is any adjuvant enumerated herein. Each possibility represents a separate embodiment of the present invention.

[00103] In another embodiment, this invention provides a vaccine comprising a peptide of the present invention. In another embodiment, the vaccine further comprises a carrier. In another embodiment, the vaccine further comprises an adjuvant. In another embodiment, the vaccine further comprises a combination of a carrier and an adjuvant. In another embodiment, the vaccine is an antigen presenting cell (APC) associated with a mixture of peptides of the present invention. Each possibility represents a separate embodiment of the present invention.

[00104] In another embodiment, this invention provides an immunogenic composition comprising a peptide of the present invention. In another embodiment, the immunogenic composition further comprises a carrier. In another embodiment, the immunogenic composition further comprises an adjuvant. In another embodiment, the immunogenic composition further comprises a combination of a carrier and an adjuvant. Each possibility represents a separate embodiment of the present invention.

[00105] In another embodiment, the term "vaccine" refers to a material or composition that, when introduced into a subject, provides a prophylactic or therapeutic response for a particular disease, condition, or symptom of same. In another embodiment, this invention comprises peptide-based vaccines, wherein the peptide comprises any embodiment listed herein, including immunomodulating compounds such as cytokines, adjuvants, etc.

[00106] In another embodiment, a vaccine of methods and compositions of the present invention further comprises an adjuvant. In one embodiment, the adjuvant is Montanide ISA 51. Montanide ISA 51 contains a natural metabolizable oil and a refined emulsifier. In another embodiment, the adjuvant is GM-CSF. Recombinant GM-CSF is a human protein grown, in one embodiment, in a yeast (*S. cerevisiae*) vector. GM-CSF promotes clonal expansion and differentiation of hematopoietic progenitor cells, APC, and dendritic cells and T cells.

[00107] In another embodiment, the adjuvant is a cytokine. In another embodiment, the adjuvant is a growth factor. In another embodiment, the adjuvant is a cell population. In another embodiment, the adjuvant is QS21. In another embodiment, the adjuvant is Freund's incomplete adjuvant. In another

embodiment, the adjuvant is aluminum phosphate. In another embodiment, the adjuvant is aluminum hydroxide. In another embodiment, the adjuvant is BCG. In another embodiment, the adjuvant is alum. In another embodiment, the adjuvant is an interleukin. In another embodiment, the adjuvant is a chemokine. In another embodiment, the adjuvant is any other type of adjuvant known in the art. In another embodiment, the vaccine comprises two of the above adjuvants. In another embodiment, the vaccine comprises more than two of the above adjuvants. Each possibility represents a separate embodiment of the present invention.

[00108] In another embodiment, the present invention provides a cell comprising a peptide of the present invention. In another embodiment, the cell is an antigen-presenting cell (APC). In another embodiment, the present invention provides a vaccine comprising an APC of the present invention.

[00109] In another embodiment, the present invention provides a nucleic acid molecule encoding a peptide of the present invention. In another embodiment, the present invention provides a vaccine comprising a nucleic acid molecule of the present invention.

[00110] In another embodiment, the present invention provides a vector comprising a nucleic acid molecule of the present invention. In another embodiment, the present invention provides a vaccine comprising a vector of the present invention.

[00111] In other embodiments, a vaccine or composition of the present invention comprises any of the embodiments of peptides of the present invention and combinations thereof. Each possibility represents a separate embodiment of the present invention.

[00112] In another embodiment, a composition of the present invention contains two peptides that are derived from the same protein fragment, each containing a different HLA class I heteroclitic peptide. In another embodiment, the two HLA class I heteroclitic peptides contain mutations in different HLA class I molecule anchor residues. In another embodiment, the two HLA class I heteroclitic peptides contain different mutations in the same anchor residue(s). Each possibility represents a separate embodiment of the present invention.

[00113] In another embodiment, the peptides in a composition of the present invention bind to two distinct HLA class II molecules. In another embodiment, the peptides bind to three distinct HLA class II molecules. In another embodiment, the peptides bind to four distinct HLA class II molecules. In another embodiment, the peptides bind to five distinct HLA class II molecules. In another

embodiment, the peptides bind to more than five distinct HLA class II molecules. In another embodiment, the peptides in the composition bind to the same HLA class II molecules.

[00114] In another embodiment, each of the peptides in a composition of the present invention binds to a set of HLA class II molecules. In another embodiment, each of the peptides binds to a distinct set of HLA class II molecules. In another embodiment, the peptides in the composition bind to the same set of HLA class II molecules. In another embodiment, two of the peptides bind to a distinct but overlapping set of HLA class II molecules. In another embodiment, two or more of the peptides bind to the same set of HLA class II molecules, while another of the peptides binds to a distinct set. In another embodiment, two or more of the peptides bind to an overlapping set of HLA class II molecules, while another of the peptides binds to a distinct set.

[00115] In another embodiment, the peptides in a composition of the present invention bind to two distinct HLA class I molecules. In another embodiment, the peptides bind to three distinct HLA class I molecules. In another embodiment, the peptides bind to four distinct HLA class I molecules. In another embodiment, the peptides bind to five distinct HLA class I molecules. In another embodiment, the peptides bind to more than five distinct HLA class I molecules. In another embodiment, the peptides in the composition bind to the same HLA class I molecules.

[00116] In another embodiment, each of the peptides in a composition of the present invention binds to a set of HLA class I molecules. In another embodiment, each of the peptides binds to a distinct set of HLA class I molecules. In another embodiment, the peptides in the composition bind to the same set of HLA class I molecules. In another embodiment, two of the peptides bind to a distinct but overlapping set of HLA class I molecules. In another embodiment, two or more of the peptides bind to the same set of HLA class I molecules, while another of the peptides binds to a distinct set. In another embodiment, two or more of the peptides bind to an overlapping set of HLA class I molecules, while another of the peptides binds to a distinct set.

[00117] In one embodiment, a "set of HLA class II molecules" or "set of HLA class I molecules" refers to the HLA molecules encoded by different alleles at a particular locus. In another embodiment, the term refers to HLA molecules with a particular binding specificity. In another embodiment, the term refers to HLA molecules with a particular peptide consensus sequence. In another embodiment, the term refers to a superfamily of HLA class II molecules. Each possibility represents a separate embodiment of the present invention.

[00118] Each of the above compositions and types of compositions represents a separate embodiment of the present invention.

[00119] In another embodiment, any embodiments described herein, regarding peptides, vaccines and compositions of this invention can be employed in any of the methods of this invention. Each combination of peptide, vaccine, or composition with a method represents an embodiment thereof.

[00120] In another embodiment, the present invention provides a method of treating a subject with a bcr-abl-expressing cancer, the method comprising administering to the subject a peptide of the present invention, thereby treating a subject with a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of treating a subject with a bcr-abl-expressing cancer, the method comprising administering to the subject a vaccine of the present invention, thereby treating a subject with a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of treating a subject with a bcr-abl-expressing cancer, the method comprising administering to the subject an immunogenic composition of the present invention, thereby treating a subject with a bcr-abl-expressing cancer.

[00121] In another embodiment, the present invention provides a method of suppressing or halting the progression of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject a peptide of the present invention, thereby suppressing or halting the progression of a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of suppressing or halting the progression of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject a vaccine of the present invention, thereby suppressing or halting the progression of a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of suppressing or halting the progression of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject an immunogenic composition of the present invention, thereby suppressing or halting the progression of a bcr-abl-expressing cancer

[00122] In another embodiment, the present invention provides a method of reducing the incidence of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject a peptide of the present invention, thereby reducing the incidence of a bcr-abl-expressing cancer in a subject. In another embodiment, the present invention provides a method of reducing the incidence of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject a vaccine of the present invention, thereby reducing the incidence of a bcr-abl-expressing cancer in a subject. In another embodiment, the present invention provides a method of reducing the incidence of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject an immunogenic

composition of the present invention, thereby reducing the incidence of a bcr-abl-expressing cancer in a subject.

[00123] In another embodiment, the present invention provides a method of reducing the incidence of relapse of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject a peptide of the present invention, thereby reducing the incidence of relapse of a bcr-abl-expressing cancer in a subject. In another embodiment, the present invention provides a method of reducing the incidence of relapse of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject a vaccine of the present invention, thereby reducing the incidence of relapse of a bcr-abl-expressing cancer in a subject. In another embodiment, the present invention provides a method of reducing the incidence of relapse of a bcr-abl-expressing cancer in a subject, the method comprising administering to the subject an immunogenic composition of the present invention, thereby reducing the incidence of relapse of a bcr-abl-expressing cancer in a subject

[00124] In another embodiment, the present invention provides a method of overcoming a T cell tolerance of a subject to a bcr-abl-expressing cancer, the method comprising administering to the subject a peptide of the present invention, thereby overcoming a T cell tolerance to a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of overcoming a T cell tolerance of a subject to a bcr-abl-expressing cancer, the method comprising administering to the subject a vaccine of the present invention, thereby overcoming a T cell tolerance to a bcr-abl-expressing cancer. In another embodiment, the present invention provides a method of overcoming a T cell tolerance of a subject to a bcr-abl-expressing cancer, the method comprising administering to the subject an immunogenic composition of the present invention, thereby overcoming a T cell tolerance to a bcr-abl-expressing cancer

[00125] In another embodiment, the present invention provides a method of treating a subject having a bcr-abl-expressing cancer, comprising (a) inducing in a donor formation and proliferation of human cytotoxic T lymphocytes (CTL) that recognize a malignant cell of the cancer by a method of the present invention; and (b) infusing the human CTL into the subject, thereby treating a subject having a cancer.

[00126] In another embodiment, the present invention provides a method of treating a subject having a bcr-abl-expressing cancer, comprising (a) inducing *ex vivo* formation and proliferation of human CTL that recognize a malignant cell of the cancer by a method of the present invention, wherein the human

immune cells are obtained from a donor; and (b) infusing the human CTL into the subject, thereby treating a subject having a cancer.

[00127] In another embodiment, the present invention provides a method of inducing formation and proliferation of bcr-abl-specific CTL, the method comprising contacting a lymphocyte population with a vaccine of the present invention, thereby inducing formation and proliferation of bcr-abl-specific CTL. In another embodiment, the present invention provides a method of inducing formation and proliferation of bcr-abl-specific CTL, the method comprising contacting a lymphocyte population with a peptide of the present invention, thereby inducing formation and proliferation of bcr-abl-specific CTL. In another embodiment, the present invention provides a method of inducing formation and proliferation of bcr-abl-specific CTL, the method comprising contacting a lymphocyte population with an immunogenic composition of the present invention, thereby inducing formation and proliferation of bcr-abl-specific CTL. In another embodiment, the target cell is a cell of a bcr-abl-expressing leukemia. In another embodiment, the target cell is a cell of a bcr-abl-expressing cancer.

[00128] In another embodiment, the present invention provides a method of inducing in a subject formation and proliferation of bcr-abl-specific CTL, the method comprising contacting the subject with a vaccine of the present invention, thereby inducing in a subject formation and proliferation of bcr-abl-specific CTL. In another embodiment, the present invention provides a method of inducing in a subject formation and proliferation of bcr-abl-specific CTL, the method comprising contacting the subject with a peptide of the present invention, thereby inducing in a subject formation and proliferation of bcr-abl-specific CTL. In another embodiment, the present invention provides a method of inducing in a subject formation and proliferation of bcr-abl-specific CTL, the method comprising contacting the subject with an immunogenic composition of the present invention, thereby inducing in a subject formation and proliferation of bcr-abl-specific CTL. In another embodiment, the target cell is a cell of a bcr-abl-expressing leukemia. In another embodiment, the target cell is a cell of a bcr-abl-expressing cancer.

[00129] In another embodiment, the present invention provides a method of inducing formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein, the method comprising contacting a lymphocyte population with a vaccine of the present invention, thereby inducing formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein. In another embodiment, the present invention provides a method of inducing formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte

specific for the bcr-abl protein, the method comprising contacting a lymphocyte population with a peptide of the present invention, thereby inducing formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein. In another embodiment, the present invention provides a method of inducing formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein, the method comprising contacting a lymphocyte population with a immunogenic composition of the present invention, thereby inducing formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein.

[00130] In another embodiment, the present invention provides a method of inducing in a subject formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein, the method comprising administering to the subject with a vaccine of the present invention, thereby inducing in a subject formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein. In another embodiment, the present invention provides a method of inducing in a subject formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein, the method comprising administering to the subject with a peptide of the present invention, thereby inducing in a subject formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein. In another embodiment, the present invention provides a method of inducing in a subject formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein, the method comprising administering to the subject with a immunogenic composition of the present invention, thereby inducing in a subject formation and proliferation of both (a) a CD8+ lymphocyte specific for a bcr-abl protein; and (b) a CD4+ lymphocyte specific for the bcr-abl protein.

[00131] In another embodiment, this invention provides a method of generating a heteroclitic immune response in a subject, wherein the heteroclitic immune response is directed against a bcr-abl-expressing cancer, the method comprising administering to the subject a peptide of the present invention, thereby generating a heteroclitic immune response. In another embodiment, this invention provides a method of generating a heteroclitic immune response in a subject, wherein the heteroclitic immune response is directed against a bcr-abl-expressing cancer, the method comprising administering to the subject a vaccine of the present invention, thereby generating a heteroclitic immune response. In another embodiment, this invention provides a method of generating a heteroclitic immune response in

a subject, wherein the heteroclitic immune response is directed against a bcr-abl-expressing cancer, the method comprising administering to the subject an immunogenic composition of the present invention, thereby generating a heteroclitic immune response.

[00132] Each method represents a separate embodiment of the present invention.

[00133] An immunogenic composition of the present invention comprises, in another embodiment, an APC associated with a peptide of the present invention. In another embodiment, the immunogenic composition comprises an APC associated with a mixture of peptides of the present invention. In another embodiment, the immunogenic composition consists of an APC associated with a peptide of the present invention. In another embodiment, the immunogenic composition consists of an APC associated with a mixture of peptides of the present invention. Each possibility represents a separate embodiment of the present invention.

[00134] In another embodiment, a target cell of an immune response elicited by a method of the present invention presents the peptide of the present invention, or a corresponding bcr-abl fragment, on an HLA class I molecule. In another embodiment, the HLA molecule is an HLA class I molecule. In another embodiment, the HLA molecule is an HLA class II molecule. In another embodiment, the peptide or a fragment thereof is presented on both an HLA class I molecule and an HLA class II molecule. In other embodiments, the HLA class I molecule is any HLA class I subtype or HLA class I molecule known in the art. In other embodiments, the HLA class I molecule is any HLA class I subtype or HLA class I molecule enumerated herein. In other embodiments, the HLA class II molecule is any HLA class II subtype or HLA class II molecule known in the art. In other embodiments, the HLA class II molecule is any HLA class II subtype or HLA class II molecule enumerated herein. In another embodiment, the immune response against the peptide or fragment is a heteroclitic immune response. Each possibility represents a separate embodiment of the present invention.

[00135] In another embodiment, the bcr-abl-expressing cancer is a bcr-abl-expressing leukemia. In another embodiment, the bcr-abl-expressing cancer is acute myeloid leukemia (AML). In another embodiment, the bcr-abl-expressing cancer is chronic myeloid leukemia (CML). In another embodiment, the bcr-abl-expressing cancer is acute lymphoblastic leukemia (ALL). In another embodiment, the bcr-abl-expressing cancer is any other bcr-abl-expressing cancer known in the art. Each possibility represents a separate embodiment of the present invention.

[00136] In another embodiment, multiple peptides of this invention are used to stimulate an immune response in methods of the present invention.

[00137] As provided herein, heteroclitic peptides that elicit antigen-specific $CD8^+$ T cell responses can be created using methods of the present invention (Examples 1-2 and 11-14). As provided in Examples 3-4, peptides that elicit $CD4^+$ T cell responses to multiple HLA class II molecules can be identified. $CD4^+$ T cells recognize peptides bound to the HLA class II molecule on APC. In another embodiment, antigen-specific $CD4^+$ T cell responses assist in induction and maintenance of $CD8^+$ cytotoxic T cell (CTL) responses (Examples 7-10 and 16-19).

[00138] In another embodiment, peptides of the present invention exhibit an enhanced ability to elicit CTL responses, due to their ability to bind both HLA class I and HLA class II molecules. In another embodiment, vaccines of the present invention have the advantage of activating or eliciting both $CD4^+$ and $CD8^+$ T cells that recognize bcr-abl antigens. In another embodiment, activation or eliciting both $CD4^+$ and $CD8^+$ T cells provides a synergistic anti-bcr-abl immune response, relative to activation of either population alone. In another embodiment, the enhanced immunogenicity of peptides of the present invention is exhibited in individuals of multiple HLA class II subtypes, due to the ability of peptides of the present invention to bind multiple HLA class II subtypes. Each possibility represents a separate embodiment of the present invention.

[00139] In another embodiment, activated $CD4^+$ cells enhance immunity by licensing dendritic cells, thereby sustaining the activation and survival of the cytotoxic T cells. In another embodiment, activated $CD4^+$ T cells induce tumor cell death by direct contact with the tumor cell or by activation of the apoptosis pathway. Mesothelioma tumor cells, for example, are able to process and present antigens in the context of HLA class I and class II molecules.

[00140] The methods disclosed herein will be understood by those in the art to enable design of other bcr-abl-derived peptides that bind other HLA class I and HLA class II molecules. The methods further enable design of vaccines combining bcr-abl -derived peptides of the present invention. Each possibility represents a separate embodiment of the present invention.

[00141] In another embodiment, peptides, vaccines, and/or immunogenic compositions of the present invention have the advantage of activating or eliciting bcr-abl-specific $CD4^+$ T cells containing multiple different HLA class II alleles. In another embodiment, the vaccines have the advantage of activating or eliciting bcr-abl-specific $CD4^+$ T cells in a substantial proportion of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 10% of the population. In

another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 15% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 20% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 25% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 30% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 35% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 40% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 45% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 50% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 55% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 60% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 70% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 75% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 80% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 85% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 90% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in 95% of the population. In another embodiment, the peptides activate bcr-abl-specific $CD4^+$ T cells in greater than 95% of the population. In another embodiment, the vaccines activate or elicit bcr-abl-specific $CD4^+$ T cells in a substantial proportion of a particular population (e.g. American Caucasians). Each possibility represents a separate embodiment of the present invention.

[00142] In another embodiment, peptides, vaccines, and/or immunogenic compositions of the present invention elicit enhanced bcr-abl-specific CTL responses in individuals carrying both the HLA class I molecule and the HLA class II molecule whose binding motifs are present in the peptide. In another embodiment, due to the binding of multiple HLA class I molecules and/or multiple HLA class II molecules, the peptides elicit enhanced bcr-abl-specific CTL responses in a substantial proportion of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 10% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 15% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 20% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 25% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 30% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 35% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 40% of the population. In

another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 45% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 50% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 55% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 60% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 70% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 75% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 80% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 85% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 90% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in 95% of the population. In another embodiment, the peptides elicit enhanced bcr-abl-specific CTL responses in greater than 95% of the population. In another embodiment, the vaccines activate or elicit bcr-abl-specific $CD4^+$ T cells in a substantial proportion of a particular population (e.g. American Caucasians). Each possibility represents a separate embodiment of the present invention.

[00143] In another embodiment, methods of the present invention provide for an improvement in an immune response that has already been mounted by a subject. In one embodiment, methods of the present invention comprise administering the peptide, composition, or vaccine 2 or more times. In another embodiment, the peptides are varied in their composition, concentration, or a combination thereof. In another embodiment, the peptides provide for the initiation of an immune response against bcr-abl in a subject in which an immune response against bcr-abl has not already been initiated. In another embodiment, the CTL that are induced proliferate in response to presentation of the peptide on the APC or cancer cell. In other embodiments, reference to modulation of the immune response involves, either or both the humoral and cell-mediated arms of the immune system, which is accompanied by the presence of Th2 and Th1 T helper cells, respectively, or in another embodiment, each arm individually.

[00144] In other embodiments, the methods affecting the growth of a tumor result in (1) the direct inhibition of tumor cell division, or (2) immune cell mediated tumor cell lysis, or both, which leads to a suppression in the net expansion of tumor cells. Each possibility represents a separate embodiment of the present invention.

[00145] Inhibition of tumor growth by either of these two mechanisms can be readily determined by one of ordinary skill in the art based upon a number of well known methods. In one embodiment,

tumor inhibition is determined by measuring the actual tumor size over a period of time. In another embodiment, tumor inhibition can be determined by estimating the size of a tumor (over a period of time) utilizing methods well known to those of skill in the art. More specifically, a variety of radiologic imaging methods (e.g., single photon and positron emission computerized tomography; see generally, "Nuclear Medicine in Clinical Oncology," Winkler, C. (ed.) Springer-Verlag, New York, 1986), can be utilized to estimate tumor size. Such methods can also utilize a variety of imaging agents, including for example, conventional imaging agents (e.g., Gallium-67 citrate), as well as specialized reagents for metabolite imaging, receptor imaging, or immunologic imaging (e.g., radiolabeled monoclonal antibody specific tumor markers). In addition, non-radioactive methods such as ultrasound (see, "Ultrasonic Differential Diagnosis of Tumors", Kossoff and Fukuda, (eds.), Igaku-Shoin, New York, 1984), can also be utilized to estimate the size of a tumor.

[00146] In addition to the *in vivo* methods for determining tumor inhibition discussed above, a variety of *in vitro* methods can be utilized in order to determine *in vivo* tumor inhibition. Representative examples include lymphocyte mediated anti-tumor cytolytic activity determined for example, by a ^{51}Cr release assay (Examples), tumor dependent lymphocyte proliferation (Ioannides, et al., J. Immunol. 146(5):1700-1707, 1991), *in vitro* generation of tumor specific antibodies (Herlyn, et al., J. Immunol. Meth. 73:157-167, 1984), cell (e.g., CTL, helper T-cell) or humoral (e.g., antibody) mediated inhibition of cell growth *in vitro* (Gazit, et al., Cancer Immunol Immunother 35:135-144, 1992), and, for any of these assays, determination of cell precursor frequency (Vose, Int. J. Cancer 30:135-142 (1982), and others.

[00147] In another embodiment, methods of suppressing tumor growth indicate a growth state that is curtailed compared to growth without contact with, or exposure to a peptide of this invention. Tumor cell growth can be assessed by any means known in the art, including, but not limited to, measuring tumor size, determining whether tumor cells are proliferating using a ^{3}H-thymidine incorporation assay, or counting tumor cells. "Suppressing" tumor cell growth refers, in other embodiments, to slowing, delaying, or stopping tumor growth, or to tumor shrinkage. Each possibility represents a separate embodiment of the present invention.

[00148] Methods of determining the presence and magnitude of an immune response are well known in the art. In one embodiment, lymphocyte proliferation assays, wherein T cell uptake of a radioactive substance, e.g. ^{3}H-thymidine is measured as a function of cell proliferation. In other embodiments, detection of T cell proliferation is accomplished by measuring increases in interleukin-2 (IL-2)

production, Ca^{2+} flux, or dye uptake, such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium. Each possibility represents a separate embodiment of the present invention.

[00149] In another embodiment, CTL stimulation is determined by means known to those skilled in the art, including detection of cell proliferation, cytokine production and others. Analysis of the types and quantities of cytokines secreted by T cells upon contacting ligand-pulsed targets can be a measure of functional activity. Cytokines can be measured by ELISA, ELISPOT assays or fluorescence-activated cell sorting (FACS) to determine the rate and total amount of cytokine production. (Fujihashi K. et al. (1993) J. Immunol. Meth. 160:181; Tanguay S. and Killion J. J. (1994) Lymphokine Cytokine Res. 13:259).

[00150] In another embodiment, CTL activity is determined by ^{51}Cr-release lysis assay. Lysis of peptide-pulsed ^{51}Cr-labeled targets by antigen-specific T cells can be compared for target cells pulsed with control peptide. In another embodiment, T cells are stimulated with a peptide of this invention, and lysis of target cells expressing the native peptide in the context of MHC can be determined. The kinetics of lysis as well as overall target lysis at a fixed timepoint (e.g., 4 hours) are used, in another embodiment, to evaluate ligand performance. (Ware C. F. et al. (1983) J Immunol 131: 1312).

[00151] Methods of determining affinity of a peptide for an HLA molecule are well known in the art. In one embodiment, affinity is determined by TAP stabilization assays (Examples).

[00152] In another embodiment, affinity is determined by competition radioimmunoassay. In one embodiment, the following protocol is utilized: Target cells are washed two times in PBS with 1% bovine serum albumin (BSA; Fisher Chemicals, Fairlawn, NJ). Cells are resuspended at 10^7/ml on ice, and the native cell surface bound peptides are stripped for 2 minutes at 0° C using citrate-phosphate buffer in the presence of 3 mg/ml $beta_2$ microglobulin. The pellet is resuspended at 5 x 10^6 cells/ml in PBS/1% BSA in the presence of 3 mg/ml $beta_2$ microglobulin and 30 mg/ml deoxyribonuclease, and 200 ml aliquots are incubated in the presence or absence of HLA-specific peptides for 10 min at 20°C, then with ^{125}I-labeled peptide for 30 min at 20°C. Total bound ^{125}I is determined after two washes with PBS/2% BSA and one wash with PBS. Relative affinities are determined by comparison of escalating concentrations of the test peptide versus a known binding peptide.

[00153] In another embodiment, a specificity analysis of the binding of peptide to HLA on surface of live cells (e.g. SKLY-16 cells) is conducted to confirm that the binding is to the appropriate HLA molecule and to characterize its restriction. This includes, in another embodiment, competition with excess unlabeled peptides known to bind to the same or disparate HLA molecules and use of target

cells which express the same or disparate HLA types. This assay is performed, in one embodiment, on live fresh or 0.25% paraformaldehyde-fixed human PBMC, leukemia cell lines and EBV-transformed T-cell lines of specific HLA types. The relative avidity of the peptides found to bind MHC molecules on the specific cells are assayed by competition assays as described above against ^{125}I-labeled peptides of known high affinity for the relevant HLA molecule, e.g., tyrosinase or HBV peptide sequence.

[00154] In another embodiment, a peptide of methods and compositions of the present invention comprises a non-classical amino acid such as: 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Kazmierski et al. (1991) J. Am Chem. Soc. 113:2275-2283); (2S,3S)-methyl-phenylalanine, (2S,3R)-methyl-phenylalanine, (2R,3S)-methyl-phenylalanine and (2R,3R)-methyl-phenylalanine (Kazmierski and Hruby (1991) Tetrahedron Lett. 32(41): 5769-5772); 2-aminotetrahydronaphthalene-2-carboxylic acid (Landis (1989) Ph.D. Thesis, University of Arizona); hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Miyake et al. (1984) J. Takeda Res. Labs. 43:53-76) histidine isoquinoline carboxylic acid (Zechel et al. (1991) Int. J. Pep. Protein Res. 38(2):131-138); and HIC (histidine cyclic urea), (Dharanipragada et al.(1993) Int. J. Pep. Protein Res. 42(1):68-77) and ((1992) Acta. Crst., Crystal Struc. Comm. 48(IV):1239-124).

[00155] In another embodiment, a peptide of methods and compositions of the present invention comprises an AA analog or is a peptidomimetic, which, in other embodiments, induces or favors specific secondary structures. Such peptides comprise, in other embodiments, the following: LL-Acp (LL-3-amino-2-propenidone-6-carboxylic acid), a ß-turn inducing dipeptide analog (Kemp et al. (1985) J. Org. Chem. 50:5834-5838); ß-sheet inducing analogs (Kemp et al. (1988) Tetrahedron Lett. 29:5081-5082); ß-turn inducing analogs (Kemp et al. (1988) Tetrahedron Left. 29:5057-5060); .alpha.-helix inducing analogs (Kemp et al. (1988) Tetrahedron Left. 29:4935-4938); .gamma.-turn inducing analogs (Kemp et al. (1989) J. Org. Chem. 54:109:115); analogs provided by the following references: Nagai and Sato (1985) Tetrahedron Left. 26:647-650; and DiMaio et al. (1989) J. Chem. Soc. Perkin Trans. p. 1687; a Gly-Ala turn analog (Kahn et al. (1989) Tetrahedron Lett. 30:2317); amide bond isostere (Jones et al. (1988) Tetrahedron Left. 29(31):3853-3856); tretrazol (Zabrocki et al. (1988) J. Am. Chem. Soc. 110:5875-5880); DTC (Samanen et al. (1990) Int. J. Protein Pep. Res. 35:501:509); and analogs taught in Olson et al. (1990) J. Am. Chem. Sci. 112:323-333 and Garveyet al. (1990) J. Org. Chem. 55(3):936-940. Conformationally restricted mimetics of beta turns and beta bulges, and peptides containing them, are described in U.S. Pat. No. 5,440,013, issued Aug. 8, 1995 to Kahn.

[00156] In other embodiments, a peptide of this invention is conjugated to one of various other molecules, as described hereinbelow, which can be via covalent or non-covalent linkage (complexed),

the nature of which varies, in another embodiment, depending on the particular purpose. In another embodiment, the peptide is covalently or non-covalently complexed to a macromolecular carrier, (e.g. an immunogenic carrier), including, but not limited to, natural and synthetic polymers, proteins, polysaccharides, polypeptides (amino acids), polyvinyl alcohol, polyvinyl pyrrolidone, and lipids. In another embodiment, a peptide of this invention is linked to a substrate. In another embodiment, the peptide is conjugated to a fatty acid, for introduction into a liposome (U.S. Pat. No. 5,837,249). In another embodiment, a peptide of the invention is complexed covalently or non-covalently with a solid support, a variety of which are known in the art. In another embodiment, linkage of the peptide to the carrier, substrate, fatty acid, or solid support serves to increase an elicited an immune response.

[00157] In other embodiments, the carrier is thyroglobulin, an albumin (e.g. human serum albumin), tetanus toxoid, polyamino acids such as poly (lysine: glutamic acid), an influenza protein, hepatitis B virus core protein, keyhole limpet hemocyanin, an albumin, or another carrier protein or carrier peptide; hepatitis B virus recombinant vaccine, or an APC. Each possibility represents a separate embodiment of the present invention.

[00158] In another embodiment, the term "amino acid" refers to a natural or, in another embodiment, an unnatural or synthetic AA, and can include, in other embodiments, glycine, D- or L optical isomers, AA analogs, peptidomimetics, or combinations thereof.

[00159] In another embodiment, the terms "cancer," "neoplasm," "neoplastic" or "tumor," are used interchangeably and refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. Primary cancer cells (that is, cells obtained from near the site of malignant transformation) can be readily distinguished from non-cancerous cells by well-established techniques, particularly histological examination. The definition of a cancer cell, as used herein, includes not only a primary cancer cell, but also any cell derived from a cancer cell ancestor. This includes metastasized cancer cells, and in vitro cultures and cell lines derived from cancer cells. In one embodiment, a tumor is detectable on the basis of tumor mass; e.g., by such procedures as CAT scan, magnetic resonance imaging (MRI), X-ray, ultrasound or palpation, and in another embodiment, is identified by biochemical or immunologic findings, the latter which is used to identify cancerous cells, as well, in other embodiments.

[00160] Methods for synthesizing peptides are well known in the art. In one embodiment, the peptides of this invention are synthesized using an appropriate solid-state synthetic procedure (see for example, Steward and Young, Solid Phase Peptide Synthesis, Freemantle, San Francisco, Calif. (1968);

Merrifield (1967) Recent Progress in Hormone Res 23: 451). The activity of these peptides is tested, in other embodiments, using assays as described herein.

[00161] In another embodiment, the peptides of this invention are purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. In another embodiment, immuno-affinity chromatography is used, whereby an epitope is isolated by binding it to an affinity column comprising antibodies that were raised against that peptide, or a related peptide of the invention, and were affixed to a stationary support.

[00162] In another embodiment, affinity tags such as hexa-His (Invitrogen), Maltose binding domain (New England Biolabs), influenza coat sequence (Kolodziej et al. (1991) Meth. Enzymol. 194:508-509), glutathione-S-transferase, or others, are attached to the peptides of this invention to allow easy purification by passage over an appropriate affinity column. Isolated peptides can also be physically characterized, in other embodiments, using such techniques as proteolysis, nuclear magnetic resonance, and x-ray crystallography.

[00163] In another embodiment, the peptides of this invention are produced by *in vitro* translation, through known techniques, as will be evident to one skilled in the art. In another embodiment, the peptides are differentially modified during or after translation, e.g., by phosphorylation, glycosylation, cross-linking, acylation, proteolytic cleavage, linkage to an antibody molecule, membrane molecule or other ligand, (Ferguson et al. (1988) Ann. Rev. Biochem. 57:285-320).

[00164] In one embodiment, the peptides of this invention further comprise a detectable label, which in one embodiment, is fluorescent, or in another embodiment, luminescent, or in another embodiment, radioactive, or in another embodiment, electron dense. In other embodiments, the dectectable label comprises, for example, green fluorescent protein (GFP), DS-Red (red fluorescent protein), secreted alkaline phosphatase (SEAP), beta-galactosidase, luciferase, ^{32}P, ^{125}I, ^{3}H and ^{14}C, fluorescein and its derivatives, rhodamine and its derivatives, dansyl and umbelliferone, luciferin or any number of other such labels known to one skilled in the art. The particular label used will depend upon the type of immunoassay used.

[00165] In another embodiment, a peptide of this invention is linked to a substrate, which, in one embodiment, serves as a carrier. In one embodiment, linkage of the peptide to a substrate serves to increase an elicited an immune response.

[00166] In one embodiment, peptides of this invention are linked to other molecules, as described herein, using conventional cross-linking agents such as carbodimides. Examples of carbodimides are 1-cyclohexyl-3-(2-morpholinyl-(4-ethyl) carbodiimide (CMC), 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDC) and 1-ethyl-3-(4-azonia-44-dimethylpentyl) carbodiimide.

[00167] In other embodiments, the cross-linking agents comprise cyanogen bromide, glutaraldehyde and succinic anhydride. In general, any of a number of homo-bifunctional agents including a homo-bifunctional aldehyde, a homo-bifunctional epoxide, a homo-bifunctional imido-ester, a homo-bifunctional N-hydroxysuccinimide ester, a homo-bifunctional maleimide, a homo-bifunctional alkyl halide, a homo-bifunctional pyridyl disulfide, a homo-bifunctional aryl halide, a homo-bifunctional hydrazide, a homo-bifunctional diazonium derivative and a homo-bifunctional photoreactive compound can be used. Also envisioned, in other embodiments, are hetero-bifunctional compounds, for example, compounds having an amine-reactive and a sulfhydryl-reactive group, compounds with an amine-reactive and a photoreactive group and compounds with a carbonyl-reactive and a sulfhydryl-reactive group.

[00168] In other embodiments, the homo-bifunctional cross-linking agents include the bifunctional N-hydroxysuccinimide esters dithiobis(succinimidylpropionate), disuccinimidyl suberate, and disuccinimidyl tartarate; the bifunctional imido-esters dimethyl adipimidate, dimethyl pimelimidate, and dimethyl suberimidate; the bifunctional sulfhydryl-reactive crosslinkers 1,4-di-[3'-(2'-pyridyldithio)propionamido]butane, bismaleimidohexane, and bis-N-maleimido-1,8-octane; the bifunctional aryl halides 1,5-difluoro-2,4-dinitrobenzene and 4,4'-difluoro-3,3'-dinitrophenylsulfone; bifunctional photoreactive agents such as bis-[b-(4-azidosalicylamido)ethyl]disulfide; the bifunctional aldehydes formaldehyde, malondialdehyde, succinaldehyde, glutaraldehyde, and adipaldehyde; a bifunctional epoxide such as 1,4-butaneodiol diglycidyl ether; the bifunctional hydrazides adipic acid dihydrazide, carbohydrazide, and succinic acid dihydrazide; the bifunctional diazoniums o-tolidine, diazotized and bis-diazotized benzidine; the bifunctional alkylhalides N1N'-ethylene-bis(iodoacetamide), N1N'-hexamethylene-bis(iodoacetamide), N1N'-undecamethylene-bis(iodoacetamide), as well as benzylhalides and halomustards, such as a1a'-diiodo-p-xylene sulfonic acid and tri(2-chloroethyl)amine, respectively.

[00169] In other embodiments, hetero-bifunctional cross-linking agents used to link the peptides to other molecules, as described herein, include, but are not limited to, SMCC (succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate), MBS (m-maleimidobenzoyl-N-hydroxysuccinimide ester), SIAB (N-succinimidyl(4-iodoacteyl)aminobenzoate), SMPB (succinimidyl-4-(p-

maleimidophenyl)butyrate), GMBS (N-(.gamma.-maleimidobutyryloxy)succinimide ester), MPBH (4-(4-N-maleimidopohenyl) butyric acid hydrazide), M2C2H (4-(N-maleimidomethyl) cyclohexane-1-carboxyl-hydrazide), SMPT (succinimidyloxycarbonyl-a-methyl-a-(2-pyridyldithio)toluene), and SPDP (N-succinimidyl 3-(2-pyridyldithio)propionate).

[00170] In another embodiment, the peptides of the invention are formulated as non-covalent attachment of monomers through ionic, adsorptive, or biospecific interactions. Complexes of peptides with highly positively or negatively charged molecules can be accomplished, in another embodiment, through salt bridge formation under low ionic strength environments, such as in deionized water. Large complexes can be created, in another embodiment, using charged polymers such as poly-(L-glutamic acid) or poly-(L-lysine), which contain numerous negative and positive charges, respectively. In another embodiment, peptides are adsorbed to surfaces such as microparticle latex beads or to other hydrophobic polymers, forming non-covalently associated peptide-superantigen complexes effectively mimicking cross-linked or chemically polymerized protein, in other embodiments. In another embodiment, peptides are non-covalently linked through the use of biospecific interactions between other molecules. For instance, utilization of the strong affinity of biotin for proteins such as avidin or streptavidin or their derivatives could be used to form peptide complexes. The peptides, according to this aspect, and in one embodiment, can be modified to possess biotin groups using common biotinylation reagents such as the N-hydroxysuccinimidyl ester of D-biotin (NHS-biotin), which reacts with available amine groups.

[00171] In another embodiment, the peptides are linked to carriers. In another embodiments, the peptides are any that are well known in the art, including, for example, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly (lysine:glutamic acid), influenza, hepatitis B virus core protein, hepatitis B virus recombinant vaccine and the like. Each possibility represents a separate embodiment of the present invention.

[00172] In another embodiment, the peptides of this invention are conjugated to a lipid, such as P3 CSS. In another embodiment, the peptides of this invention are conjugated to a bead.

[00173] In another embodiment, the compositions of this invention further comprise immunomodulating compounds. In other embodiments, the immunomodulating compound is a cytokine, chemokine, or complement component that enhances expression of immune system accessory or adhesion molecules, their receptors, or combinations thereof. In some embodiments, the immunomodulating compound include interleukins, for example interleukins 1 to 15, interferons alpha, beta or gamma, tumour necrosis factor, granulocyte-macrophage colony stimulating factor

(GM-CSF), macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), chemokines such as neutrophil activating protein (NAP), macrophage chemoattractant and activating factor (MCAF), RANTES, macrophage inflammatory peptides MIP-1a and MIP-1b, complement components, or combinations thereof. In other embodiments, the immunomodulating compound stimulate expression, or enhanced expression of OX40, OX40L (gp34), lymphotactin, CD40, CD40L, B7.1, B7.2, TRAP, ICAM-1, 2 or 3, cytokine receptors, or combination thereof.

[00174] In another embodiment, the immunomodulatory compound induces or enhances expression of co-stimulatory molecules that participate in the immune response, which include, in some embodiments, CD40 or its ligand, CD28, CTLA-4 or a B7 molecule. In another embodiment, the immunomodulatory compound induces or enhances expression of a heat stable antigen (HSA) (Liu Y. et al. (1992) J. Exp. Med. 175:437-445), chondroitin sulfate-modified MHC invariant chain (Ii-CS) (Naujokas M. F. et al. (1993) Cell 74:257-268), or an intracellular adhesion molecule 1 (ICAM-1) (Van R. H. (1992) Cell 71:1065-1068), which assists, in another embodiment, co-stimulation by interacting with their cognate ligands on the T cells.

[00175] In another embodiment, the composition comprises a solvent, including water, dispersion media, cell culture media, isotonic agents and the like. In one embodiment, the solvent is an aqueous isotonic buffered solution with a pH of around 7.0. In another embodiment, the composition comprises a diluent such as water, phosphate buffered saline, or saline. In another embodiment, the composition comprises a solvent, which is non-aqueous, such as propyl ethylene glycol, polyethylene glycol and vegetable oils.

[00176] In another embodiment, the composition is formulated for administration by any of the many techniques known to those of skill in the art. For example, this invention provides for administration of the pharmaceutical composition parenterally, intravenously, subcutaneously, intradermally, intramucosally, topically, orally, or by inhalation.

[00177] In another embodiment, the vaccine comprising a peptide of this invention further comprises a cell population, which, in another embodiment, comprises lymphocytes, monocytes, macrophages, dendritic cells, endothelial cells, stem cells or combinations thereof, which, in another embodiment are autologous, syngeneic or allogeneic, with respect to each other. In another embodiment, the cell population comprises a peptide of the present invention. In another embodiment, the cell population takes up the peptide. Each possibility represents a separate embodiment of the present invention.

[00178] In one embodiment, the cell populations of this invention are obtained from *in vivo* sources, such as, for example, peripheral blood, leukopheresis blood product, apheresis blood product, peripheral lymph nodes, gut associated lymphoid tissue, spleen, thymus, cord blood, mesenteric lymph nodes, liver, sites of immunologic lesions, e.g. synovial fluid, pancreas, cerebrospinal fluid, tumor samples, granulomatous tissue, or any other source where such cells can be obtained. In one embodiment, the cell populations are obtained from human sources, which are, in other embodiments, from human fetal, neonatal, child, or adult sources. In another embodiment, the cell populations of this invention are obtained from animal sources, such as, for example, porcine or simian, or any other animal of interest. In another embodiment, the cell populations of this invention are obtained from subjects that are normal, or in another embodiment, diseased, or in another embodiment, susceptible to a disease of interest.

[00179] In another embodiment, the cell populations of this invention are separated via affinity-based separation methods. Techniques for affinity separation include, in other embodiments, magnetic separation, using antibody-coated magnetic beads, affinity chromatography, cytotoxic agents joined to a monoclonal antibody or use in conjunction with a monoclonal antibody, for example, complement and cytotoxins, and "panning" with an antibody attached to a solid matrix, such as a plate, or any other convenient technique. In other embodiment, separation techniques include the use of fluorescence activated cell sorters, which can have varying degrees of sophistication, such as multiple color channels, low angle and obtuse light scattering detecting channels, impedance channels, etc. In other embodiments, any technique that enables separation of the cell populations of this invention can be employed, and is to be considered as part of this invention.

[00180] In one embodiment, the dendritic cells are from the diverse population of morphologically similar cell types found in a variety of lymphoid and non-lymphoid tissues, qualified as such (Steinman (1991) Ann. Rev. Immunol. 9:271-296). In one embodiment, the dendritic cells used in this invention are isolated from bone marrow, or in another embodiment, derived from bone marrow progenitor cells, or, in another embodiment, from isolated from/derived from peripheral blood, or in another embodiment, derived from, or are a cell line.

[00181] In one embodiment, the cell populations described herein are isolated from the white blood cell fraction of a mammal, such as a murine, simian or a human (See, e.g., WO 96/23060). The white blood cell fraction can be, in another embodiment, isolated from the peripheral blood of the mammal.

[00182] Methods of isolating dendritic cells are well known in the art. In one embodiment, the DC are isolated via a method which includes the following steps: (a) providing a white blood cell fraction

obtained from a mammalian source by methods known in the art such as leukophoresis; (b) separating the white blood cell fraction of step (a) into four or more subfractions by countercurrent centrifugal elutriation; (c) stimulating conversion of monocytes in one or more fractions from step (b) to dendritic cells by contacting the cells with calcium ionophore, GM-CSF and IL-13 or GM-CSF and IL-4, (d) identifying the dendritic cell-enriched fraction from step (c); and (e) collecting the enriched fraction of step (d), preferably at about 4° C.

[00183] In another embodiment, the dendritic cell-enriched fraction is identified by fluorescence-activated cell sorting, which identifies, in another embodiment, at least one of the following markers: HLA-DR, HLA-DQ, or B7.2, and the simultaneous absence of the following markers: CD3, CD14, CD16, 56, 57, and CD 19, 20.

[00184] In another embodiment, the cell population comprises lymphocytes, which are, in one embodiment, T cells, or in another embodiment, B cells. The T cells are, in other embodiments, characterized as NK cells, helper T cells, cytotoxic T lymphocytes (CTL), TILs, naïve T cells, or combinations thereof. It is to be understood that T cells which are primary, or cell lines, clones, etc. are to be considered as part of this invention. In one embodiment, the T cells are CTL, or CTL lines, CTL clones, or CTLs isolated from tumor, inflammatory, or other infiltrates.

[00185] In another embodiment, hematopoietic stem or early progenitor cells comprise the cell populations used in this invention. In one embodiment, such populations are isolate or derived, by leukapheresis. In another embodiment, the leukapheresis follows cytokine administration, from bone marrow, peripheral blood (PB) or neonatal umbilical cord blood. In one embodiment the stem or progenitor cells are characterized by their surface expression of the surface antigen marker known as $CD34^+$, and exclusion of expression of the surface lineage antigen markers, Lin-.

[00186] In another embodiment, the subject is administered a peptide, composition or vaccine of this invention, in conjunction with bone marrow cells. In another embodiment, the administration together with bone marrow cells embodiment follows previous irradiation of the subject, as part of the course of therapy, in order to suppress, inhibit or treat cancer in the subject.

[00187] In one embodiment, the phrase "contacting a cell" or "contacting a population" refers to a method of exposure, which can be, in other embodiments, direct or indirect. In another embodiment, such contact comprises direct injection of the cell through any means well known in the art, such as microinjection. It is also envisaged, in another embodiment, that supply to the cell is indirect, such as

via provision in a culture medium that surrounds the cell, or administration to a subject, via any route well known in the art, and as described herein.

[00188] In one embodiment, CTL generation of methods of the present invention is accomplished *in vivo*, and is effected by introducing into a subject an antigen presenting cell contacted *in vitro* with a peptide of this invention (See for example Paglia et al. (1996) J. Exp. Med. 183:317-322).

[00189] In another embodiment, the peptides of methods and compositions of the present invention are delivered to antigen-presenting cells (APC).

[00190] In another embodiment, the peptides are delivered to APC in the form of cDNA encoding the peptides. In one embodiment, the term "antigen-presenting cells" refers to dendritic cells (DC), monocytes/macrophages, B lymphocytes or other cell type(s) expressing the necessary MHC/co-stimulatory molecules, which effectively allow for T cell recognition of the presented peptide. In another embodiment, the APC is a cancer cell. Each possibility represents a separate embodiment of the present invention.

[00191] In another embodiment, the CTL are contacted with two or more APC populations. In another embodiment, the two or more APC populations present different peptides. Each possibility represents a separate embodiment of the present invention.

[00192] In another embodiment, techniques that lead to the expression of antigen in the cytosol of APC (e.g. DC) are used to deliver the peptides to the APC. Methods for expressing antigens on APC are well known in the art. In one embodiment, the techniques include (1) the introduction into the APC of naked DNA encoding a peptide of this invention, (2) infection of APC with recombinant vectors expressing a peptide of this invention, and (3) introduction of a peptide of this invention into the cytosol of an APC using liposomes. (See Boczkowski D. et al. (1996) J. Exp. Med. 184:465-472; Rouse et al. (1994) J. Virol. 68:5685-5689; and Nair et al. (1992) J. Exp. Med. 175:609-612).

[00193] In another embodiment, foster antigen presenting cells such as those derived from the human cell line 174xCEM.T2, referred to as T2, which contains a mutation in its antigen processing pathway that restricts the association of endogenous peptides with cell surface MHC class I molecules (Zweerink et al. (1993) J. Immunol. 150:1763-1771), are used, as exemplified herein.

[00194] In one embodiment, any of the methods described herein is used to elicit CTL, which are elicited *in vitro*. In another embodiment, the CTL are elicited *ex-vivo*. In another embodiment, the CTL are elicited *in* vitro. The resulting CTL, are, in another embodiment, administered to the subject,

thereby treating the condition associated with the peptide, an expression product comprising the peptide, or a homologue thereof. Each possibility represents a separate embodiment of the present invention.

[00195] In another embodiment, the method entails introduction of the genetic sequence that encodes the peptides of this invention. In one embodiment, the method comprises administering to the subject a vector comprising a nucleotide sequence, which encodes a peptide of the present invention (Tindle, R. W. et al. Virology (1994) 200:54). In another embodiment, the method comprises administering to the subject naked DNA which encodes a peptide, or in another embodiment, two or more peptides of this invention (Nabel, et al. PNAS-USA (1990) 90: 11307). In another embodiment, multi-epitope, analogue-based cancer vaccines are utilized (Fikes et al, ibid). Each possibility represents a separate embodiment of the present invention.

[00196] Nucleic acids can be administered to a subject via any means as is known in the art, including parenteral or intravenous adminstration, or in another embodiment, by means of a gene gun. In another embodiment, the nucleic acids are administered in a composition, which correspond, in other embodiments, to any embodiment listed herein.

[00197] Vectors for use according to methods of this invention can comprise, in another embodiment, any vector that facilitates or allows for the expression of a peptide of this invention. In another embodiment, "vectors" includes attenuated viruses, such as vaccinia or fowlpox, such as described in, e.g., U.S. Pat. No. 4,722,848, incorporated herein by reference. In another embodiment, the vector is BCG (Bacille Calmette Guerin), such as described in Stover et al. (Nature 351:456-460 (1991)). Other vectors useful for therapeutic administration or immunization of the peptides of the invention, e.g., Salmonella typhi vectors and the like, will be apparent to those skilled in the art from the description herein.

[00198] In one embodiment, the vector further encodes for an immunomodulatory compound, as described herein. In another embodiment, the subject is administered an additional vector encoding same, concurrent, prior to or following administration of the vector encoding a peptide of this invention to the subject.

[00199] In another embodiment, the peptides, compositions and vaccines of this invention are administered to a subject, or utilized in the methods of this invention, in combination with other anti-cancer compounds and chemotherapeutics, including monoclonal antibodies directed against alternate cancer antigens, or, in another embodiment, epitopes that consist of an AA sequence which

corresponds to, or in part to, that from which the peptides of this invention are derived.

[00200] Various embodiments of dosage ranges are contemplated by this invention. In one embodiment, the dosage is 20 μg per peptide per day. In another embodiment, the dosage is 10 μg/peptide/day. In another embodiment, the dosage is 30 μg/peptide/day. In another embodiment, the dosage is 40 μg/peptide/day. In another embodiment, the dosage is 60 μg/peptide/day. In another embodiment, the dosage is 80 μg/peptide/day. In another embodiment, the dosage is 100 μg/peptide/day. In another embodiment, the dosage is 150 μg/peptide/day. In another embodiment, the dosage is 200 μg/peptide/day. In another embodiment, the dosage is 300 μg/peptide/day. In another embodiment, the dosage is 400 μg/peptide/day. In another embodiment, the dosage is 600 μg/peptide/day. In another embodiment, the dosage is 800 μg/peptide/day. In another embodiment, the dosage is 1000 μg/peptide/day.

[00201] In another embodiment, the dosage is 10 μg/peptide/dose. In another embodiment, the dosage is 30 μg/peptide/dose. In another embodiment, the dosage is 40 μg/peptide/dose. In another embodiment, the dosage is 60 μg/peptide/dose. In another embodiment, the dosage is 80 μg/peptide/dose. In another embodiment, the dosage is 100 μg/peptide/dose. In another embodiment, the dosage is 150 μg/peptide/dose. In another embodiment, the dosage is 200 μg/peptide/dose. In another embodiment, the dosage is 300 μg/peptide/dose. In another embodiment, the dosage is 400 μg/peptide/dose. In another embodiment, the dosage is 600 μg/peptide/dose. In another embodiment, the dosage is 800 μg/peptide/dose. In another embodiment, the dosage is 1000 μg/peptide/dose.

[00202] In another embodiment, the dosage is 10-20 μg/peptide/dose. In another embodiment, the dosage is 20-30 μg/peptide/dose. In another embodiment, the dosage is 20-40 μg/peptide/dose. In another embodiment, the dosage is 30-60 μg/peptide/dose. In another embodiment, the dosage is 40-80 μg/peptide/dose. In another embodiment, the dosage is 50-100 μg/peptide/dose. In another embodiment, the dosage is 50-150 μg/peptide/dose. In another embodiment, the dosage is 100-200 μg/peptide/dose. In another embodiment, the dosage is 200-300 μg/peptide/dose. In another embodiment, the dosage is 300-400 μg/peptide/dose. In another embodiment, the dosage is 400-600 μg/peptide/dose. In another embodiment, the dosage is 500-800 μg/peptide/dose. In another embodiment, the dosage is 800-1000 μg/peptide/dose.

[00203] In another embodiment, the total amount of peptide per dose or per day is one of the above

amounts. In another embodiment, the total peptide dose per dose is one of the above amounts.

[00204] Each of the above doses represents a separate embodiment of the present invention.

EXPERIMENTAL DETAILS SECTION

EXAMPLE 1: BINDING OF HLA-A0201 AND -A0301 BY SYNTHETIC PEPTIDE ANALOGUES DERIVED FROM WT1

MATERIALS AND EXPERIMENTAL METHODS

Peptides

[00205] Peptides were synthesized by Genemed Synthesis Inc, CA using fluorenylmethoxycarbonyl chemistry and solid phase synthesis, and were purified by high pressure liquid chromatography (HPLC). The quality of the peptides was assessed by HPLC analysis, and the expected molecular weight was measured using matrix-assisted laser desorption mass spectrometry. Peptides were sterile and > 90% pure. The peptides were dissolved in DMSO and diluted in PBS at pH 7.4 or saline solution to yield a concentration of 5 milligrams per milliliter (mg/ml) and were stored at -80° C. For *in vitro* experiments, an irrelevant control peptide, HLA A24 consensus, was used.

Peptide sequence analysis

[00206] Peptide sequence analysis was performed using 2 databases. The first was the software of the Bioinformatics & Molecular Analysis Section (National Institutes of Health, Washington, DC) (Parker KC et al, Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J Immunol 152: 163-175, 1994), which ranks 9-mer or 10-mer peptides on a predicted half-time dissociation coefficient from HLA class I molecules. The second database, SYFPEITHI prediction software, is described in Rammensee HG et al (SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 50: 213-219, 1999).

Cell lines

[00207] Cell lines were cultured in RPMI 1640 medium supplemented with 5% FCS, penicillin, streptomycin, 2mM glutamine and 2-mercaptoethanol at 37 °C in humidified air containing 5% CO_2. T2 is a human cell line lacking TAP1 and TAP2 and therefore unable to present peptides derived from cytosolic proteins. Raji cells are a human Burkitt lymphoma cells that exhibit a high level of TAP expression.

T2 assay for peptide binding and stabilization of HLA A0201 molecules

[00208] T2 cells lack TAP function and consequently are defective in properly loading class I molecules with antigenic peptides generated in the cytosol. The association of exogenously added peptides with thermolabile, empty HLA-A2 molecules stabilizes them and results in an increase in the level of surface HLA-A0201 recognizable by specific mAb such as BB7.2. T2 cells (TAP$^-$, HLA-A0201$^+$) were incubated overnight at 27° C at a concentration of 1 x 10^6 cells/ml in FCS-free RPMI medium supplemented with 5 µg/ml human $ß_2$m (Sigma, St Louis, MO) in the absence (negative control) or presence of either a positive reference tyrosinase peptide or test peptides at various final concentrations (50, 10, 1, and 0.1 micrograms (µg)/ml). Following a 4-hour incubation with 5 µg/ml brefeldin A (Sigma), T2 cells were labeled for 30 minutes at 4 °C with a saturating concentration of anti-HLA-A2.1 (BB7.2) mAb, then washed twice. Cells were then incubated for 30 minutes, 4° C with a saturating concentration of FITC-conjugated goat IgG F(ab')2 anti-mouse Ig (Caltag, San Francisco, CA), washed twice, fixed in PBS/1% paraformaldehyde and analyzed using a FACS Calibur® cytofluorometer (Becton Dickinson, Immunocytometry Systems, San Jose, CA).

[00209] The mean intensity of fluorescence (MIF) observed for each peptide concentration (after dividing by the MIF in the absence of peptide) was used as an indication of peptide binding and expressed as a "fluorescence index." Stabilization assays were performed similarly. Following initial evaluation of peptide binding at time 0, cells were washed in RPMI complete medium to remove free peptides and incubated in the continuous presence of 0.5 µg/ml brefeldin-A for 2, 4, 6 or 8 hours.

[00210] The number of stable peptide-HLA-A2.1 complexes was estimated as described above by immunofluorescence. The half time of complexes is an estimate of the time required for a 50% reduction of the MIF value at time = 0.

RESULTS

[00211] Peptides having predicted affinity for HLA-A0201 and HLA-A0301 molecules were identified from the WT1 sequence. These WT1 native peptides were modified to generate heteroclitic peptides with increased predicted binding to HLA-A0201 and HLA-A0301 molecules, as shown in Tables 1-2. Several of the heteroclitic peptides significantly stabilized HLA-A0201 and HLA-A0301 molecules in thermostabilization assays using a TAP 1/2 negative cell line (T2) and Raji HLA-A0301 cells. Specifically, WT1-A1, B1, and C1 exhibited similar or increased binding compared to the corresponding native peptides WT1-A, B, and C. WT1-D1 exhibited similar or increased binding compared to corresponding native peptide WT1-D (Figure 1A). A comparison of HLA A0301 binding of A3 WT1-A, -B, -C, and -D with each of their respective three analogues

demonstrated similar binding (Figures 1B-5E).

[00212] Thus, heteroclitic WT1 peptides of the present invention exhibit enhanced binding to HLA class I molecules.

TABLE 1

HLA 0201-binding native peptides from WT1 and synthetic analogues

Name	Sequence	SEQ ID NO:	BIMAS score
WT1 A (native)	RMFPNAPYL	5	313
WT1 A1 (analogue)	**Y**MFPNAPYL	6	1444
WT1 B (native)	SLGEQQYS V	7	285
WT1 B1 (analogue)	**Y**LGEQQYSV	8	1311
WT1 C (native)	ALLPAVPS L	9	181
WT1 C1 (analogue)	**Y**LLPAVPSL	10	836
WT1 D (native)	NLGATLKGV	11	159
WT1 D1 (analogue)	**Y**LGATLKGV	12	735
WT1 E (native)	DLNALLPAV	13	11
WT1 E1 (analogue)	**Y**LNALLPAV	14	735
WT1 F (native)	GVFRGIQDV	15	51
WT1 F1 (analogue)	G**LR**RGIQDV	16	12
WT1 G (native)	KRYFKLSHL	17	1
WT1 G1 (analogue)	K**L**YFKLSHL	18	550
WT1 H (native)	ALLLRTPY S	19	1
WT1 H1 (analogue)	ALLLRTPY**V**	20	1415
WT1 J (native)	CMTWNQMNL	21	15
WT1 J1 (analogue)	**Y**MTWNQMNL	22	70

TABLE 2

HLA 0201-binding native peptides from WT1 and synthetic analogues

Name	Sequence	SEQ ID	BIMAS score

A3 WT1 A (native)	NMHQRNMTK	23	40
A3 WT1 A1 (analogue)	NM**Y**QRNMTK	24	200
A3WT1 A2 (analogue)	NMHQR**V**MTK	25	120
A3 WT1 A3 (analogue)	NM**Y**QR**V**MTK	26	600
A3 WT1 B (native)	QMNLGATLK	27	20
A3WT1 B1 (analogue)	QM**Y**LGATLK	28	100
A3WT1 B2 (analogue)	QMNLG**V**TLK	29	60
A3WT1 B3 (analogue)	QM**Y**LG**V**TLK	30	300
A3WT1 C (native)	FMCAYPGCNK	31	30
A3WT1 C1 (analogue)	FM**Y**AYPGCNK	32	150
A3 WT1 C2 (analogue)	FMCAYP**F**CNK	33	90
A3 WT1 C3 (analogue)	FM**Y**AYP**F**CNK	34	450
A3WT1 D (native)	KLSHLQMHSR	35	18
A3WT1 D1 (analogue)	KL**Y**HLQMHSR	36	90
A3 WT1 D2 (analogue)	KLSHLQMHS**K**	37	90
A3 WT1 D3 (analogue)	KL**Y**HLQMHS**K**	38	450

EXAMPLE 2: INDUCTION OF IMMUNE RESPONSES AGAINST SYNTHETIC PEPTIDE ANALOGUES DERIVED FROM WT1

MATERIALS AND EXPERIMENTAL METHODS

Peptide stimulations

[00213] PBMC were purified from HLA-A0201 positive healthy donors and CML patients by centrifugation in Ficoll-Paque centrifugation medium (Amersham Biosciences). Peripheral blood dendritic cells (DC) were generated as follows: Monocyte-enriched PBMC fractions were isolated, using a plastic adherence technique, from total PBMC. The plastic-adherent cells were cultured further in RPMI 1640 medium (Invitrogen) containing 1-5% autologous plasma, 1000 units per milliliter (U/mL) recombinant human interleukin (IL)-4 (Schering-Plough, N.J.), and 1000 U/mL recombinant human granulocyte-macrophage colony- stimulating factor (GM-CSF) (Immunex, Seattle).

[00214] On days 2 and 4 of incubation, fresh culture medium supplemented with IL-4 and GM-CSF was added. On day 6, half of the medium was exchanged for culture medium containing IL-4, GM-CSF, 10 ng/mL recombinant human tumor necrosis factor (TNF)-alpha (R&D system) and 500 ng/ml

trimeric soluble CD40L (Immunex, Seattle). On day 9, cells were harvested and used as APC for antigen stimulation. The cells expressed DC-associated antigens, such as CD80, CD83, CD86, and HLA class I and class II on their cell surfaces.

[00215] T lymphocytes were isolated from the same donors by use of negative selection by depletion with an anti-CD11b, anti-CD56 and CD19 monoclonal antibody (Miltenyi, CA). 1 x 10^6 T lymphocytes were cultured with 1 x 10^5 autologous DC in RPMI 1640 containing 5% heat-inactivated human autologous plasma with 10 μg/mL peptide and 2 μg/ml β_2 microglobulin, 5 ng/mL recombinant human IL-7 (Genzyme), and 0.1 ng/ml IL-12 in 24 well plates.

[00216] After culture for 3 days, 20 U/ml of recombinant IL-2 (Sandoz Pharmaceutical) was added. After 10 days, 1 x 10^6 cells were stimulated again by adding 2 x 10^5 autologous magnetically isolated $CD14^+$ monocytes together with 10 ng/ml IL-7, 20 U/ml IL-2, and 10 μg/mL peptide. In some cases, after culture for another 7 days, the cells were stimulated a third time in the same manner. After the last stimulation, $CD8^+$ T cells were isolated magnetically, and cytotoxicity and gamma-IFN secretion of these cells were determined.

RESULTS

[00217] To determine the ability of heteroclitic WT1 peptides to generate immune responses against native and heteroclitic WT peptides, the $CD3^+$ PBMC subpopulation of a healthy donor was isolated and stimulated with autologous monocyte-derived, peptide-pulsed DC, then re-stimulated with peptide-pulsed $CD14^+$ monocytes. The presence of activated, antigen-specific T cells was then determined using pulsed, HLA-matched leukemic cell lines. Several analogue peptides generated greater immune responses (i.e. increased T cell precursor frequency, in comparison with the native peptides) by IFN gamma ELISPOT (Figure 2A) and chromium release assay (Figure 2B). Similar results were observed using $CD3^+$ (Figures 3B-D) and $CD8^+$ (Figure 3A) subpopulations of donors. Moreover, $CD8^+$ T cells stimulated with the heteroclitic WT1 peptides cross-reacted with the native WT1 peptides and were able to kill HLA-matched CML blasts (Figures 4A-B).

[00218] Thus, heteroclitic WT1 peptides of the present invention are able to generate T cells that (a) secrete inflammatory cytokines and (b) perform cytolysis in response to cells presenting WT1 peptides. In addition, the T cells generated by the heteroclitic WT1 peptides recognize both native and heteroclitic WT1 peptides

EXAMPLE 3: SELECTION OF SYNTHETIC WT1 PEPTIDES THAT BIND HLA CLASS

II MOLECULES

[00219] In order to identify WT1 peptides that bind to many different HLA class II molecules with relative high affinities, allele frequencies of HLA-DRB in the North American Caucasian population were determined, using the information available from the NCBI's MHC database (http://www.ncbi.nlm.nih.gov/mhc/MHC). Using the SYFPEITHI epitope prediction algorithm, two peptides that were predicted to bind the HLA-DRB molecules with relatively high affinities were identified from the WT1 sequence (Table 3).

[00220] Table 3: WT1 native peptides predicted binding to HLA-DR alleles based on SYFPEITHI algorithm (0 (low)- 28 (high)).

Peptide identifier	SEQ ID No:	DRB 101	DRB 301	DRB 401	DRB 701	DRB 1101	DRB 1501
Allele frequency		17.9 %	18.6 %	13.8 %	25.5 %	10.4 %	15.9 %
427	1	15	7	12	8	7	4
427 long	2	15	17	20	14	10	24
331	3	28	2	28	18	25	10
331 long	4	28	11	28	18	25	20

[00221] AA sequences of the peptides in Table 3 are LVRHHNMHQRNMTKL (427); RSDELVRHHNMHQRNMTKL (427 long); NKRYFKLSHLQMHSR (331); and PGCNKRYFKLSHLQMHSRKHTG (331 long).

[00222] Thus, HLA class II-binding WT1 peptides of the present invention bind to HLA class II molecules in a large percentage of the population.

EXAMPLE 4: HLA CLASS II MOLECULE-BINDING, WT1 PEPTIDES STIMULATE CD4$^+$ T CELLS

MATERIALS AND EXPERIMENTAL METHODS

Preparation of DC and CD4$^+$ effector cells

[00223] PBMC were Ficoll-purified from blood and resuspended at 5 x 10^6/ ml in Ex-Vivo-15® medium (BioWhittaker, Walkersville, MD) containing 1% autologous plasma. After a 2-hour incubation at 37 °C, the non-adherent fraction was harvested and washed repeatedly with PBS, then resuspended in media containing 1 x 10^3 IU/ml GM-CSF and 0.0032 IU/ml IL-4. On day 2 and 4, the

same media was added as re-feed (i.e., ½ the volume of media, containing enough cytokines for the entire dish, was added). On day 5, 10 μg/ml of peptide was added.

[00224] On day 6, a maturation cocktail of cytokines was added, and cells were cultured for another 48 hours. The maturation cocktail consisted of: 4 x 10^2 IU/ml IL-1-beta, 0.0032 IU/ml IL-4, 1 x 10^3 IU/ml IL-6, 1 x 10^3 IU/ml GMCSF, 10 μg/ml TNF-alpha, and 1 μg/ml PGE2.

[00225] On day 7, the DC were harvested and washed twice with RPMI, counted, aliquoted and resuspended at 1 x 10^6/ml in X-vivo 15 media (without serum). Peptides were added to a final concentration of 10 μg/ml, and incubated for 2 h, 37 °C and 5% CO2, gently re-suspending every 15 minutes, then washed twice in HBSS and re-suspended in RPMI + 5% autologous plasma at an appropriate concentration depending on the number of effectors isolated in the next step.

[00226] In addition, on day 7, additional PBMC were used to generate additional DC and $CD3^+$ cells. DC were isolated from the adherent fraction and prepared as described above for the second stimulation of the effector cells on day 14. $CD3^+$ cells were isolated from the non-adherent fraction by negative selection and stimulated with the previously prepared DC by re-suspending the $CD3^+$ cells at a concentration of 2 x 10^6 cells/ml in RPMI + 5% autologous plasma, and adding DC at an effector:DC ratio of 20:1 and 10 ng/ml IL-15. Cells were then plated in 2-ml and co-incubated at 37 °C and 5% CO_2 for 1 week.

[00227] On day 14, the $CD3^+$ cells were stimulated a second time with the second batch of DC in the same manner, except that 1 x 10^6 cells/ml were mixed with DC at an effector:DC ratio of 50:1. On day 18, the same media was added as re-feed. On day 20, the DC from the previous generation were defrosted and incubated in maturation cytokines in X-vivo15 media. On day 21, the ELISPOT assay was conducted.

ELISPOT assay

[00228] Plates were pre-wet with 30 μl/well 70% alcohol, shaking the plates to ensure coverage of the entire surface area, washed 3 times with 150 μl/well sterile PBS, then incubated overnight at 4°C with 10 μg/ml coating antibody (anti-INF clone) in PBS, 100 μl/well, wrapped in aluminum foil. Plates were then washed 2 times with 150 μl/well PBS and 1 time with RPMI/ 10% autologous plasma (AP), then blocked with 150 μl/well RPMI/5% AP for 2 hours at 37°C. PBMC were suspended in RPMI/5% AP at 1 x 10^6/ml. 1 x 10^5 cells and 2 μg of the appropriate peptides were added per well, and the volume brought up to 200 μl/well with media. 1 μl/well of 2.5mg/ml stock of PHA was added to the

control wells. Plates were wrapped in aluminum foil and incubated for 20 hours at 37°C.

[00229] To develop, plates were washed 3 times with PBS/0.05%Tween 2 and 3 times with PBS. 100µl/well anti-INF-gamma-Biotin (Clone 7-B6-1), diluted 1:500 in PBS/0.5% BSA, was added, and plates were incubated for 2 hours at 37°C. After 1 hour and 30 minutes, Avidin-peroxidase Complex (ABC) (Vectastain Elite Kit, Vector) was prepared by adding 1 drop of reagent A and 1 drop of reagent B to 10 ml of PBS/0.1% Tween20, and was stored at room temperature (rt) wrapped in aluminum foil. Plates were washed 3 times with PBS/0.05% Tween and 3 times with PBS, then 100 µl/well of Avidin-peroxidase Complex was added and plates incubated for 1 hour at rt wrapped in aluminum foil, then washed 3 times with PBS/0.05 % Tween-20, followed by 3 times with PBS. 100µl/well of substrate was added, plates were incubated for 4 minutes at rt in the dark, and the reaction was stopped with water. Wells were dried, and plate stored overnight in the dark at rt, then spots were counted with an ELISPOT reader.

Preparation of substrate

[00230] To prepare solution # 1: (acetate buffer), 23.4 ml dd H_20, 2.3 ml 0.1 N Acetic Acid, and 5.5 ml 0.1N Sodium Acetate were mixed. To prepared solution # 2, 1 tablet of AEC (Sigma) was dissolved in 2.5 ml of dimethylformamide. Then 1.25ml of solution #2 was mixed with 23.7 ml of solution #1, 13µl of 30% H_2O_2 was added, and the resulting solution mixed well and filtered using a 0.45µm filter.

RESULTS

[00231] To determine the ability of the HLA class II-binding WT1 peptides of the present invention to stimulate $CD4^+$ T cells, the $CD4^+$ PBMC subpopulations of healthy donors were isolated and stimulated with autologous monocyte-derived, peptide-pulsed DC, then re-stimulated with peptide-pulsed $CD14^+$ monocytes. Antigen-specific $CD4^+$ T cells recognizing each of the HLA class II-binding WT1 peptides were generated, as shown by IFN-γ ELISPOT (Figure 5). As expected, cells stimulated with RAS (irrelevant control peptide) or with APC alone did not produce IFN-γ over background levels.

[00232] Thus, HLA class II-binding WT1 peptides of the present invention are able to generate T cells that recognize cells presenting WT1 peptides.

EXAMPLE 5: IDENTIFICATION AND TESTING OF HETEROCLITIC HLA CLASS II WT1 PEPTIDES

[00233] HLA class II molecule-binding WT1 peptides are modified by mutation of HLA class II

molecule anchor residues, using methods and algorithms described herein, to generate heteroclitic HLA class II WT1 peptides. The HLA binding and immunogenicity of the heteroclitic peptides are tested using methods known in the art (e.g. methods described in the previous Examples). The heteroclitic peptides are found to bind HLA class II molecules and stimulate WT1-specific immune responses. Various heteroclitic peptides exhibit enhanced affinity for HLA class II molecules and/or expanded repertoire of HLA class II molecules that are bound, relative to the WT1 peptides from which the heteroclitic peptides were derived. The heteroclitic peptides are used to increase the immunogenicity of vaccines of the present invention and the range of subjects for which the vaccines are effective.

EXAMPLE 6: IDENTIFICATION OF ADDITIONAL HLA CLASS II MOLECULE-BINDING WT1 PEPTIDES; MUTATION OF SAME TO CONTAIN HETEROCLITIC CLASS I MOLECULE EPITOPES

[00234] A WT1 peptide spanning residues 122-140, having the sequence SGQARMFPNAPYLPSCLES (SEQ ID No: 39) was generated and designated "WT1 122." Binding affinity of WT1 122 for common HLA DRB molecules was predicted using the SYFPEITHI epitope prediction algorithm (Rammensee H et al, SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics. 1999 Nov;50(3-4):213-9). 4 of the 6 HLA-DR types showed improved predicted binding relative to a shorter peptide, WT1 124, having the sequence QARMFPNAPYLPSCL (SEQ ID No: 40) (Table 4). In addition, a peptide termed "WT1 122A1" was generated, comprising the $CD8^+$ heteroclitic WT1A1 peptide YMFPNAPYL (Example 1; SEQ ID No: 6) nestled inside the elongated $CD4^+$ peptide epitope and having the sequence SGQAYMFPNAPYLPSCLES (SEQ ID No: 41). WT1 122A1 also exhibited improved predicted binding over WT1 124 to a broad array of HLA-DR types (Table 4). The average score of WT1 122A1 was 19, with a binding score over 14 (the halfway mark) for all 6 HLA-DR types, compared to an average score of 12 with only one HLA-DR type over 14. Predicted WT1 122A1 binding to the HLA-DR types was also superior to a shorter peptide containing the WT1A1 peptide, "124A1," having the sequence QAYMFPNAPYLPSCL (SEQ ID No: 42).

[00235] In addition, a WT1 peptide spanning residues 247-261, having the sequence GATLKGVAAGSSSSVKWT (SEQ ID No: 43) was generated and designated "WT1 244." Binding affinity of WT1 244 for common HLA DRB molecules was predicted as described above for WT 122. Several HLA-DR types showed improved predicted binding relative to a shorter peptide, WT1 247, having the sequence LKGVAAGSSSSVKWT (SEQ ID No: 64) (Table 4).

[00236] Table 4. Predicted binding identification of WT1 peptides to class 2 HLA-DR types.

Peptide sequence	Name	SYFPEITHI Score (high 28- low 0)					
		DRB-101	DRB-301	DRB-401	DRB-701	DRB-1101	DRB-1501
QARMFPNAPYLPSCL	124	12	12	8	8	14	18
SGQARMFPNAPYLPSCLES	122	22	18	22	16	16	18
QAYMFPNAPYLPSCL	124A1	22	12	8	8	14	18
SGQAYMFPNAPYLPSCLES	122A1	27	17	22	18	16	18
GATLKGVAAGSSSSVKWT	244	31	11	20	24	18	18
LKGVAAGSSSSVKWT	247	22	11	20	24	6	18
Frequency of HLA in US Caucasian population		17.9%	18.6%	13.8%	25.5%	10.4%	15.9%

EXAMPLE 7: WT1-EXPRESSING CELLS PROCESS AND PRESENT PEPTIDES COMPRISING A HETEROCLITIC MHC CLASS I PEPTIDE ASSOCIATED WITH AN MHC CLASS II PEPTIDE

[00237] Cross-priming studies were performed to determine whether WT1-expressing cells process and present peptides comprising a heteroclitic MHC class I peptide that is associated with an MHC class II peptide and/or the corresponding native peptides. Total tumor lysates were prepared from 3 different cell lines: 697 ($WT1^+$, HLA $A0201^+$), an e1a2 leukemia cell line; JMN ($WT1^+$, HLA $A0201^+$) a biphasic mesothelioma cell line, and as a control, MeWo ($WT1^-$, HLA $A0201^+$), a malignant melanoma cell line. DCs from healthy $A0201^+$ donors were incubated for 18 hours with the tumor lysates and used to stimulate autologous $CD3^+$ T cells. Following 3 stimulations, the T cells were tested for their reactivity to autologous DCs pulsed with the WT1 peptides. T cells that had been stimulated with WT1 positive tumor lysates recognized the individual HLA class II peptides (Figure 6A-B), while T cells stimulated by DCs pulsed with MeWo lysate did not stimulate WT1 specific T cells. In addition, T cells stimulated with DCs pulsed with 697 tumor lysate recognized the native

short class I peptide WT1A (126-134) and the analog WT1A1 peptide. These experiments were repeated in 5 separate donors. Stimulated T cells recognized WT1DR peptide 328 and WT1DR peptide 122A1 in 3/5 experiments and recognized WT1DR 427 in all experiments. Therefore, despite the low expression of WT1 transcript in the mesothelioma cell lines, WT1 CD4 epitopes of the present invention were processed and presented by HLA class II molecules of mesothelioma cells.

[00238] These findings show that peptides comprising a heteroclitic MHC class I peptide that is associated with an MHC class II peptide are (a) taken up and presented by APC in an antigenic form; and (b) are presented by APC exposed to WT1-expressing tumor cells; and (c) APC exposed to WT1 122 and 122A1 peptides elicit the formation of T cells that recognize WT1-expressing tumor cells. Thus, WT1-expressing cells, including mesothelioma and leukemia cells, process and present peptides comprising a heteroclitic MHC class I peptide that is associated with an MHC class II peptide.

EXAMPLE 8: STIMULATION WITH WT1 122 OR 122A1 STIMULATES THE PRODUCTION OF ANTIGEN-SPECIFIC $CD4^+$ AND $CD8^+$ T CELLS; $CD8^+$ T CELLS ELICITED BY WT1 122A1 ALSO RECOGNIZE THE NATIVE ANTIGEN

MATERIALS AND EXPERIMENTAL METHODS

[00239] $CD3^+$ cells from healthy donors were isolated and stimulated 2 times with peptide, and then recognition of $WT1^+$ JMN cells or $WT1^-$ Mewo cells, either alone or with the indicated peptides, was determined by gamma IFN ELISPOT, using the methods described in Example 4.

RESULTS

[00240] T cells were stimulated with autologous, monocyte-derived DC pulsed with WT1 122, 122A1, or negative control peptide, re-stimulated with $CD14^+$ monocytes pulsed with the same peptide, then assayed for formation of antigen-specific T cells by IFN-γ ELISPOT. Stimulation with WT1 122 or 122A1, but not negative control peptide, generated $CD4^+$ T cells that recognized targets pulsed with peptides containing the respective $CD4^+$ epitopes, but not targets pulsed with negative control peptide (Figure 7A-B).

[00241] In addition, both WT1DR 122 and WT1DR 122A1 were able to activate $CD8^+$ T cells against the native short epitope WT1A (amino acids 126-134 (Figure 7C); WT1DR 122A1 was a more potent stimulator.

[00242] These stimulation experiments were reproduced in 7 different healthy donors, each with a

different HLA-DRB1 type. Up to 15 separate experiments were performed with each WT1DR peptide. WT1DR 328 stimulated peptide specific T cell responses in 11 / 15 experiments; WT1 DR 423 in 3 / 14 experiments; WT1DR 122 in 2 / 5 experiments; and WT1DR 122A1 stimulated T cells that recognized WT1DR 122A1 and WT1DR 122 peptide in 6 / 9 experiments.

[00243] Thus, stimulation with WT1 122 or 122A1 generates antigen-specific $CD4^+$ and $CD8^+$ T cells. In addition, stimulation with WT1 122A1 generates $CD8^+$ T cells that recognize the heteroclitic $CD8^+$ peptide and its native counterpart, whether buried in a longer peptide (WT1 122 or WT1 122A1, respectively) or alone.

EXAMPLE 9: ANTIGEN-SPECIFIC $CD4^+$ T CELLS GENERATED BY PEPTIDES COMPRISING A HETEROCLITIC MHC CLASS I PEPTIDE ASSOCIATED WITH AN MHC CLASS II PEPTIDE RECOGNIZE WT1-EXPRESSING TUMOR CELLS

[00244] To test whether antigen-specific $CD4^+$ T cells generated by peptides comprising a heteroclitic MHC class I peptide that is associated with an MHC class II peptide recognize WT1-expressing tumor cells, peptide-stimulated T cells were challenged in an IFN-gamma ELISPOT with $WT\text{-}1^+$ and - negative tumor cells. A sufficient amount of WT1 peptide was presented on the surface of the $WT1^+$ mesothelioma tumor cell for T cells stimulated with individual WT1DR peptides to recognize mesothelioma tumor cells, compared to the control WT1 negative melanoma cells (Figure 8, left panel). In another experiment, T cells were stimulated by the mutated WT1DR 122A1 and challenged with pulsed and unpulsed targets. When control WT1 negative target cells were pulsed with additional WT1DR 122A1 peptide, IFN-gamma production increased. When WT1 positive target cells were pulsed with additional WT1DR 122A1 peptide, production did not increase, showing that a maximal response was achieved with the native processed peptides (Figure 8, right panel). Thus, vaccination with peptides comprising a heteroclitic MHC class I peptide that is associated with an MHC class II peptide results in generation of antigen-specific T cells with activity against WT1-expressing tumors.

EXAMPLE 10: ANTIGEN-SPECIFIC $CD8^+$ T CELLS GENERATED BY PEPTIDES COMPRISING A HETEROCLITIC MHC CLASS I PEPTIDE ASSOCIATED WITH AN MHC CLASS II PEPTIDE RECOGNIZE WT1-EXPRESSING TUMOR CELLS

[00245] To determine whether antigen-specific CD8+ T cells generated by peptides comprising a heteroclitic MHC class I peptide that is associated with an MHC class II peptide recognize WT1-expressing tumor cells, $CD3^+$ cells from healthy donors were isolated and stimulated with autologous, monocyte-derived DC pulsed with WT1DR 122A1, WT1DR 122, or negative control peptide, re-

stimulated with $CD14^+$ monocytes pulsed with the same peptide, then assayed by IFN-γ ELISPOT for formation of antigen-specific T cells that recognized $WT1^+$ JMN cells.

[00246] WT1DR 122A1, but not WT1DR 122, stimulated a sufficient number of $CD8^+$ cells to be cytotoxic to 697, a $WT1^+$ leukemia cell line. The $CD8^+$ T cells did not recognize SKLY16, a WT1 negative B cell lymphoma, unless it was pulsed with WT1A (Figure 9, left panel), showing antigen specificity of the immune response. Similar results were observed in 3/4 different $A0201^+$ donors, each with a distinct HLA-DRB1 type. As expected, the negative control peptides generated no antigen-specific $CD8^+$ T cells. In other experiments, $CD3^+$ T cells generated by stimulation with WT1 122A1 or WT1A1 recognized JMN cells but not negative control MeWo cells, whether alone or pulsed with WT1 122A1 peptide (Figure 9, right panel). In contrast, $CD4^+$ cells stimulated with WT1DR 122A1 showed no cytotoxicity to either WT1+ mesothelioma or WT1- melanoma cells. Human T cells stimulated 2 times with either the native WT1A or the analog WT1A1 peptide were able to lyse human $WT1^+$ mesothelioma cell lines compared to $WT1^-$ control cell lines (9.2% lysis of MeWo vs. 19% lysis of JMN for WT1A stimulated T cells; 22.2% lysis of MeWo vs. 44.8% lysis of JMN for WT1A1 stimulated T cells).

[00247] These findings show that vaccination with WT1 122A1 results in generation of antigen-specific T cells with activity against WT1-expressing tumors. These findings also show that peptides comprising a heteroclitic MHC class I peptide that is associated with an MHC class II peptide stimulate both $CD4^+$ and $CD8^+$ T cells, including antigen-specific T cell responses against the native peptide.

EXAMPLE 11: IDENTIFICATION AND GENERATION OF BCR-ABL BREAKPOINT PEPTIDES WITH A HIGH PROBABILITY OF HLA A0201 BINDING

[00248] AA sequences of the human b3a2 and b2a2 fusion proteins were scanned for peptides with potential binding capacity for HLA A0201, a subtype encompassing 95% of the prevalence of the HLA-A02 allele. HLA-A0201 is expressed in about 40% of the Caucasian population.

[00249] Single or double AA substitutions were introduced at HLA A0201 preferred residues (positions 1, 2, 6 and 9, see underlined residues in Table 5) of a peptide that does not exhibit the consensus HLA 0201 binding motifs but has weak avidity to MHC, to yield sequences that had comparatively high binding scores predicted for HLA A0201 molecules. Substitutions were determined using the software of the Bioinformatics & Molecular Analysis Section (National Institutes of Health, Washington, DC) (Parker KC, et al. J Immunol 1994;152(1):163-75; available at http://bimas.dcrt.nih.gov/cgi-

bin/molbio/ken_parker_comboform), which ranks 9-mer or 10-mer peptides on a predicted half life dissociation coefficient from HLA class I molecules. The BIMAS score is based on the calculation of the theoretical half-life of the MHC-I/β_2-microglobulin/peptide complex, which is a measure of peptide-binding affinity. The program uses information about HLA-I peptides of 8–10 amino acids in length. The higher the binding affinity of a peptide to the MHC, the higher the likelihood that this peptide represents an epitope. The BIMAS algorithm assumes that each amino acid in the peptide contributes independently to binding to the class I molecule. Dominant anchor residues, which are critical for binding, have coefficients in the tables that are significantly higher than 1. Unfavorable amino acids have positive coefficients that are less than 1. If an amino acid is not known to make either a favorable or unfavorable contribution to binding, then is assigned the value 1. All the values assigned to the amino acids are multiplied and the resulting running score is multiplied by a constant to yield an estimate of half-time of dissociation.

[00250] Several analogue peptides were designed, whereby one or both anchor amino acids or additional amino acids adjacent to anchor amino acids were modified. The predicted half life for binding to HLA A0201 was greater than 240 minutes in four synthetic peptides and less than 240 in seven. All the native peptides were predicted to have a half life of less than one minute. Most substitutions affected the primary or secondary anchor motifs (leucine in position 2 or valine in position 9 or 6) but in some cases, a tyrosine was substituted in position 1, which stabilized the binding of the position 2 anchor residue. Also depicted in Table 5 are the predicted half lives according to another software program, SYFPEITHI (Rammensee HG, et al., Immunogenetics 1995; 41(4): 178-228).

[00251] Table 5. The AA sequences of native breakpoint peptides and synthetic analogues and their predicted score for binding to HLA A0201, generated by two BIMAS and SYFPEITHI.

Name/ type	Sequence	BIMAS score	SYFPEITHI score	SEQ ID NO:
p210-b3a2				
CMLA2 native	SSKALQRPV	0.003	12	44
p210F (analogue)	YLKALQRPV	2.240	22	45
CMLA3 native	KQSSKALQR	0.005	3	46
p210A (analogue)	KQSSKALQV	24.681	13	47
p210B (analogue)	KLSSKALQV	243.432	23	48
p210Cn native	KALQRPVAS	0.013	10	49
p210C (analogue)	KLLQRPVAV	900.689	26	50
p210Dn native	TGFKQSSKA	0.120	7	51
p210D (analogue)	TLFKQSSKV	257.342	23	52
p210E (analogue)	YLFKQSSKV	1183.775	25	53
p210-b2a2				
b2a2A native	LTINKEEAL	0.247	20	54
b2a2 A1 (analogue)	LLINKEEAL	17.795	26	55
b2a2 A2 (analogue)	LTINKVEAL	21.996	24	56
b2a2 A3 (analogue)	YLINKEEAL	48.151	26	57
b2a2 A4 (analogue)	YLINKEEAV	156.770	26	58
b2a2 A5 (analogue)	YLINKVEAL	110.747	30	59
HLA A24 consensus peptide	VYFFLPDHL			60
positive control influenza matrix peptide	GILGFVFTL			61

Residues in bold (K in the b3a2 and E in b2a2) represent the amino acid at the fusion breakpoint. Residues underlined represent modifications from the native sequence.

EXAMPLE 12: BINDING OF HLA-A0201 BY SELECTED PEPTIDES

[00252] To determine the capacity of the mutated peptides to bind and stabilize MHC class I molecules on the live cell surface, direct measurement of the strength of the interaction between the peptides and the HLA-A0201 molecule was performed, with a conventional binding and stabilization assay that uses the antigen-transporting deficient (TAP2 negative) HLA-A0201 human T2 cells, as described in Example 1. Seven out eleven peptides designed to have higher binding scores exhibited a relatively high binding affinity for HLA A0201 molecules as measured by the T2 assay (Figure 10, left panel). A correlation between binding scores and binding affinity was established, thus demonstrating the utility of the computer generated binding scores for predicting peptides that will bind to MHC class I molecules on live cells.

[00253] Some of these peptides demonstrated the same order of binding affinity as that of the influenza matrix viral antigen, which is among the most potent known antigens for CTL induction. In only four cases was a good correlation between computer predicted half-life and T2 stabilization not found. One of the peptides derived from b3a2, p210C, was mutated from a native peptide that did not have a good prediction score. Nevertheless, the native sequence was able to bind HLA A0201 weakly and at the same level that the previously described CMLA2 peptide. To design p210C, a neutral alanine was substituted for a leucine in position two and a serine was substituted for a valine in position nine. p210C has a high BIMAS score that correlated with T2 binding assay data (Figure 10, left panel). p210F is a peptide derived from CMLA2 (Table 5), shown to be a weak binder in the T2 assay. In this case, the two serine residues in positions 1 and 2 were substituted for a tyrosine and a leucine, respectively, with the intent of increasing peptide binding and stabilization to HLA A0201, while retaining the amino-acids for the TCR interaction. The BIMAS prediction was increased 700-fold, and high avidity for HLA A0201 molecules was demonstrated by binding to T2 cells. Of the peptides derived from b2a2, all were generated from a peptide that was not predicted to binding avidly to HLA A0201. Three new synthetic peptides, b2a2 A3-A5 (Table 5) bound well to HLA A0201 molecules (Figure 10, right panel). These three peptides have a tyrosine-leucine sequence substitution at position 1 and 2 and also a valine substitution in position 6 or 9 that are reflected in increased binding to HLA A0201.

[00254] These results show that heteroclitic peptides of the present invention exhibit increased MHC molecule binding, and therefore exhibit increased immunogenicity. The results further show that methods of the present invention for identifying and designing heteroclitic peptides work for bcr-abl peptides as well as WT1 peptides, and thus can be successfully used for various antigens.

EXAMPLE 13: PEPTIDE ANALOGUE DISSOCIATION FROM HLA A0201

[00255] The immunogenicity of peptide antigens is, under the conditions utilized herein, related to their low dissociation rate from MHC molecule-peptide complexes. The stability of complexes formed between HLA-A0201 and the b3a2 analogue peptides was therefore assayed with T2 cells, as a function of time. Overnight incubation of T2 cells with saturating amounts of HLA-A0201 binding peptides and human β_2 microglobulin resulted in increased surface HLA-A0201 expression. After removal of unbound peptide and addition of brefeldin A to inhibit protein synthesis, the number of HLA-A0201 molecules remaining at the T2 cell surface was determined. The stability of each peptide/HLA-A0201 complex was then normalized relative to that observed for the tyrosinase D peptide or HIV gag peptide (peptides with known high affinity and half life). HLA-A0201 complexes

with p210C, p210D, p210E and p210F formed complexes that were stable over 6-8 hours. In contrast, p210A and p210B were less stable, reaching background levels in less than 1 hour of incubation.

[00256] These results confirm the results of the previous Examples, showing that methods of the present invention for identifying and designing heteroclitic peptides can be successfully used for various antigens.

EXAMPLE 14: P210 PEPTIDE STIMULATION OF CD8$^+$ IMMUNE RESPONSES; T CELLS GENERATED BY SYNTHETIC ANALOGUES RECOGNIZED NATIVE SEQUENCES

[00257] The next experiment measured the ability of bcr-abl peptides to induce reactive precursor T cells with cognate T cell receptors. Using an optimized T cell-expansion system, with monocyte-derived DC, CD14$^+$ cells as APC, and purified CD3$^+$ T cells, synthetic b3a2 and b2a2 analogues were evaluated for their ability to stimulate peptide-specific CTL. Cells from ten healthy HLA A0201 donors and 4 patients with chronic phase CML were assayed. The peptides used were heteroclitic peptides p210A, p210B, p210C, p210D, and p210E, and CMLA3, p210Cn, p201Dn, and CMLA2, the native sequences corresponding to p210A-B, p210C, p210D, and p210E, respectively (Table 5).

[00258] Cells from 5 / 10 healthy donors responded to immunization, generating T cells that secreted IFN-gamma when challenged with peptide-pulsed T2 cells as targets. p210C and p210F generated the most consistent and significant immune-responses (Figure 11); p210D and p210E also produced an immune response in some donors tested. Responses were observed after the second or third round of peptide stimulation, either after CD8$^+$ isolation or in CD3$^+$ T cells not subject to further purification. Spot numbers were consistently higher with peptides that bound with higher affinity to HLA 0201 molecules in the T2 assay. By contrast, no immune response was generated against p210A and p210B, consistent with their reduced affinity for MHC.

[00259] In addition, the T cells elicited by p210C and p210F vaccination were able to recognize their respective native sequences (Figure 11). For example, the peptide CMLA2, the native sequence corresponding to p210F, is a weak MHC binder, and is expressed in the surface of CML blasts.

[00260] Immune responses to the heteroclitic peptide p210C were also observed in two of the CML patients. After two rounds of stimulation with p210C, CD8$^+$ cells recognized T2 pulsed with the synthetic peptide with a frequency of nearly 400 spot-forming cells (SCF) per $1x10^5$ cells, and recognized the native peptide on T2 cells with a frequency of 200 SFC per 1×10^8 (Figure 12).

[00261] b2a2-derived peptides A3, A4 and A5 also generated a significant immune response, as measured by gamma-IFN secretion by $CD3^+$ T cells (Figure 13A-B), with the response against A3 the most consistent between donors. A3-generated T cells recognized the native sequence as well, despite the fact that the native sequence is a weak HLA binder (Bocchia M, Wentworth PA, et al, Blood. 1995; 85(10): 2680-4).

[00262] To determine whether the *in vitro*-generated T cells were capable of cytolysis, T cell lines obtained after several stimulations with p210C and b2a2A3 were assayed by chromium-51 release assays using peptide-pulsed target cell lines. The cells were able to kill T2 cells pulsed with the heteroclitic peptides. In addition, the cells were able to recognize and kill cells expressing the native peptide from which the heteroclitic peptide was derived (Figures 14-15). As expected, the cells did not lyse T2 cells without peptide or T2 cells with control peptide, showing the specificity of the assay.

[00263] These results confirm the results of the previous Examples, showing that:

(a) heteroclitic peptides of the present invention exhibit increased immunogenicity relative to the corresponding unmutated ("native") sequences in both healthy subjects and CML subjects;

(b) T cells generated with the heteroclitic peptides recognize MHC molecules bearing the native peptides, even when the native peptide is a weak binder, and lyse target cells bearing the corresponding peptides; and

(c) methods of the present invention for identifying and designing heteroclitic peptides can be successfully used for various antigens.

EXAMPLE 15: IDENTIFICATION OF HLA CLASS II MOLECULE-BINDING BCR-ABL PEPTIDES

[00264] Bcr-abl peptides (e.g. bcr-abl breakpoint peptides) are tested for ability to bind HLA class II molecules, and thus stimulate $CD4^+$ T cells, as described in Example 6. In other experiments, b3a2-bcr-abl derived peptides (e.g. IVHSATGFKQSSKALQRPVASDFEP; SEQ ID No: 62) are utilized. In another experiments, b2a2-bcr-abl derived peptides (e.g. VHSIPLTINKEEALQRPVASDFE; SEQ ID No: 63) are utilized. Wild-type bcr-abl peptides with ability to bind HLA class II molecules are identified.

EXAMPLE 16: MUTATION OF BCR-ABL HLA CLASS II PEPTIDES TO CONTAIN HETEROCLITIC HLA CLASS I MOLECULE EPITOPES

[00265] Bcr-abl peptides identified in the previous Example are mutated to contain buried heteroclitic HLA class I molecule epitopes, as described in Example 6. In some experiments, the heteroclitic HLA class I molecule epitope introduced into the peptides is one of the sequences set forth in SEQ ID No: 45, 47-48, 50, 52-53, and 55-59.

EXAMPLE 17: BCR-ABL PEPTIDES CONTAINING BURIED HLA CLASS I EPITOPES ARE IMMUNOGENIC

[00266] The immunogenicity of bcr-abl peptides containing buried, heteroclitic HLA class I epitopes is tested, as described in Examples 7-8. The peptides exhibit the ability to stimulate bcr-abl-specific T cells. Moreover, addition of the peptides to naive PMBC generates antigen-specific $CD4^+$ T cells and $CD8^+$ T cells. Both the $CD4^+$ T cells and the $CD8^+$ T cells recognize both the heteroclitic bcr-abl peptides and their native counterparts. Thus, bcr-abl peptides of the present invention are immunogenic.

EXAMPLE 18: BCR-ABL PEPTIDES CONTAINING BURIED HLA CLASS I EPITOPES RECOGNIZE ANTIGEN-PRESENTING CELLS EXPOSED TO BCR-ABL-EXPRESSING TUMOR CELLS

[00267] Antigen-specific $CD4^+$ T cells from the previous Example are incubated with DC pulsed with tumor lysate from CML cells, then target recognition is measured by IFN-γ ELISPOT. $CD4^+$ T cells generated by vaccination with bcr-abl peptides of the present invention recognize the peptide-pulsed DC, but not DC pulsed with bcr-abl-negative tumor cells. Thus, vaccination with bcr-abl peptides of the present invention results in generation of antigen-specific T cells with activity against bcr-abl-expressing tumors.

EXAMPLE 19: ANTIGEN-SPECIFIC $CD8^+$ T CELLS GENERATED BY BCR-ABL PEPTIDES OF THE PRESENT INVENTION RECOGNIZE BCR-ABL-EXPRESSING TUMOR CELLS

[00268] Antigen-specific $CD8^+$ T cells are generated by incubating PBMC with either bcr-abl peptides containing buried, heteroclitic HLA class I epitopes, the corresponding wild-type bcr-abl peptides, the heteroclitic HLA class I epitope alone, or negative control peptide, as described in Example 8, then are incubated with bcr-abl-expressing tumor cells or bcr-abl-negative tumor cells and target recognition is determined by IFN-γ ELISPOT or *in vitro* cytolysis assay. $CD8^+$ T cells generated by vaccination with either (a) peptides containing buried, heteroclitic HLA class I epitopes or (b) the heteroclitic HLA

class I epitope alone recognize the DC, but not DC pulsed with bcr-abl-negative tumor cells, while vaccination with the corresponding wild-type bcr-abl peptides is less effective. Moreover, peptides containing buried, heteroclitic HLA class I epitopes are more potent and/or stronger than the heteroclitic HLA class I epitope alone in induction of antigen-specific $CD8^+$ T cells.

[00269] These findings show that vaccination with bcr-abl peptides containing buried, heteroclitic HLA class I epitopes results in generation of antigen-specific T cells with activity against bcr-abl-expressing tumors. These findings also show that peptides comprising a heteroclitic MHC class I peptide associated with an MHC class II peptide stimulate cytotoxic T cell responses more potently and/or strongly than the heteroclitic MHC class I peptide alone. Thus, peptides of the present invention exhibit protective activity against bcr-abl-expressing cancers.

WHAT IS CLAIMED IS:

1. An isolated, mutated bcr-abl peptide, comprising:

a. a binding motif of a human leukocyte antigen (HLA) Class II molecule; and

b. a binding motif of an HLA class I molecule, having a point mutation in one or more anchor residues of said binding motif of an HLA class I molecule,

wherein said isolated, mutated bcr-abl peptide is 11-30 amino acids in length.

2. The isolated, mutated bcr-abl peptide of claim 1, wherein said length is 16-22 amino acids in length.

3. The isolated, mutated bcr-abl peptide of claim 1, wherein said length is 19 amino acids.

4. The isolated, mutated bcr-abl peptide of claim 1, wherein said point mutation increases an affinity of said isolated, mutated bcr-abl peptide for said HLA class I molecule.

5. The isolated, mutated bcr-abl peptide of claim 1, wherein said binding motif of an HLA class I molecule is contained within said binding motif of an HLA class II molecule.

6. The isolated, mutated bcr-abl peptide of claim 1, wherein said binding motif of an HLA class I molecule overlaps with said binding motif of an HLA class II molecule.

7. The isolated, mutated bcr-abl peptide of claim 1, wherein said HLA class II molecule is an HLA-DR molecule.

8. The isolated, mutated bcr-abl peptide of claim 7, wherein said isolated, mutated bcr-abl peptide binds to an additional HLA-DR molecule, wherein said HLA-DR molecule and said additional HLA-DR molecule are encoded by separate HLA-DR alleles.

9. The isolated, mutated bcr-abl peptide of claim 1, wherein said HLA class II molecule is an HLA-DRB molecule.

10. The isolated, mutated bcr-abl peptide of claim 9, wherein said isolated, mutated bcr-abl peptide binds to an additional HLA-DRB molecule, wherein said HLA-DRB molecule and said additional HLA-DRB molecule are encoded by separate HLA-DRB alleles.

11. The isolated, mutated bcr-abl peptide of claim 1, wherein said HLA class I molecule is an HLA-A molecule.

12. The isolated, mutated bcr-abl peptide of claim 1, wherein said isolated, mutated bcr-abl peptide is counterpart of an unmutated bcr-abl peptide having a sequence selected from IVHSATGFKQSSKALQRPVASDFEP (SEQ ID No: 62) and VHSIPLTINKEEALQRPVASDFE (SEQ ID No: 63).

13. The isolated, mutated bcr-abl peptide of claim 1, wherein said binding motif of an HLA class I molecule has one of the sequences set forth in SEQ ID No: 45, 47-48, 50, 52-53, and 55-59.

14. A vaccine comprising the isolated, mutated bcr-abl peptide of claim 1 and an adjuvant.

15. The vaccine of claim 14, wherein said adjuvant is QS21.

16. A method of treating a subject with a bcr-abl-expressing leukemia, the method comprising administering to said subject the vaccine of claim 14, thereby treating a subject with a bcr-abl-expressing leukemia.

17. The method of claim 16, wherein said leukemia is acute myeloid leukemia, chronic myeloid leukemia, or acute lymphoblastic leukemia.

18. A method of reducing an incidence of a bcr-abl-expressing leukemia, or its relapse, in a subject, the method comprising administering to said subject the vaccine of claim 14, thereby reducing an incidence of a bcr-abl-expressing leukemia, or its relapse, in a subject.

19. The method of claim 18, wherein said leukemia is acute myeloid leukemia, chronic myeloid leukemia, or acute lymphoblastic leukemia.

20. A method of inducing formation and proliferation of a bcr-abl-specific cytotoxic T lymphocyte (CTL), the method comprising contacting a lymphocyte population with the vaccine of claim 14, thereby inducing formation and proliferation of a bcr-abl-specific CTL.

21. A method of inducing formation and proliferation of (a) a $CD8^+$ lymphocyte specific for a bcr-abl protein; and (b) a $CD4^+$ lymphocyte specific for said bcr-abl protein, the method comprising contacting a lymphocyte population with the vaccine of claim 14, thereby inducing formation and proliferation of (a) a $CD8^+$ lymphocyte specific for a bcr-abl protein; and (b) a $CD4^+$ lymphocyte specific for said bcr-abl protein.

22. An immunogenic composition comprising the isolated, mutated bcr-abl peptide of claim 1.

23. An antigen-presenting cell comprising the isolated, mutated bcr-abl peptide of claim 1.

24. A vaccine comprising the antigen-presenting cell of claim 23.

25. A nucleic acid molecule encoding the isolated, mutated bcr-abl peptide of claim 1.

26. A vaccine comprising the nucleic acid molecule of claim 24.

27. A vector comprising the nucleic acid molecule of claim 24.

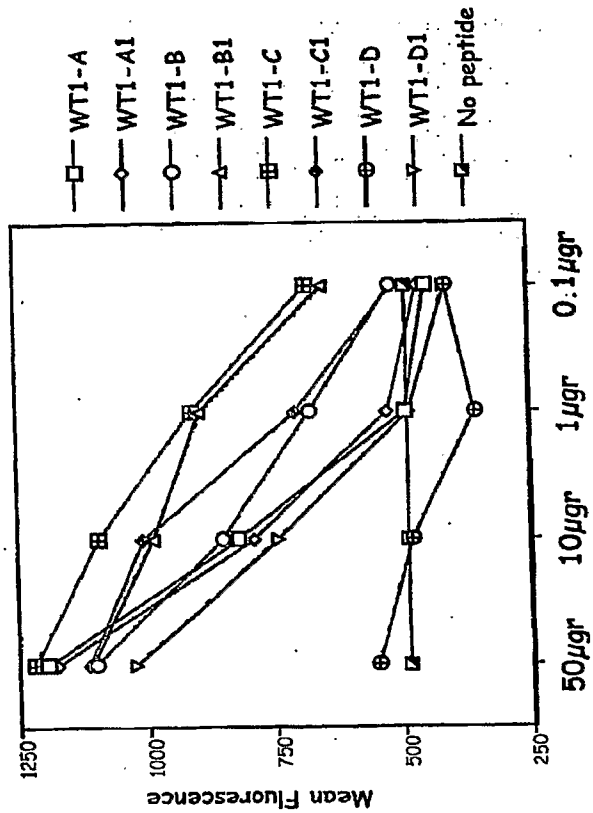

WT1-A
WT1-A1
WT1-B
WT1-B1
WT1-C
WT1-C1
WT1-D
WT1-D1
No peptide
Mean Fluorescence
1250
1000
750
500
250
50µgr
10µgr
1µgr
0.1µgr

Figure 1A

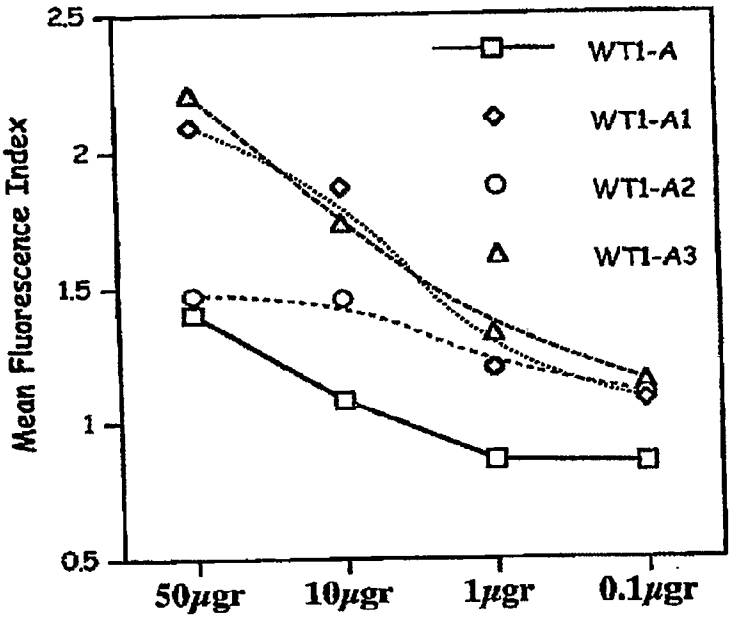


Figure 1B

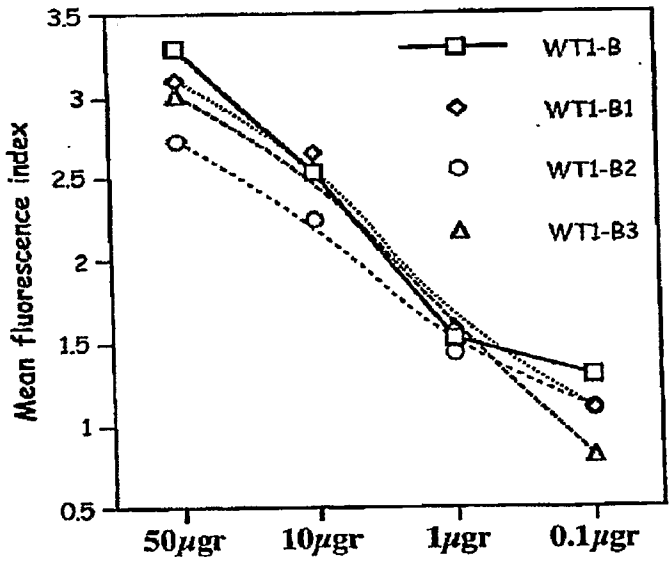


Figure 1C

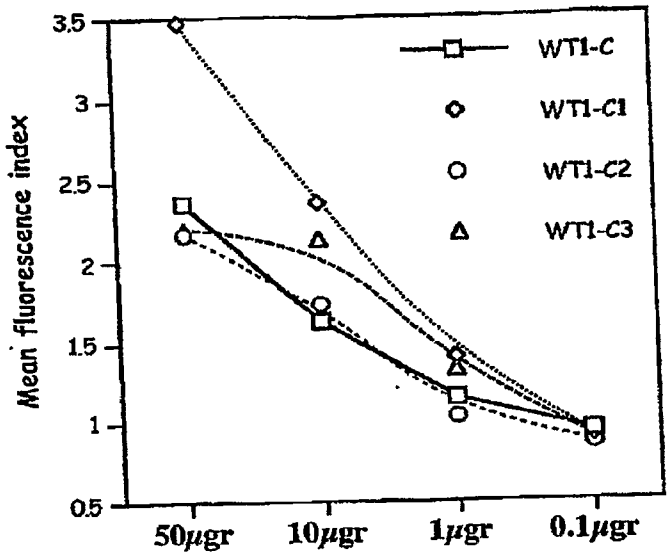


Figure 1D

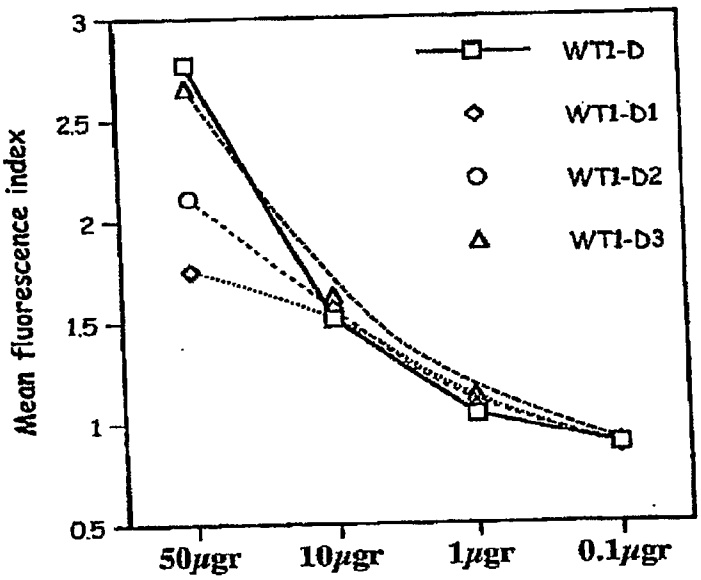


Figure 1E

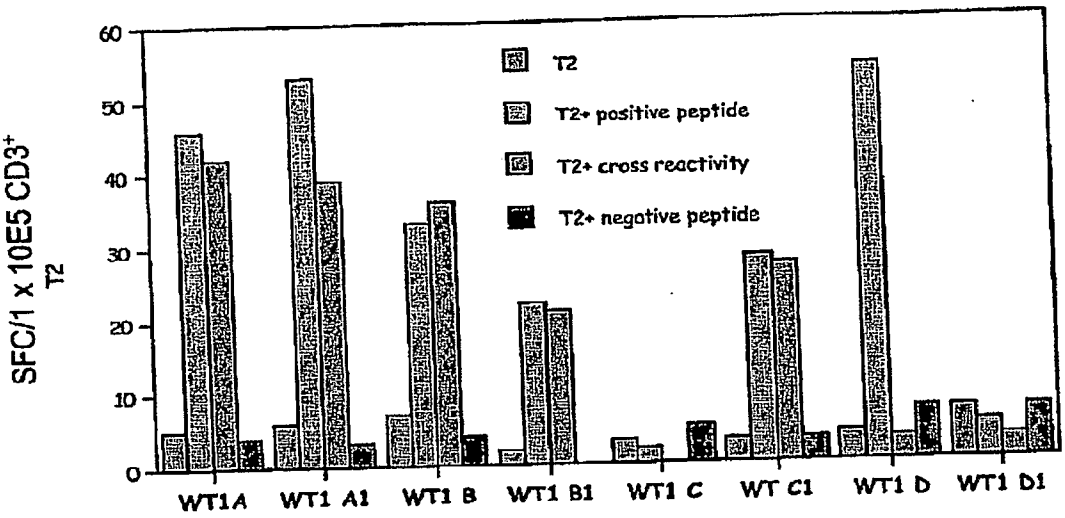


Figure 2A

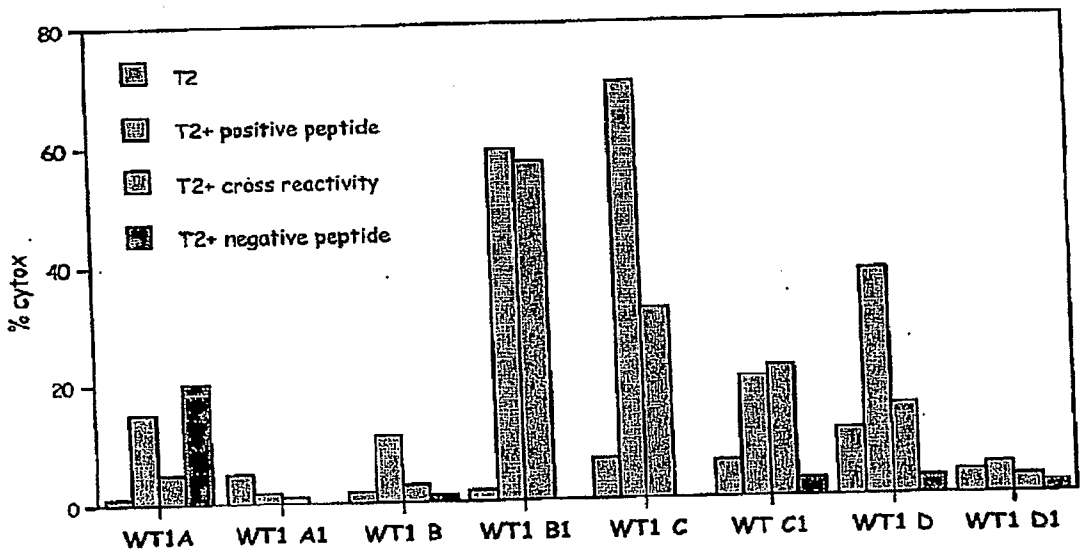


Figure 2B

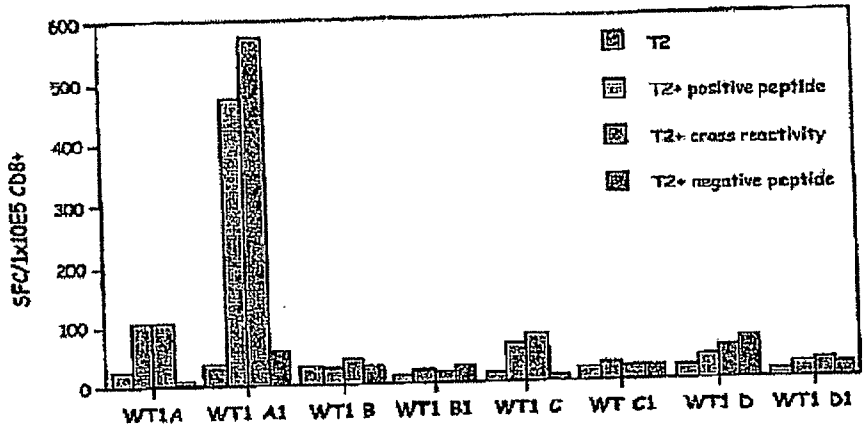


Figure 3A

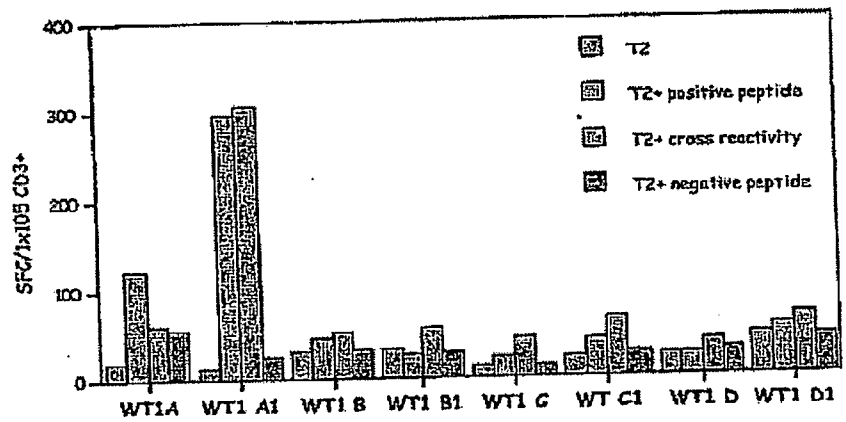


Figure 3B

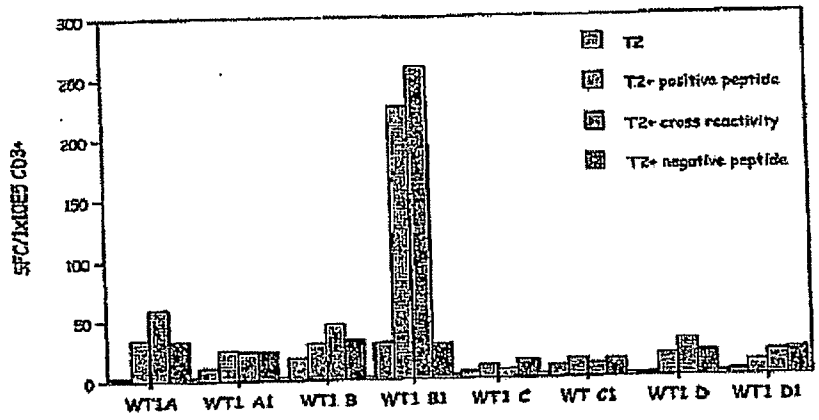


Figure 3C

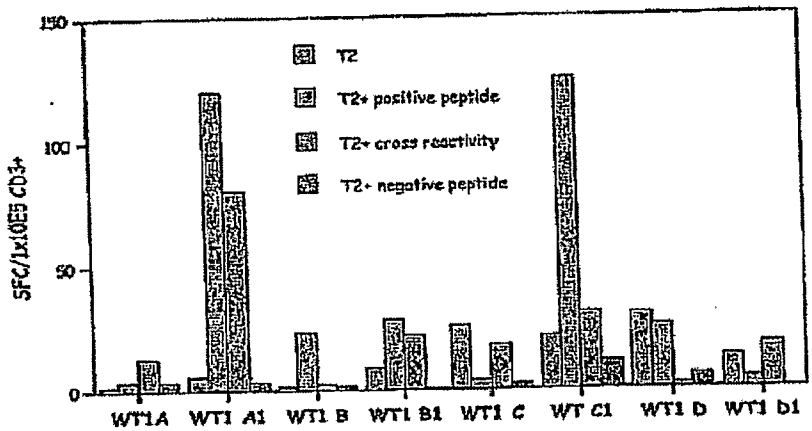


Figure 3D

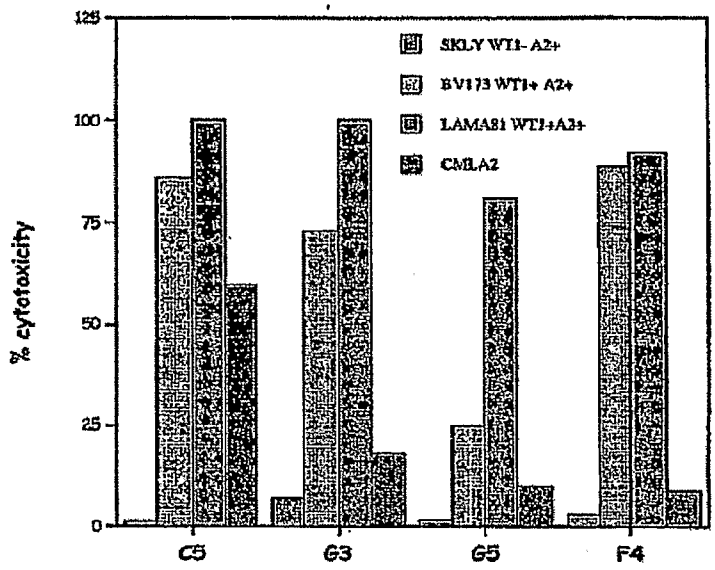


Figure 4A

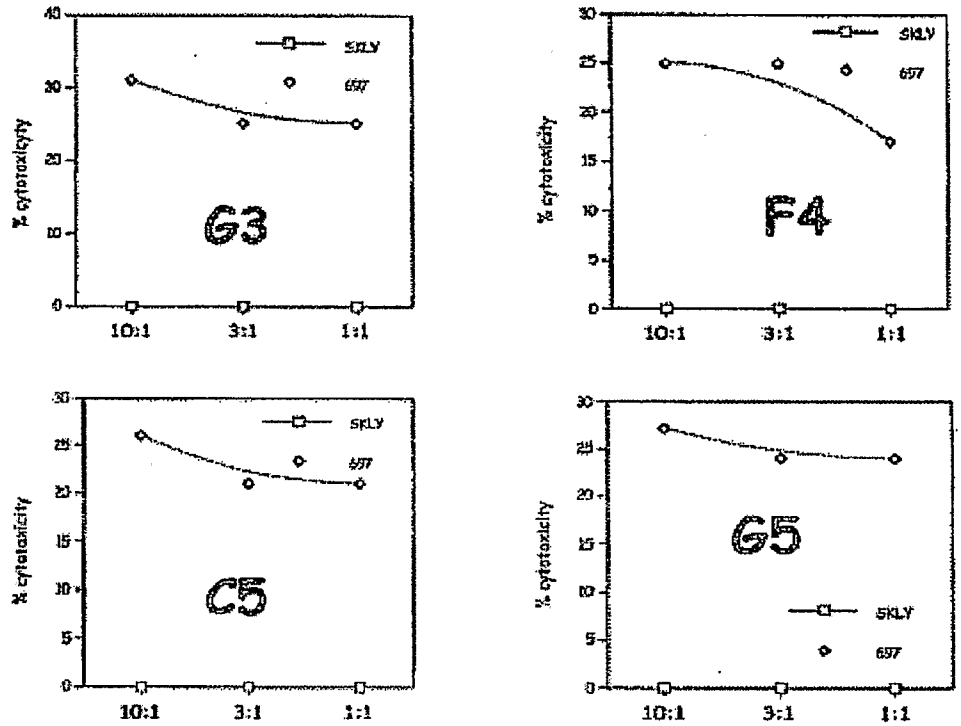


Figure 4B

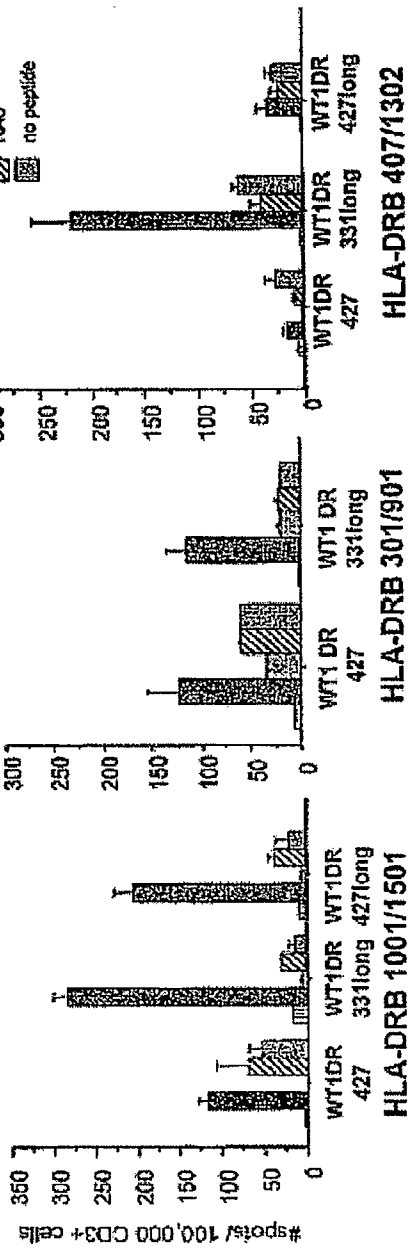


Figure 5, part 1

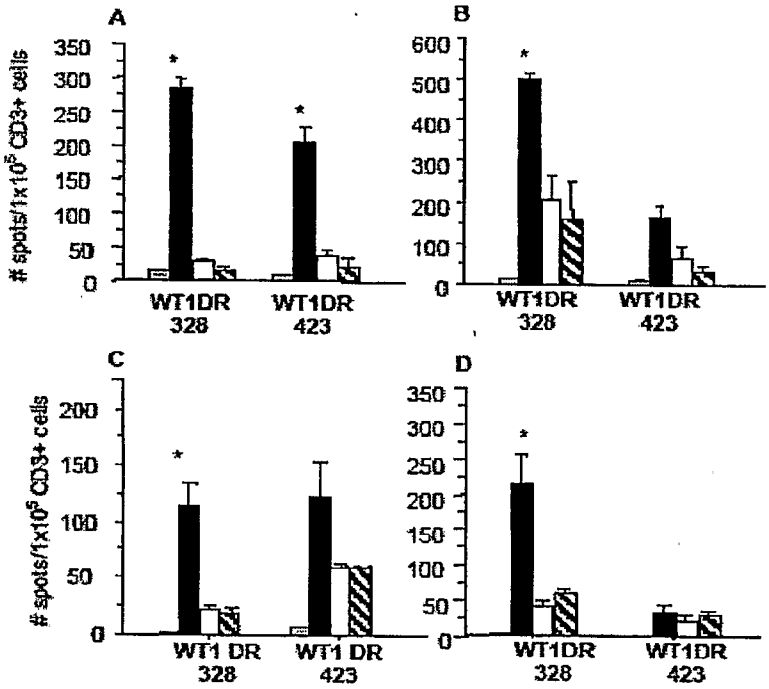


Figure 5, part 2

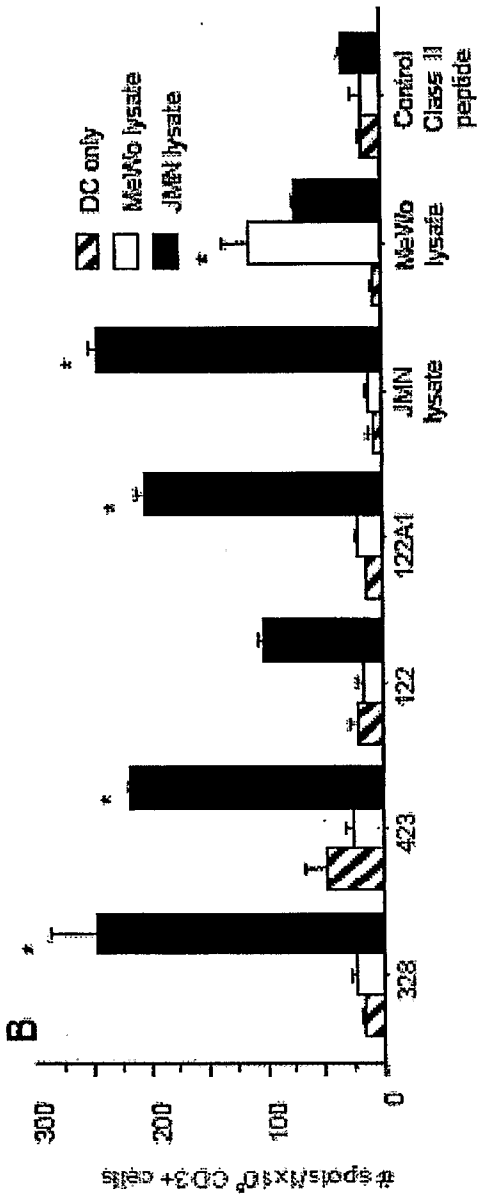

B
spots/Mx10^6 CD3+ cells
300
200
100
0
328
423
122
122A1
JMN lysate
MeWo lysate
Control Class II peptide
DC only
MeWo lysate
JMN lysate

Figure 6A

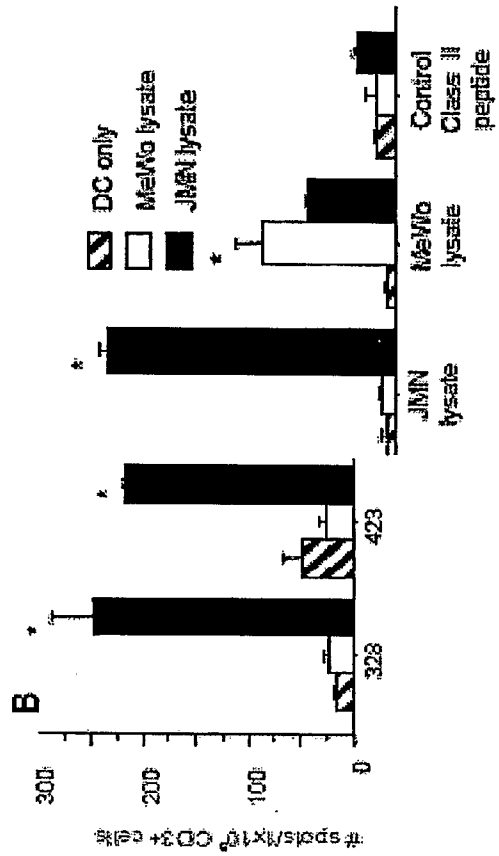

B
spots/1x10^5 CD3+ cells
300
200
100
0
328
423
DC only
MelMo lysate
JMN lysate
JMN lysate
MelMo lysate
Control Class II peptide

Figure 6B

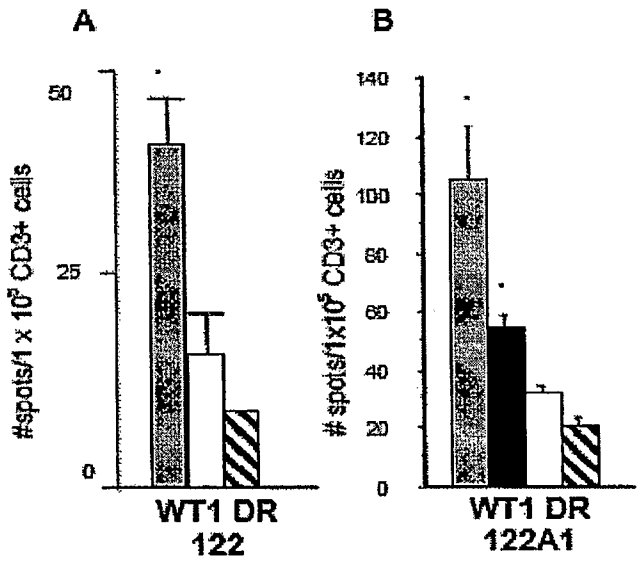

A
B
50
25
0
#spots/1 x 10^5 CD3+ cells
140
120
100
80
60
40
20
0
spots/1x10^5 CD3+ cells
WT1 DR
122
WT1 DR
122A1

Figure 7A-B

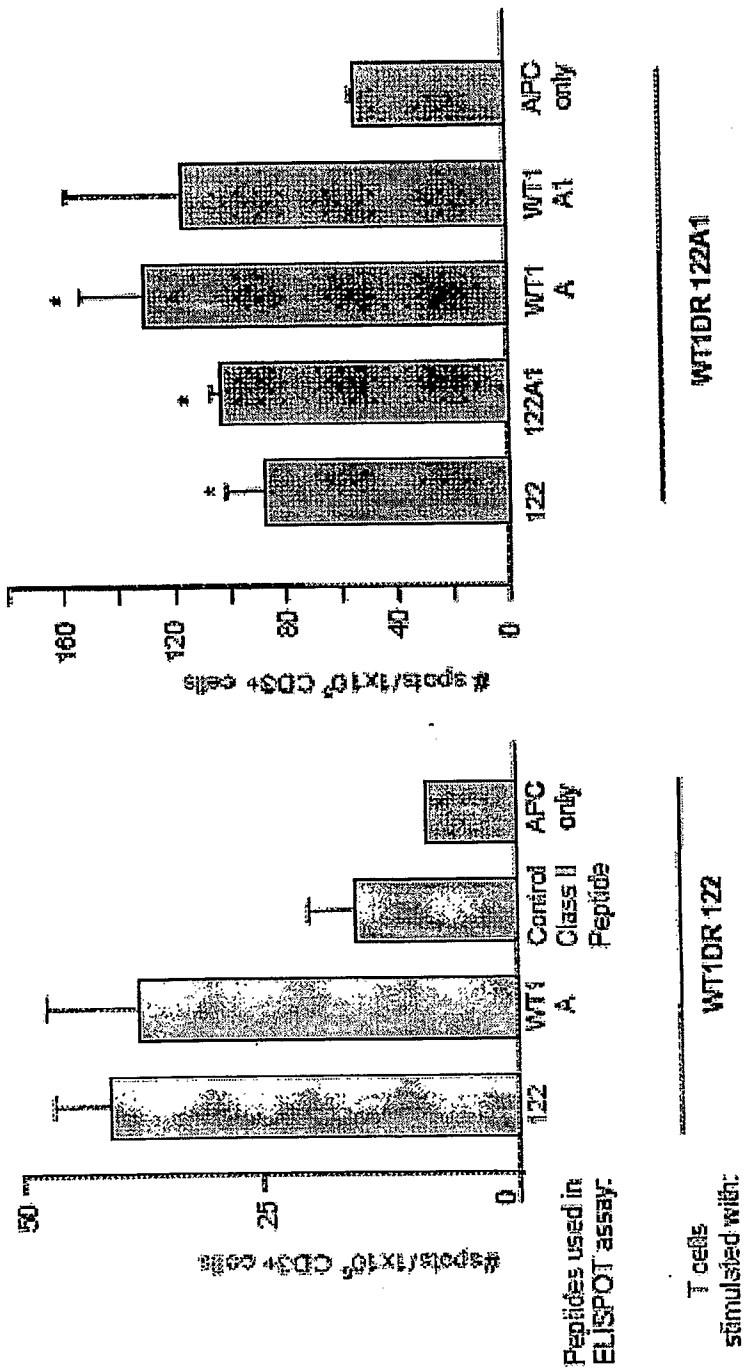

#spots/1x10^5 CD3+ cells
0
25
50
122
WT1 A
Control Class II Peptide
APC only
Peptides used in ELISPOT assay:
T cells stimulated with:
WT1DR 122
#spots/1x10^5 CD3+ cells
0
40
80
120
160
122
122A1
WT1 A
WT1 A1
APC only
WT1DR 122A1

Figure 7C

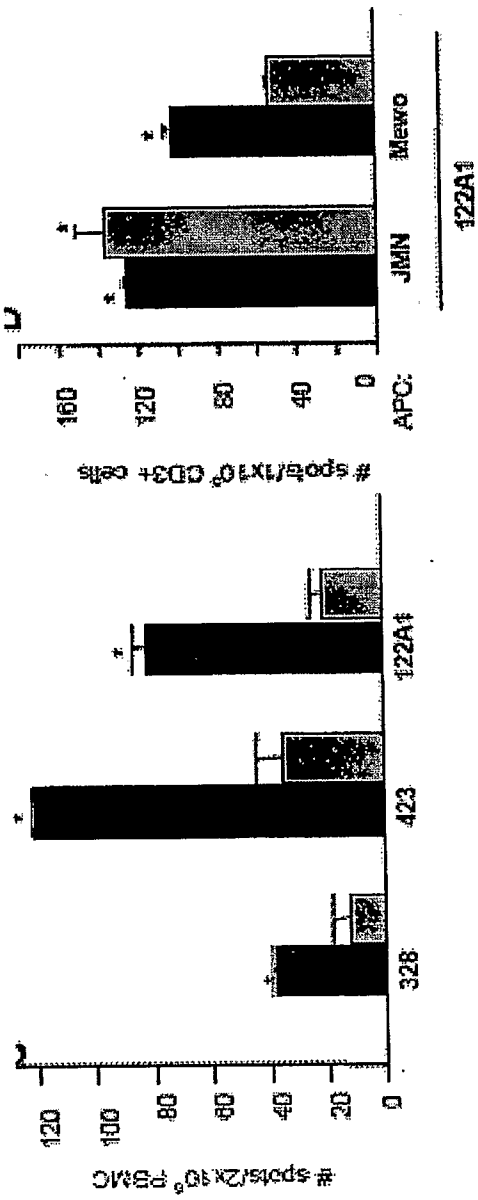

J
spots/1x10^5 CD3+ cells
160
120
80
40
0
APC:
JMN
Mewo
122A1
I
spots/2x10^5 PBMC
120
100
80
60
40
20
0
328
423
122A1

Figure 8

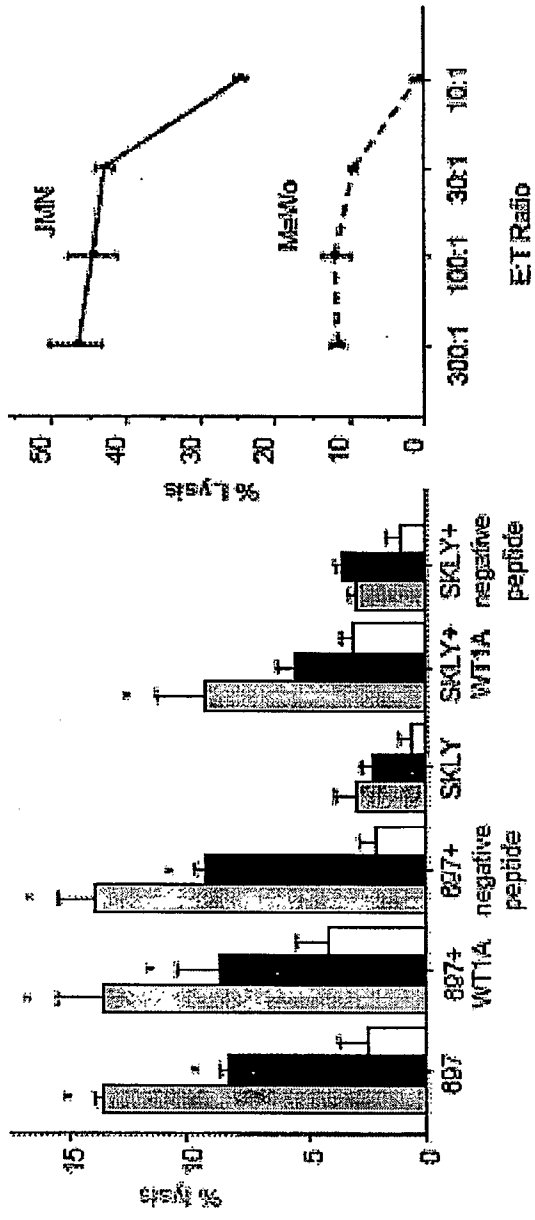

% lysis
15
10
5
0
697
697+ WT1A
697+ negative peptide
SKLY
SKLY+ WT1A
SKLY+ negative peptide
% Lysis
50
40
30
20
10
0
JMN
MeMo
300:1
100:1
30:1
10:1
E:T Ratio

Figure 9

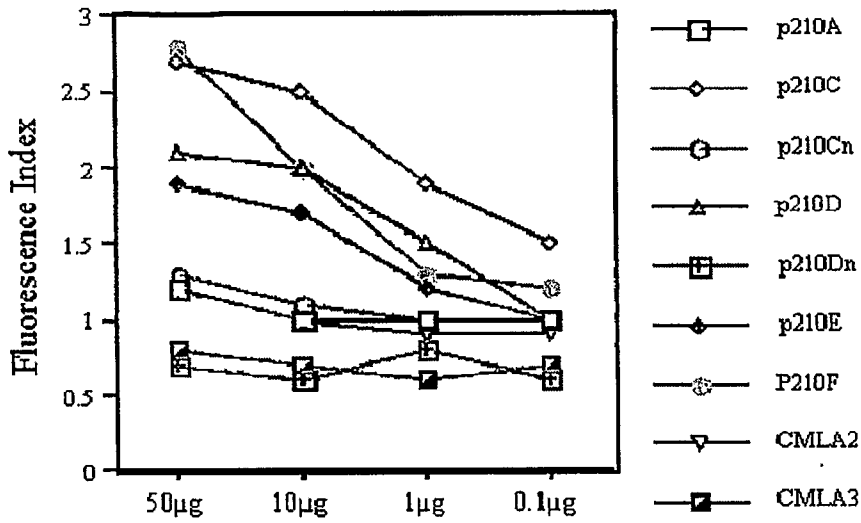


Figure 10A

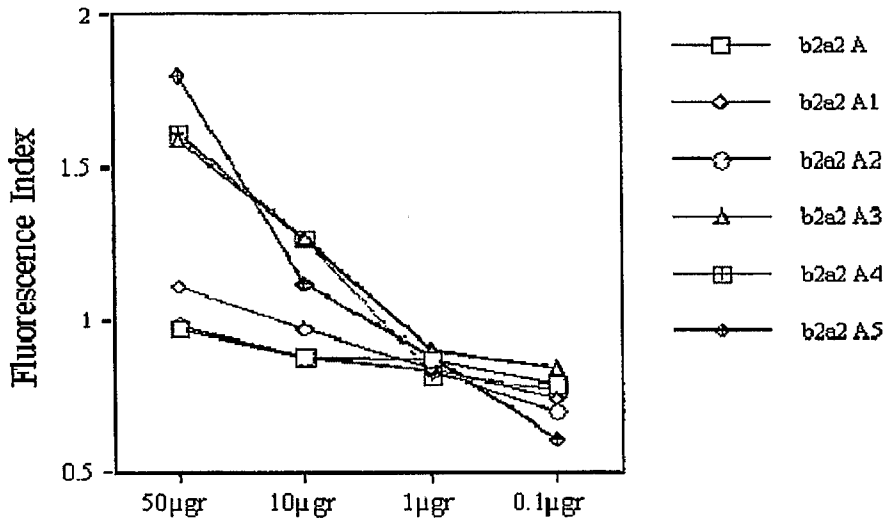


Figure 10B

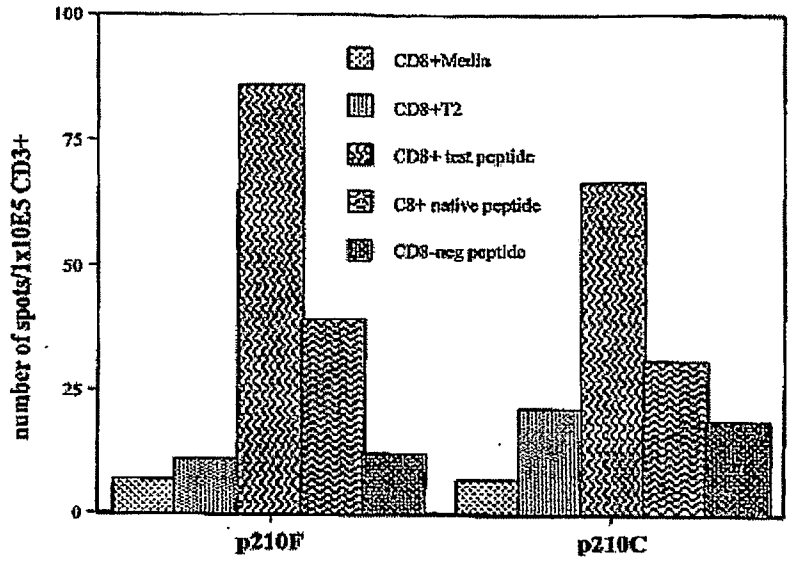


Figure 11

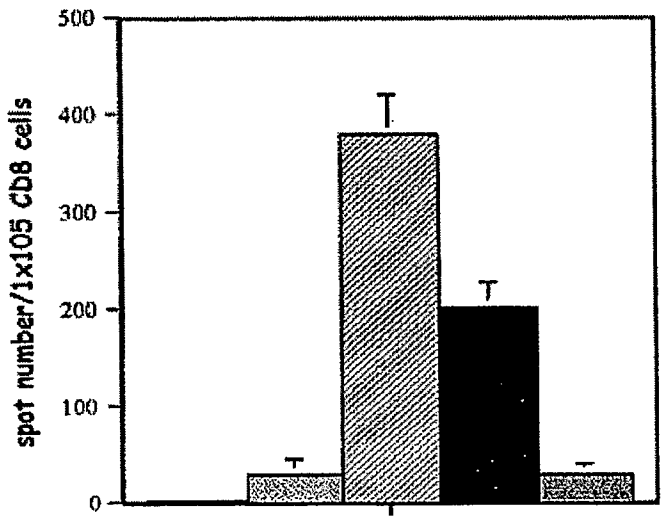


Figure 12

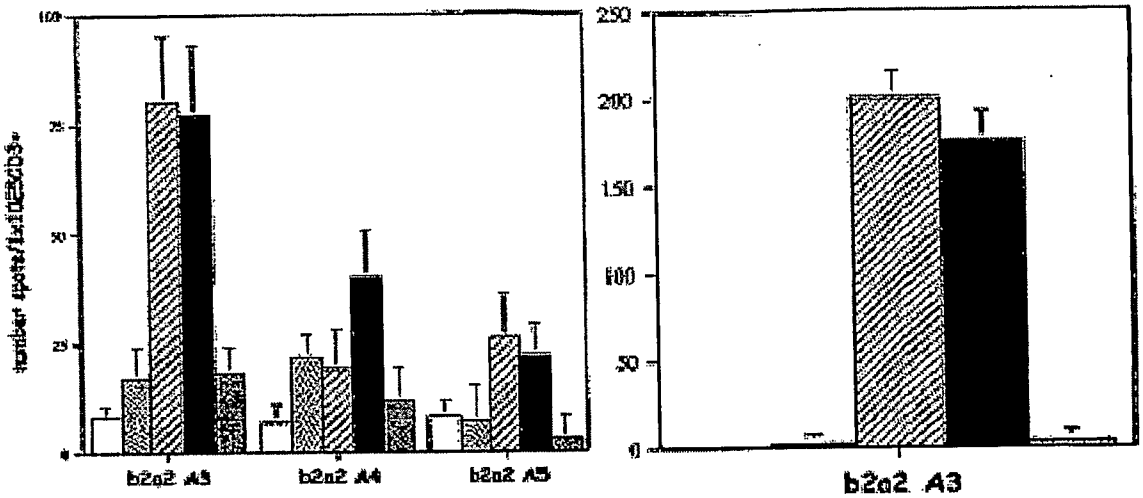


Figure 13

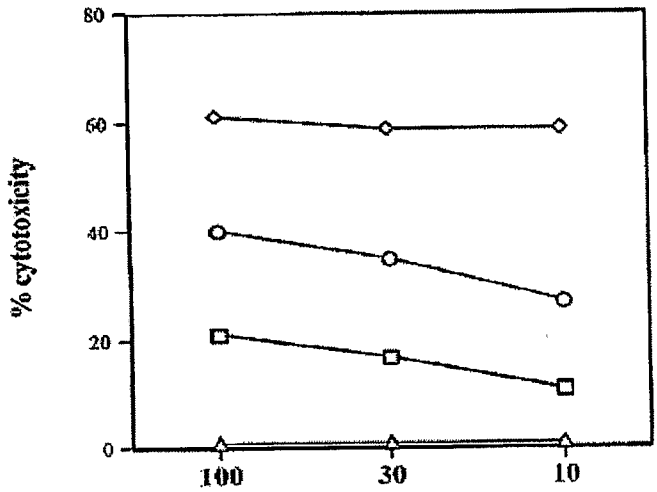


Figure 14

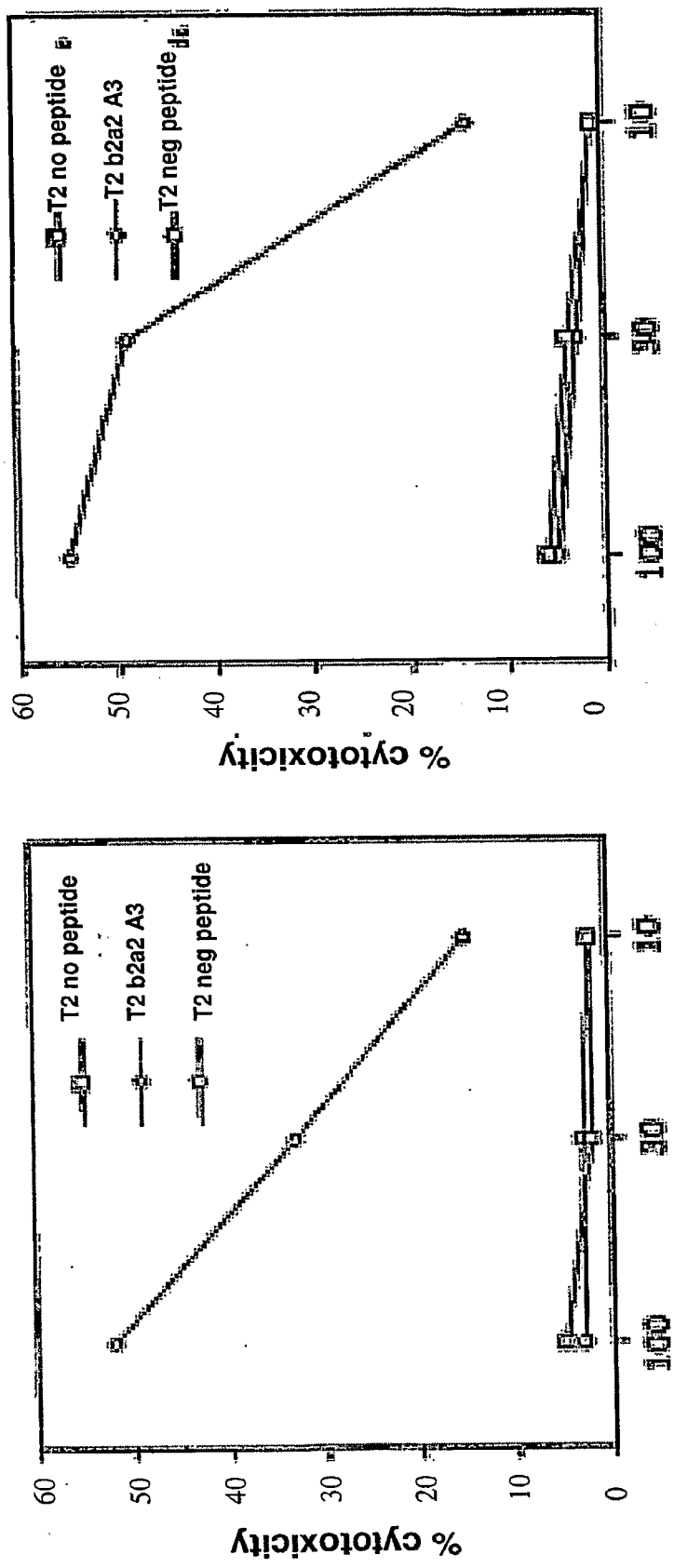

% cytotoxicity
60
50
40
30
20
10
0
T2 no peptide
T2 b2a2 A3
T2 neg peptide
100
10
1
% cytotoxicity
60
50
40
30
20
10
0
T2 no peptide
T2 b2a2 A3
T2 neg peptide
100
10
1

Figure 15