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(54) SOLUBLE PD-1 VARIANTS, FUSION CONSTRUCTS, AND USES THEREOF

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A61P 31/14	(2006.01)
A61P 31/20	(2006.01)
A61P 31/16	(2006.01)
C07K 14/725	(2006.01)

(2006.01)

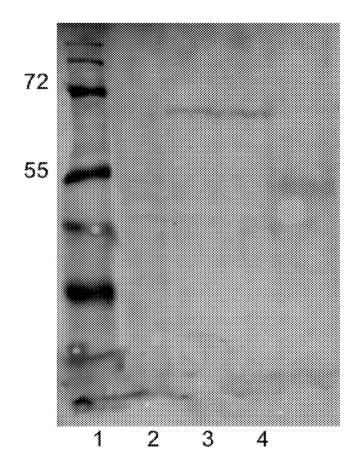
(57) ABSTRACT

A61P 37/04

The subject invention provides novel soluble PD-1 (sPD-1) proteins, nucleic acids, and fusion constructs thereof, for enhancing humoral and cell-mediated immunity of a subject. Also provided are therapeutic compositions comprising the sPD-1 proteins, nucleic acids, and fusion constructs of the subject invention. In a preferred embodiment, the therapeutic composition is formulated as a vaccine composition. Advantageously, the sPD-1 proteins, nucleic acids, and therapeutic compositions provide protective immunity against pathogenic infection including HIV infection. In additon, the subject invention can be used in the prevention and/or treatment of tumor or cancer.

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mapdi-p34-fo mapdi-tqVa-p34-fo p34-fo	655 ***********************************			****	77 73.0 800.0873	(1), (1), (1) , (1), (1), (1), (1), (1), (1), (1), (1)		:: V###	.	∷ %

FIG. 1A



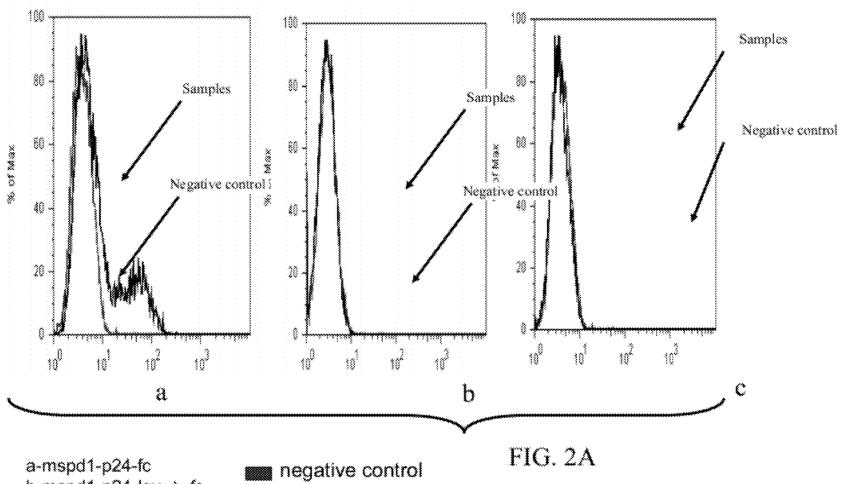
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2--- negative control 3--- mspd1-p24-fc

4--- mspd1-lgV△-p24-fc

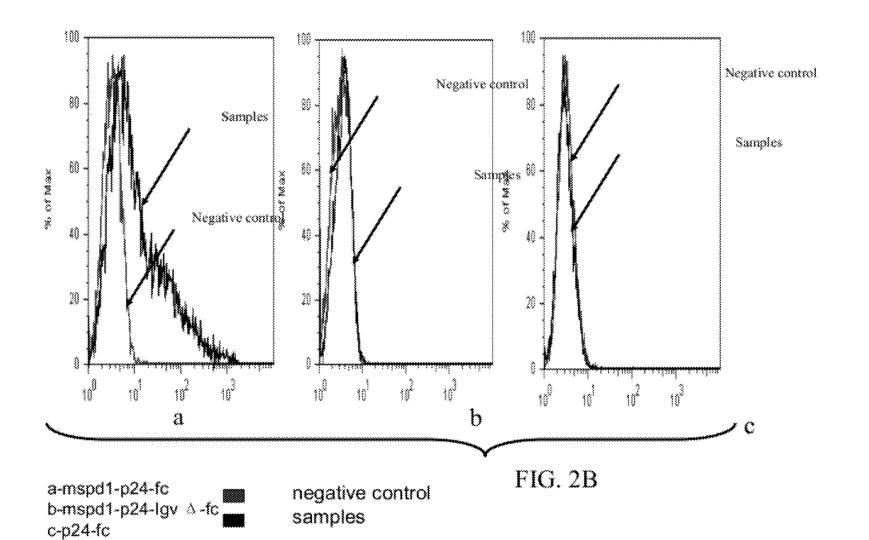
5--- p24-fc

FIG. 1B



b-mspd1-p24-lgv △-fc c-p24-fc

samples



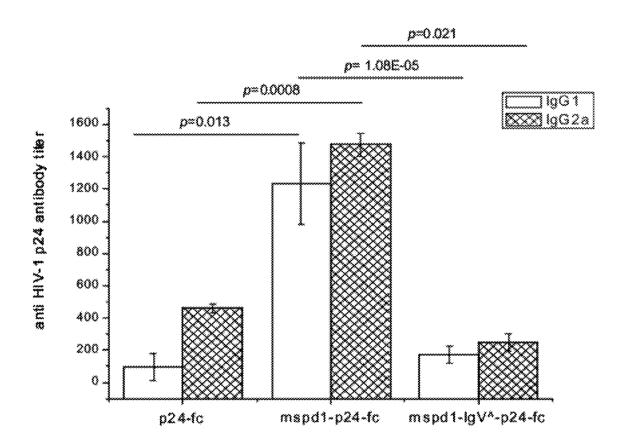


FIG. 3A

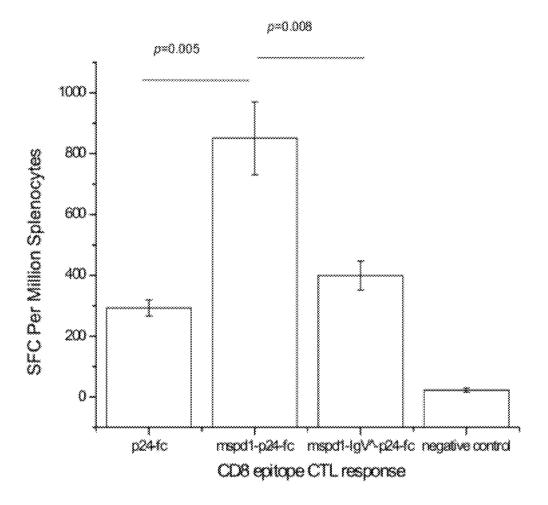
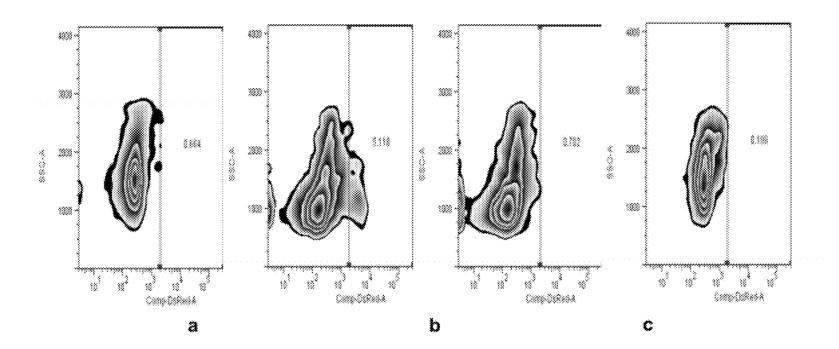


FIG. 3B

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a-p24-fc; b-mspd1-p24-fc; c-mspd1-lgv △-p24-fc; d-PBS

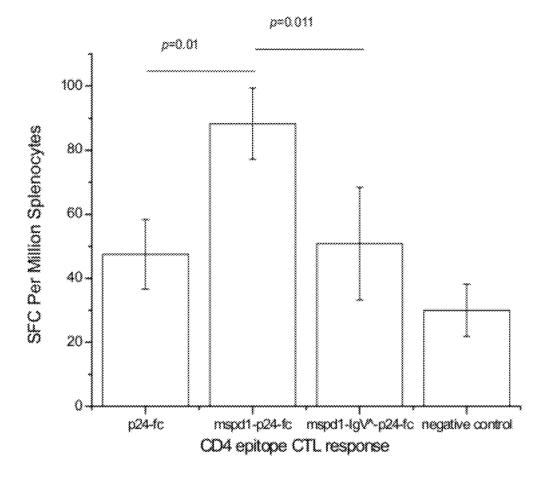
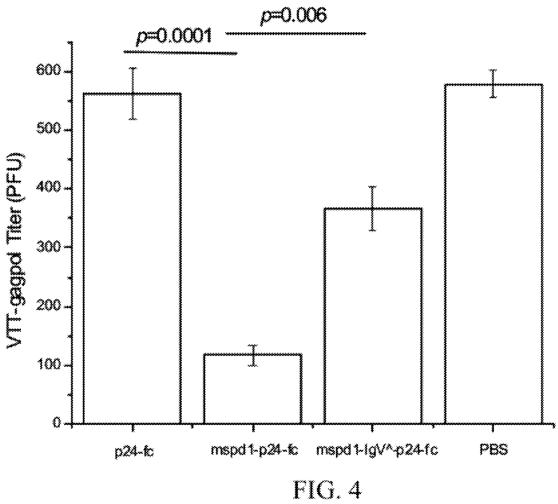


FIG. 3D



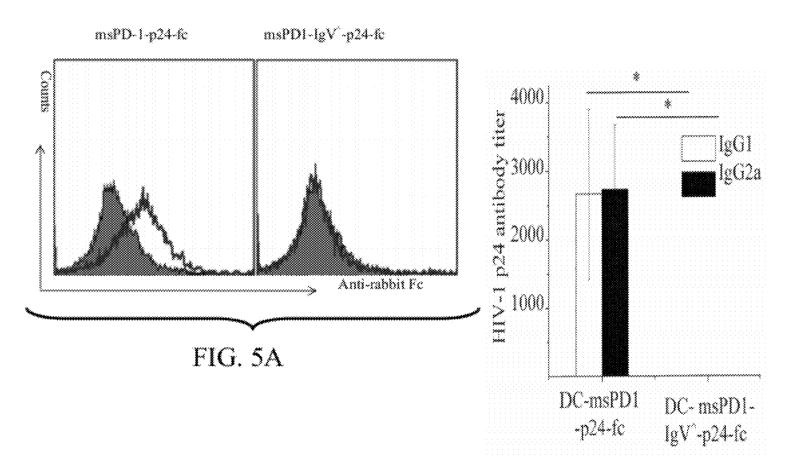
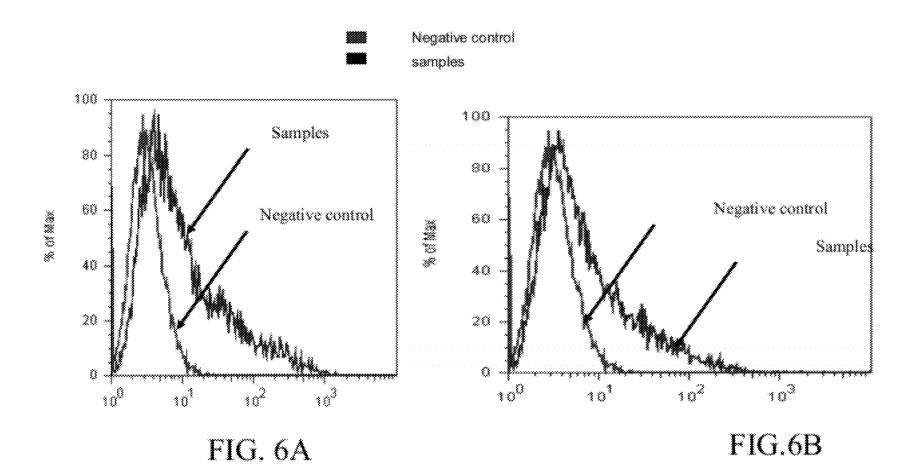


FIG. 5B



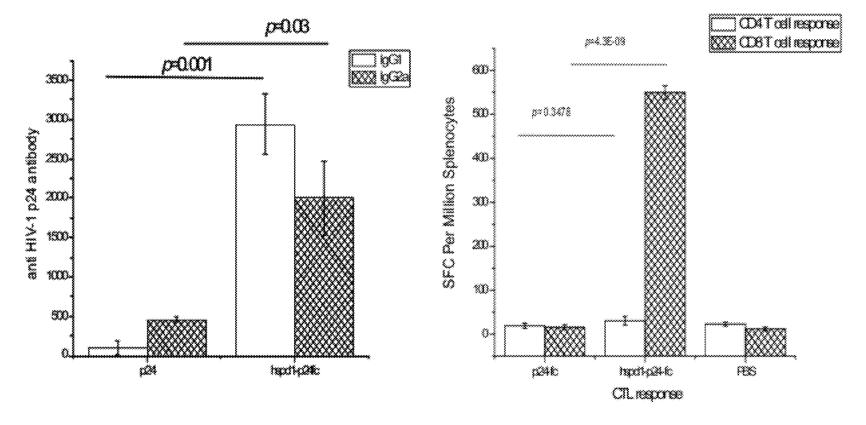
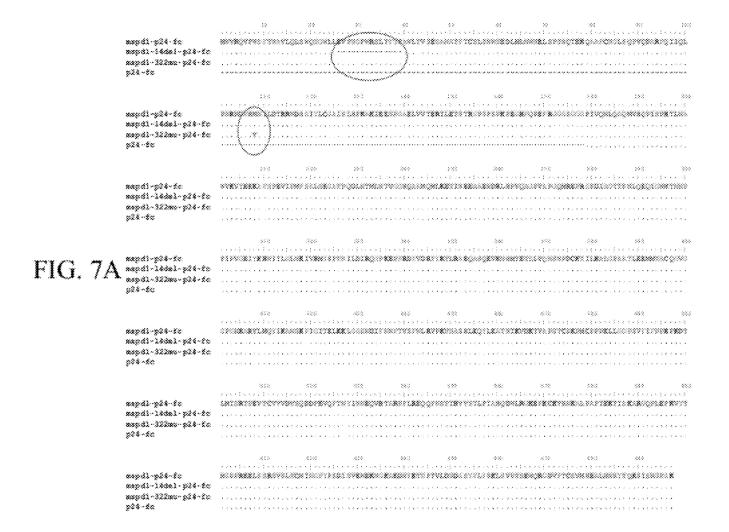
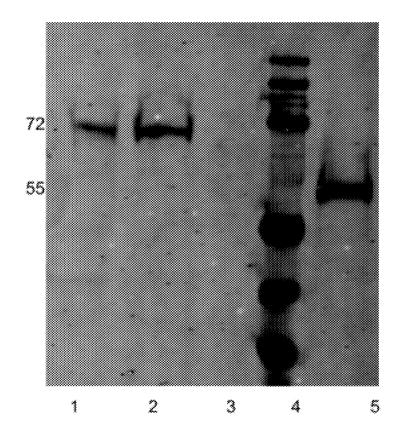


FIG. 6C FIG. 6D





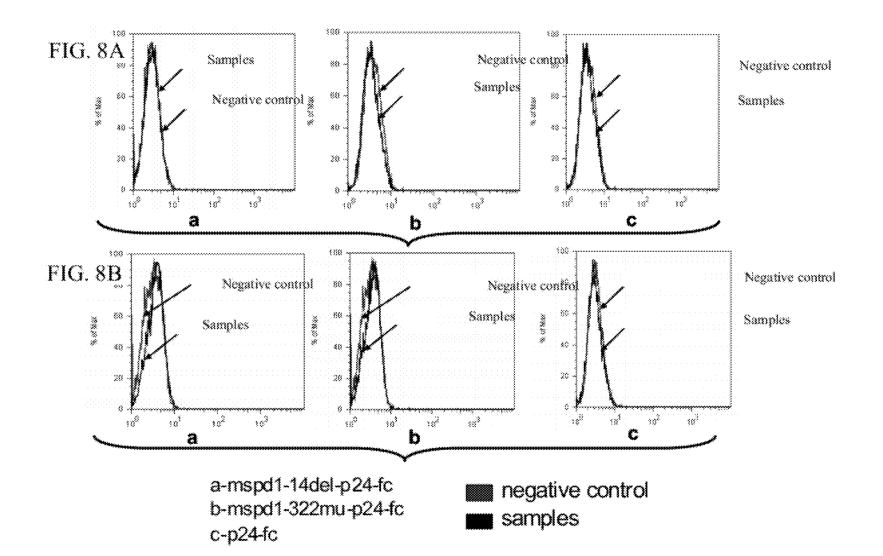
1-- mspd1-322mu-p24-fc

2--- mspd1-14del-p24-fc

3— negative control

4— marker 5— p24-fc

FIG. 7B



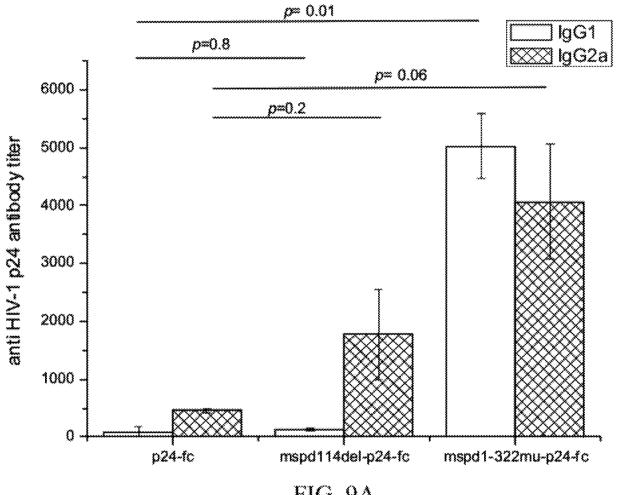


FIG. 9A

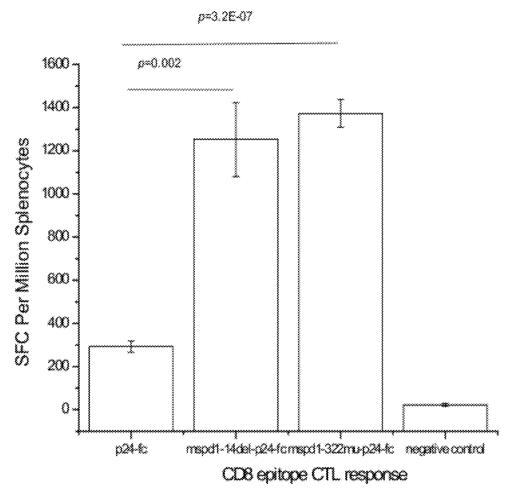
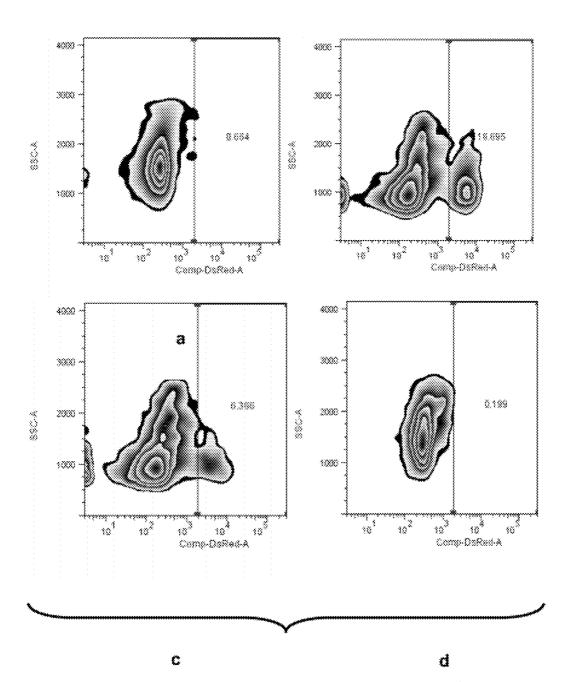


FIG. 9B



a-p24-fc b-mspd1-14del-p24-fc c-mspd1-322mu-p24-fc d-PBS

FIG. 9C

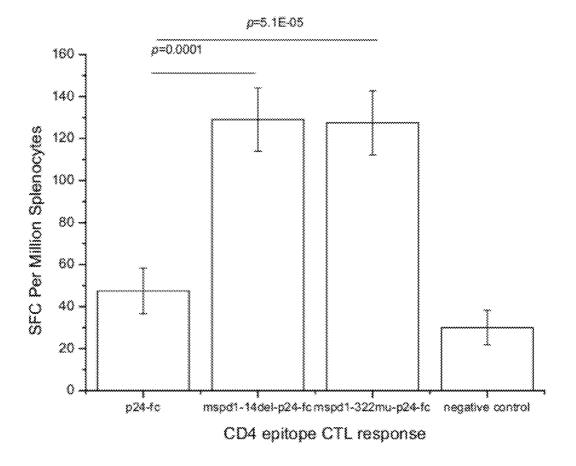


FIG. 9D

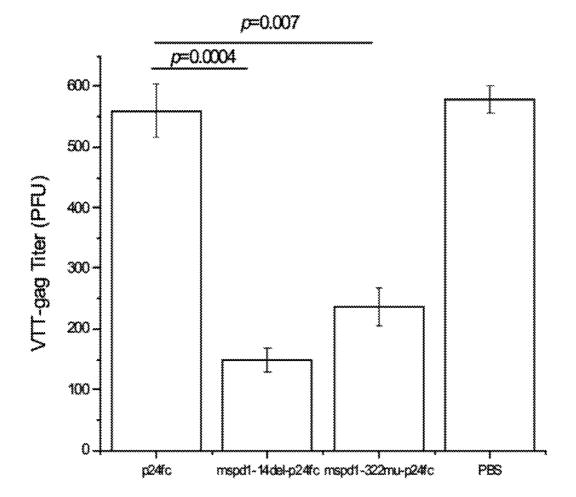
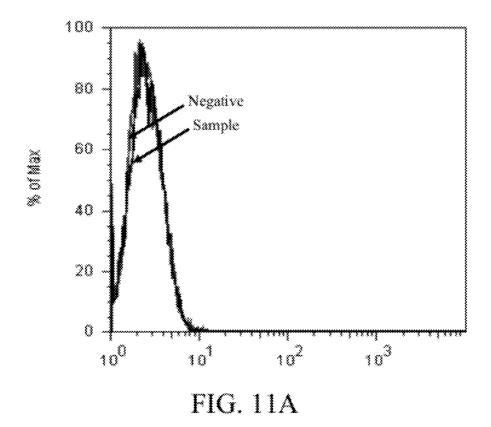


FIG. 10



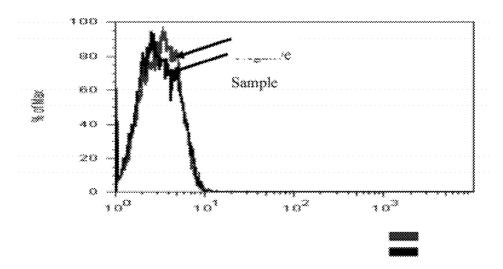
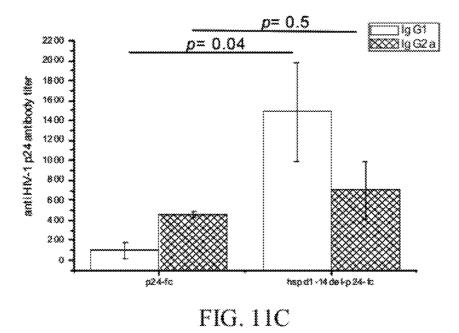


FIG. 11B negative sample



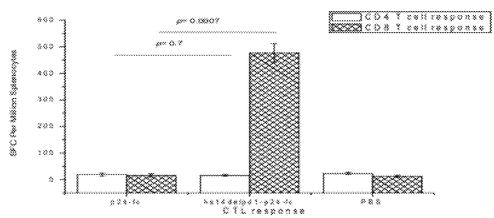


FIG. 11D

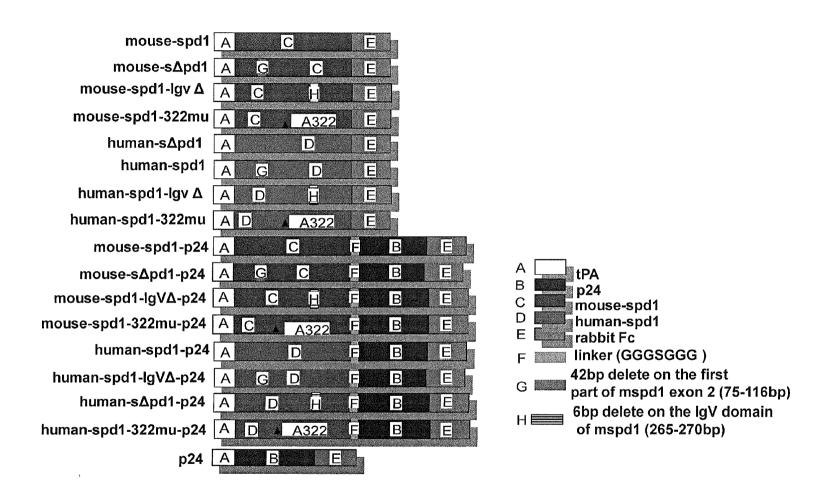


FIG. 12

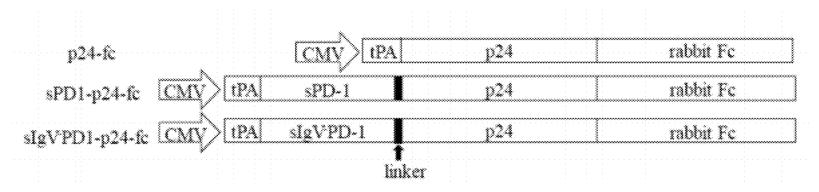


FIG. 13A

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and the state of t				.76 % % % %	
• Immunization	1		Analysis:	antibody and 1-cell responses	
			······································		
A (maabe)	2	6	Q		
V (NEEKS)	2	V	Q.		

FIG. 13B

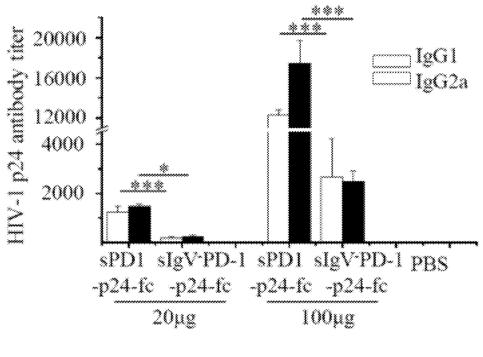


FIG. 13C

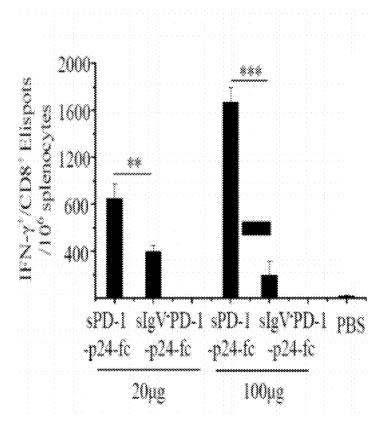
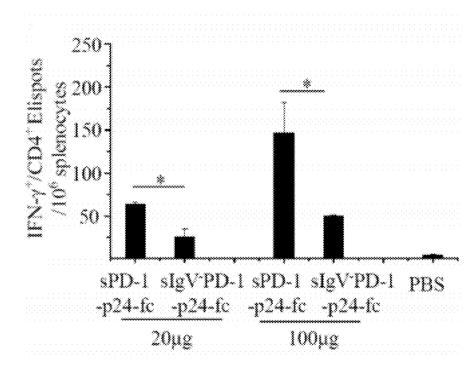


FIG. 13D



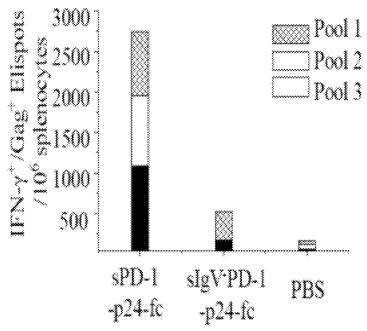
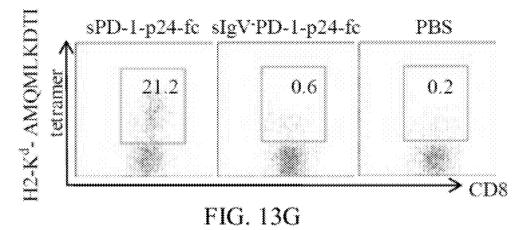
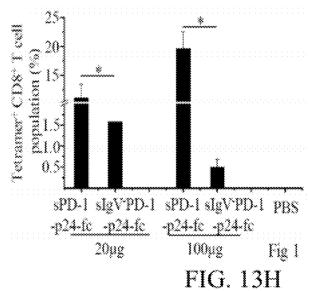
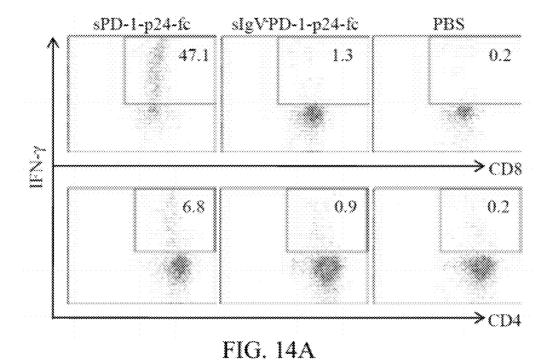


FIG. 13E

FIG. 13F







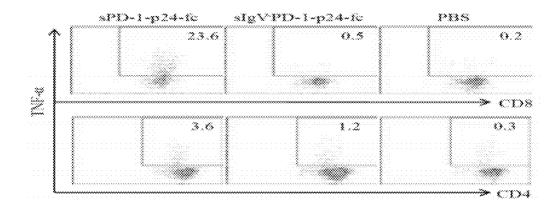
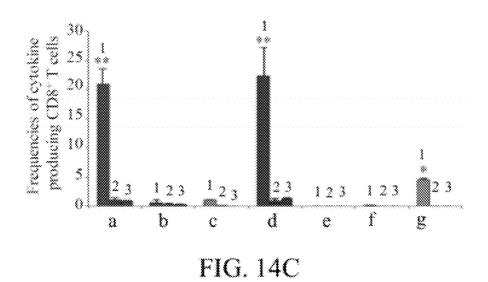


FIG. 14B



moducing CD4 Tools frequencies of cytoking 3 3 2 2 3 3 2.3 2 3 23 2 3 2 3 b d ř g C C a

FIG. 14D

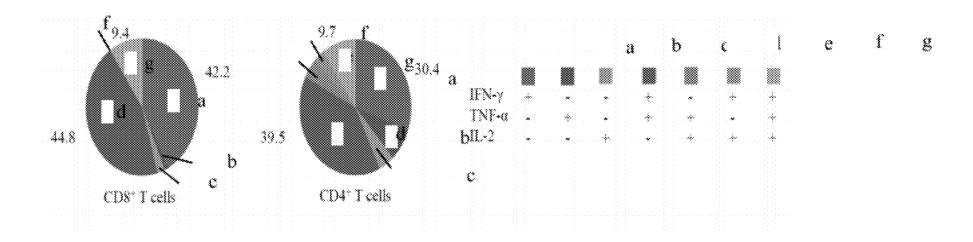


FIG. 14E

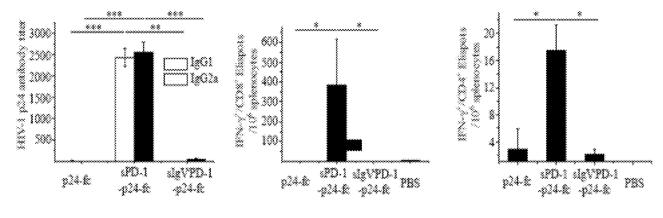
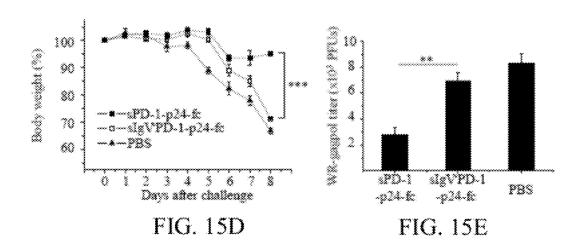


FIG. 15A

FIG. 15B

FIG. 15C



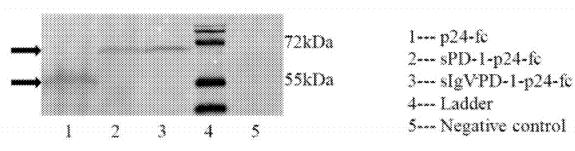
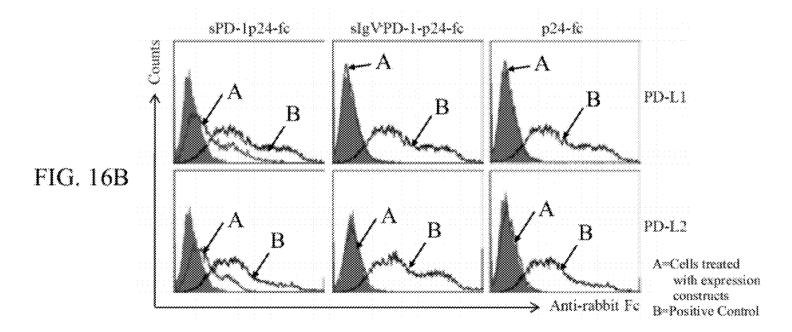
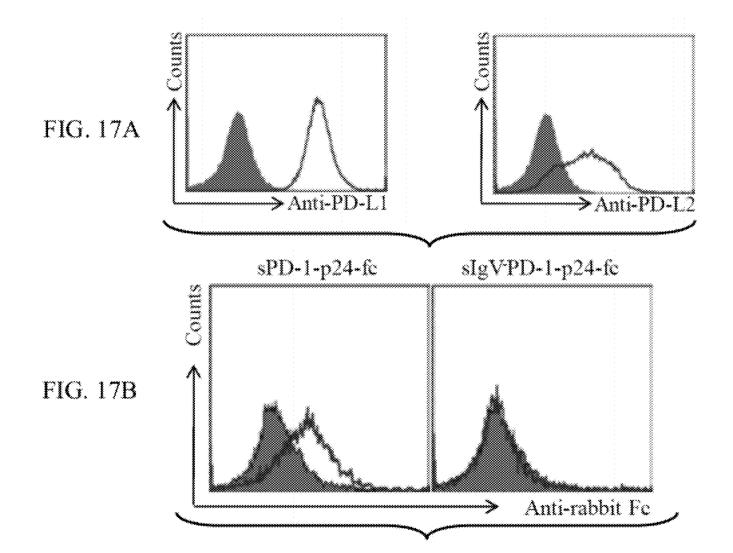
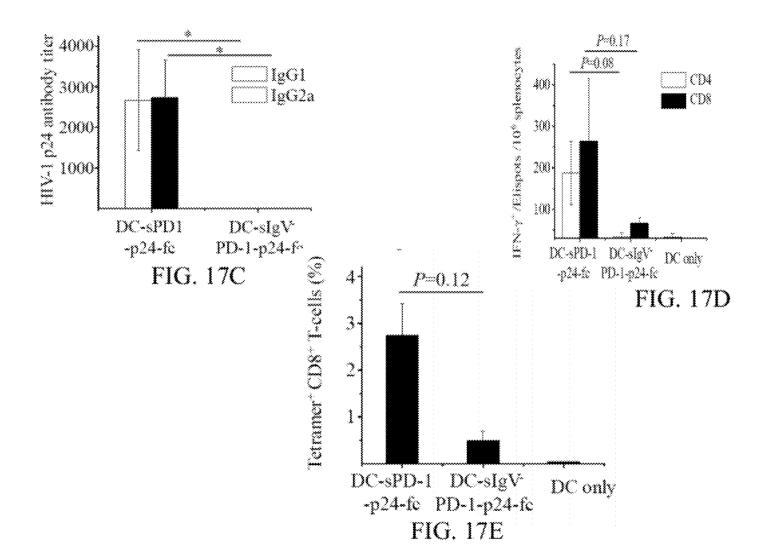


FIG. 16A







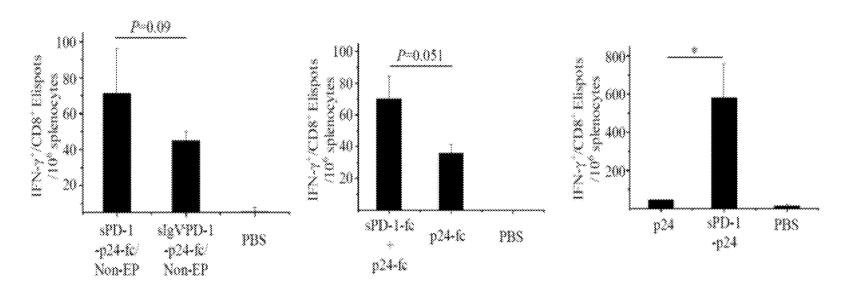
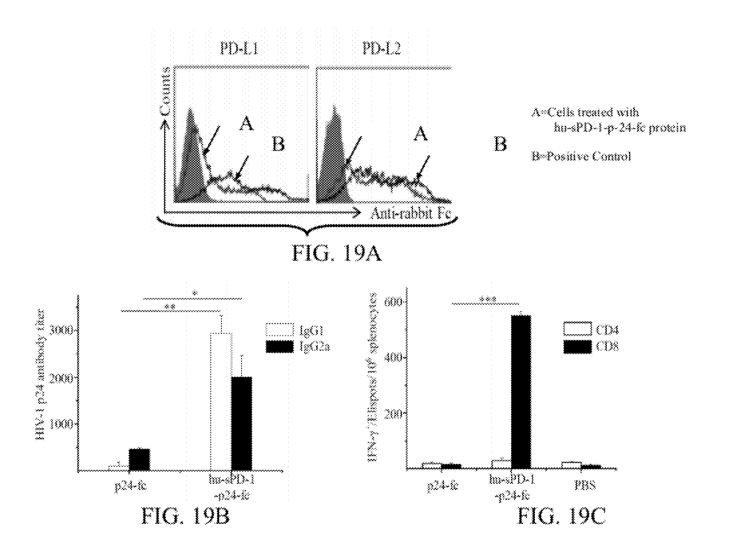


FIG. 18A FIG. 18B FIG. 18C



SOLUBLE PD-1 VARIANTS, FUSION CONSTRUCTS, AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. provisional application Ser. No. 61/412,557, filed Nov. 11, 2010, which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Programmed death 1 (PD-1), expressed primarily on T cells, is a receptor for B7-H1 molecule (also known as programmed death ligand 1 (PD-L1)) and B7-DC molecule (also known as programmed death ligand 2 (PD-L2)). PD-L1 is expressed on many different cell types, whereas PD-L2 is expressed only on antigen-presenting cells such as B cells, dendritic cells and macrophages.

[0003] The PD-1/PD-L pathway, which transmits negative signals to immune cells, plays a critical role in the modulation of immune responses during infection and cancer. The interaction of PD-1 with PD-L1/L2 inhibits T cell function during HIV infection. A recent study suggested that the blockade of PD-1 during chronic simian immunodeficiency virus (SIV) infection by anti PD-1 antibody resulted in enhanced B cell responses as well as rapid expansion and restoration of SIV-specific polyfunctional CD8 T cells. Other studies suggested that the blockade of the PD-1/PD-L pathway facilitates the restoration of humoral and cell-mediated immune responses during LCMV and HBV infection.

[0004] Human immunodeficiency virus type I (HIV-1) has contributed to an estimated 25 million deaths since it was first recognized in 1981. Currently, over 33 million people worldwide are living with the virus. One of the existing HIV vaccine compositions, obtained by fusing HIV-1 p24 to DEC-205 antibody, enhances CD4 T cell immune responses and cytokine release. In addition, this vaccine composition confers protection against vaccinia-gag viral challenge. However, this HIV vaccine composition does not improve Th1 CD8 T cell response. Thus, improved HIV-1 vaccine compositions that enhance host immunity and protect against HIV infection are needed. As will be clear from the disclosure that follows, these and other benefits are provided by the present invention.

BRIEF SUMMARY OF THE INVENTION

[0005] The subject invention provides soluble PD-1 (sPD-1) proteins and nucleic acids, and therapeutic compositions comprising sPD-1 proteins and nucleic acids, for enhancing immunity of a subject. In one embodiment, the sPD-1 proteins, nucleic acids, and compositions are formulated as a vaccine composition.

[0006] One aspect of the subject invention provides sPD-1 protein variants. In an embodiment, the sPD-1 protein variant is mspd1-14de1, which has an amino acid sequence comprising SEQ ID NO: 11. In an embodiment, the sPD-1 protein variant is mspd1-322 mu, which has an amino acid sequence comprising SEQ ID NO: 15. In an embodiment, the sPD-1 protein variant is hspd1-14de1, which was found in healthy Chinese people. The hspd1-14de1 variant has an amino acid sequence comprising SEQ ID NO: 25.

[0007] Another aspect of the invention provides nucleic acid molecules that encode the sPD-1 proteins of the subject invention. In an embodiment, the nucleic acid molecule

encodes mspd1-14de1, and has a sequence comprising SEQ ID NO: 12. In an embodiment, the nucleic acid molecule encodes mspd1-322mu, and has a sequence comprising SEQ ID NO: 16. In an embodiment, the nucleic acid molecule encodes hspd1-14de1, and has a sequence comprising SEQ ID NO: 26.

[0008] In addition, the subject invention provides sPD-1 fusion proteins. In specific embodiments, the subject sPD-1 fusion protein comprises SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 23, or SEQ ID NO: 27. The subject invention also provides sPD-1 fusion nucleic acid molecules. In specific embodiments, the subject sPD-1 fusion DNA comprises SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 24, or SEQ ID NO: 28.

[0009] Another aspect of the subject invention provides methods for the prevention and/or treatment of pathogenic infection, cancer or tumor, and other diseases in which induction of antigen-specific protective immunity would be beneficial. Advantageously, the methods of the subject invention enhance host humoral and cell-mediated immunity. The method comprises administering to a subject in need of such treatment an effective amount of a fusion protein or fusion nucleic acid molecule of the subject invention. In a preferred embodiment, the subject method can be used in the prevention and treatment of HIV or other pathogen infection. In addition, the methods can be used in the prevention and/or treatment of tumor or cancer.

[0010] The subject invention further provides for therapeutic or pharmaceutical compositions. In an embodiment, the composition comprises a therapeutically effective amount of a protein and/or nucleic acid molecule of the subject invention and, optionally, a pharmaceutically acceptable carrier. In a preferred embodiment, the therapeutic composition is a vaccine composition.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1A shows alignment of amino acid sequences of mspd1-p24-Fc, mspd1-IgV Δ -p24-Fc and p24-Fc fusion proteins. FIG. 1B shows Western blot analysis of mspd1-p24-Fc, mspd1-IgV Δ -p24-Fc and p24-Fc. Proteins are detected by anti-rabbit Fc antibody.

[0012] FIG. 2 shows the binding ability of msPD1-p24-Fc fusion proteins to sPD-1 ligands. (A) shows the binding ability of mspd1-p24-Fc, mspd1-IgV Δ -p24-Fc and p24-Fc to mouse PD-L1, respectively. (B) shows the binding ability of mspd1-p24-Fc, mspd1-IgV Δ -p24-Fc and p24-Fc to mouse PD-L2, respectively.

[0013] FIG. 3 shows that wild-type sPD1 DNA elicits humoral and cell-mediated immune responses against HIV p24. (A) shows serum levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with p24-Fc, mspd1-p24-Fc and mspd1-IgVΔ-p24-Fc fusion DNA, respectively. Bars represent the average values of three samples (±standard deviations). (B) shows the number of IFN-γ-secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) for CD8 T cells. Bars represent the average values of three samples (±standard deviations). (C) shows images of splenocytes isolated from immunized mice. To analyze p24-specific immune response, splenocytes were stained with H2d-Kd-AMQM-LKDTI-PE tetramer for CD8 T cell population analysis. (D) shows the number of IFN-y-secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWIILGL) for CD4 T cells. Bars represent the average values of three samples

(±standard deviations). Data represent three experiments on the same batch of immunized mice.

[0014] FIG. 4 shows that immunization with wild-type msPD 1 fusion DNA protects mice against viral infection. Balb/c mice immunized with p24-Fc, mspd1-p24-Fc or mspd1-IgV Δ -p24-Fc were challenged with 4×10^7 PFU of vaccinia VTT-HIV-gagpol intranasally three weeks after the last immunization. The mice were sacrificed 3 days after viral challenge.

[0015] Viral titers in lungs were evaluated by plaque-forming assay in Vero cells. Bars represent the average values of five samples (±standard deviations).

[0016] FIG. 5 shows that targeting DCs using sPD-1-p24-fc induces enhanced p24-specific antibody and T cell responses. (A) Expression of PD-L1 and PD-L2 on purified CD11c+ BM-DCs isolated from Balb/c mice were confirmed by flow cytometric analysis using anti-mouse PD-L1 or L2 antibodies (solid line, not shaded). Cells stained with isotype antibody control are shown as shaded histogram. (B) BM-DCs treated with purified msPD-1-p24-fc and msPD1-IgVΔ-p24-fc proteins to examine binding. Proteins bound to DCs were detected by flow cytometry using an anti-rabbit Fc-FITC antibody (solid line, not shaded) in parallel to DCs without treatment of proteins as negative control (shaded). 2×10⁶ DCs treated with 20 μg of msPD-1-p24-fc or msPD1-IgV Δ -p24-fc proteins were introduced to Balb/c mouse by tail vein injection once every three weeks for a total experimental duration of six weeks. Mice that received untreated CD11c+ DCs served as control. (C) Mice sera were collected and analyzed for the presence of IgG1 and IgG2a antibodies specific against HIV-1 p24 by ELISA. (D) IFN-γ producing CD8+ and CD4⁺ cells were measured by ELISpot assay in mice splenocytes stimulated using specific peptides gagAI and gag26, respectively. H2-Kd-AMQMLKDTI-PE tetramer staining was performed on isolated splenocytes and analyzed by flow cytometry as a column graph of data from groups of immunized mice. Bars represent the mean values of two replicate mice with standard error depicted by error bars. Data are representative of two independent immunization experiments. *P<0.05.

[0017] FIG. 6 shows that hspd1-p24-Fc elicits humoral and cell-mediated immune responses against HIV-1 p24. (A) shows that hsPD-1-p24-Fc binds to mouse PD-L1. (B) shows that hsPD-1-p24-Fc binds to mouse PD-L2. (C) shows high sera levels of anti-p24 lgG1 and lgG2a antibodies in mice immunized with hspd1-p24-Fc, when compared to mice immunized with p24-Fc. (D) shows the numbers of IFN-y-secreting splenocytes specific for p24 epitope gagAI (AM-QMLKDTI) for CD8 T cells and the numbers of IFN-y-secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWIILGL) for CD4 T cells. Bars represent the average values of three samples (±standard deviations)

[0018] FIG. 7A shows alignment of amino acid sequences of mspd1-p24-Fc, mspd1-14de1-p24-Fc, mspd1-322mu-p24-Fc, and p24-fc fusion proteins. FIG. 7B shows Western blot analysis of mspd1-14de1-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc. Proteins are detected by anti-rabbit Fc anti-body.

[0019] FIG. 8 shows the binding ability of mspd1-14de1-p24-Fc, mspd1-322mu-p24-Fc, and p24-fc fusion proteins to sPD-1 ligands, respectively. (A) shows the binding ability of mspd1-14de1-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion proteins to PD-L1, respectively. (B) shows the binding

ability of mspd1-14de1-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion proteins to PD-L2, respectively.

[0020] FIG. 9 shows that variant sPD1 DNA elicits humoral and cell-mediated immune responses against HIV p24. (A) shows serum levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with mspd1-14de1-p24-Fc, mspd1-322mup24-Fc, and p24-Fc fusion DNA, respectively. Bars represent the average values of three samples (±standard deviations). (B) shows the number of IFN-γ-secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) for CD8 T cells. Bars represent the average values of three samples (±standard deviations). (C) shows images of splenocytes isolated from immunized mice. To analyze p24-specific immune response, splenocytes were stained with H2d-K^d-AMQMLKDTI-PE tetramer for CD8 T cell population analysis. (D) shows the number of IFN-y-secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWIILGL) for CD4 T cells. Bars represent the average values of three samples (±standard deviations). Data represent three experiments on the same batch of immunized mice.

[0021] FIG. 10 shows that immunization with variant msPD1 fusion DNA protects mice against viral infection. Balb/c mice immunized with mspd1-14de1-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion DNA were challenged with 4×10⁷ PFU of vaccinia VTT-HIV-gagpol intranasally three weeks after the last immunization. The mice were sacrificed 3 days after viral challenge. Viral titers in lungs were evaluated by plaque-forming assay in Vero cells. Bars represent the average values of five samples (±standard deviations)

[0022] FIG. 11 shows that hspd1-14de1-p24-Fc elicits humoral and cell-mediated immune responses against HIV-1 p24. (A) shows that hspd1-14de1-p24-Fc does not bind to mouse PD-L1. (B) shows that hspd1-14de1-p24-Fc does not bind to mouse PD-L2. (C) shows high sera levels of anti-p24 IgG1 and IgG2a antibodies in mice immunized with hspd1-14de1-p24-Fc, when compared to mice immunized with p24-Fc. (D) shows the numbers of IFN-γ-secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) for CD8 T cells and the numbers of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWIILGL) for CD4 T cells. Bars represent the average values of three samples (±standard deviations).

[0023] FIG. 12 shows the structures of various clones useful according to the subject invention.

[0024] FIG. 13 shows the induction of potent p24-specific immune responses by sPD-1-p24-fc vaccination. (A) Schematic representation of constructs encompassing the soluble form of PD-1 or with two amino acid deletions essential for binding with PD-L1/L2 (sIgV-PD-1), p24 and rabbit Fc under the CMV promoter. Rabbit Fc was used as a tag for purification purposes. (B) Mouse immunization schedule is depicted. Balb/c mice were immunized with sPD-1-p24-fc, sIgV-PD-1-p24-fc and p24-fc at week 0, 3 and 6 at a low dose of 20 μg or a high dose of 100 µg i.m. with EP. Mice that received PBS only served as a negative control. Mice sera and splenocytes were collected two weeks after the final immunization for analysis of antibody and T cell responses, respectively. (C) Detection of specific IgG1 and IgG2a antibodies against HIV-1 Gag p24 by ELISA two weeks post immunization. (D) Number of IFN-γ-secreting CD8+ and (E) CD4+ T cells measured by ELISpot in specific response to HIV-1 Gag p24 epitopes gagAI and gag26, respectively. (F) IFN-γ⁺ secreting cells in response to stimulation using three different peptide

pools derived from 59 peptides that spans the whole HIV-1 Gag p24. (G) Representative H2-Kd-AMQMLKDTI-PE tetramer staining of CD8⁺ T cell population is shown in flow cytometric plots or data amalgamated into a column graph (H). Data are representative of three independent immunization experiments. *P<0.05, **P<0.01, ***P<0.001.

[0025] FIG. 14 shows polyfunctionality of sPD1-p24-fc induced T cells. Balb/c mice were immunized with sPD1p24-fc and sIgV-PD1-p24-fc at a dose of 100 µg i.m./EP. Mice that received PBS alone served as control. Splenocytes were collected and analyzed by flow cytometry following intracellular staining using antibodies against IFN- γ^+ , TNF- α , and IL-2. (A) Scatter plots indicating CD8+ or CD4+ T cells positive for IFN- γ^+ and (B) TNF- α . (C) Column graphs depicting single, double or triple positive CD8+ or (D) CD4+ T cells for the cytokines IFN- γ^+ , TNF- α , and IL-2. (E) Pie chart analysis representing subpopulations of total cytokine secreting CD8+ or CD4+ T cells positive for combinations of IFN- γ , TNF- α , and IL-2. Columns represent the mean values of three replicate mice with standard error as error bars. Data are representative of two independent immunization experiments. *P<0.05, **P<0.01.

[0026] FIG. 15 shows that vaccination with sPD-1-p24-fc induces specific long lasting and protective immunity. Sera and splenocytes derived from mice 30 weeks after immunization were isolated and examined for antibody and CD8+ and CD4+ T cell responses. (A) Specific IgG1 and IgG2a antibodies against HIV-1 Gag p24 detected by ELISA. ELISpot assays using specific HIV-1 Gag p24 epitope for (B) CD8+T cells and (C) CD4+T cells was performed to test the ability of T cells to produce IFN-γ. Mice previously immunized with a dose of 100 µg DNA vaccines were challenged with 2×10⁵ PFUs of virulent WRgagpol three weeks post immunization to examine immune protection. Each group contained up to 5 mice. (D) Immunized mice were weighed daily for eight days after vaccinia challenge. (E) Virus titers in the lungs of immunized mice were evaluated by plaque formation on Vero cell monolayers.

[0027] FIG. 16 shows expression and binding characteristics of DNA vaccine constructs. (A) DNA vaccines encoding sPD-1, the mutated form slgV-PD-1, p24 and fc were tested for protein expression by Western blotting. Lower sized band represents p24-fc, while the higher sized band represents sPD-1-p24-fc or slgV-PD-1-p24-fc. (B) 293T cells were transiently transfected with PD-L1 or PD-L2 expression vectors, and the binding profiles of recombinant proteins were examined. Flow cytometric signals were obtained by treating the cells with purified proteins from the constructs followed by detection using anti-rabbit Fc-FITC antibody. Controls included transfected 293T cells stained with anti-rabbit Fc-FITC antibody (negative, shaded) or anti-mouse PD-L1 or L2 antibodies (positive, solid line, not shaded).

[0028] FIG. 17 shows that targeting dendritic cells (DCs) using sPD-1-p24-fc induces enhanced p24-specific antibody and T cell responses. (A) Expression of PD-L1 and PD-L2 on purified CD11c+ BM-DCs isolated from Balb/c mice was confirmed by flow cytometric analysis using anti-mouse PD-L1 or L2 antibodies (solid line, not shaded). Cells stained with isotype antibody control are shown as shaded histogram. (B) BM-DCs treated with purified sPD-1-p24-fc and sIgV-PD-1-p24-fc proteins to examine binding. Proteins bound to DCs were detected by flow cytometry using an anti-rabbit Fc-FITC antibody (solid line, not shaded) in parallel to DCs without treatment of proteins as negative control (shaded).

2x10⁶ DCs treated with 20 μg of sPD-1-p24-fc or sIgV-PD-1-p24-fc proteins were introduced to Balb/c mouse by tail vein injection once every three weeks for a total experimental duration of six weeks. Mice that received untreated CD11c+ DCs served as control. (C) Mice sera were collected and analyzed for the presence of IgG1 and IgG2a antibodies specific against HIV-1 p24 by ELISA. (D) IFN-γ producing CD8⁻ and CD4⁺ cells were measured by ELISpot assay in mice splenocytes stimulated using specific peptides gagAI and gag26, respectively. H2-Kd-AMQMLKDTI-PE tetramer staining was performed on isolated splenocytes and analyzed by flow cytometry as a column graph of data from groups of immunized mice (F). Bars represent the mean values of two replicate mice with standard error depicted by error bars. Data are representative of two independent immunization experiments. *P<0.05.

[0029] FIG. 18 characterizes sPD-1-p24-fc DNA vaccination. CD8+ T cell ELISpot assay of immunization strategy by i.m. (A) without electroporation (EP), (B) with purified p24-fc and/or sPD-1-fc with EP, or (c) using DNA vaccines without rabbit Fc tag with EP. All data points represent the mean ±standard error as error bars. *P<0.05.

[0030] FIG. 19 shows that human sPD-1-p24-fc elicits similar p24-specific immunity in mice. (A) Binding profiles of hu-sPD-1-p24-fc protein to murine PD-1 ligands transiently expressed on 293T cells. Flow cytometric signals were obtained by treating the cells with husPD-1-p24-fc protein followed by anti-rabbit Fc-FITC antibody for detection. Controls included transfected 293T cells stained with anti-rabbit Fc-FITC antibody (negative, shaded) or anti-mouse PD-L1or L2- FITC antibodies (positive, solid line, not shaded). Balb/c mice were immunized with hu-sPD-1- p24-fc and p24-fc at a dose of 20 µg i.m./EP, or received PBS only serving as a negative control. (B) Detection of specific IgG1 and IgG2a antibodies against HIV-1 Gag p24 by ELISA two weeks post immunization in mice sera. (C) Frequencies of IFN-γ-secreting CD8+ and CD4+ T cells in mice splenocytes measured by ELISpot assay in specific response to HIV-1 Gag p24 epitopes specific for CD4+ and CD8+ T cells, respectively. Columns represent the mean values of three replicate mice with standard error as error bars. Data are representative of two independent immunization experiments. *P<0.05, **P<0.01, ***P<0.001.

BRIEF DESCRIPTION OF THE SEQUENCES

[0031] SEQ ID NO: 1 is an amino acid sequence of the wild-type soluble extracellular domain of mouse PD-1 (mouse spd1).

[0032] SEQ ID NO: 2 is a nucleic acid sequence of the wild-type mouse spd1 DNA.

[0033] SEQ ID NO: 3 is an amino acid sequence of HIV p24 useful according to the subject invention.

[0034] SEQ ID NO: 4 is a nucleic acid sequence of HIV p24 DNA useful according to the subject invention.

[0035] SEQ ID NO: 5 is an amino acid sequence of rabbit Fc domain useful to the subject invention.

[0036] SEQ ID NO: 6 is a nucleic acid sequence of rabbit Fc DNA useful to the subject invention.

[0037] SEQ ID NO: 7 is an amino acid sequence of mspd1- $\text{IgV}\Delta$

[0038] SEQ ID NO: 8 is a nucleic acid sequence of mspd1-IgV Δ DNA.

[0039] SEQ ID NO: 9 is an amino acid sequence of mspd1-IgV Δ -p24-Fc fusion protein.

[0040] SEQ ID NO: 10 is a nucleic acid sequence of mspd1-IgV Δ -p24-Fc fusion DNA.

[0041] SEQ ID NO: 11 is an amino acid sequence of mspd1-14de1.

[0042] SEQ ID NO: 12 is a nucleic acid sequence of mspd1-14de1 DNA.

[0043] SEQ ID NO: 13 is an amino acid sequence of mspd1-14de1-p24-Fc fusion protein.

[0044] SEQ ID NO: 14 is a nucleic acid sequence of mspd1-14de1-p24-Fc fusion DNA.

[0045] SEQ ID NO: 15 is an amino acid sequence of mspd1-322mu.

[0046] SEQ ID NO: 16 is a nucleic acid sequence of mspd1-322mu DNA.

[0047] SEQ ID NO: 17 is an amino acid sequence of mspd1-322mu-p24-Fc fusion protein.

[0048] SEQ ID NO: 18 is a nucleic acid sequence of mspd1-322mu-p24-Fc fusion DNA.

[0049] SEQ ID NO: 19 is an amino acid sequence of mspd1-p24-Fc fusion protein.

[0050] SEQ ID NO: 20 is a nucleic acid sequence of mspd1-p24-Fc fusion DNA.

[0051] SEQ ID NO: 21 is an amino acid sequence of the wild-type soluble extracellular domain of human PD-1 (human spd1).

[0052] SEQ ID NO: 22 is a nucleic acid sequence of the wild-type human spd1DNA.

[0053] SEQ ID NO: 23 is an amino acid sequence of hspd1-p24-Fc fusion protein.

[0054] SEQ ID NO: 24 is a nucleic acid sequence of hspd1-p24-Fc fusion DNA.

[0055] SEQ ID NO: 25 is an amino acid sequence of hspd1-14de1.

[0056] SEQ ID NO: 26 is a nucleic acid sequence of hspd1-14de1 DNA.

[0057] SEQ ID NO: 27 is an amino acid sequence of hspd1-14de1-p24-Fc fusion protein.

[0058] SEQ ID NO: 28 is a nucleic acid sequence of hspd1-14de1-p24-Fc fusion DNA.

[0059] SEQ ID NO: 29 is an amino acid sequence of a linker sequence useful according to the subject invention.

[0060] SEQ ID NO: 30 is an amino acid sequence of a linker sequence useful according to the subject invention.

[0061] SEQ ID NO: 31 is an amino acid sequence of a linker sequence useful according to the subject invention.

[0062] SEQ ID NO: 32 is an amino acid sequence of a linker sequence useful according to the subject invention.

[0063] SEQ ID NO: 33 is an amino acid sequence of a linker sequence useful according to the subject invention.

[0064] SEQ ID NO: 34 is an amino acid sequence of a linker sequence useful according to the subject invention.

[0065] SEQ ID NO: 35 is an amino acid sequence of a

linker sequence useful according to the subject invention.

[0066] SEQ ID NO: 36 is an amino acid sequence of a

linker sequence useful according to the subject invention. [0067] SEQ ID NO: 37 is an amino acid sequence of a

linker sequence useful according to the subject invention. [0068] SEQ ID NO: 38 is an amino acid sequence of a linker sequence useful according to the subject invention.

[0069] SEQ ID NO: 39 is an amino acid sequence useful according the subject invention.

[0070] SEQ ID NO: 40 is an amino acid sequence useful according the subject invention.

DETAILED DISCLOSURE OF THE INVENTION

[0071] The subject invention provides soluble PD-1 (sPD-1) proteins and nucleic acids, and therapeutic compositions comprising soluble PD-1 proteins and nucleic acids, useful for inducing antigen-specific protective immunity against infection and cancer. In one embodiment, the subject sPD-1 proteins, nucleic acids, and compositions are formulated as a vaccine composition. In an embodiment, the subject invention provides novel fusion proteins mspd1-p24Fc, mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc, and nucleic acid molecules encoding these fusion proteins.

[0072] The subject invention is based on the findings that the immune regulatory PD-1/PD-L pathway down-regulates HIV-1-specific CD8+T cells responses. The present inventors discovered a natural variant of PD-1 present in healthy people that does not interact with either PD-L1 or PD-L2 (the ligands of PD-1). In addition, a point mutation, which is essential for PD-1 and its ligands interaction, is discovered.

[0073] In one embodiment, the subject invention provides a novel DNA vaccine design that mimics the binding of programmed death-1 (PD-1) to its ligands expressed on dendritic cells (DCs) for functional activation, by fusing soluble PD-1 with an antigen of interest. Intramuscular immunization via electroporation (EP) of the fusion DNA vaccine elicited robust anti-Gag antibody titers in mice, with both IgG1 (Th2) and IgG2a (Th1) responses detected. High frequencies of Gag-specific, broadly reactive and polyfunctional T cells, especially CD8+ T cells were elicited following immunization. These responses were dose-dependent, long lasting and conferred protection against intranasal challenge with virulent vaccinia-Gag virus. Specifically, mspd-p24fc, mspd1-14de1-p24fc and mspd1-322mu-p24fc enhance HIV-1 Gagspecific immune responses, as determined by the number of IFN-γ expressed CD4 and CD8 T cells using Elispot assays. Thus, soluble PD-1-based DNA/EP vaccination of the subject invention offers an easy, repeatable and effective way to induce durable and protective CD8+ cell immunity, which has important implications for vaccine development and gene therapy.

[0074] In one embodiment, the mspd1-14de1 protein variant is obtained by deleting amino acids 26-39 of the wild-type mspd1(Amino acids 26-39 are the first 14 amino acids encoded by the second exon of the wild-type mouse PD-1 gene. These 14 amino acids of mspd1have the same sequence as the first 14 amino acids encoded by the second exon of the human hspd1-14de1 homologue). The mspd1-322mu protein variant is obtained by changing amino acid residue 108 of the wild-type PD-1 protein from Met to Val. The hspd1-14de1 variant, which is derived from a natural isoform of human PD-1, has a deletion of amino acids 26-39 of the wild-type hspd1 (encoded by the first part of the second exon of the wild-type human PD-1 gene).

[0075] The mspd1-p24Fc fusion protein binds to PD-1 ligands PD-L1 and PD-L2, and the binding of PD-1 to PD-L can be blocked by anti-PD-L1/L2 antibodies. It is postulated that the binding of mspd1-p24Fc fusion protein inhibits the PD-1/PD-L pathway, which transmits negative signals to immune cells. In comparison, none of mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc fusion proteins binds to PD-L1 or PD-L2. This indicates that amino acid

residues 26-39 encoded by DNA in exon 2 of spd1 and amino acid residue 108 Met of mspd1are important for PD-L binding.

[0076] Advantageously, the administration of mspd1p24Fc, mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc fusion proteins, or fusion DNA thereof, enhanced HIV-1 Gag-specific immune responses. As shown in FIGS. 3-6 and 8-11, administration of mspd1-p24Fc, mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc DNA significantly increased anti-p24 IgG1 (Th2) and IgG2a (Th1) antibody titers. In addition, the administration of mspd1-p24Fc, mspd1-14de1-p24Fc, mspd1-322mu-p24Fc, and hspd1-14de1-p24Fc DNA also significantly increased the number of IFN-y-expressing CD4 and CD8 T cells in mice. Specifically, mice immunized mspd1-p24Fc, mspd1-14de1-p24Fc, or mspd1-322mup24Fc DNA had significantly reduced titers of challenge virus upon vaccinia virus-gagpol (VTT-gagpol) challenges. [0077] In comparison, mspd1-IgVΔ-p24-Fc, which is obtained by deleting amino acids 89-90 of the mouse PD-1 protein, does not bind to PD-1 ligands PD-L1 and PD-L2. In addition, the administration of mspd1-IgVΔ-p24-Fc DNA does not enhance humoral or cell-mediated immunity in mice. Further, the administration of mspd1-IgVΔ-p24-Fc DNA does not reduce HIV viral titers upon vaccinia virusgagpol (MVTT-gagpol) challenges.

PD-1 Variants and Fusion Constructs

[0078] A first aspect of the subject invention provides sPD-1 protein variants. In one embodiment, the sPD-1 protein variant is obtained by deleting amino acid residues 26-39 of a wild-type sPD-1 protein. The wild-type sPD-1 protein is preferably of mammalian origin (such as a wild-type mouse, rabbit, non-human primates, or pig PD-1 protein), more preferably, of human origin.

[0079] In an embodiment, the sPD-1 protein variant is mspd1-14de1, which has an amino acid sequence comprising SEQ ID NO: 11. In an embodiment, the sPD-1 protein variant is mspd1-322mu, which has an amino acid sequence comprising SEQ ID NO: 15. In an embodiment, the sPD-1 protein variant is hspd1-14de1, which has an amino acid sequence comprising SEQ ID NO: 25.

[0080] In certain embodiments, the subject invention encompasses PD-1 protein variants that are homologous to mspd1-14de1 (SEQ ID NO: 11), mspd1-322mu (SEQ ID NO: 15), or hspd1-14de1 (SEQ ID NO: 25). In an embodiment, the sPD-1 protein variant has an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 11. In an embodiment, the sPD-1 protein variant has an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 15. In an embodiment, the sPD-1 protein variant has an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 25. In an embodiment, the PD-1 protein variant does not comprise SEQ ID NO:7.

[0081] A second aspect of the subject invention provides nucleic acid molecules that encode the sPD-1 proteins of the subject invention. The nucleic acid molecules encompass DNA molecules (e.g. genomic DNA and cDNA) and RNA molecules. In addition, the subject nucleic acid molecules may be single-stranded or double-stranded.

[0082] In one embodiment, the nucleic acid molecule encodes a sPD-1 protein, which is obtained by deleting amino acid residues 26-39 of a wild-type sPD-1 protein (such as a

wild-type human, mouse, or rabbit sPD-1 protein). In an embodiment, the nucleic acid molecule encodes mspd1-14de1, and has a sequence comprising SEQ ID NO: 12. In an embodiment, the subject nucleic acid molecule encodes mspd1-322mu, and has a sequence comprising SEQ ID NO: 16. In an embodiment, the subject nucleic acid molecule encodes hspd1-14de1, and has a sequence comprising SEQ ID NO: 26.

[0083] In certain embodiments, the subject invention encompasses nucleic acid molecules that are homologous to nucleic acids encoding mspd1-14de1, mspd1-322mu, or hspd1-14de1. In an embodiment, the nucleic acid molecule has a sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 99.5% identical to SEQ ID NO: 12, SEQ ID NO: 16, or SEQ ID NO: 26. In an embodiment, the sPD-1 nucleic acid molecule does not comprise SEQ ID NO: 8.

[0084] A third aspect of the invention provides PD-1 fusion proteins. In one embodiment, the subject invention provides PD-1 fusion proteins, comprising a sPD-1 protein fragment fused with an antigenic protein fragment. In a further embodiment, the sPD-1 fusion protein comprises a Fc domain. In one embodiment, the soluble PD-1 protein is linked to the antigen via a linker sequence. In an alternative embodiment, the PD-1 fusion protein comprises a PD-1 protein fused with a Fc domain, optionally via a linker sequence.

[0085] In an embodiment, the sPD-1 fusion protein comprises the wild-type mouse soluble PD-1 protein (mspd), which has an amino acid sequence comprising SEQ ID NO: 1. In an embodiment, the sPD-1 fusion protein comprises the wild-type human sPD-1 protein (hspd1), which has an amino acid sequence comprising SEQ ID NO: 21. In an embodiment, the sPD-1 fusion protein is a variant mouse sPD-1 protein mspd1-14de1, which has an amino acid sequence comprising SEQ ID NO: 11. In an embodiment, the sPD-1 fusion protein is a variant mouse sPD-1 protein mspd1-322mu, which has an amino acid sequence comprising SEQ ID NO: 15. In an embodiment, the sPD-1 protein is a variant human sPD-1 protein (hspd1-14de1), and has an amino acid sequence comprising SEQ ID NO: 25.

[0086] The antigenic protein fragment can be derived from an immunogenic fragment of viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HSV including HSV-1 and HSV-2, KSHV, HPV including HPV-6, HPV-11, HPV-16, and HPV-18, respiratory syncytial virus, rhinovirus, hepatitis viruses including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, anpapillomavirus, pneumococcus, streptococcus, staphylococcus, neisseria, E. coli, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, flavivirus, dengue virus, Mycobacteria tuberculosis, and Plasmodium falciparu; and pathogens causing diseases including, but not limited to, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rabies, chickenpox, diphtheria, anthrax, plague, encephalitis, pneumonia, typhus, typhoid fever, lyme disease, cholera, shigella, leishmania, leprosy, toxoplasmosis, coccidiomycosis, schistosomiasis, and malaria. The antigenic protein fragment can also be derived from tumor or cancer cells.

[0087] In one embodiment, the soluble PD-1, its variants, and fusion proteins thereof serve as molecular or protein

adjuvants to enhance immune response. Additionally, nucleic acid molecules encoding the soluble PD-1, its variants, and fusion proteins thereof can also be administered to a subject to enhance immune response.

[0088] In an embodiment, the antigenic protein fragment is derived from an immunogenic fragment of an HIV protein domain including, but not limited to, p24, gag, pol, nef, tat, rev, gp120, and gp41. In an embodiment, the antigen protein is derived from HIV p24. In a specific embodiment, the antigen protein comprises SEQ ID NO: 3. In a further embodiment, the sPD-1 fusion protein further comprises a Fc domain. In an embodiment, the sPD-1 fusion protein comprises a rabbit Fc domain for protein purification purpose.

[0089] The term "Fc domain" encompasses the full length and fragments of native human and animal Fc and Fc variant molecules and sequences, including for example, IgG, IgM, IgD, IgE, IgA and subtypes such as for example IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. As with Fc variants and native Fc's, the term "Fc domain" includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

[0090] In an embodiment, the antigenic protein fragment is derived from a tumor antigen.

[0091] The term "Fc variant" refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage receptor. Fc domains include molecules having two or more polypeptide chains associated covalently, noncovalently, or by both covalent and non-covalent interactions. IgG molecules typically form dimers; IgM, pentamers; IgD, dimers; and IgA, monomers, dimers, trimers, or tetramers. Multimers may be formed by exploiting the sequence and resulting activity of the native Ig source of the Fc or by derivatizing (as defined below) such a native Fc.

[0092] The Fc domain within the scope of the invention can be of antibodies of any isotype, including IgG, IgA, IgE, IgD, and IgM. IgG isotype antibodies can be further subdivided into IgG1, IG2, IgG3, and IgG4 subtypes. IgA antibodies can be further subdivided into IgA1 and IgA2 subtypes. In a specific embodiment, the Fc domain is IgG1.

[0093] In a further embodiment, the sPD-1 fusion protein of the subject invention comprises a linker sequence that links the soluble PD-1 domain to the antigen. In addition, the Fc domain can also be linked to the fusion protein via a linker sequence. Linker sequence is typically a peptide chain. The length of the peptide may be, for example, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50 or more amino acid residues, but typically is between 5 and 25 residues.

[0094] Depending upon the length and side chain composition, a linker may have, but need not have, greater than average flexibility. Flexibility can be calculated using algorithms known in the art. In an embodiment, the linker sequence is SEQ ID NO: 29. Examples of useful linkers include, but are not limited to, 9Gly (SEQ ID NO: 30), 9Glu (SEQ ID NO: 31), 9Ser (SEQ ID NO: 32), 5GlyCys2ProCys (SEQ ID NO: 33), 4Gly3Ser (SEQ ID NO: 34), Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn (SEQ ID NO: 35), Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn (SEQ ID NO: 36), Gly Asp Leu Ile Tyr Arg Asn Gln Lys (SEQ ID NO: 37), and 9GlyProSerCysValProLeuMetArg-CysGlyGlyCysCysAsn (SEQ ID NO: 38).

[0095] In a specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 13. In another specific embodiment, the subject sPD-1 fusion protein comprises

SEQ ID NO: 17. In another specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 19. In another specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 23. In another specific embodiment, the subject sPD-1 fusion protein comprises SEQ ID NO: 27.

[0096] In addition, the subject invention provides sPD-1 fusion nucleic acid constructs, comprising a nucleic acid molecule encoding the subject sPD-1 fusion protein. In one embodiment, the sPD-1 fusion construct comprises a nucleic acid molecule encoding a sPD-1 protein fused with a nucleic acid encoding a protein antigen. In a further embodiment, the PD-1 fusion construct comprises a Fc DNA. In one embodiment, the soluble PD-1 DNA is linked to the antigen DNA via a linker sequence. Optionally, the Fc DNA is linked to the sPD-1-antigen DNA via a linker DNA sequence.

[0097] The antigenic nucleic acid molecule of the subject invention encodes immunogenic fragments of viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HSV including HSV-1 and HSV-2, KSHV, HPV including HPV-6, HPV-11, HPV-16, and HPV-18, respiratory syncytial virus, rhinovirus, hepatitis viruses including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, meningococcus, anpapillomavirus, pneumococcus, streptococcus, staphylococcus, E. coli, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, flavivirus, dengue virus, Mycobacteria tuberculosis, and Plasmodium falciparu; and pathogens causing diseases including, but not limited to, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rabies, chickenpox, diphtheria, anthrax, plague, encephalitis, pneumonia, typhus, typhoid fever, lyme disease, cholera, shigella, leishmania, leprosy, toxoplasmosis, coccidiomycosis, schistosomiasis, and malaria.

[0098] In an embodiment, the antigenic nucleic acid molecule encodes a tumor antigen. In an embodiment, the fusion nucleic acid molecule comprises the wild-type mouse PD-1 (mspd1) DNA (SEQ ID NO: 2). In an embodiment, the fusion nucleic acid molecule comprises the wild-type human PD-1 (hspd1) DNA (SEQ ID NO: 22). In an embodiment, the fusion nucleic acid molecule comprises a variant mouse PD-1 DNA that has a sequence of SEQ ID NO: 12 or SEQ ID NO: 16. In an embodiment, the fusion nucleic acid molecule comprises a variant human PD-1 DNA that has a sequence of SEQ ID NO: 26. In a specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 14.

[0099] In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 18. In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 20. In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 24. In another specific embodiment, the subject PD-1 fusion DNA comprises SEQ ID NO: 28. In certain embodiments, the PD-1 protein or nucleic acid of the subject invention is typically substantially free of other components, such as other biological molecules, proteins or peptides, nucleic acids, lipids and carbohydrates. The term "substantially free of," as used herein, encompasses preparations of the subject invention having less than about 20%, 10% and preferably less than 5% (by dry weight) con-

taminating factors (such as biological molecules, proteins or peptides, nucleic acids, lipids and carbohydrates and other cellular components).

[0100] If desired, the subject proteins and nucleic acid molecules can be modified by any suitable process. Strategies for protein optimization are sometimes carried out using random mutagenesis. In these cases positions are chosen randomly, or amino acid changes are made using simplistic rules. For example all residues may be mutated to alanine, referred to as alanine scanning In addition, substitution of amino acids other than those specifically exemplified or naturally present in a fusion protein of the invention are also within the scope of the subject invention. For example, non-natural amino acids can be substituted for the amino acids of the fusion protein, so long as the fusion protein having the substituted amino acids retains substantially the same functional activity as the fusion protein in which amino acids have not been substituted.

[0101] Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ -amino butyric acid, 2-amino butyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, τ -butylglycine, τ -butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form.

[0102] The subject invention also concerns variants of nucleic acid molecules that encode functional fusion proteins of the invention. Variant sequences include those sequences wherein one or more nucleotides of the sequence have been substituted, deleted, and/or inserted.

[0103] The nucleotides that can be substituted for natural nucleotides of DNA have a base moiety that can include, but is not limited to, inosine, 5-fluorouracil, 5-bromouracil, hypoxanthine, 1-methylguanine, 5-methylcytosine, and tritylated bases. The sugar moiety of the nucleotide in a sequence can also be modified and includes, but is not limited to, arabinose, xylulose, and hexose. In addition, the adenine, cytosine, guanine, thymine, and uracil bases of the nucleotides can be modified with acetyl, methyl, and/or thio groups. Sequences containing nucleotide substitutions, deletions, and/or insertions can be prepared and tested using standard techniques known in the art.

[0104] Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (1990). BLAST searches can be performed with the NBLAST program, score=100, wordlength=12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul et al. (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. See NCBI/NIH website.

[0105] The subject invention also contemplates those nucleic acid molecules having sequences which are sufficiently homologous with the nucleic acid sequences exemplified herein so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis et al., 1982). As used herein, "stringent" conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25 C below the melting temperature (Tm) of the DNA hybrid in 6× SSPE, 5× Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature, Tm, is described by the following formula (Beltz et al., 1983):

Tm=81.5 C+16.6 Log [Na+]+0.41(% G+C)-0.61(% formamide)-600/length of duplex in base pairs.

[0106] Washes are typically carried out as follows:

[0107] (1) Twice at room temperature for 15 minutes in $1 \times$ SSPE, 0.1% SDS (low stringency wash).

[0108] (2) Once at Tm-20 C for 15 minutes in 0.2× SSPE, 0.1% SDS (moderate stringency wash).

[0109] Further, the subject invention provides expression constructs comprising PD-1 nucleic acid molecules or fusion constructs thereof. Expression constructs of the invention generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements.

[0110] An expression construct of the invention can comprise a promoter sequence operably linked to a nucleic acid sequence encoding a peptide of the invention. Multiple copies of promoters or multiple promoters can be used in an expression construct of the invention. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

[0111] For expression in animal cells, an expression construct of the invention can comprise suitable promoters that can drive transcription of the polynucleotide sequence. For mammalian cells, suitable promoters include for example, Pcmv, actin promoter, metallothionein promoter, NF-kappaB promoter, EGR promoter, SRE promoter, IL-2 promoter, NFAT promoter, osteocalcin promoter, SV40 early promoter and SV40 late promoter, Lck promoter, BMP5 promoter, and TRP-1 promoter.

Protection against Pathogenic Infection and Cancer

[0112] Another aspect of the subject invention provides methods for the prevention and/or treatment of pathogenic infection and/or cancer. Advantagously, the methods of the subject invention induce antigen-specific humoral and cell-mediated immunity. In one embodiment, the method comprises administering, to a subject in need of such treatment, an effective amount of a fusion protein or fusion nucleic acid molecule of the subject invention.

[0113] In an embodiment, the subject invention provides a method of inducing protective immunity against pathogenic infection and/or cancer. In a specific embodiment, the method comprises administering a composition comprising a fusion nucleic acid molecule, wherein the fusion nucleic acid molecule comprises a nucleic acid encoding an antigen of interest; a sPD-1 nucleic acid encoding a wild-type soluble PD1 protein, a nucleic acid encoding a spd1-14 de1 protein of the

invention, or a nucleic acid encoding a spd1-322 del protein of the invention; and, optionally, a nucleic acid encoding Fc domain and a linker nucleic acid sequence that links the sPD-1 nucleic acid and the antigen nucleic acid. In one embodiment, the composition is administered by intramuscular injection via electroporation (EP).

[0114] In another specific embodiment, the method comprises administering a composition comprising a fusion protein, wherein the fusion protein comprises an antigen of interest; a soluble PD-1 protein selected from a wild-type soluble PD1 protein, a spd1-14de1 protein of the invention, or a spd1-322 de1 protein of the invention; and, optionally, a Fc domain and a linker sequence that links the sPD-1 protein and the antigen protein.

[0115] The methods can be used for prevention and/or treatment of infection and other diseases where induction of antigen-specific humoral and cell-mediated immunity is beneficial. In a specific embodiment, the subject invention can be used in the prevention and/or treatment of tumor or cancer.

[0116] The term "treatment" or any grammatical variation thereof (e.g., treat, treating, and treatment etc.), as used herein, includes but is not limited to, ameliorating or alleviating a symptom of a disease or condition, reducing, suppressing, inhibiting, lessening, or affecting the progression, severity, and/or scope of a condition.

[0117] The term "prevention" or any grammatical variation thereof (e.g., prevent, preventing, and prevention etc.), as used herein, includes but is not limited to, delaying the onset of symptoms, preventing relapse to a disease, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, or a combination thereof. Prevention, as used herein, does not require complete inhibition or elimination of symptoms.

[0118] The term "effective amount," as used herein, refers to an amount that is capable of treating or ameliorating a disease or condition or otherwise capable of producing an intended therapeutic effect.

[0119] The term "subject," as used herein, describes an organism, including mammals such as primates, to which treatment with the compositions according to the subject invention can be provided. Mammalian species that can benefit from the disclosed methods of treatment include, but are not limited to, apes, chimpanzees, orangutans, humans, monkeys; and other animals such as dogs, cats, horses, cattle, pigs, sheep, goats, chickens, mice, rats, guinea pigs, and hamsters.

[0120] In certain embodiments, in case of prevention of pathogenic infection or cancer, the sPD-1-based composition of the invention is administered to a subject that does not suffer from the pathogenic infection or cancer type to be prevented, or a subject that does not exhibit symptoms of the

[0121] In one embodiment, the subject invention can be used in the prevention and/or treatment of infection by viral, bacterial, fungal, or other microbial pathogens including, but not limited to, human immunodeficiency virus (HIV), HSV including HSV-1 and HSV-2, KSHV, HPV including HPV-6, HPV-11, HPV-16, and HPV-18, respiratory syncytial virus, rhinovirus, hepatitis viruses including hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, and hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, meningococcus, anpapillomavirus, pneumococcus, streptococcus, staphylococcus, E. coli, cytomegalovirus

pathogenic infection or cancer type to be prevented.

(CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, dengue virus, *Mycobacteria tuberculosis*, and *Plasmodium falciparu*; and pathogens causing diseases including, but not limited to, pertussis, polio, measles, mumps, rubella, smallpox, zoster, anthrax, tetanus, rabies, chickenpox, diphtheria, anthrax, plague, encephalitis, pneumonia, typhus, typhoid fever, lyme disease, cholera, *shigella*, *leishmania*, leprosy, toxoplasmosis, coccidiomycosis, schistosomiasis, and malaria.

[0122] In a specific embodiment, the subject invention can be use to prevent and/or treat HIV infection. In certain embodiments, the method comprises administering to a subject in need of such treatment an effective amount of a fusion protein, comprising an amino acid sequence selected from SEQ ID NOs: 13, 17, 19, 23, and 27. In specific embodiments, the subject method comprises administering to a subject in need of such treatment an effective amount of a fusion DNA, comprising a nucleic acid sequence selected from SEQ ID NOs: 14, 18, 20, 24, and 28.

[0123] In additon, the methods can be used in the prevention and/or treatment of diseases where enhanced humoral and cell-mediated immunity is beneficial. In an embodiment, the subject invention can be used in the prevention and/or treatment tumor or cancer.

[0124] In one embodiment, the sPD-1 protein useful for the prevention and/or treatment of tumor comprises an antigenic fragment derived from cancer or tumor cells. Soluble PD-1 proteins useful for the prevention and/or treatment of tumor or cancer also include, for example, the wild-type mspd-1 (SEQ ID NO:1), the wild-type hspd1 (SEQ ID NO: 21), mspd1-14de1 (SEQ ID NO: 11), mspd1-322mu (SEQ ID NO: 15), hspd1-14de1 (SEQ ID NO: 25), or fusion proteins thereof. Additionally or alternatively, the PD-1 protein useful for the prevention and/or treatment of tumor or cancer comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99%, or 99.5% identical to the wild-type mspd-1 (SEQ ID NO:1), the wild-type hspd1 (SEQ ID NO: 21), mspd1-14de1 (SEQ ID NO: 15), hspd1-14de1 (SEQ ID NO: 25), or fusion proteins thereof.

[0125] In specific embodiments, sPD-1 nucleic acid molecules useful for the prevention and/or treatment of tumor or cancer include, for example, the wild-type mspd-1 DNA (SEO)

[0126] ID NO: 2), the wild-type hspd1 DNA (SEQ ID NO: 22), mspd1-14de1 DNA (SEQ ID NO: 12), mspd1-322mu DNA (SEQ ID NO: 16), hspd1-14de1 DNA (SEQ ID NO: 26), or fusion DNA thereof.

[0127] Additionally or alternatively, the sPD-1 nucleic acid molecule useful for the prevention and/or treatment of tumor or cancer comprises a sequence that is at least 90%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical to the wild-type mspd-1 DNA (SEQ ID NO:2), the wild-type hspd1 DNA (SEQ ID NO: 22), mspd1-14de1 DNA (SEQ ID NO: 12), mspd1-322mu DNA (SEQ ID NO: 16), hspd1-14de1 DNA (SEQ ID NO: 26), or fusion DNA thereof.

Therapeutic Compositions and Routes of Administration

[0128] The subject invention further provides for therapeutic or pharmaceutical compositions. In an embodiment, the composition comprises a therapeutically effective amount of a protein and/or nucleic acid molecule of the subject invention and, optionally, a pharmaceutically acceptable carrier.

[0129] In one embodiment, the proteins and/or nucleic acid molecules are formulated into a vaccine composition for

administration to subjects having certain risks of pathogenic infection. A vaccine composition is an antigenic preparation that comprises one or more immunogenic antigens used to produce active immunity to a disease. In addition, the compositions of the subject invention can be administered to a subject with existing infection, and provide for customized vaccine schedules and compositions to prevent or minimize worsening of the diseases.

[0130] The subject invention contemplates therapeutic compositions useful for practicing the therapeutic methods described herein. The therapeutic composition can be any form of pharmaceutical format, including injectable formulations such as liquid and lyophilized injections.

[0131] In a specific embodiment, a therapeutically effective amount of a protein and/or nucleic acid molecule of the subject invention is typically an amount such that when administered in a physiologically tolerable composition is sufficient to achieve a plasma concentration of from about 0.01 microgram (ug) per milliliter (mL) to about 200 ug/mL. Stated differently, the dosage can vary from about 0.1 mg/kg to about 300 mg/kg, preferably from about 0.2 mg/kg to about 200 mg/kg, most preferably from about 0.5 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or several days.

[0132] Suitable non-toxic pharmaceutically acceptable carriers for use with the agent will be apparent to those skilled in the art of pharmaceutical formulation. See, for example, *Remington's Pharmaceutical Sciences*, seventeenth edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, Pa. (1985). Suitable carriers include ethanol, dimethyl sulfoxide, glycerol, silica, alumina, starch, sorbitol, inosital, xylitol, D-xylose, mannitol, powdered cellulose, microcrystalline cellulose, talc, colloidal silicon dioxide, calcium carbonate, magnesium cabonate, calcium phosphate, calcium aluminum silicate, aluminum hydroxide, sodium starch phosphate, lecithin, and equivalent carriers and diluents. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

[0133] Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The therapeutic composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

[0134] The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary, depending on the type of the condition and the subject to be treated. In general, a therapeutic composition contains from about 5% to about 95% active ingredient (w/w). More specifically, a therapeutic composition contains from about 20% (w/w) to about 80%, or about 30% to about 70%, active ingredient (w/w).

[0135] The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically such compositions are prepared as injectables either as liquid solutions or suspensions; however,

solid forms suitable for solution, or suspensions, in liquid prior to use also can be prepared. The preparation also can be emulsified.

[0136] The therapeutic composition of the subject invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of a polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

[0137] As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a mammal. [0138] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients, e.g., compound, carrier suitable for administration.

[0139] The compositions of the subject invention can be administered to the subject being treated by standard routes, including oral, inhalation, or parenteral administration including intravenous, subcutaneous, topical, transdermal, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intracardiac, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection, infusion, and electroporation, as well as co-administration as a component of any medical device or object to be inserted (temporarily or permanently) into a subject.

[0140] In a preferred embodiment, the microparticles of the subject invention can be formulated for parenteral administration. The preparation of an aqueous composition that contains one or more agents, such as a protein or nucleic acid molecule of the subject invention, will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and the preparations can also be emulsified.

[0141] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0142] Sterile injectable solutions are prepared by incorporating the active ingredients in the required amount in the appropriate solvent followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions,

the preferred methods of preparation are vacuum drying and freeze-drying techniques, which yield a powder of the active ingredient, plus any additional desired ingredient from a previously sterile-filtered solution thereof

[0143] In addition, the nucleic acid molecules and compositions of the subject invention can be delivered in vivo into a host cell by methods known in the art. In one embodiment, the nucleic acid molecules and compositions of the subject invention can be introduced in vivo via a viral vector such as adeno-associated virus (AAV), herpes simplex virus (HSV), retrovirus, papillomavirus, adenovirus, and Epstein-Barr virus (EBV). In addition, the nucleic acid molecules and compositions of the subject invention can also be introduced in vivo via lipofection (DNA transfection via liposomes prepared from synthetic cationic lipids) (Felgner et al., 1987). Synthetic cationic lipids (LIPOFECTIN, Invitrogen Corp., La Jolla, Calif.) can be used to prepare liposomes to encapsulate the nucleic acid molecules of the invention. The nucleic acid molecules of the subject invention can also be introduced in vivo as naked DNA using methods known in the art, such as transfection, microinjection, electroporation, calcium phosphate precipitation, and by biolistic methods.

EXAMPLES

[0144] Following are examples that illustrate embodiments for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1

Construction of Mouse sPD-1 Vaccine Candidates

[0145] This Example illustrates the construction of mouse sPD-1-p24 fusion constructs. To construct mouse sPD-1-p24-Fc construct, PVAX vector that carries the wild-type msPD-1 gene and p24 gene was fused with rabbit Fc DNA. The vector and the p24-Fc DNA were linked by a linker encoding GGGSGGG (SEQ ID NO: 29). The transcription is under the control of promoter Pcmv.

[0146] Mouse sPD1 protein variant mspd1-IgV Δ was obtained by deleting amino acids 89-90 of the mouse PD-1 protein, which forms the C"D loop of the IgV domain and is essential for PD-1 and PD-L1/L2 interaction. PVAX vector carrying mspd1-IgV Δ was linked with p24-rabbit Fc DNA via a linker encoding GGGSGGG (SEQ ID NO: 29) linker sequence. In addition, p24-Fc fusion construct was obtained by linking the PVAX vector carrying p24 with rabbit Fc DNA. The transcription is under the control of promoter Pcmv.

[0147] FIG. 1A shows alignment of amino acid sequences of msPd1-p24-Fc, mspd1-IgV Δ -p24-Fc, and p24-fc fusion proteins. FIG. 1B shows Western blot results of various fusion constructs useful according to the subject invention. Briefly, 293T cells were transfected with various fusion constructs using polyethylenimine (PEI) and the supernatants were collected 72 hours post transfection. Proteins were detected by anti-rabbit Fc antibody. FIG. 1B shows that msPd1-p24-Fc and mspd1-IgV Δ -p24-Fc are about 72KD in size, while p24-Fc is about 50KD in size. The results also show that msPd1-p24-Fc, mspd1-IgV Δ -p24-Fc, and p24-Fc fusion proteins are soluble.

Example 2

Binding Ability of Mouse sPD-1 Fusion Protein to sPD-1 Ligands

[0148] This Example shows the binding ability of msPD-1 fusion proteins to mouse sPD-1 ligands. Briefly, 293T cells

were transfected with PD-L (PD-L1 and PD-L2). The binding of sPD-1 proteins to PD-1 ligands was detected by FITC-anti rabbit Fc antibody using flow cytometer, and the results were analyzed by flowJo.

[0149] The results, as shown in FIGS. 2A-B, reveal that mspd1-p24-Fc binds to mouse PD-1 ligands PD-L1 and PD-L2. In contrast, the variant mspd1-IgVΔ fusion protein does not bind to mouse PD-1 ligands. In addition, p24-Fc does not affect the interaction between PD-1 and PD-L1/L2.

Example 3

Induction of Humoral and Cell-Mediated Immune Responses by Wild-Type Mouse sPD1 Vaccine

[0150] This Example shows that the wild-type msPD1-p24-Fc potently induces humoral and cell-mediated immune responses. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20ug mouse DNA vectors encoding msPd1-p24-Fc, mspd1-IgVΔ-p24-Fc, or p24-Fc via intramuscular electroporation. Mice that received PBS served as controls.

[0151] Two weeks after the last immunization, mice sera were collected and contacted with HIV-1 p24 viral proteins. The levels of anti-p24 IgG1 and IgG2a antibodies were measured by ELISA. The level of anti-p24 antibody in control samples is not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titer is defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution. The results show that mice immunized with mspd1-p24-Fc had high IgG1 and IgG2a titers, when compared to mice immunized with p24-Fc or mspd1-IgV Δ -p24-Fc.

[0152] To examine p24-specific immune responses, the number of IFN-γ-secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) (SEQ ID NO: 39) for CD8 T cells and the number of splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWIILGL) (SEQ ID NO: 40) for CD4 T cells was determined using ELIspot assay. In addition, splenocytes isolated from immunized vaccinated mice were subject to H2d-Kd-AMQMLKDTI-PE tetramer staining, and CD8 T cell and CD4 T cell population was analyzed.

[0153] The results show that mice immunized with mspd1-p24-Fc had high anti-p24 antibody titers (FIG. 3A) and high number of IFN- γ -secreting splenocytes (FIGS. 3B and 3D), when compared to mice immunized with p24-Fc or mspd1-IgV Δ -p24-Fc. Splenocytes isolated from mice immunized with mspd1-p24-Fc contained about five-fold higher H2d-Kd-AMQMLKDTI tetramer-positive cells (FIGS. 3C), when compared to mice immunized with p24-Fc or mspd1-IgV Δ -p24-Fc.

Example 4

Reduction of VTT-HIV-Gagpol Titers in Mice Immunized with Wild-Type Mouse sPD1 Fusion Protein

[0154] This Example shows that immunization with the wide-type msPD1 fusion protein protects against viral infection. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20ug mouse DNA encoding msPd1-p24-Fc, mspd1-IgV Δ -p24-Fc, or p24-Fc via intramuscular electroporation. Mice that received PBS served as

controls. Three weeks after immunization, mice were challenged with 4×10⁷PFU vaccinia VTT-HIV-gagpol intranasally. The mice were sacrificed 3 days after viral challenge and viral titers in the lungs were evaluated by plaque assay. The results show that mice immunized with mspd1-p24-Fc exhibited significantly reduced VTT-HIV-gagpol titers upon viral challenge (FIG. 4).

[0155] FIG. 5 shows that targeting dendritic cells using sPD-1-p24-fc induces enhanced p24-specific antibody and T cell responses.

Example 5

Induction of Humoral and Cell-Mediated Immune Responses by Wild-Type Human sPD1 Vaccine

[0156] Human sPD-1-p24-Fc was constructed by fusing PVAX vector carrying hsPD-1-p24 with rabbit Fc DNA. The vector and the p24-Fc DNA were linked by a linker encoding GGGSGGG (SEQ ID NO: 29). The transcription is under the control of promoter Pcmv.

[0157] To analyze the binding ability of hsPD-1-p24-Fc to sPD-1 ligands, 293T cells were transfected with mouse PD-L1 and PD-L2, respectively. The binding of sPD-1 proteins to PD-1 ligands was detected by mouse sPD-1-Fc proteins and FITC-anti rabbit Fc antibody using flow cytometer, and the results were analyzed by flowJo. The results, as shown in FIGS. 6A and 6B, reveal that hspd1-p24-Fc fusion protein binds to PD-1 ligands.

[0158] To examine the induction of immune responses by hsPD-1-p24-Fc, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 µg mouse DNA encoding hsPD1-p24-Fc or p24-Fc via intramuscular electroporation. Mice that received PBS served as controls.

[0159] Two weeks after the last immunization, mice sera were collected. The levels of anti-p24 IgG1 and IgG2a antibodies were measured by ELISA. The levels of anti-p24 antibody in control samples is not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titer is defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution. The results, as shown in FIG. **6**C, reveal that mice immunized with hspd1-p24-Fc had high IgG1 and IgG2a titers, when compared to mice immunized with p24-Fc.

[0160] To examine p24-specific immune response, the number of IFN-γ-secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) (SEQ ID NO: 39) for CD8 T cells and the number of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWIILGL) (SEQ ID NO: 40) for CD4 T cells was determined by ELIspot assay. Bars represent the average values of three samples (±standard deviations). The results, as shown in FIG. 6D, reveal that wild-type hsPD1 (hspd1-p24-Fc) binds to mouse PD-L1 and PD-L2, and potently elicits humoral and cell-mediated immune responses.

Example 6

Construction of Mouse sPD-1 Variant Vaccine Candidates

[0161] This Example illustrates the construction of variant msPD-1 vaccine candidates. Mouse sPD1 variants, mspd1-14de1 and mspd1-322mu, were constructed (FIG. 7A). The

mspd1-14de1 variant is obtained by deleting amino acids 26-39 of the wild-type mspd1 (encoded by the first part of the second exon of the wild-type mouse PD-1 gene). The mspd1-322mu variant is obtained by changing amino acid residue 108 of the wild-type mouse PD-1 protein from Met to Val.

[0162] Mouse sPD-1 fusion constructs were obtained by fusing PVAX vector carrying mspd1variant-p24 with rabbit Fc DNA. The PVAX vector and the p24-Fc DNA were linked by a linker encoding GGGSGGG (SEQ ID NO: 29). The transcription is under the control of promoter Pcmv.

[0163] FIG. 7A shows alignment of amino acid sequences of msPd1-p24-Fc, mspd1-14de1-p24-Fc, mspd1-322mu-p24-Fc, and p24-Fc fusion proteins. FIG. 7B shows Western blot results of various fusion proteins useful according to the subject invention. Briefly, 293T cells were transfected with various fusion constructs using polyethylenimine (PEI) and the supernatants were collected 72 hours post transfection. Proteins were detected by anti-rabbit Fc antibody. FIG. 7B shows that mspd1-14de1-p24-Fc, and mspd1-322mu-p24-Fc are about 72KD in size, while p24-Fc is about 50KD in size. The results also show that mspd1-14de1-p24-Fc, and mspd1-322mu-p24-Fc fusion proteins are soluble.

Example 7

Binding Ability of Mouse sPD-1 Variant Fusion Proteins to sPD-1 Ligands

[0164] This Example shows that msPD-1 variant fusion proteins, mspd1-14de1-p24-Fc and mspd1-322mu-p24-Fc, do not bind to mouse sPD-1 ligands PD-L1 and PD-L2 (FIG. 8). Briefly, 293T cells were transfected with PD-L (PD-L1 and PD-L2). The binding of sPD-1 proteins to PD-1 ligands was detected by mouse sPD-1-p24-Fc proteins and FITC-anti rabbit Fc antibody using flow cytometer, and the results were analyzed by flowJo.

Example 8

Induction of Humoral and Cell-Mediated Immune Responses by Variant sPD 1 Vaccines

[0165] This Example shows that msPD 1 variants potently elicit humoral and cell-mediated immune responses. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 µg mouse DNA vectors encoding mspd1-14de1-p24-Fc, mspd1-322mu-p24-Fc, or p24-Fc via intramuscular electroporation. Mice that received PBS served as controls

[0166] Two weeks after the last immunization, mice sera were collected. The levels of anti-p24 IgG1 and IgG2a antibodies were measured by ELISA. The level of anti-p24 antibody in control samples is not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody endpoint titer is defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution. The results show that mice immunized with mspd1-14de1-p24-Fc or mspd1-322mu-p24-Fc had high IgG1 and IgG2a titers, when compared to mice immunized with p24-Fc.

[0167] To examine p24-specific immune response, the number of IFN-γ-secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) (SEQ ID NO: 39) for CD8 T cells and the number of IFN-γ-secreting splenocytes specific

for p24 epitope gag26 (TSNPPIPVGDIYKRWIILGL) (SEQ ID NO: 40) for CD4 T cells was determined by ELIspot assay. [0168] The results show that mice immunized with mspd1-14de1-p24-Fc or mspd1-322mu-p24-Fc had high anti-p24 antibody titers (FIG. 9A) and high number of IFN-γ-secreting splenocytes (FIGS. 9B and 9D). Splenocytes isolated from mice immunized with mspd1-14de1-p24-Fc or mspd1-322mu-p24-Fc contained higher H2d-Kd-AMQMLKDTI tetramer-positive cells (FIG. 9C), when compared to mice immunized with p24-Fc.

Example 9

Reduction of VTT-HIV-Gagpol Titers in Mice Immunized with Variant Mouse sPD 1 Fusion Proteins

[0169] This Example shows that immunization with variant mspd1-14de1-p24-Fc or mspd1-322mu-p24-Fc protects against HIV infection. Briefly, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 µg mouse DNA encoding mspd1-14de1-p24-fc or mspd1-322mu-p24-Fc via intramuscular electroporation. Three weeks after immunization, mice were challenged with 4×10⁷PFU of VTT-HIV-gagpol intranasally. Mice that received PBS served as controls.

[0170] The mice were sacrificed 3 days after viral challenge and viral titers in lungs were evaluated by plaque assay. The results show that mice immunized with mspd1-14de1-p24-Fc or mspd1-322mu-p24-Fc exhibited significantly reduced VTT-HIV-gagpol titers upon viral challenge (FIG. **10**).

Example 10

Induction of Humoral and Cell-Mediated Immune Responses by Variant Human sPD1 Vaccine

[0171] In this Example, variant hsPD1 construct, hsPD1-14de1-p24-Fc, was constructed. To analyze the binding ability of hsPD1-14de1-p24-Fc protein to mouse PD-L1 and PD-L2, 293T cells were transfected with PD-L1 and PD-L2, respectively. The binding of hsPD1-14de1-p24-Fc protein to PD-1 were detected by FITC-anti rabbit Fc antibody using flow cytometer, and the results were analyzed by flowJo. The results, as shown in FIGS. 11A and 11B, reveal that hspd1-p24-Fc fusion protein binds to PD-1 ligands.

[0172] To examine the induction of immune responses by hsPD1-14de1-p24-Fc, Balb/c mice were primed at week 0 and boosted at week 3 and week 6 with 20 μ m/mice DNA encoding hsPD1-14de1-p24-Fc or p24-Fc via intramuscular electroporation. Mice that received PBS served as controls.

[0173] Two weeks after the last immunization, mice sera were collected. The levels of anti-p24 IgG1 and IgG2a anti-bodies were determined by ELISA. The levels of anti-p24 antibody in control samples are not shown because the absorbance readouts of these samples fell below the cutoff values for determining antibody titers. The anti-p24 antibody end-point titer is defined as the reciprocal of the highest dilution of a test sample that produces a reading of at least two-fold greater than that of the control sample with the same dilution.

greater than that of the control sample with the same dilution. [0174] To examine p24-specific immune response, the number of IFN-γ-secreting splenocytes specific for p24 epitope gagAI (AMQMLKDTI) (SEQ ID NO: 39) for CD8 T cells and the number of IFN-γ-secreting splenocytes specific for p24 epitope gag26 (TSNPPIPVGDIYKRWIILGL) (SEQ ID NO: 40) for CD4 T cells was determined by ELIspot assay.

Although hspd1-14de1-p24-Fc protein does not bind to PD-1, the results show that hspd1-14de1-p24-Fc significantly enhanced humoral and cell-mediated immune responses upon HIV viral challenge (FIGS. 11C and 11D).

Example 11

Antigen Targeting to Dendritic Cells by sPD-1-Based Vaccine Amplifies CD8⁺ T Cell Immunity

[0175] This Example shows that sPD-1-based vaccine improves CD8+ T cell immunity by targeting vaccine antigens to dendritic cells (DCs), while blocking the negative effects of the PD-1/PD-L pathway on T cell function simultaneously.

[0176] HIV-1 Gag p24 was chosen as a test antigen because it has been commonly used in other DC targeting strategies as a mode1 immunogen^{7,8}. Three DNA vaccines, p24-fc, sPD-1-p24-fc, and sIgV-PD-1-p24-fc, were designed (FIGS. 13*a* and 16*a*). sIgV-PD-1-p24-fc differs from sPD-1-p24-fc by two essential amino acids in the functional IgV domain of sPD-1, rendering it unable to bind PD-1 ligands14.

[0177] The results show that PD-L 1 and PD- L2 interact with recombinant sPD-1-p24-fc protein, but do not interact with sIgV-PD-1-p24-fc or p24-fc proteins (FIG. 16b).

[0178] In addition, Balb/c mice bone marrow (BM) derived CD1 1 c+DCs that expresses PD-L1 and PD-L2 (FIG. 17a) binds to sPD-1- p24-fc, but does not bind to sIgV-PD-1-p24-fc (FIG. 17b).

[0179] 2×10⁶ BM-DCs were pulsed with 20 g sPD-1-p24-fc or control proteins, and infused back into Balb/c mice via the tail vein in accordance to a standard immunization schedule^{15,16} (FIG. 13b). Compared to sIgV-PD-1-p24-fc, sPD-1-p24-fc-pulsed BM-DCs elicited higher levels of anti-p24 IgG1 (Th2) and IgG2a (Th1) antibody responses (p<0.05; FIG. 17c). Increased levels of p24-specific CD8+ T cell immunity was also evident as determined by IFN-γ ELISpot (FIG. 17d) and H2-Kd-AMQMLKDTI (Gag-AI) tetramer assays ^{17,18} (FIG. 17e). The results show that the sPD-1-based protein vaccine induced p24 specific CD8+ T cell immunity by targeting dendritic cells.

[0180] The results also show that sPD-1-p24-fc can be used as a DNA vaccine against infection. The present inventions have previously shown that intramuscular (i.m.)/EP enhances the immunogenicity of DNA vaccines consistently 13,19,20 .

[0181] In this Example, i.m. sPD-1-p24-fc/EP vaccination was conducted, using a vaccine dose of 20 µg or 100 µg (FIG. 13b). The results show that sPD-1-p24-fc/EP elicited significantly higher levels of IgG1 (4-fold; p<0.01) and IgG2a (8-fold; p<0.01) antibody responses, when compared to the sIgV-PD-1-p24-fc/EP control (FIG. 13c), in addition to potent and dose-dependent anti-Gag CD8+ (p<0.001) and CD4⁺ (p<0.05)T cell responses as determined by IFN-γ⁺ ELISpots (FIGS. 13d and 13e). Specifically, approximately 700 and 1600 ELISpots/10⁶ splenocytes were found against the CD8⁻-specific Gag-AI epitope at the doses 20 μg and 100 μg, respectively. This greatly contrasts with the 200-300 ELISpots/10⁶ splenocytes against the same epitope elicited by 1 mg/i.m. ADVAX (a codon-optimized HIV DNA vaccine) or 106 TCID50/i.m. ADMVA (a vaccinia MVA-vectored HIV-1 vaccine) as previously described by the present

[0182] The p24-specific T cell immunity was not confined to the single Gag-AI epitope. Approximately 800-1000 ELISpots/106 splenocytes was reactive to each of the three

non-overlapping peptide pools spanning the entire p24 protein, indicating a broad breadth in anti-Gag Gag T cell responses following vaccination with sPD-1-p24-fc/EP (FIG. 13F). Additionally, over 12.7% and 22% of CD8+T cells were positive for H2-Kd-Gag- AI tetramer binding in the 20 µg and 100 μg sPD-1-p24-fc/EP dose groups respectively, which is significantly higher than that of the sIgV-PD-1-p24-fc/EP group (p<0.05, FIGS. 3g and 13h), and is comparable to those observed in Balb/c mice using a heterologous prime-boost protocol with two live vectors, L. monocytogenes and Ad5 18 . [0183] In addition, this Example investigates the ability of p24-specific T cell populations to secrete IFN-γ, TNF-α and IL-2 in response to antigen stimulation. Compared to sIgV-PD-1-p24-fc/EP, sPD-1-p24-fc/EP elicited substantially higher frequencies of p24- specific CD8⁺ T cells producing IFN- γ (47.1%) and TNF- α (23.6%), and elevated frequencies of p24-specific CD4¹ T cells producing IFN-γ (6.8%) and TNF- α (3.6%) (FIGS. 14a and 14b). The results show that the proportion of effector-producing CD8+ and CD4+ T cell populations was similar in the order of IFN- γ +/TNF- α + >IFN- γ +>IFN- γ +/TNF- α +/IL-2+ (FIGS. 14c and 14d). Upon analyzing total cytokine-producing p24-specific CD8+ T cells, high frequency of cells secreting IFN-y (42.2%), IFN- γ /TNF- α (44.8%) and IFN- γ /TNF- α /IL-2 (9.4%) are indicative of enhanced vaccine potency (FIG. 14e).

[0184] To characterize sPD-1-p24-fc/EP vaccination and investigate its underlying mechanism(s) of immune induction, additional experiments were performed. Specifically, this Example compared sPD-1-p24-fc DNA vaccination with or without EP at the 20 μ g dose. Without EP, sPD-1-p24-fc induced 10-fold less IFN- γ +-secreting CD8+T cells than sPD-1-p24-fc/EP (FIGS. 18a and 13d), likely due to the omission of EP's effective recruitment of DCs to the site 3 . In addition, the lack of statistical difference between sPD-1-p24-fc and sIgV-PD-1-p24-fc induced CD8+ T cells when delivered without EP (FIG. 18a) indicates that sPD-1 alone does not have a strong adjuvant effect.

[0185] In another experiment, mice were co-immunized with a mixture of 20 μ g of sPD-1-fc and p24-fc by i.m./EP, and no statistical difference between these two groups in their IFN- γ^+ /CD8+ T cell response was found (FIG. 18b), indicating that de novo synthesis of sPD-1-fc alone was insufficient to potentiate immunogenicity. This shows the importance of DC-targeting via fusion of the antigen to sPD-1.

[0186] To exclude a role of rabbit-Fc in enhancing p24-specific immunity, the rabbit Fc fragment was removed from sPD-1-p24-fc and p24-fc to generate sPD-1-p24 and p24 DNA vaccines for immunization. In corroboration to sPD-1-p24-fc/EP, sPD-1-p24/EP induced significantly higher levels of IFN-γ+/CD8+ T cell response than p24/EP (FIG. 18c). Also, there was no statistical difference between sPD-1-p24-fc/EP and sPD-1-p24/EP in their ability to induce p24-specific IFN-γ+/CD8+ T cell responses (FIGS. 13b and 18c).

[0187] In another experiment, a human (hu-)sPD-1-p24-fc vaccine was used for comparative study, as it is known that hu-sPD-1 cross-reacts with murine PD-L1 and PD-L223 (FIG. 19a). The results show that hu-sPD-1-p24-fc/EP induced significantly greater levels of p24-specific IFN- γ^+ /CD8+T cell and antibody responses, when compared to p24-fc/EP in Balb/c mice (FIGS. 19b and 19c). Anti-human PD-1 responses were also induced due to the sequence divergence from murine PD-1, which may account for the difference between murine sPD-1-p24-fc/EP and hu-sPD-1-p24-fc/EP in the observed immunogenicity profile (i.e. p24-specific

CD4+T cell response was weak in mice immunized with hu-sPD-1-p24-fc/EP) (FIG. 13e and FIG. 19c).

[0188] To determine whether sPD-1-p24-fc/EP elicited long-lived p24-specific memory T cell responses, groups of mice 7.5 months were sacrificed after the third immunization with 20 μ g DNA vaccine. Besides persistent anti-p24 IgG1 and IgG2a antibody responses (FIG. 15a), p24-specific CD8+ (p<0.05) and CD4+ (p<0.05) memory T cell responses were sustained in mice immunized with sPD-1-p24-fc/EP compared with controls (FIGS. 15b and 15c).

[0189] To investigate if cellular immunity elicited by sPD-1-p24-fc/EP leads to protection, Balb/c mice immunized with DNA vaccines at a dose of 100 μg (FIG. 13b) were challenged intranasally with 2×10⁵ PFUs of a virulent strain of vaccinia modified to express HIV-1 gag and pol (WRgagpol). Eight days post-challenge, a significant reduction in virus titers in the lungs was observed in mice vaccinated with sPD-1-p24fc/EP compared to controls (p<0.01; FIG. 15d). Mice immunized with the placebo or sIgV-PD-1-p24-fc/EP showed >25% body weight loss within eight days after virus inoculation in contrast to mice immunized with sPD-1-p24-fc/EP that survived the challenge with <7% body weight loss (FIG. 15e). Since there were no anti-vaccinia neutralizing antibodies involved, the results indicated that p24-specific T cell immunity induced by sPD-1-p24-fc/EP provided significant protection against mucosal challenge by a virulent virus.

[0190] To summarize, this Example demonstrates that targeting of HIV-1 p24 to DCs via sPD-1 as a DNA vaccine enhanced the magnitude, breadth and polyfunctionality of specific CD8+T cell immunity. The sPD-1-based DNA vaccine can be used for inducing protective and long-lasting CD8+T cell immunity against pathogenic infections including HIV-1, tuberculosis, and malaria.

Material and Methods

[0191] Construction of sPD-1-Based Vaccine and Controls [0192] Three DNA vaccines, sPD-1-p24-fc, slgV-PD-1-p24-fc, and p24-fc, were constructed in the background of pVAX1 (FIG. 13a). The coding sequence for the extracellular domain of murine PD-1 (sPD-1) was obtained by nested PCR from mouse cDNA 10,26, and the HIV-1 p24 fragment was amplified from a primary isolate HIV-102HNsq4 of a Chinese patient without codon-optimization²⁷. To increase the flexibility of the fusion protein, a linker was applied between the sPD-1 and HIV-1 p24 gene.

[0193] A mutant form of sPD-1 (sIgV-PD-1) was also cloned following the same strategy as wild type PD-1. sIgV-PD1 does not react with PD-1 ligands 4128 due to a two essential amino acid (position 89-90) in-frame deletion in the IgV domain. Plasmid expressing HIV-1 p24 alone served as a control.

[0194] All of the plasmids contained a rabbit Fc tag to facilitate protein purification and characterization. DNA transfection into (HEK-)293T cell was performed using Polyethylenimine (PEI), and protein expression was detected by Western blotting assay using anti-rabbit Fc antibody.

[0195] Recombinant proteins were purified from the transfected cell supernatants by affinity chromatography using Protein G Sepharose (Invitrogen), and protein concentration was measured by Micro BCA Protein Assay Kit (Thermo Scientific).

Binding Characteristics of sPD-1 Fusion Proteins

[0196] 10^6 293T cells transiently expressing PD-L1 and PD-L2 were incubated with 2 μ g of purified sPD-1-p24-fc,

sIgV-PD-1-p24-fc or p24-fc fusion protein. Goat anti-rabbit IgG (H+L)-FITC (Invitrogen) was used to capture the positive cells. Transfected 293T cells stained by FITC-rat antimouse PD-L1 or PD-L2 antibodies (eBioscience) and FITC-rat IgG1 isotype served as positive and negative controls, respectively. Data was acquired on FACSCalibur instrument (BD Biosciences) and analyzed using BD CellQuest software

Mouse Immunization

[0197] All animal experiments were approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. 5-8 weeks old female Balb/c mice were bred under standard pathogen-free conditions in the Laboratory Animal Unit, University of Hong Kong. Mice were housed in cages under standard conditions with regulated temperature and humidity, fed with pelted food and tap water, and cared for according to the criteria outlined in the Guide for the Care and Use of Laboratory Animal.

[0198] The immunization procedure was similar to the previous protocols described in 15,16 (FIG. 13b). Mice received three DNA immunizations by intramuscular (i.m.) injection with or without EP given every three weeks at a dose of 20 μg or 100 μg per mouse. Two weeks after the final immunization, mice were sacrificed, and sera and spleen cells (splenocytes) were collected for immune response analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

[0199] Specific antibody responses were assessed by ELISA as previously described 15,16. Briefly, high affinity, protein-binding ELISA plates (BD Bioscieces) were coated with HIV-1 p24 protein (Abcam). Serial diluted sera were then added and antibodies detected by goat-radish peroxidase (HRP)-labeled anti-mouse IgG1 or IgG2a antibody (Sigma). Relative antibody titer was expressed as the reciprocal highest dilution of samples producing at least two-fold greater optical density readout over that of the control serum sample at the same dilution.

Evaluation of HIV-I Gag p24-Specific T Cell Responses

[0200] IFN-γ-producing T cells were evaluated by an ELISpot assay (Millipore) as previously described 15,16. $2\mu g/ml$ of HIV-1 p24 peptide or peptide pools (at a final concentration of $2\mu g/ml$ for each peptide, donated by NIH) were used to stimulate splenocytes in vitro. Peptide pool consisting of 59-members of Gag p24 libraries were divided into 3 pools of 19-20 peptides that span from amino acids 1-87 (pool 1), 77-167 (pool 2) and 157-231 (pool 3). Peptide gagAI (AMQMLKDTI) is specific for CD8+ T cells, whereas peptide gag26 (TSNPPIPVGDIYKRWIILGL) is specific for CD4+ T cells^{15,16}.

[0201] Cells stimulated by 500 ng/ml PMA plus 1 μ g/ml calcium ionocycin or left in media only served as positive and negative controls, respectively. Cells were stimulated at 37° C., 5% CO2, and 100% humidity for 20 h. Spots were identified by an immunospot reader and image analyzer (Thermo Scientific).

[0202] For intracellular cytokine staining (ICS), splenocytes were stimulated with HIV-1 p24 peptide pool (2 μ g/ml for each peptide) in the presence of co-stimulatory anti-CD28 antibody (2 μ g/ml, eBioscience) for 20-24 h at 37° C. 10 μ g/ml Brefeldin A (BFA; Sigma) was added for the last 5 h to accumulate intracellular cytokines Cells were washed and incubated with 2.4G2 mAb for 15 min at 4° C. to block Fcy.

After surface staining with anti-mouse CD3-APC/cy7, CD4-PE/cy5, CD8-Percp/cy5.5 antibodies (eBioscience), cells were permeabilized in 100 µl Fixation/Permeabilization solution (BD Biosciences) for 20 min at 4° C., washed with Perm/WashTM buffer (BD), and then stained intracellularly with anti-IFN- γ -PE, anti-IL-2-PE/cy7, anti-TNF- α -FITC (eBioscience). Tetramer positive CD8+T cell population was evaluated using phycoerythrin (PE)-conjugated major histocompatibility complex (MHC) class I tetramer H2d-Kd-AM-QMLKDTI (Beckman Coulter). Flow cytometric data were acquired and analyzed on a BD Arial III flow cytometer (BD Biosciences).

Mouse Immunization of Antigen Pulsed-Dendritic Cells

[0203] Bone marrow DCs (BM- DCs) from Balb/c mice were enriched by Dynabeads Mouse DC Enrichment Kit (Invitrogen). Two million CD11c+ BM-DCs were co-cultured with 20 μ g of purified sPD-1-p24-fc or sIgV-PD-1-p24-fc proteins for 1 h at 4° C. Cells were then washed extensively with PBS and transduced into mice via tail vein injection. Untreated DCs alone served as control. Immunization procedure and immune responses analysis were the same as described above.

Vaccinia Viral Challenges

[0204] Immunized mice were challenged intranasally with 2×10^5 PFUs vaccinia strain Western Reserve (WR) virus modified to express HIV-1 gag and pol genes. Animal body weight was monitored daily. Groups of animals were also sacrificed 8 days post challenge to measure viral titers in their lungs. Lung homogenates were prepared by physical disruption, and virus titers in the lungs were determined by a plaqueforming assay on monolayer Vero cells and monitored for cytopathic effect.

Statistical Analysis

[0205] All statistical analyses were performed using the paired one-tailed Student's t test. P values less than 0.05 were considered statistically significant. Data were presented as mean values±the standard error of at least three independent experiments.

[0206] All references, including publications, patent applications and patents, cited herein are hereby incorporated by reference to the same extent as if each reference was individually and specifically indicated to be incorporated by reference and was set forth in its entirety herein.

[0207] The terms "a" and "an" and "the" and similar referents as used in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. [0208] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (e.g., all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by "about," where appropriate).

[0209] The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to

better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the invention unless as much is explicitly stated.

[0210] The description herein of any aspect or embodiment of the invention using terms such as "comprising", "having", "including" or "containing" with reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that "consists of", "consists essentially of", or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context (e.g., a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

[0211] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

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Ile Ile Gln Leu Pro Asn Arg His Asp Phe His Met Asn Ile Leu Asp
Thr Arg Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile Ser Leu
His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val Val
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Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr
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Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala
Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp
Asp Arg Leu His Pro Val Gln Ala Gly Pro Val Ala Pro Gly Gln Met
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Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Asn Leu Gln
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Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu
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Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met
Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro
Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln
Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln
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Asn Ser Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala
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Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Pro
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Gly His Lys Ala Arg Val Leu
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Phe Trp Leu Arg V 35	al Pro Lys Val Ser Ala Ser 40	His Leu Glu Gln Tyr 45	
Leu Glu Ala Thr A 50	sn Thr Lys Val Asp Lys Thi 55	Val Ala Pro Ser Thr	
Cys Ser Lys Pro M 65	et Cys Pro Pro Pro Glu Leu 70 75	Leu Gly Gly Pro Ser 80	
Val Phe Ile Phe P	ro Pro Lys Pro Lys Asp Thi 5 90	Leu Met Ile Ser Arg 95	
Thr Pro Glu Val T	hr Cys Val Val Val Asp Val 105	. Ser Gln Asp Asp Pro 110	
Glu Val Gln Phe T 115	hr Trp Tyr Ile Asn Asn Glu 120	Gln Val Arg Thr Ala 125	
Arg Pro Pro Leu A 130	rg Glu Gln Gln Phe Asn Sei 135	Thr Ile Arg Val Val	
	le Ala His Gln Asp Trp Let 150 155		
	is Asn Lys Ala Leu Pro Ala 65 170	Pro Ile Glu Lys Thr 175	
Ile Ser Lys Ala A 180	rg Gly Gln Pro Leu Glu Pro 185	Lys Val Tyr Thr Met 190	
Gly Pro Pro Arg G 195	lu Glu Leu Ser Ser Arg Ser 200	Val Ser Leu Thr Cys 205	
Met Ile Asn Gly P 210	he Tyr Pro Ser Asp Ile Sen 215	Val Glu Trp Glu Lys 220	
Asn Gly Lys Ala G 225	lu Asp Asn Tyr Lys Thr Thi 230 239		
	yr Phe Leu Tyr Ser Lys Leu 45 250	Ser Val Pro Thr Ser 255	
Glu Trp Gln Arg G	ly Asp Val Phe Thr Cys Sei 265	Val Met His Glu Ala 270	

Leu His Asn His Tyr Thr Gln Lys Ser Ile Ser His Ser Pro Gly Lys

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Glu	Gly	Arg	Phe	Gln 165	Pro	Glu	Phe	Arg	Gly 170	Gly	Gly	Ser	Gly	Gly 175	Gly
Pro	Ile	Val	Gln 180	Asn	Leu	Gln	Gly	Gln 185	Met	Val	His	Gln	Pro 190	Ile	Ser
Pro	Arg	Thr 195	Leu	Asn	Ala	Trp	Val 200	Lys	Val	Ile	Glu	Glu 205	Lys	Ala	Phe
Ser	Pro 210	Glu	Val	Ile	Pro	Met 215	Phe	Ser	Ala	Leu	Ser 220	Glu	Gly	Ala	Thr
Pro 225	Gln	Asp	Leu	Asn	Thr 230	Met	Leu	Asn	Thr	Val 235	Gly	Gly	His	Gln	Ala 240
Ala	Met	Gln	Met	Leu 245	ГÀа	Glu	Thr	Ile	Asn 250	Glu	Glu	Ala	Ala	Glu 255	Trp
Asp	Arg	Leu	His 260	Pro	Val	Gln	Ala	Gly 265	Pro	Val	Ala	Pro	Gly 270	Gln	Met
Arg	Glu	Pro 275	Arg	Gly	Ser	Asp	Ile 280	Ala	Gly	Thr	Thr	Ser 285	Asn	Leu	Gln
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Ile 305	Tyr	Lys	Arg	Trp	Ile 310	Ile	Leu	Gly	Leu	Asn 315	ГЛа	Ile	Val	Arg	Met 320
Tyr	Ser	Pro	Thr	Ser 325	Ile	Leu	Asp	Ile	Arg 330	Gln	Gly	Pro	ГЛа	Glu 335	Pro
Phe	Arg	Asp	Tyr 340	Val	Asp	Arg	Phe	Tyr 345	Lys	Thr	Leu	Arg	Ala 350	Glu	Gln
Ala	Ser	Gln 355	Glu	Val	ГÀа	Asn	Trp 360	Met	Thr	Glu	Thr	Leu 365	Leu	Val	Gln
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Gly	His	Lys	Ala	Arg 405	Val	Leu	Met	Gln	Tyr 410	Ile	Lys	Ala	Asn	Ser 415	Lys
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Phe	Asn	Asn 435	Phe	Thr	Val	Ser	Phe 440	Trp	Leu	Arg	Val	Pro 445	Lys	Val	Ser
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Lys 465	Thr	Val	Ala	Pro	Ser 470	Thr	Сув	Ser	Lys	Pro 475	Met	Cys	Pro	Pro	Pro 480
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Asp	Val	Ser 515	Gln	Asp	Asp	Pro	Glu 520	Val	Gln	Phe	Thr	Trp 525	Tyr	Ile	Asn
Asn	Glu 530	Gln	Val	Arg	Thr	Ala 535	Arg	Pro	Pro	Leu	Arg 540	Glu	Gln	Gln	Phe
Asn 545	Ser	Thr	Ile	Arg	Val 550	Val	Ser	Thr	Leu	Pro 555	Ile	Ala	His	Gln	Asp 560

Trp Leu Arg Gly Lys Glu Phe Lys Cys Lys Val His Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Arg Gly Gln Pro Leu Glu Pro Lys Val Tyr Thr Met Gly Pro Pro Arg Glu Glu Leu Ser Ser Arg Ser Val Ser Leu Thr Cys Met Ile Asn Gly Phe Tyr Pro Ser Asp Ile Ser Val Glu Trp Glu Lys Asn Gly Lys Ala Glu Asp Asn Tyr Lys Thr Thr Pro Thr Val Leu Asp Ser Asp Gly Ser Tyr Phe Leu Tyr Ser 645 650 Lys Leu Ser Val Pro Thr Ser Glu Trp Gln Arg Gly Asp Val Phe Thr 665 Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser 675 680 685 Ile Ser His Ser Pro Gly Lys 690 <210> SEQ ID NO 10 <211> LENGTH: 2104 <212> TYPE: DNA <213 > ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: mspd1-IgV-p24-Fc <400> SEQUENCE: 10 atgtgggtcc ggcaggtacc ctggtcattc acttgggctg tgctgcagtt gagctggcaa tcagggtggc ttctagaggt ccccaatggg ccctggaggt ccctcacctt ctacccagcc tggctcacag tgtcagaggg agcaaatgcc accttcacct gcagcttgtc caactggtcg gaggatetta tgetgaactg gaacegeetg agteecagea accagactga aaaacaggee gccttctgta atggtttgag ccaacaggat gcccgcttcc agatcataca gctgcccaac 300 aggeatgact tecacatgaa cateettgae acaeggegea atgaeagtgg catetacete tgtggggcca tctccctgca ccccaaggca aaaatcgagg agagccctgg agcagagctc 420 gtggtaacag agagaatcct ggagacctca acaagatatc ccagcccctc gcccaaacca 480 gaaggccggt ttcaaccgga attccggggt ggtggtggtt caggaggagg acctatagtg 540 caaaacctcc aggggcaaat ggtacatcag cccatatcac ctagaacttt aaatgcatgg 600 gtaaaagtaa tagaagagaa ggcttttagt ccagaagtaa tacccatgtt ttcagcatta 660 tcagaaggag ccaccccaca agatttaaac accatgctaa acacagtggg gggacatcaa 720 gcagccatgc aaatgttaaa agaaaccatc aatgaggaag ctgcagaatg ggatagattg 780 catccaqtqc aqqcaqqqcc aqttqcacca qqccaqatqa qaqaaccaaq qqqaaqtqac 840 atagcaggaa ctactagtaa tetteaggag caaataggat ggatgacaaa taatecacet 900 atcccagtag gagaaatcta taaaagatgg ataatcctgg ggttaaataa aatagtaaga 960 1020 atqtataqcc ctaccaqcat tctqqacata aqacaaqqac caaaqqaacc ctttaqaqac tatgtagacc ggttctataa aactctaaga gccgagcaag cttcacaaga ggtaaaaaaat tggatgacag aaaccttgtt ggtccaaaat tcgaacccag attgtaagac tattttaaaa gcattgggac cagcagctac actagaagaa atgatgacag catgtcaggg agtggggga

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Gly Ala Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp \$35\$ \$40\$ \$45\$

Leu Met Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Gln Thr Glu Lys $50 \hspace{1.5cm} 55 \hspace{1.5cm} 60 \hspace{1.5cm}$

Gln Ala Ala Phe Cys Asn Gly Leu Ser Gln Pro Val Gln Asp Ala Arg 65 70 70 75 80

Phe Gln Ile Ile Gln Leu Pro Asn Arg His Asp Phe His Met Asn Ile 85 90 95

Leu Asp Thr Arg Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile 100 \$105\$

Ser Leu His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu 115 120 125

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Ser Pro Lys Pro Glu Gly Arg Phe Gln Pro Glu Phe Arg Gly Gly Gly 145 $$ 150 $$ 155 $$ 160

Ser Gly Gly Gly

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tato	ccaç	gcc (cctc	gecea	aa a	ccaga	aaggo	c cgg	gttto	caac	cgga	aatt	ccg (gggtg	ggtggt	
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Phe	Gln	Ile	Ile	Gln 85	Leu	Pro	Asn	Arg	His 90	Asp	Phe	His	Met	Asn 95	Ile	
Leu	Asp	Thr	Arg 100	Arg	Asn	Asp	Ser	Gly 105	Ile	Tyr	Leu	Сла	Gly 110	Ala	Ile	
Ser	Leu	His 115	Pro	Lys	Ala	Lys	Ile 120	Glu	Glu	Ser	Pro	Gly 125	Ala	Glu	Leu	
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Ser 145	Pro	Lys	Pro	Glu	Gly 150	Arg	Phe	Gln	Pro	Glu 155	Phe	Arg	Gly	Gly	Gly 160	
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Gln	Pro	Ile	Ser 180	Pro	Arg	Thr	Leu	Asn 185	Ala	Trp	Val	Lys	Val 190	Ile	Glu	
Glu	Lys	Ala	Phe	Ser	Pro	Glu	Val	Ile	Pro	Met	Phe	Ser	Ala	Leu	Ser	

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Glu	Gly 210	Ala	Thr	Pro	Gln	Asp 215	Leu	Asn	Thr	Met	Leu 220	Asn	Thr	Val	Gly
Gly 225	His	Gln	Ala	Ala	Met 230	Gln	Met	Leu	Lys	Glu 235	Thr	Ile	Asn	Glu	Glu 240
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Ser	Asn	Leu 275	Gln	Glu	Gln	Ile	Gly 280	Trp	Met	Thr	Asn	Asn 285	Pro	Pro	Ile
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Pro	ГЛа	Glu	Pro	Phe 325	Arg	Asp	Tyr	Val	Asp 330	Arg	Phe	Tyr	Lys	Thr 335	Leu
Arg	Ala	Glu	Gln 340	Ala	Ser	Gln	Glu	Val 345	ГЛа	Asn	Trp	Met	Thr 350	Glu	Thr
Leu	Leu	Val 355	Gln	Asn	Ser	Asn	Pro 360	Asp	Сла	Lys	Thr	Ile 365	Leu	Lys	Ala
Leu	Gly 370	Pro	Ala	Ala	Thr	Leu 375	Glu	Glu	Met	Met	Thr 380	Ala	CÀa	Gln	Gly
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Ala	Asn	Ser	ГЛа	Phe 405	Ile	Gly	Ile	Thr	Glu 410	Leu	Lys	ГÀа	Leu	Gly 415	Gly
Ser	Asn	Asp	Ile 420	Phe	Asn	Asn	Phe	Thr 425	Val	Ser	Phe	Trp	Leu 430	Arg	Val
Pro	Lys	Val 435	Ser	Ala	Ser	His	Leu 440	Glu	Gln	Tyr	Leu	Glu 445	Ala	Thr	Asn
Thr	Lys 450	Val	Asp	Lys	Thr	Val 455	Ala	Pro	Ser	Thr	Cys 460	Ser	Lys	Pro	Met
Cys 465	Pro	Pro	Pro	Glu	Leu 470	Leu	Gly	Gly	Pro	Ser 475	Val	Phe	Ile	Phe	Pro 480
Pro	Lys	Pro	ГЛа	Asp 485	Thr	Leu	Met	Ile	Ser 490	Arg	Thr	Pro	Glu	Val 495	Thr
Cys	Val	Val	Val 500	Asp	Val	Ser	Gln	Asp 505	Asp	Pro	Glu	Val	Gln 510	Phe	Thr
Trp	Tyr	Ile 515	Asn	Asn	Glu	Gln	Val 520	Arg	Thr	Ala	Arg	Pro 525	Pro	Leu	Arg
Glu	Gln 530	Gln	Phe	Asn	Ser	Thr 535	Ile	Arg	Val	Val	Ser 540	Thr	Leu	Pro	Ile
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Asn	ГЛа	Ala	Leu	Pro 565	Ala	Pro	Ile	Glu	Lys 570	Thr	Ile	Ser	Lys	Ala 575	Arg
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Glu	Leu	Ser 595	Ser	Arg	Ser	Val	Ser 600	Leu	Thr	Сув	Met	Ile 605	Asn	Gly	Phe
Tyr	Pro	Ser	Asp	Ile	Ser	Val	Glu	Trp	Glu	Lys	Asn	Gly	Lys	Ala	Glu

Asp Asn Tyr Lys Thr Thr Pro Thr Val Leu Asp Ser Asp Gly Ser Tyr Phe Leu Tyr Ser Lys Leu Ser Val Pro Thr Ser Glu Trp Gln Arg Gly Asp Val Phe Thr Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Ile Ser His Ser Pro Gly Lys 680 <210> SEQ ID NO 14 <211> LENGTH: 2068 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: mspd1-14del-p24-Fc <400> SEOUENCE: 14 atgtgggtcc ggcaggtacc ctggtcattc acttgggctg tgctgcagtt gagctggcaa 60 tcaqqqtqqc ttctaqcctq qctcacaqtq tcaqaqqqaq caaatqccac cttcacctqc 120 agettqteca actqqteqqa qqatettatq etqaactqqa accqeetqaq teccaqeaac 180 cagactgaaa aacaggccgc cttctgtaat ggtttgagcc aacccgtcca ggatgcccgc 240 ttccaqatca tacaqctqcc caacaqqcat qacttccaca tqaacatcct tqacacacqq 300 cgcaatgaca gtggcatcta cctctgtggg gccatctccc tgcaccccaa ggcaaaaatc gaggagagcc ctggagcaga gctcgtggta acagagagaa tcctggagac ctcaacaaga tatcccagcc cctcgcccaa accagaaggc cggtttcaac cggaattccg gggtggtggt ggttcaggag gaggacctat agtgcaaaac ctccaggggc aaatggtaca tcagcccata tcacctagaa ctttaaatgc atgggtaaaa gtaatagaag agaaggcttt tagtccagaa gtaataccca tgttttcagc attatcagaa ggagccaccc cacaagattt aaacaccatg 660 ctaaacacag tggggggaca tcaagcagcc atgcaaatgt taaaagaaac catcaatgag 720 gaagctgcag aatgggatag attgcatcca gtgcaggcag ggccagttgc accaggccag 780 atgagagaac caaggggaag tgacatagca ggaactacta gtaatcttca ggagcaaata 840 ggatggatga caaataatcc acctatccca gtaggagaaa tctataaaag atggataatc 900 ctggggttaa ataaaatagt aagaatgtat agccctacca gcattctgga cataagacaa 960 ggaccaaagg aaccetttag agactatgta gaccggttet ataaaactet aagageegag 1020 caagetteac aagaggtaaa aaattggatg acagaaacet tgttggteca aaattegaac 1080 ccagattgta agactatttt aaaagcattg ggaccagcag ctacactaga agaaatgatg 1140 acagcatgtc agggagtggg gggacctggc cataaagcaa gagttttgat cctgatgcag 1200 1260 tacatcaaqq ccaacaqtaa qttcatcqqa atcaccqaqc ttaaqaaqct qqqaqqctca aacgacatat tcaacaactt cacagtgtcc ttctggttgc gggttcccaa ggtctctgct 1320 agccacctcg aacaatacct ggaggccacc aacaccaaag tggacaagac cgttgcgccc 1380 tcgacatgca gcaagcccat gtgcccaccc cctgaactcc tggggggacc gtctgtcttc

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Asn Ala Thr Phe Thr Cys Ser Leu Ser Asn Trp Ser Glu Asp Leu Met
Leu Asn Trp Asn Arg Leu Ser Pro Ser Asn Gln Thr Glu Lys Gln Ala
Ala Phe Cys Asn Gly Leu Ser Gln Pro Val Gln Asp Ala Arg Phe Gln
Ile Ile Gln Leu Pro Asn Arg His Asp Phe His Val Asn Ile Leu Asp
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Thr Arg Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile Ser Leu
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His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val Val
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Thr Glu Arg Ile Leu Glu Thr Ser Thr Arg Tyr Pro Ser Pro Ser Pro
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Thr Arg Arg Asn Asp Ser Gly Ile Tyr Leu Cys Gly Ala Ile Ser Leu 115 120 125														
His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val Val 130 135 140														
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Lys Pro Glu Gly Arg Phe Gln Pro Glu Phe Arg Gly Gly Ser Gly 165 170 175														
Gly Gly Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Pro 180 185 190														
Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys 195 200 205														
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Glu Trp Asp Arg Leu His Pro Val Gln Ala Gly Pro Val Ala Pro Gly 260 265 270														

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Gly 305	Glu	Ile	Tyr	ГÀа	Arg 310	Trp	Ile	Ile	Leu	Gly 315	Leu	Asn	Lys	Ile	Val 320
Arg	Met	Tyr	Ser	Pro 325	Thr	Ser	Ile	Leu	Asp	Ile	Arg	Gln	Gly	Pro 335	Lys
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Glu	Gln	Ala 355	Ser	Gln	Glu	Val	Lys 360	Asn	Trp	Met	Thr	Glu 365	Thr	Leu	Leu
Val	Gln 370	Asn	Ser	Asn	Pro	Asp 375	Cys	Lys	Thr	Ile	Leu 380	Lys	Ala	Leu	Gly
Pro 385	Ala	Ala	Thr	Leu	Glu 390	Glu	Met	Met	Thr	Ala 395	Cys	Gln	Gly	Val	Gly 400
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Asp	Ile	Phe 435	Asn	Asn	Phe	Thr	Val 440	Ser	Phe	Trp	Leu	Arg 445	Val	Pro	Tàa
Val	Ser 450	Ala	Ser	His	Leu	Glu 455	Gln	Tyr	Leu	Glu	Ala 460	Thr	Asn	Thr	TÀa
Val 465	Asp	Lys	Thr	Val	Ala 470	Pro	Ser	Thr	Сув	Ser 475	Lys	Pro	Met	Сув	Pro 480
Pro	Pro	Glu	Leu	Leu 485	Gly	Gly	Pro	Ser	Val 490	Phe	Ile	Phe	Pro	Pro 495	Lys
Pro	Lys	Asp	Thr 500	Leu	Met	Ile	Ser	Arg 505	Thr	Pro	Glu	Val	Thr 510	Сув	Val
Val	Val	Asp 515	Val	Ser	Gln	Asp	Asp 520	Pro	Glu	Val	Gln	Phe 525	Thr	Trp	Tyr
Ile	Asn 530	Asn	Glu	Gln	Val	Arg 535	Thr	Ala	Arg	Pro	Pro 540	Leu	Arg	Glu	Gln
Gln 545	Phe	Asn	Ser	Thr		_	Val			Thr 555		Pro	Ile	Ala	His 560
Gln	Asp	Trp	Leu	Arg 565	Gly	ГÀа	Glu	Phe	Lys 570	Cys	ГÀа	Val	His	Asn 575	Lys
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Pro	Leu	Glu 595	Pro	Lys	Val	Tyr	Thr 600	Met	Gly	Pro	Pro	Arg 605	Glu	Glu	Leu
Ser	Ser 610	Arg	Ser	Val	Ser	Leu 615	Thr	Суз	Met	Ile	Asn 620	Gly	Phe	Tyr	Pro
Ser 625	Asp	Ile	Ser	Val	Glu 630	Trp	Glu	Lys	Asn	Gly 635	Lys	Ala	Glu	Asp	Asn 640
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His Pro Lys Ala Lys Ile Glu Glu Ser Pro Gly Ala Glu Leu Val Val 130 135 140														
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Pro 385	Ala	Ala	Thr	Leu	Glu 390	Glu	Met	Met	Thr	Ala 395	CÀa	Gln	Gly	Val	Gly 400
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Asp	Ile	Phe 435	Asn	Asn	Phe	Thr	Val 440	Ser	Phe	Trp	Leu	Arg 445	Val	Pro	Tàa
Val	Ser 450	Ala	Ser	His	Leu	Glu 455	Gln	Tyr	Leu	Glu	Ala 460	Thr	Asn	Thr	ГЛа
Val 465	Asp	Lys	Thr	Val	Ala 470	Pro	Ser	Thr	Cys	Ser 475	ГÀа	Pro	Met	CÀa	Pro 480
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Ala	Leu	Pro	Ala 580	Pro	Ile	Glu	Lys	Thr 585	Ile	Ser	Lys	Ala	Arg 590	Gly	Gln
Pro	Leu	Glu 595	Pro	Lys	Val	Tyr	Thr 600	Met	Gly	Pro	Pro	Arg 605	Glu	Glu	Leu
Ser	Ser 610	Arg	Ser	Val	Ser	Leu 615	Thr	Cys	Met	Ile	Asn 620	Gly	Phe	Tyr	Pro
Ser 625	Asp	Ile	Ser	Val	Glu 630	Trp	Glu	Lys	Asn	Gly 635	Lys	Ala	Glu	Asp	Asn 640
Tyr	Lys	Thr	Thr	Pro 645	Thr	Val	Leu	Asp	Ser 650	Asp	Gly	Ser	Tyr	Phe 655	Leu
Tyr	Ser	Lys	Leu 660	Ser	Val	Pro	Thr	Ser 665	Glu	Trp	Gln	Arg	Gly 670	Asp	Val
Phe	Thr	Сув 675	Ser	Val	Met	His	Glu 680	Ala	Leu	His	Asn	His 685	Tyr	Thr	Gln
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<212> TYPE: DNA

<213 > ORGANISM: Artificial Sequence

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Leu Ala Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg 65 \phantom{000}70\phantom{000} 70 \phantom{0000}75\phantom{000} 80
Phe Arg Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val
Val Arg Ala Arg Ash Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile 100 $105$
Ser Leu Ala Pro Lys Ala Gl<br/>n Ile Lys Glu Ser Leu Arg Ala Glu Leu 115 120 125
Arg Val Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro
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Gly Ala Thr Gly Gly Thr Thr Cys Thr Thr Ala Gly Cys Cys Cys Thr 65 70 75 80
Gly Cys Thr Cys Gly Thr Gly Gly Thr Gly Ala Cys Cys Gly Ala Ala
Gly Gly Gly Gly Ala Cys Ala Ala Cys Gly Cys Cys Ala Cys Cys Thr 100 \hspace{1.5cm} 105 \hspace{1.5cm} 110 \hspace{1.5cm}
Thr Cys Ala Cys Cys Thr Gly Cys Ala Gly Cys Thr Thr Cys Thr Cys 115 120 125
Cys Ala Ala Cys Ala Cys Ala Thr Cys Gly Gly Ala Gly Ala Gly Cys
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Thr Thr Cys Gly Thr Gly Cys Thr Ala Ala Cys Thr Gly Gly Thr Ala Cys Cys Gly Cys Ala Thr Gly Ala Gly Cys Cys Cys Cys Ala Gly $165 \\ 170 \\ 175$ Cys Ala Ala Cys Cys Ala Gly Ala Cys Gly Gly Ala Cys Ala Ala Gly Cys Thr Gly Gly Cys Cys Gly Cys Cys Thr Thr Cys Cys Cys Gly Ala Gly Gly Ala Cys Cys Gly Cys Ala Gly Cys Cys Ala Gly Cys Cys 210 215 220 Cys Gly Gly Cys Cys Ala Gly Gly Ala Cys Thr Gly Cys Cys Gly Cys 225 230235235 Thr Thr Cys Cys Gly Thr Gly Thr Cys Ala Cys Ala Cys Ala Ala Cys 250 Thr Gly Cys Cys Cys Ala Ala Cys Gly Gly Gly Cys Gly Thr Gly Ala 265 Cys Thr Thr Cys Cys Ala Cys Ala Thr Gly Ala Gly Cys Gly Thr Gly $275 \\ 280 \\ 285$ Gly Thr Cys Ala Gly Gly Gly Cys Cys Cys Gly Gly Cys Gly Cys Ala 290 \$295\$Ala Thr Gly Ala Cys Ala Gly Cys Gly Gly Cys Ala Cys Cys Thr Ala 305 310315315 Cys Gly Cys Ala Gly Ala Thr Cys Ala Ala Ala Gly Ala Gly Ala Gly 355 \$360\$Cys Cys Thr Gly Cys Gly Gly Gly Cys Ala Gly Ala Gly Cys Thr Cys $370 \hspace{1cm} 375 \hspace{1cm} 380 \hspace{1cm}$ Ala Gly Gly Gly Thr Gly Ala Cys Ala Gly Ala Gly Ala Gly Ala Ala 395 Gly Gly Cys Ala Gly Ala Ala Gly Thr Gly Cys Cys Cys Ala Cys 410 Ala Gly Cys Cys Cys Ala Cys Cys Cys Cys Cys Ala Gly Cys Cys Cys Cys Cys Cys $420 \hspace{1cm} 425 \hspace{1cm} 430 \hspace{1cm}$ Gly Cys Cys Ala Gly Cys Cys Gly Gly Ala Ala Thr Thr Cys Cys Gly Gly Gly Gly Thr Gly Gly Thr Gly Gly Thr Gly Gly Thr Thr Cys Ala 465 470 470 475 Gly Gly Ala Gly Gly Ala Gly Gly Ala 485 <210> SEQ ID NO 27 <211> LENGTH: 681 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: hspd1-14del-p24-Fc

<400> SEQUENCE: 27

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

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	Asp	Ile	Phe	Asn 420	Asn	Phe	Thr	Val	Ser 425	Phe	Trp	Leu	Arg	Val 430	Pro	Lys		
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	Pro	Lys	Asp	Thr	Leu 485	Met	Ile	Ser	Arg	Thr 490	Pro	Glu	Val	Thr	Сув 495	Val		
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	Tyr	Ser	Lys	Leu	Ser 645	Val	Pro	Thr	Ser	Glu 650	Trp	Gln	Arg	Gly	Asp 655	Val		
	Phe	Thr	Сув	Ser 660	Val	Met	His	Glu	Ala 665	Leu	His	Asn	His	Tyr 670	Thr	Gln		
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	cgca	atga	aca ç	gegge	cacct	ca co	ctct	gtggg	g gco	catct	ccc	tgg	cccc	caa 🤅	gacgo	cagatc	3	360

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We claim:

- 1. A soluble PD-1 protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 15, and SEQ ID NO: 25.
- $\mathbf{2}$. A PD-1 nucleic acid molecule encoding a soluble PD-1 protein of claim $\mathbf{1}$.
- 3. A soluble PD-1 fusion protein, comprising a soluble PD-1 protein fragment and an antigenic protein fragment, wherein the soluble PD-1 protein fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 21, SEQ ID NO: 11, SEQ ID NO: 15, and SEQ ID NO: 25.
- 4. The soluble PD-1 fusion protein of claim 3, wherein the antigenic protein fragment is derived from an immunogenic protein fragment of a viral, bacterial, or fungal pathogen, or cancer or tumor cells.
- **5**. The PD-1 fusion protein of claim **4**, wherein the pathogen is selected from human immunodeficiency virus (HIV), HSV, respiratory syncytial virus, rhinovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, anpapillomavirus, *pneumococcus, streptococcus, staphylococcus, E. coli*, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, flavivirus, dengue virus, *Mycobacteria tuberculosis*, or *Plasmodium falciparum*.
- **6.** The PD-1 fusion protein of claim **4**, wherein the antigenic protein fragment is derived from HIV p24.
- 7. The PD-1 fusion protein of claim 3, further comprising a Fc domain.
- **8.** The PD-1 fusion protein of claim **7**, further comprising a linker sequence, wherein the linker sequence links the soluble PD-1 domain and the antigen.
- **9.** The PD-1 fusion protein of claim **3**, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 17, 19, 23, and 27.
- 10. A sPD-1 fusion nucleic acid molecule encoding a PD-1 fusion protein of claim 3.
- 11. The sPD-1 fusion nucleic acid molecule of claim 10, wherein the nucleic acid molecule comprises a sequence selected from the group consisting of SEQ ID NOs: 14, 18, 20, 24, and 28.

- 12. A vaccine composition comprising the sPD-1 fusion nucleic acid molecule of claim 10.
- 13. A method for preventing or treating pathogenic infection and/or tumor or cancer, comprising administering, to a subject in need of such prevention or treatment, an effective amount of a fusion nucleic acid of claim 10.
- 14. The method of claim 13, wherein the pathogenic infection is caused by a pathogen selected from human immunodeficiency virus (HIV), HSV, respiratory syncytial virus, rhinovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza virus, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, anpapillomavirus, pneumococcus, streptococcus, staphylococcus, E. coli, cytomegalovirus (CMV), respiratory syncytial virus, parainfluenza virus, adenovirus, flavivirus, dengue virus, Mycobacteria tuberculosis, or Plasmodium falciparum.
- 15. The method of claim 14, wherein the fusion nucleic acid comprises an antigenic nucleic acid fragment encoding HIV p24 and the pathogenic infection is HIV infection.
- 16. The method of claim 15, wherein the fusion nucleic acid comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 18, 20, 24, and 28.
- 17. The method of claim 13, wherein the fusion nucleic acid is delivered by injection.
- **18**. The method of claim **17**, wherein the fusion nucleic acid is delivered via electroporation.
- 19. A method for preventing or treating pathogenic infection and/or tumor or cancer, comprising administering, to a subject in need of such prevention or treatment, an effective amount of a fusion protein of claim 3.
- 20. The method of claim 19, wherein the pathogenic infection is caused by a pathogen selected from human immunodeficiency virus (HIV), HSV, respiratory syncytial virus, rhinovirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, hepatitis F virus, hepatitis G virus, oncoviruses, human T-lymphotropic virus Type I (HTLV-1), influenza, bovine leukemia virus (BLV), Epstein-Barr virus, rotavirus, anpapillomavirus, *streptococcus, staphylococcus, E. coli, shigella, cy*tomegalovirus (CMV), respiratory syncytial virus, adenovirus, flavivirus, *Mycobacteria tuberculosis*, or *Plasmodium falciparum*.

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