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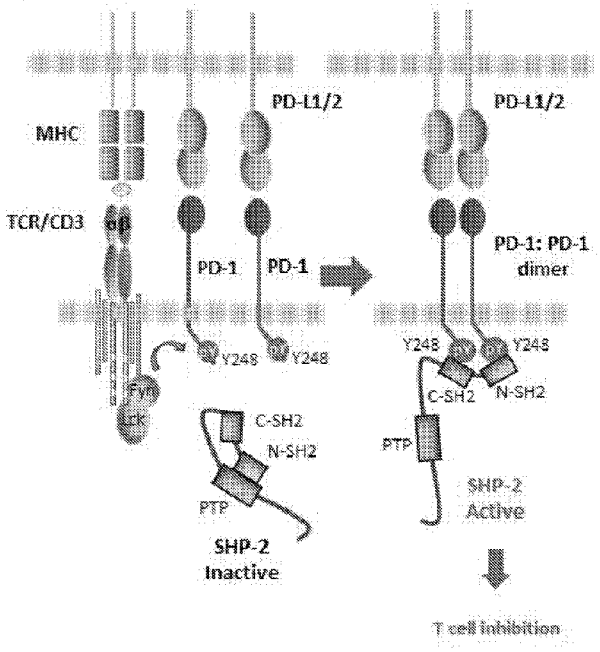
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(54) Title: MODULATION OF PD-1

FIG. 5F



(57) Abstract: The present disclosure provides compositions and methods for modulating immune checkpoint genes, for example, programmed death receptor (PD-1).

[Continued on next page]

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MODULATION OF PD-1

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/779,526, filed on December 14, 2018, the entire contents of which are incorporated herein.

FIELD OF THE DISCLOSURE

The disclosure is directed to compositions and methods for modulating immune checkpoint genes, for example, programmed death receptor-1 (PD-1).

GOVERNMENT INTEREST

This invention was made with government support under Grant No. RO1CA183605 and RO1CA183605S1 awarded by the National Institute of Health. The government has certain rights in the invention.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created about December 10, 2019, is named "BID-008PC_ST25.txt" and is about 16,398 bytes in size.

BACKGROUND

Immune checkpoint therapy, which often targets regulatory pathways in T cells to enhance anti-tumor immune responses, has led to important clinical advances and provides a new defense, at least, against cancer. The programmed death receptor (PD-1) has been shown to be involved in regulating the balance between T cell activation and T cell tolerance. Currently, antibodies against PD-1 or against its ligand PD-L1 are available for clinical uses; however, therapeutic responses have been observed only in a small number of patients, whereas the majority of patients have only transient responses or no response at all. No currently-available PD-1-related therapeutic compounds has been tested for its ability to trigger PD-1 dimerization or disrupt PD-1 dimerization. Accordingly, there exists a need for therapeutics that either disrupt PD-1 dimerization for enhancing a T cell response or trigger PD-1 dimerization for suppressing T cell responses. The present disclosure fulfills these needs and further provides other related advantages.

SUMMARY

Programmed cell death-1 (PD-1) is an inhibitory checkpoint receptor of the B7-CD28 family, which is upregulated upon T cell activation and plays a key role in peripheral T cell tolerance (*i.e.,* an inhibition of T cell activity). PD-1 also restrains anti-viral and anti-tumor T cell responses and is used by tumors to evade immune attack. PD-1-mediated inhibition relies on its interaction with SHP-2 by a previously-unknown mechanism. The cytoplasmic tail of PD-1 has one immunoreceptor tyrosine-based inhibitory motif (ITIM) at Y223 and one immunoreceptor tyrosine-based switch motif (ITSM) at Y248, which has an indispensable role in PD-1-mediated inhibitory function, yet their function in relation to SHP-2 remained unclear.

Disclosed herein is data showing that SHP-2 - *via* its amino terminal (N)-SH2 and carboxy terminal (C)-SH2 domains - bridges a first phosphorylated ITSM-Y248 residue on one PD-1 polypeptide and a second phosphorylated ITSM-Y248 residue on a second PD-1 polypeptide, thereby, forming a PD-1: PD-1 dimer. SHP-2's interaction with the two phosphorylated ITSM-Y248 residues (but not with two ITIM-Y223 residues or with one ITIM-Y223 residue and one ITSM-Y248 residue) activates SHP-2 and inhibits IL-2 production. Accordingly, the present invention relates, in part, to exploiting the geometry of the PD-1: SHP-2 interaction in methods, compounds, and compositions, *e.g.,* for treating cancer, which enhance T cell responses by disrupting PD-1 dimerization and in methods, compounds, and compositions, *e.g.,* for treating an autoimmune disease or disorder of for treating inflammation, which suppress T cell responses (*i.e.,* inducing T cell tolerance) by triggering PD-1 dimerization.

In one aspect, the present invention provides a method for treating cancer in a patient in need thereof, comprising: (a) selecting an agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity; and (b) administering the agent to the patient.

In embodiments, the agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity is a small molecule or peptide agent.

In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SHP-2 polypeptide, *e.g.,* is capable of disrupting an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2. In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 by preventing binding of the SH2 domain with a motif of the PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, *e.g.,* a motif comprising an immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 by preventing binding of an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) with the PD-1 polypeptide and/or by preventing binding of a

carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20) with the PD-1 polypeptide. In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and is capable of disrupting an interaction between a second PD-1 polypeptide and an carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20). In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between the first PD-1 polypeptide and the N-SH2 domain by preventing binding of the N-SH2 domain with a motif of the first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and is capable of disrupting an interaction between the second PD-1 polypeptide and the C-SH2 domain by preventing binding of the C-SH2 domain with a motif of the second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248. In embodiments, the motif of the first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and the motif of the second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, each comprises a portion of immunoreceptor tyrosine-based switch motif (ITSM). As used herein, the term "portion" includes a full-length ITSM or a fraction thereof in which the fraction retains the ability to bind a SH2 domain. The tyrosine 248 position is relative to the wild-type, human PD-1 polypeptide sequence: Accession Number: Q15116; SEQ ID NO: 21.

In embodiments, the small molecule or peptide agent is capable of binding to a SH2 domain of SHP-2. In embodiments, the small molecule or peptide agent is capable of binding to an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) or to a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20). In embodiments, the peptide agent is capable of binding to an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and to a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20). In embodiments, the peptide agent comprises at least one motif of a PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248. In embodiments, the motif comprises a portion of an immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent comprises at least two motifs of a PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, wherein the at least two motifs are separated by a linker. In embodiments, each of the at least two motifs of a PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, comprises a portion of an immunoreceptor tyrosine-based switch motif (ITSM). As used herein, the term "portion of an ITSM" includes a full-length ITSM or a fraction thereof in which the fraction retains the ability to bind a SH2 domain. In embodiments, the linker comprises at least 3 amino acids and/or the linker is at least about 35 Angstroms long.

In embodiments, the peptide agent, *e.g.,* that is capable of binding two SH2 domains, comprises: (a) a first amino acid sequence of SEQ ID NO: 1, 22, or 23, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, (b) a second amino acid sequence of SEQ ID NO: 1, 22, or 23, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, and (c) a linker comprising at least 3 amino acids between the first amino acid sequence and the second amino acid sequence, optionally, the linker separates the two amino acid sequences by at least about 35 Angstroms. In

embodiments, the peptide agent comprises the amino acid sequence of SEQ ID NO: 3, 9, 10, or 11, optionally comprising a mutation of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids. In embodiments, the mutation is not of the tyrosine 248, with reference to SEQ ID NO: 21. In embodiments, the peptide agent comprises or consists of SEQ ID NO: 3, 9, 10, or 11. In embodiments, the peptide agent comprises the amino acid sequence of SEQ ID NO: 3, 9, 10, 11, or 24, optionally comprising a mutation of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids. In embodiments, the mutation is not of the tyrosine 248, with reference to SEQ ID NO: 21. In embodiments, the peptide agent comprises or consists of SEQ ID NO: 3, 9, 10, 11, or 24.

In embodiments, the peptide agent which reduces PD-1 dimer activity is capable of binding to at least a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248; in this embodiment, the peptide agent lacks a phosphatase domain or comprises a non-functional phosphatase domain. In embodiments, the small molecule or peptide agent is capable of binding to the at least first PD-1 polypeptide at or near its immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent is capable of binding to the first PD-1 polypeptide and to an at least second PD-1 polypeptide. In embodiments, the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, or the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20. In embodiments, the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20. In embodiments, the first amino acid sequence and the second amino acid sequence are separated by a linker, *e.g.,* a linker comprising at least 3 amino acids and/or a linker that is at least about 35 Angstroms long. The arginine at amino acid position 32 in SEQ ID NO: 19 and the arginine at amino acid position 28 in SEQ ID NO: 20, respectively correspond to arginine 32 and arginine 138 of the wild-type, human SHP-2 polypeptide sequence: Accession Number: Q06124; SEQ ID NO: 18.

SEQ ID NO: 18 has the amino acid sequence of:

MTSRRWFHPNITGVEAENLLLTRGVDGSFLARPSKSNPGDFTLSVRRNGAVTHIKIQNTGDYYDLYGGEKF
ATLAELVQYYMEHHGQLKEKNGDVIELKYPLNCADPTSERWFHGHLSGKEAEKLLTEKGKHGSFLVRESQS

HPGDFVLSVRTGDDKGESNDGKSKVTHVMIRCQELKYDVGGGERFDSLTDLVEHYKKNPMVETLGTVLQL
KQPLNTTRINAAEIESRVRELSKLAETTDKVKQGFWEEFETLQQQECKLLYSRKEGQRQENKNKNRYKNILP
FDHTRVVLHDGDPNEPVSDYINANIIMPEFETKCNNSKPKKSYIATQGCLQNTVNDFWRMVFQENSRVIVMT
TKEVERGKSKCVKYWPDEYALKEYGVMRVRNVKESAAHDYTLRELKLSKVGQALLQGNTERTVWQYHFR
TWPDHGVPSDPGGVLDFLEEVHHKQESIMDAGPVVVHCSAGIGRTGTFIVIDILIDIIREKGVDCDIDVPKTIQ
MVRSQRSGMVQTEAQYRFIYMAVQHYIETLQRRIEEEQKSKRKGHEYTNIKYSLADQTSGDQSPLPPCTPT
PPCAEMREDSARVYENVGLMQQQKSFR

SEQ ID NO: 19 has the amino acid sequence of:

MTSRRWFHPNITGVEAENLLLTRGVDGSFLARPSKSNPGDFTLSVRRNGAVTHIKIQNTGDYYDLYGGEKF
ATLAELVQYYMEHHGQLKEKNGDVIELKYPLNCA

SEQ ID NO: 20 has the amino acid sequence of:

RWFHGHLSGKEAEKLLTEKGKHGSFLVRESQSHPGDFVLSVRTGDDKGESNDGKSKVTHVMIRCQELKYD
VGGGERFDSLTDLVEHYKKNPMVETLGTVLQLKQPLNC

SEQ ID NO: 21 has the amino acid sequence of:

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYR
MSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLR
AELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLK
EDPSAVPVFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPE
DGHCSWPL

In embodiments, the patient is undergoing treatment with an immune checkpoint immunotherapy selected from an agent that modulates one or more PD-1, programmed death-ligand 1 (PD-L1), or programmed death-ligand 2 (PD-L2).

In embodiments, the method further comprises administering an agent that modulates one or more of PD-1, PD-L1, or PD-L2. In embodiments, the administering of the agent that modulates one or more of PD-1, PD-L1, or PD-L2 and the administering of the agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity is sequential or simultaneous.

In embodiments, the agent that modulates PD-1 is an antibody or antibody format specific for PD-1. In embodiments, the antibody or antibody format specific for PD-1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific

antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody. In embodiments, the antibody or antibody format specific for PD-1 is selected from nivolumab, pembrolizumab, and pidilizumab.

In embodiments, the agent that modulates PD-L1 is an antibody or antibody format specific for PD-L1. In embodiments, the antibody or antibody format specific for PD-L1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody. In embodiments, the antibody or antibody format specific for PD-L1 is selected from atezolizumab, avelumab, durvalumab, and BMS-936559.

In embodiments, the agent that modulates PD-L2 is an antibody or antibody format specific for PD-L2. In embodiments, the antibody or antibody format specific for PD-L2 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

In embodiments, the administering of the agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity is by intratumoral, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or direct injection into cancer tissue.

In embodiments, the administering of the agent that modulates one or more of PD-1, PD-L1, or PD-L2 is by intratumoral, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or direct injection into cancer tissue.

In embodiments, the patient is predicted to be poorly responsive or non-responsive to the immune checkpoint immunotherapy or has presented as poorly responsive or non-responsive to the immune checkpoint immunotherapy.

In embodiments, the method reduces and/or mitigates one or more side effects of the immune checkpoint immunotherapy. In embodiments, the side effect is selected from decreased appetite, rashes, fatigue, pneumonia, pleural effusion, pneumonitis, pyrexia, nausea, dyspnea, cough, constipation, diarrhea, immune-mediated pneumonitis, colitis, hepatitis, endocrinopathies, hypophysitis, iridocyclitis, and nephritis. In embodiments, the method reduces the dose of the immune checkpoint immunotherapy. In embodiments, the method reduces number of administrations of the immune checkpoint immunotherapy. In embodiments, the method increases a therapeutic window of the immune checkpoint immunotherapy.

In embodiments, the method elicits a potent immune response in less-immunogenic tumors.

In embodiments, the method converts a tumor with reduced inflammation ("cold tumor") to a responsive, inflamed tumor ("hot tumor"). In embodiments, the method makes the cancer responsive or more responsive to a combination therapy of the immune checkpoint immunotherapy and one or more chemotherapeutic agents and/or radiotherapy. In embodiments, the chemotherapeutic agent is selected from one or more of daunorubicin, doxorubicin, epirubicin, idarubicin, adriamycin, vincristine, carmustine, cisplatin, 5-fluorouracil, tamoxifen, prodasone, sandostatine, mitomycin C, foscarnet, paclitaxel, docetaxel, gemcitabine, fludarabine, carboplatin, leucovorin, tamoxifen, goserelin, ketoconazole, leuprolide flutamide, vinblastine, vindesine, vinorelbine, camptothecin, topotecan, irinotecan hydrochloride, etoposide, mitoxantrone, teniposide, amsacrine, merbarone, piroxantrone hydrochloride, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine (Ara-C), trimetrexate, acivicin, alanosine, pyrazofurin, pentostatin, 5-azacitidine, 5-azacitidine, 5-Aza-5-Aza-2'-deoxycytidine, adenosine arabinoside (Ara-A), cladribine, ftorafur, UFT (combination of uracil and florafur), 5-fluoro-2'-deoxyuridine, 5-fluorouridine, 5'-deoxy-5-fluorouridine, hydroxyurea, dihydrolenchiorambucil, tiazofurin, oxaliplatin, melphalan, thiotepa, busulfan, chlorambucil, plicamycin, dacarbazine, ifosfamide phosphate, cyclophosphamide, pipobroman, 4-ipomeanol, dihydrolenperone, spiromustine, geldenamycin, cytochalasins, depsipeptide, 4'-cyano-3-(4-(*e.g.,* ZOLADEX), and 4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-3-methyl-3'-(trifluorometh- yl)propionanilide. In embodiments, the patient is predicted to be poorly responsive or non-responsive to the immune checkpoint immunotherapy based on expression of one or more of PD-1, PD-L1, or PD-L2, in the patient's biological specimen. In embodiments, the patient is predicted to be poorly responsive or non-responsive to an agent that modulates one or more of PD-1, PD-L1, and PD-L2 based on low on expression of PD-1, PD-L1, and PD-L2 in a tumor specimen from the patient. In embodiments, the patient is predicted to be poorly responsive or non-responsive to an agent that modulates one or more of PD-1, PD-L1, and PD-L2 based on a tumor proportion score (TPS) of less than about 49% for PD-L1 staining.

In embodiments, the cancer is selected from one or more of basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade

diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (*e.g.,* that associated with brain tumors), and Meigs' syndrome.

In another aspect, the present invention provides a method for treating an autoimmune disease or disorder or for treating inflammation in a patient in need thereof, comprising: (a) selecting an agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity; and (b) administering the agent to the patient.

In embodiments, the agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity is a small molecule or peptide agent. In embodiments, the small molecule or peptide agent is capable of stimulating an interaction between a PD-1 polypeptide and a SHP-2 polypeptide. In embodiments, the small molecule or peptide agent is capable of stimulating an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and an interaction between a second PD-1 polypeptide and a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20).

In embodiments, the peptide agent is capable of binding to a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and to an at least second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248. In embodiments, the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20. In embodiments, the first amino acid sequence and the second amino acid sequence are separated by a linker, *e.g.,* a linker comprising at least 3 amino acids and/or a linker that is at least about 35 Angstroms long.

In embodiments, the peptide agent comprises: (a) a first amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, (b) a second amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, and (c) a linker comprising at least 3 amino acids

between the first amino acid sequence and the second amino acid sequence, optionally, the linker separates the two amino acid sequences by at least about 35 Angstroms.

In embodiments, the peptide agent further comprises a functional phosphatase domain. In embodiments, the functional phosphatase domain comprises a protein tyrosine phosphatase (PTP) domain. As used herein, a phosphatase domain is any portion, *e.g.,* a catalytic domain, of a phosphatase enzyme that is capable of removing a phosphate group from a phosphorylated amino acid, *e.g.,* a phosphorylated tyrosine. In embodiments, the peptide agent comprises the amino acid sequence of SEQ ID NO: 18. In embodiments, the peptide agent does not comprise of the amino acid sequence of SEQ ID NO: 18.

In embodiments, wherein the autoimmune disease or disorder is selected from multiple sclerosis, diabetes mellitus, lupus, celiac disease, Crohn's disease, ulcerative colitis, Guillain-Barre syndrome, scleroderms, Goodpasture's syndrome, Wegener's granulomatosis, autoimmune epilepsy, Rasmussen's encephalitis, Primary biliary sclerosis, Sclerosing cholangitis, Autoimmune hepatitis, Addison's disease, Hashimoto's thyroiditis, Fibromyalgia, Menier's syndrome; transplantation rejection (*e.g.,* prevention of allograft rejection) pernicious anemia, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, Sjogren's syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, and Grave's disease.

In embodiments, the inflammation is acute inflammation, chronic inflammation, respiratory disease, atherosclerosis, restenosis, asthma, allergic rhinitis, atopic dermatitis, septic shock, rheumatoid arthritis, inflammatory bowel disease, inflammatory pelvic disease, pain, ocular inflammatory disease, celiac disease, Leigh Syndrome, Glycerol Kinase Deficiency, Familial eosinophilia (FE), autosomal recessive spastic ataxia, laryngeal inflammatory disease; Tuberculosis, Chronic cholecystitis, Bronchiectasis, Silicosis and other pneumoconioses.

In embodiments, the patient is undergoing treatment or has undergone treatment with an immunosuppressive agent.

In embodiments, wherein the method further comprises administering an immunosuppressive agent. In embodiments, the administering of the agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity and the administering of the immunosuppressive agent is sequential or simultaneous.

In embodiments, the immunosuppressive agent is a steroidal anti-inflammatory agent or a non-steroidal anti-inflammatory agent (NSAID), selected from salicylic acid, acetyl salicylic acid, methyl salicylate, glycol salicylate, salicylmides, benzyl-2,5-diacetoxybenzoic acid, ibuprofen, fulindac, naproxen, ketoprofen, etofenamate, phenylbutazone, and indomethacin. In embodiments, the immunosuppressive agent is a steroid, such as a corticosteroids selected from hydroxyltriamcinolone, alpha-methyl dexamethasone, beta-methyl betamethasone, beclomethasone dipropionate, betamethasone benzoate, betamethasone dipropionate, betamethasone valerate,

clobetasol valerate, desonide, desoxymethasone, dexamethasone, diflorasone diacetate, diflucortolone valerate, fluadrenolone, fluclorolone acetonide, flumethasone pivalate, fluosinolone acetonide, fluocinonide, flucortine butylester, fluocortolone, fluprednidene (fluprednylidene) acetate, flurandrenolone, halcinonide, hydrocortisone acetate, hydrocortisone butyrate, methylprednisolone, triamcinolone acetonide, cortisone, cortodoxone, flucetonide, fludrocortisone, difluorosone diacetate, fluradrenolone acetonide, medrysone, amcinafel, amcinafide, betamethasone and the balance of its esters, chloroprednisone, clocortelone, clescinolone, dichlorisone, difluprednate, flucloronide, flunisolide, fluoromethalone, fluperolone, fluprednisolone, hydrocortisone, meprednisone, paramethasone, prednisolone, prednisone, beclomethasone dipropionate. In embodiments, the immunosuppressive agent is a cytostatic such as alkylating agents, antimetabolites (*e.g.,* azathioprine, methotrexate), cytotoxic antibiotics, antibodies (*e.g.,* basiliximab, daclizumab, and muromonab), anti-immunophilins (*e.g.,* cyclosporine, tacrolimus, sirolimus), inteferons, opioids, TNF binding proteins, mycophenolates, and small biological agents (*e.g.,* fingolimod, myriocin).

In yet another aspect, the present invention provides a method of making an agent effective for the treatment of a cancer, comprising: (a) identifying the agent by screening for a disruption or decrease of SHP-2-mediated programmed cell death protein-1 (PD-1) dimerization or of PD-1 dimer activity; and (b) formulating the agent for administration to a patient having a cancer.

In embodiments, the agent is a small molecule or peptide agent. In embodiments, the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and a SHP-2 polypeptide. In embodiments, the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2. In embodiments, the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 which prevents their interaction at the immunoreceptor tyrosine-based switch motif (ITSM) of the PD-1 polypeptide. In embodiments, the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 which prevents their interaction at tyrosine 248 of PD-1. In embodiments, the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19), *e.g.,* at the arginine at amino acid position 32 of SEQ ID NO: 19, or disrupts or decreases an interaction between the PD-1 polypeptide and a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20), *e.g.,* at the arginine at amino acid position 28 of SEQ ID NO: 20. In embodiments, the small molecule or peptide agent disrupts or decreases an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19), *e.g.,* at the arginine at amino acid position 32 of SEQ ID NO: 19, and disrupts or decreases an interaction between a second PD-1 polypeptide and an carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20), *e.g.,* at the arginine at amino acid position 28 of SEQ ID NO: 20. In embodiments, the peptide agent that disrupts or decreases PD-1 dimer activity is capable of binding to at least a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248; in this embodiment,

the peptide agent lacks a phosphatase domain or comprises a non-functional phosphatase domain. In embodiments, the peptide agent is capable of binding to the at least first PD-1 polypeptide at or near its immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent is capable of binding to the first PD-1 polypeptide and to an at least second PD-1 polypeptide. In embodiments, the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM)

In embodiments, the peptide agent comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, or the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20. In embodiments, the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20. In embodiments, the first amino acid sequence and the second amino acid sequence are separated by a linker, *e.g.,* a linker comprising at least 3 amino acids and/or a linker that is at least about 35 Angstroms long.

In an aspect, the present invention provides a method of making an agent effective for the treatment of an autoimmune disease or disorder of for the treatment of inflammation, comprising: (a) identifying the agent by screening for a stimulation or increase of SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity; and (b) formulating the agent for administration to a patient having a autoimmune disease or disorder or inflammation.

In embodiments, the agent which stimulates or increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity is a small molecule or peptide agent. In embodiments, the small molecule or peptide agent is capable of stimulating an interaction between a PD-1 polypeptide and a SHP-2 polypeptide. In embodiments, the small molecule or peptide agent is capable of stimulating an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and an interaction between a second PD-1 polypeptide and a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20). In embodiments, the peptide agent is capable of binding to a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and to an at least second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248. In embodiments, the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent comprises an

amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20. In embodiments, the first amino acid sequence and the second amino acid sequence are separated by a linker, *e.g.,* a linker comprising at least 3 amino acids and/or a linker that is at least about 35 Angstroms long.

In embodiments, the peptide agent comprises: (a) a first amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, (b) a second amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, and (c) a linker comprising at least 3 amino acids between the first amino acid sequence and the second amino acid sequence, optionally, the linker separates the two amino acid sequences by at least about 35 Angstroms. In embodiments, the peptide agent further comprises a functional phosphatase domain. In embodiments, the functional phosphatase domain comprises a protein tyrosine phosphatase (PTP) domain. As used herein, a phosphatase domain is any portion of a phosphatase enzyme that is capable of removing a phosphate groups from phosphorylated amino acid, *e.g.,* a phosphorylated tyrosine. In embodiments, the peptide agent comprises the amino acid sequence of SEQ ID NO: 18. In embodiments, the peptide agent does not comprise the amino acid sequence of SEQ ID NO: 18.

In another aspect, the present invention provides a method for predicting a cancer patient response to an immune checkpoint immunotherapy, comprising determining the presence of SHP-2-mediated PD-1 dimerization in a biological sample from the patient, wherein the presence of SHP-2-mediated PD-1 dimerization is indicative of an inhibitory immune signal and a likelihood of responding to the immune checkpoint immunotherapy.

In embodiments, the immune checkpoint immunotherapy is an agent that modulates one or more of PD-1, PD-L1, or PD-L2. In embodiments, the agent that modulates PD-1 is an antibody or antibody format specific for PD-1.

In embodiments, the antibody or antibody format specific for PD-1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody. In embodiments, the antibody or antibody format specific for PD-1 is selected from nivolumab, pembrolizumab, and pidilizumab.

In embodiments, the agent that modulates PD-L1 is an antibody or antibody format specific for PD-L1. In embodiments,

the antibody or antibody format specific for PD-L1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody. In embodiments, the antibody or antibody format specific for PD-L1 is selected from atezolizumab, avelumab, durvalumab, and BMS-936559.

In embodiments, the agent that modulates PD-L2 is an antibody or antibody format specific for PD-L2. In embodiments, the antibody or antibody format specific for PD-L2 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

In yet another aspect, the present invention provides method for treating cancer, comprising: (a) evaluating a subject's likelihood of response to an immune checkpoint immunotherapy, comprising evaluating a level of SHP-2-mediated PD-1 dimerization in a biological sample from the patient, wherein a presence or high level of SHP-2-mediated PD-1 dimerization is indicative of a cancer that is suitable for immune checkpoint immunotherapy; and (b) administering an immune checkpoint immunotherapy to the patient.

In embodiments, the immune checkpoint immunotherapy is an agent that modulates one or more of PD-1, PD-L1, or PD-L2. In embodiments, the agent that modulates PD-1 is an antibody or antibody format specific for PD-1. In embodiments, the antibody or antibody format specific for PD-1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody. In embodiments, the antibody or antibody format specific for PD-1 is selected from nivolumab, pembrolizumab, and pidilizumab.

In embodiments, the agent that modulates PD-L1 is an antibody or antibody format specific for PD-L1. In embodiments, the antibody or antibody format specific for PD-L1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody. In embodiments, the antibody or antibody format specific for PD-L1 is selected from atezolizumab, avelumab, durvalumab, and BMS-936559. In embodiments, the agent that modulates PD-L2 is an antibody or antibody format specific for PD-L2.

In embodiments, the antibody or antibody format specific for PD-L2 is selected from one or more of a monoclonal

antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

Any aspect or embodiment as disclosed herein can be combined with any other aspect or embodiment as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A to **FIG. 1D** show that phosphorylation of PD-1 immunoreceptor tyrosine-based switch motif (ITSM) Y248 by TCR proximal Src family kinases Fyn and Lck but not ZAP-70 is required for interaction with SHP-2. In **FIG. 1A**, Jurkat-PD1 cells were left unstimulated or stimulated with αCD3/CD28/IgG or αCD3/CD28/PDL1-Ig beads for the indicated times, lysates were prepared followed by immunoprecipitation with anti-PD-1 antibody, SDS PAGE and western blot with antibodies for SHP-2 and PD-1. SHP-2 expression in whole cell lysates was examined (bottom panel). In **FIG. 1B**. J-PD1 (Jurkat-PD-1) and J-PD1-Y248 (Jurkat-PD-1-Y248F) cells were either left unstimulated or stimulated with αCD3/CD28/IgG or aCD3/CD28/PDL1-Ig beads for 5 minutes, cell lysates were prepared followed by immunoprecipitation with anti-PD-1 antibody, SDS-PAGE and western blot with antibodies specific for pPD-1-Y248, SHP-2 and PD-1. In **FIG. 1C**, left panel, COS cells were co-transfected with PD-1, SHP-2, and either the kinase active (+) or inactive (-) form of either Fyn, Lck or ZAP-70. After PD-1 immunoprecipitation of cell lysates and SDS-PAGE, immunoblot was performed with antibodies for SHP-2, PD-1 or an antibody specific for phosphorylated tyrosine of PD-1 ITSM Y248. Right panel: Expression of the indicated proteins in the same whole cell lysates was assessed. In **FIG. 1D**, left panel, COS cells were co-transfected with SHP-2, Fyn kinase active and either PD-1 wild type, PD-1 Y223F, PD-1 Y248F or PD-1 Y223F/Y248F. PD-1 immunoprecipitation was performed in cell lysates followed by SDS-PAGE and immunoblot with the indicated antibodies. **FIG. 1D**, Right panel shows expression of the same proteins in the same whole cell lysates was assessed. Results are representative of five independent experiments.

FIG. 2A to FIG. 2H shows that interaction of both SH2 domains of SHP-2 is required for SHP-2 binding to PD-1. **FIG. 2A** is a schematic representation of the GST-SHP-2 fusion proteins: SHP-2 full-length (GST-SHP-2-FL); GST-SHP-2-PTP, which contains only the protein tyrosine phosphatase (PTP) domain, GST-SHP-2- DNSH2, which lacks the N-terminal SH2 domain; GST-SHP-2-N-SH2 and GST-SHP-2-C-SH2, which contain only the N-SH2 and the C-SH2 domain, respectively. In **FIG. 2B**, Jurkat-PD-1 cells were either left unstimulated or were stimulated with αCD3/CD28/IgG or aCD3/CD28/PDL1-Ig beads for 5 minutes at 37°C followed by lysate preparation. Pull down assays were performed with the indicated GST-SHP-2 fusion proteins followed by SDS-PAGE and PD-1 immunoblot. Pull down with GST alone served as negative control. In **FIG. 2C**, COS cells were transfected with kinase active Fyn, PD-1 WT and either SHP-2-WT, SHP-2-R32A or SHP-2-R138A mutants FLAG-tagged (top two panels); Fyn, PD-1-Y223F

and either SHP-2-WT, SHP-2-R32A or SHP-2-R138A mutants FLAG-tagged (middle two panels); Fyn, PD-1-Y248F and either SHP-2-WT, SHP-2-R32A or SHP-2-R138A mutants FLAG-tagged (bottom two panel). Immunoprecipitation of cell lysates was performed with anti-PD-1 antibody followed by SDS-PAGE and immunoblot with FLAG- or PD-1-specific antibodies. Expression of the indicated proteins in the same whole cell lysates was assessed. Results are representative of four separate experiments. **FIG. 2D** shows that ITSM-pY248 but not immunoreceptor tyrosine-based inhibitory motif (ITIM)-pY223 phosphopeptide binds with SHP-2 SH2 domains. For assessment of ITIM-pY223 and ITSM-pY248 binding to SHP-2 SH2 domains, SHP-2 amino acids 1-225, which only contains the SHP-2 N-SH2 and C-SH2 domains in their natural tandem sequence (referred to as SHP-2 tandem-SH2), was used. 20 μM of SHP-2 tandem-SH2 (N-SH2+C-SH2) were mixed with either ITIM-pY223 or ITSM-pY248 phosphopeptides at various molar ratios as indicated, incubated for one hour at room temperature and binding was examined by Native PAGE followed by Coomassie blue staining. **FIG. 2E** is a model of PD-1: SHP-2 interaction after PD-1 phosphorylation. **FIG. 2F** is a gel showing binding of ITIM-pY223 or ITSM-pY248 phosphopeptide to 1SHP-2 SH2 domains by native PAGE binding assay. Detection of an electrophoretic mobility shift of t-SHP-2 was preserved by a peptide containing phosphorylation of Y248 (PD-1cyto-ITIM-pITSM) but not by a peptide containing phosphorylation of Y223 (PD-1cyto-pITIM-ITSM. **FIG. 2G** is a model showing that PD-1 ITSM-pY248 serves as the high affinity binding site for one of the SHP-2 SH2 domains thereby being a pre-requisite for the binding of the second SH2 domain on PD-1 ITIM Y223 that serves the low affinity interaction site. **FIG. 2H** is a model showing that both SH2 domains interact with ITSM-pY248 and because the PD-1 molecule has only one ITSM-Y248, SHP-2 binds phosphorylated ITSM-pY248 residues in two PD-1 molecules using its N-SH2 domain for one PD-1 and its C-SH2 domain for a second PD-1 to form a PD-1 dimer.

FIG. 3A to **FIG. 3F** shows a surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) analysis of SHP-2 interaction with PD-1 ITSM-pY248. In **FIG. 3A**, PD-1 phosphotyrosyl ITSM-Y248 peptide was immobilized on CM5 BIAcore chip and binding of the indicated concentrations of purified proteins SHP2-WT (left panel), SHP2-R32A (middle Panel), and SHP2-R138A (right Panel), was tested. Sensograms of 900 seconds (15 min) association and 900 sec dissociation time were analyzed and KD values were calculated. **FIG. 3B** shows binding of the purified N-SH2 (left panel) and C-SH2 (right Panel), domains of SHP-2 on PD-1 phosphotyrosyl ITSM-Y248 peptide that was assessed in **FIG. 3A**. Results are representative of five experiments with full length SHP-2 proteins and three experiments with single SH2 domains. **FIG. 3C** (PD-1 ITSM-pY248 and PD-1 ITIM-pY223 phosphopeptides coated surface) and **FIG. 3D** (PD-1 ITIM-pY223-coated surface) show SPR of the interaction of purified SHP-2 protein with immobilized PD-1 ITSM-pY248 and PD-1 ITIM-pY223 phosphopeptides. **FIG. 3E** shows ITC of the PD-1cyto-pITIM-pITSM: t-SHP-2 interaction, which occurred at 1:1 stoichiometry. **FIG. 3F** shows ITC of the PD-1cyto-ITIM-pITSM: t-SHP-2 interaction, which occurred at 2:1 stoichiometry.

FIG. 4A to **FIG. 4G** shows that SHP-2 bridges two PD-1 polypeptides *via* PD-1 ITSM pY248. **FIG. 4A** shows that

NanoBiT proximity assay allows detection of protein: protein interaction in living cells by employing a split luciferase enzyme. PD-1-LgBiT+PD-1-SmBiT would induce luciferase activity only if two PD-1 polypeptides stably interact thereby forming an active luciferase enzyme. **FIG. 4B** shows HEK-293 cells co-transfected with PD-1-SmBiT and PD-1-LgBiT together with kinase active (+) or inactive (-) Fyn and either SHP-2 WT, control empty vector or the mutants SHP-2-R32A, SHP-2-R138A or double mutant SHP-2-R32A/R138A (DM). Complex formation between PD-1-SmBiT and PD-1-LgBiT was assessed by luciferase assay. Comparison of complex formation among the different conditions was performed by 2-way ANOVA and Tukey's multiple comparisons test (****P<0.0001, PD1sm+PD1Lg+SHP2WT+Fyn (+) versus all other samples, **P<0.005, PD1sm+PD1Lg+SHP2R32A+Fyn (+) versus PD1sm+PD1Lg+SHP2DM+Fyn (+), n=6 experiments). **FIG. 4C** shows HEK-293 cells that were co-transfected with PD-1-SmBiT and PD-1-LgBiT carrying either PD-1 WT or PD-1-Y248F, together with SHP-2 WT and either kinase active (+) of kinase inactive (-) Fyn. Complex formation between PD-1-SmBiT and PD-1-LgBiT was assessed by luciferase assay. **FIG. 4D** shows HEK-293 cells that were co-transfected with PD-1-SmBiT and PD-1-LgBiT together with SHP-2 WT and either kinase active (+) of kinase inactive (-) Fyn. hPD-L1-Ig (dimer), hPD-L1 monomer or IgG were added in the culture and complex formation between PD-1-SmBiT and PD-1-LgBiT was assessed by luciferase assay. **FIG. 4E** shows that when vehicle control (0) or increasing amounts of the allosteric SHP-2 inhibitor SHP099 were added in the culture and complex formation between PD-1-SmBiT and PD-1-LgBiT was assessed by luciferase assay. (H: baseline luciferase activity obtained when cells are transfected with PD-1-SmBiT and PD-1-LgBiT, vector and kinase (-) Fyn). **FIG. 4F** and **FIG. 4G** show, respectively, primary human T cells and Jurkat T cells transiently co-transfected with PD-1 NanoBiT constructs PD1-LgBiT and PD1-SmBiT and either SHP-2-WT, SHP-2-R32A, SHP-2-R138A or the double mutant SHP-2-DM. Cells were co-cultured with Raji-PD-L1 with or without loaded SEE and complex formation between PD-1-SmBiT and PD-1-LgBiT was assessed 6 hours later by luciferase assay. Comparisons were made as in **FIG. 4B** (****P<0.0001, SHP-2-WT+SEE versus SHP-2-DM+SEE, ***P<0.0005, SHP-2-WT+SEE versus SHP-2-R32A+SEE, **P<0.005, SHP-2-WT+SEE versus SHP-2-R138A+SEE n=3 experiments).

FIG. 5A to **FIG. 5G** show that the interaction of SHP-2 SH2 domains with two PD-1 ITSM pY248 residues is required for activation of SHP-2 phosphatase activity. **FIG. 5A** shows SHP-2-WT incubated with 20 μM DiFMUP (6,8-difluoro-4-methylumbelliferyl phosphate) as substrate in the presence of the indicated phosphotyrosyl peptides and phosphatase activity was assessed as described in Methods. A representative of n=3 experiments is shown. **FIG. 5B** shows SHP-2-WT incubated with bpITSM phosphotyrosyl peptides containing a 4-, 2- or 1-Ahx spacer; pIRSY727 was used as negative control. Phosphatase activity was assessed as in **FIG. 5A** and **FIG. 5C**. SHP-2-WT, SHP-2-R32A or SHP-2-R138A was incubated with the indicated concentrations of bpITSM phosphotyrosyl peptide containing a 4-Ahx spacer and phosphatase activity was assessed. **FIG. 5D** shows that when SHP-2-WT is incubated with 20 μM DiFMUP (6,8-difluoro-4-methylumbelliferyl phosphate) as substrate in the presence of the indicated phosphotyrosyl bpITSM peptide (0.01 μM) either alone or with increasing amounts of monophosphorylated pITIM or monophosphorylated

pITSM peptide and phosphatase activity was assessed. Results are representation of three independent experiments. **FIG. 5E** shows Jurkat T cells stably expressing PD-1-WT (J-PD-1), PD-1-Y223F (J-PD-1-Y223F) or PD-1-Y248F (J-PD-1-Y248F) were co-cultured with Raji-control or Raji-PD-L1 cells loaded with SEE. Where indicated, anti-PD-1 blocking antibody or isotype control was added in the cultures. Culture supernatants were collected at 24 hours and IL-2 production was measured. Results are expressed as % of maximum IL-2 production induced in each cell line by Raji control cells loaded with SEE (****P<0.0001, n=3 experiments). **FIG. 5F** is a model for PD-1: PD-1 bridging by SHP-2 and activation of SHP-2 phosphatase activity: An "inside-out" regulation of PD-1: PD-1 dimer complex formation is initiated by TCR-mediated activation of TCR proximal Src family kinases, such as Fyn or Lck, and requires binding of both SH2 domains of SHP-2 to PD-1 tyrosine phosphorylated on ITSM-pY248 in the cytoplasmic tail bringing together two PD-1 polypeptides. Binding of SHP-2 brings together a PD-1 dimer and results in SHP-2 phosphatase activation proximal to the TCR and inhibition of activated T cell responses. Phosphatase activity was assessed in **FIG. 5G**, and bpITSM-Ahx10 (SEQ ID NO: 24) caused a higher increase in SHP-2 activation that was measurable only when using 3x lower enzyme concentrations than in all other phosphatase assays.

FIG. 6A to **FIG. 6C** shows the regulation of SHP-2 activation and unique structural properties of SHP-2 SH2 domains. **FIG. 6A** shows that SHP-2 contains two tandem SH2 domains, N-terminal (N-SH2) and C-terminal SH2 (C-SH2), followed by a single protein tyrosine phosphatase (PTP) domain, and a C-terminal hydrophobic tail with two tyrosine phosphorylation sites. **FIG. 6B** shows that exposure of cells to a variety of extracellular stimuli triggers the binding of SHP-2 *via* its SH2 domains to tyrosine phosphorylated receptors for growth factors as well as to tyrosine-phosphorylated docking proteins such as insulin receptor substrates (IRSs). At the basal state, the N-SH2 domain of SHP-2 binds the phosphatase domain in an auto-inhibitory closed conformation and directly blocks its active phosphatase site. Interaction of the N-SH2 domain with phosphotyrosine peptide disrupts its interaction of N-SH2 with the phosphatase active site and activates the enzyme. The C-SH2 domain contributes binding energy and specificity but does not have a direct role in enzymatic activation. **FIG. 6C** shows the two SH2 domains of SHP-2 have a relatively fixed and a roughly antiparallel or roughly perpendicular orientation relative to one another, with the phosphopeptide-binding sites lying on the surface of the polypeptide and widely spaced. This relative fixed orientation of SH2 domains is stabilized by a disulphide bond and a small hydrophobic patch within the interphase that separates the phosphopeptide binding sites.

FIG. 7A to **FIG. 7C** show the generation of PD-1-expressing J-PD-1 and J-PD-1Y248F cells. **FIG. 7A** shows Jurkat T cells transfected with human PD-1 cDNA expressed in pEF6 vector and stable PD-1+ cells were generated by antibiotic selection. Cell lines were subcloned and stable clones were generated. Experiments were performed in polyclonal cell lines and clones. Surface expression of PD-1 in subclone J-PD-1 is shown. **FIG. 7B** shows Jurkat T cells transfected with human PD-1 cDNA in which tyrosine 248 was mutated to phenylalanine. Generation and selection of stable cell

lines and clones was done as in **FIG. 7A** the surface expression of PD-1 in subclone J-PD-1-Y248F is shown.

FIG. 8A shows a densitometric analysis of **FIG. 1A**. The abundance of SHP-2 normalized to that of immunoprecipitated PD-1 at each time point and expressed as the fold change over the levels obtained in unstimulated cells (defined as 1). Fold changes are compared between unstimulated and stimulated cells at each time point (*P < 0.05) or between cells stimulated with or without PD-1 ligation at each time point (*P < 0.05). Data are presented as the means ± SEM, n = 5 experiments. **FIG. 8B** shows a densitometric analysis of **FIG. 1B**. The abundance of SHP-2 and pPD1(Y248) was normalized to that of immunoprecipitated PD-1 and was expressed as the fold change over the values obtained from unstimulated J-PD1 cells (defined as 1). Fold changes in each experimental condition are compared to the values obtained from unstimulated cells (*P < 0.05) and between J-PD1 and J-PD1(Y248F) in each condition ("P < 0.05). Data are presented as the means ± SEM, n = 5 experiments. **FIG. 8C** shows a densitometric analysis of **FIG. 1C**. In cells transfected with kinase active (+) or inactive (-) Fyn, Lck or ZAP70, the abundance of SHP-2 and pPD1(Y248) normalized to that of immunoprecipitated PD-1 was expressed as the fold change over the values obtained with the kinase inactive (-) form (defined as 1). Fold changes were compared between the corresponding kinase active and inactive forms (*P < 0.05) or between the Fyn active and Lck active forms (*P < 0.05). Data are presented as the means ± SEM, n = 5 experiments. **FIG. 8D** shows a densitometric analysis of **FIG. 1D**. The abundance of SHP-2 normalized to that of immunoprecipitated PD-1 in each condition was expressed as the fold change over the values obtained in samples expressing PD-1-WT (defined as 1). Fold changes are compared to the values obtained in samples expressing PD-1-WT < 0.05) and in samples expressing PD-1-Y223F (*P< 0.05). Data are presented as the means ± SEM, n = 5 experiments.

FIG. 9 shows Jurkat-PD1 cells that were left unstimulated (0) or stimulated with beads coated with either IgG or PDL1-Ig for the indicated times, lysates were prepared followed by immunoprecipitation with anti-PD-1 antibody, SDS-PAGE and western blot with antibodies for SHP-2 and PD-1. SHP-2 expression in whole cell lysates was also examined (bottom panel).

FIG. 10A to **FIG. 10C** show that Fyn is required for PD-1(Y248) phosphorylation and interaction with SHP-2. **FIG. 10A** shows T cells that were purified from spleens and lymph nodes of WT and Fyn-KO mice and were cultured for 72h with anti-CD3 and anti-CD28 mAb and expression of PD-1 was examined by flow cytometry. **FIG. 10B** shows that after resting in RPMI and 2% FBS for 10 hours, the cells were either left unstimulated (0) or stimulated with aCD3/CD28/IgG- or aCD3/CD28/PDL1-19-coated beads for 3 minutes. Cell lysates were prepared followed by immunoprecipitation with anti-PD-1 antibody, SDS-PAGE and western blot with antibodies specific for SHP-2, pPD-1(Y248) and PD-1. Expression of PD-1 and SHP-2 in whole cell lysates was also examined. **FIG. 10C** shows a densitometric analysis of the immunoprecipitation data shown in **FIG. 10B**. The abundance of SHP-2 and pPD1(Y248) was normalized to that

of immunoprecipitated PD-1 and was expressed as fold change over the values obtained in unstimulated (0) WT cells (defined as 1). Fold changes are compared between unstimulated and stimulated cells in each strain *P < 0.05) and between WT and Fyn-KO cells in each stimulation condition (*P < 0.05). Data are presented as the means ± SEM, n = 3 experiments.

FIG. 11A to **FIG. 11C** show that Lck is required for PD-1(Y248) phosphorylation and interaction with SHP-2. **FIG. 11A** shows that the lck-deficient Jurkat T cell line was transfected with human PD-1 cDNA expressed in pEF6 vector and stable PD-1+ cells lines and clones were generated by antibiotic selection. Comparable expression levels of PD-1 in J-PD1 and JCaM1.6-PD1 stably transfected with human PD-1 was confirmed by flow cytometry. Comparable expression of CD3 and CD28 was also confirmed by flow cytometry (not shown). **FIG. 11B** shows J-PD1 and J-CaM1.6-PD1 cells that were left unstimulated (0) or stimulated with αCD3/CD28/IgG or aCD3/CD28/PDL1-Ig beads for the indicated times; lysates were prepared followed by immunoprecipitation with anti-PD-1 antibody. SDS-PAGE and western blot with antibodies for SHP-2, pPD-1(Y248) and PD-1. **FIG. 11C** shows a densitometric analysis of the data shown in **FIG. 11B**. The abundance of SHP-2 and pPD-1(Y248) was normalized to that of immunoprecipitated PD-1 at each time point and was expressed as fold change over the values obtained in unstimulated cells (defined as 1). Fold changes are compared between unstimulated and stimulated cells at each time point (*P < 0.05) or between cells activated with or without PD-1 ligation at each time point (*P < 0.05). Data are presented as the means ± SEM, n = 3 experiments.

FIG. 12A shows a densitometric analysis of **FIG. 2B**. Jurkat-PD-1 cells were either left unstimulated or were stimulated with αCD3/CD28/IgG or αCD3/CD28/PDL1-Ig beads for 5 minutes at 37°C followed by lysate preparation. Pull down assays were performed with the indicated GST-SHP-2 fusion proteins followed by SDS-PAGE and PD-1 immunoblot. The abundance of PD-1 pulled down by SHP-2-GST in each sample was normalized to that of total PD-1 and expressed as the fold change over the values obtained by GST-SHP2-FL pull down using lysates from unstimulated cells (defined as 1). Fold changes of αCD3/CD28/PDL1-Ig-stimulated samples are compared to each corresponding unstimulated (*P < 0.05) or αCD3/aCD28/Ig-stimulated sample (*P < 0.05). Data are presented as the means ± SEM, n = 4 experiments. **FIG. 12B** shows a densitometric analysis of **FIG. 2C**. COS cells were transfected with kinase active Fyn, PD-1 WT and either SHP-2-WT, SHP-2-R32A or SHP-2-R138A mutants FLAG-tagged (top panel); Fyn, PD-1-Y223F and either SHP-2-WT, SHP-2-R32A or SHP-2-R138A (middle panel); Fyn, PD-1-Y248F and either SHP-2-WT, SHP-2-R32A or SHP-2-R138A (bottom panel). Immunoprecipitation of cell lysates was performed with anti-PD-1 antibody followed by SDS-PAGE and immunoblot with FLAG- or PD-1-specific antibodies. In each sample, the abundance of SHP-2 WT (FLAG) coprecipitated with PD-1 was normalized to that of immunoprecipitated PD-1 and was expressed as fold change over the value obtained in cells transfected with SHP-2 WT and PD-1 WT (defined as 1). Fold changes of SHP-2 immunoprecipitated with PD-1 were compared among cells transfected with SHP-2 WT, SHP-2 R32A or

SHP-2 R138A (*P < 0.05). Data are presented as the means ± SEM, n = 4 experiments.

In **FIG. 12C**, primary human T cells were activated for 72h with aCD3 (100 ng/ml) and aCD28 (300 ng/ml) mAbs. The cells were then transfected with the indicated FLAG-tagged SHP-2 constructs or empty vector as described, and cell lysates were prepared and PD-1 immunoprecipitation was performed followed by SDS-PAGE and immunoblot with FLAG- or PD-1-specific antibody. Densitometric analysis of **FIG. 12C** is shown in **FIG. 12D.** The abundance of SHP-2 (FLAG) was normalized to that of immunoprecipitated PD-1 in each indicated condition and was expressed as the fold change over the values obtained in samples transfected with SHP-2-WT (defined as 1). Fold changes are compared to the values obtained in samples transfected with SHP-2 WT (213 < 0.05, n = 3 experiments). WCL: whole cell lysates.

FIG. 13A shows that PD-1 interacts with both SH2 domains of SHP-2 in primary human T cells. Human PBMC were cultured with PHA for 72 hours and T cells were purified, rested overnight in RPM, with 2.5% FBS and subsequently were either left unstimulated or were stimulated with αCD3/CD28/1gG or αCD3/CD28/PDL1-Ig beads for 5 minutes at 37°C followed by lysate preparation. Pull down assays were performed with the indicated GST-SHP-2 fusion proteins followed by SDS-PAGE and immunoblot with PD-1-specific antibody. Pull down with GST alone served as negative control. **FIG. 13B** shows a densitometric analysis of results shown in **FIG. 13A**. The abundance of PD-1 pulled down by SHP-2-GST in each sample was normalized to that of total PD-1 and was expressed as fold change over the value obtained after pull down with GST-SHP2-FL using lysates from unstimulated cells (defined as 1). Fold changes of αCD3/CD28/PDL1-Ig-stimulated samples in the pull down with each different GST-SHP-2 fusion protein are compared to the values of the corresponding unstimulated (*P < 0.05) or αCD3/αCD28/Ig-stimulated sample (*P < 0.05). Data are presented as the means ± SEM, n = 4 experiments.

FIG. 14A to FIG. 14D shows Surface plasmon resonance (SPR) Biacore analysis of SHP-2 interaction using phosphotyrosyl PD-1 pITSM-82 pY248 peptide and GST-SHP- 2 fusion proteins. **FIG. 14A** shows Phosphotyrosyl PD-1 pITSM peptide (KTPEPPVPCVPEQTE(pY)AYIVFP (SEQ ID NO: 1) immobilized on negatively charged CM5 Biacore chip of Biacore 3000 (GE Healthcare). Efficient immobilization of the phosphopeptide was confirmed by binding of phosphotyrosine specific 4G10 monoclonal antibody. In **FIG. 14B,** the GST fusion proteins GST-SHP-2-WT, GST-SHP-2-R32A, GST-SHP-2-R138A, GST-SHP-2-N-SH2 and GST-SHP-2-C-SH2 were generated, purified by affinity chromatography using glutathione-Sepharose beads and analyzed by SDS PAGE and Coomassie staining. **FIG. 14C** and **FIG. 14D**, the GST tag was removed by thrombin digestion. The cleaved untagged proteins were collected, concentrated, subjected to FPLC purification and subsequently applied to anion exchange column. The eluted fractions were subjected to SDS PAGE and Coomassie staining and immunoblotting.

FIG. 15A and **FIG. 15B** shows that SHP-2 mediates bridging of two PD-1 polypeptides in the presence of kinase active

but not kinase inactive Fyn. In **FIG. 15A**, HEK-293 cells were co transfected with PD-1-SmBiT and PD-1-LgBiT together with kinase inactive (-) Fyn and either SHP-2 WT, SHP-2-R32A, SHP-2-R138A or double mutant SHP-2-R32A/R138A (DM), or control empty vector. Complex formation between PD-1-SmBiT and PD-1-LgBiT was assessed by luciferase assay. In **FIG. 15B**, as positive and negative control for the assay, cells were transfected with SmBit+LgBit or NCsm+LgBit (provided by the NanoBit Assay system), respectively, together with empty vector for normalization of cDNA expression to experimental samples. Comparison of complex formation among the different conditions was performed by 2way ANOVA and Tukey's multiple comparisons test for **FIG. 15A** and significant differences are indicated (****p<0.0001, Student's T- test, n=6 experiments for all results.).

FIG. 16A shows HEK-293 cells, Jurkat T cells, or primary human T cells that were transfected with PD1Lg and PD1sm nanobit constructs and expression of PD-1 was assessed by flow cytometry. **FIG. 16B** shows expression of PD-1 in primary human T cells before and after stimulation for 48 hours with anti-CD3 and anti-CD28 antibodies which was assessed in parallel. Results are representative of three independent experiments.

FIG. 17A and **FIG. 17B** shows the generation of Raji-PD-L1. Raji cells were transfected with human PD-L1 cDNA and stable PD-L1+ cells were generated by antibiotic selection. Cell lines were subcloned and stable clones were generated. percentage of cells and Raji-PD-L1 (**FIG. 17B**) and Raji-control (**FIG. 17A**).

FIG. 18 shows that bisphosphorylated PD-1 ITSM pY248 induces higher SHP-2 enzymatic activity compared to bisphosphorylated IRS1. Briefly, 1.6 µg/ml of purified SHP-2-WT protein was incubated with 20 µM DiFMUP with or without the indicated phosphotyrosyl peptides at a concentration of 20 nM. Monophosphoryl peptide pIRSY727 was used as negative control. SHP-2 phosphatase activity was monitored by a fluorescent assay using 6,8-difluoro-4-methylumbelliferone (DiFMUP) as substrate. Fluorescence signal was monitored at 30 °C every 5 min for 30 min and reaction rate was calculated by the change of fluorescence signal with time by a microplate reader using excitation and emission wavelengths of 340 nm and 450 nm, respectively. Comparison of SHP-2 phosphatase activity among the different conditions was performed by 2-way ANOVA and Tukey's multiple comparisons test and significant differences are indicated (*p<0.05, n=3 experiments, ****P<0.0001, n=3 experiments).

FIG. 19 shows Jurkat T cells stably expressing PD-1-W1 (J-PD1), PD-1-Y223F (J-PD1-Y223F) or PD-1-Y248F (J-PD1-Y248F) that were co-cultured with Raji-control or Raji-PD-L1 cells loaded with SEE. Where indicated, anti-PD-1 blocking antibody or isotype control was added in the cultures. Culture supernatants were collected at 24 hours and IL-2 production was measured by ELISA (****P<0.0001, n=3 experiments).

FIG. 20A to **FIG. 20C** show dose-response curves for SHP-2 wild-type, N-SH2 domain, and C-SH2 domain binding to ITSM-pY248 determined by SPR. All SHP-2-related analytes were passed over the pITSM-pY248 ligand-immobilized

surface using standard conditions as described herein. The dissociation constant (k_d) was first determined for each analyte concentration using the BIAeval package and was then used to discriminate the association constant k_a during the association phase. Calculated K_D was then determined as k_d / k_a. The Chi-square value, where smaller values equate to better fits, are listed first for k_a, then for k_d, and were: (**FIG. 20A**) for SHP-2-FL on ITSM-pY248, 0.06 and 0.06; (**FIG. 20B**) for N-SH2 on ITSM-pY248. 0.37 and 0.16; (**FIG. 20C**) for C-SH2 on ITSM-pY248. 0.07 and 0.27. Following the curve fitting of the binding curves (top panels), the residuals ([Predicted — Observed] RU) were calculated (bottom panels), with the vast majority falling into the 0.5 - 1 RU range for variation from the fitted curves. Shading in lower panel correspond to those of curves in top panel depicting each concentration.

DETAILED DESCRIPTION OF THE DISCLOSURE

The presents disclosure relates, in part, to exploiting the geometry of the PD-1: SHP-2 interaction, which leads to SHP-2 activation in methods, compounds, and compositions which enhance T cell responses by disrupting PD-1 dimerization and in methods, compounds, and compositions which suppress T cell responses (*i.e.,* inducing T cell tolerance) by triggering PD-1 dimerization.

Disclosed herein is experimental evidence that, after PD-1 phosphorylation by TCR-proximal Src family tyrosine kinases, SHP-2 bridges a first phosphorylated immunoreceptor tyrosine-based switch motif (ITSM)-Y248 residue one one PD-1 polypeptide and a second phosphorylated ITSM-Y248 residue on a second PD-1 polypeptide *via* its amino terminal (N-SH2) and carboxy terminal (C-SH2) domains; thereby, forming a dimer of the two PD-1 polypeptides (**FIG. 2E**). Mutagenesis of the active site of either the C-SH2 domain or the N-SH2 domain abrogates the SHP-2: PD-1 interaction. Assessment of PD-1 dimerization in live cells showed that upon PD-1 phosphorylation, the PD-1: PD1 interaction occurs only in the presence of SHP-2 which has an intact N-SH2 domain and an intact C-SH2 domain. The interaction of both SH2 domains of SHP-2 with two ITSM-pY248 residues is necessary for SHP-2 catalytic activation. Indeed, the below-disclosed experimental evidence shows that a PD-1 ITSM-pY248 phosphopeptide does not induce SHP-2 phosphatase activity whereas a bisphosphorylated peptide generated by covalently joining two PD-1 ITSM-pY248 peptides with a linker (which has a length such that SHP-2's two SH2 domains can simultaneously bind the two Y248 motifs) induced rapid activation of SHP-2. In contrast, a similarly-designed bisphosphorylated peptide generated by covalently joining two PD-1 immunoreceptor tyrosine-based inhibitory motif (ITIM)-pY223 peptides or one PD-1 ITIM-pY223 and one ITSM-pY248 did not induce SHP-2 catalytic activation. The ability of a pair of PD-1 ITSM-pY248 - but not one or more PD-1 ITIM-pY223 - to induce SHP-2 activation was correlated with inhibition of antigen-mediated IL-2 production, which was abrogated when the tyrosine 248 was mutagenized to phenylalanine. These data reveal avenues for the development of methods, compounds, and compositions which selectively suppress T cell responses by triggering PD-1 dimerization or enhance T cell responses by disrupting PD-1 dimerization.

In some embodiments, the method provides an agent that inhibits PD-1: SHP-2 interaction and PD-1-mediated activation of SHP-2. Such agents compete for PD-1: SHP-2 interaction. In some embodiments, such agents include a small molecule, for example, a peptide or peptide mimetic.

In some embodiments, the peptide is a biphosphorylated ITSM. In some embodiments, the ITSM induces robust activation of SHP-2. In some embodiments, the ITSM is a cell-permeable peptide format to compete and inhibit PD-1: SHP-2 interaction, and, thus, block PD-1-mediated inhibitory effects.

In some embodiments, the method provides an agent that inhibits PD-1 dimerization, which prevents the formation of PD1-homodimer that is formed by bridging together two PD-1 polypeptides by SHP-2. In some embodiments, disruption of PD-1 homodimerization prevents the interaction with SHP-2 and PD-1 mediated inhibition of T cell responses. In some embodiments, such compositions are monomeric recombinant PD-L1 or PD-L2 polypeptide. In some embodiments, the monomeric recombinant PD-L1 or PD-L2 polypeptide bind on one but not on two PD-1 polypeptides simultaneously thereby preventing PD-1 dimerization, which is required for activation of SHP-2.

In some embodiments, the method provides an agent that promotes PD-1 dimerization. In some embodiments, the PD-1 dimerization promotes the formation of PD-1 homodimer and the interaction of SHP-2 with the phosphorylated ITSM-Y248 residues on the PD-1 homodimer.

Immune Checkpoint Genes

Immune checkpoints (IC) regulate T cell responses to maintain self-tolerance. They deliver costimulatory and coinhibitory signals to T cells. PD-L1, mainly expressed by antigen presenting cells engages its receptor PD-1 on T cells, to provide a growth inhibitory signal. Different tumors express high PD-L1 to evade immune recognition. Thus, inhibition of PD-1/PD-L1 and other IC polypeptides have become important targets in cancer immunotherapy.

Programmed Cell Death-1 (PD-1)

PD-1 is a cell-surface receptor that is a member of the CD28 family of T-cell regulators, within the immunoglobulin superfamily of receptors. The human PD-1 gene is located at chromosome 2q37, and the full-length PD-1 cDNA encodes a protein with 288 amino acid residues with 60% homology to murine PD-1. It is present on CD4− CD8− (double negative) thymocytes during thymic development and is expressed upon activation in mature hematopoietic cells such as T and B cells, NKT cells and monocytes after prolonged antigen exposure.

PD-1 promotes peripheral tolerance and restrains anti-viral and anti-tumor immunity. The cytoplasmic tail of PD-1 has one ITIM (V/L/I/XpYXX/L/V), which contains tyrosine 223 (Y223\) residue, and one ITSM (TXpYXXV/I), which contains

a tyrosine 248 (Y248) residue. The Y223 and Y248 positions are relative to the wild-type, human PD-1 polypeptide sequence: Accession Number: Q15116; SEQ ID NO: 21. Mutational studies have shown that PD-1-mediated inhibition relies on the interaction of the ITSM with SHP-2 but the mechanism by which PD-1 induces SHP-2 activation remained unclear. As disclosed herein, phosphorylation of PD-1 by TCR-proximal Src family kinases is required for interaction with SHP-2, which binds to the PD-1 ITSM-Y248 residue. This interaction is mediated by both the amino terminal SH2 (N-SH2) domain and carboxy terminal SH2 (C-SH2) domain of SHP-2, which together bridge two PD-1 polypeptides by binding on each PD-1 polypeptide's ITSM-Y248 residue. As disclosed herein, SHP-2 bridges two PD-1 polypeptides by PD-1 dimerization in live cells by NanoBiT proximity assays; these data showed that upon PD-1 phosphorylation, PD-1: PD1 interaction occurs only in the presence of SHP-2 expressing intact N-SH2 domain (SEQ ID NO: 19) and C-SH2 domain (SEQ ID NO: 20). Binding of the SH2 domains by two PD-1 polypeptides is required for activation of SHP-2 phosphatase activity. Further, it is disclosed herein that a monomeric PD-1 ITSM-pY248 phosphopeptide does not induce catalytic activation of SHP-2 whereas a phosphopeptide generated by covalently joining two PD-1 ITSM-pY248 peptides with a linker - that meets the spacing requirements between the binding sites of the two SI-IP-2 SH2 domains - induced robust activation of SHP-2. Moreover, cultures of T cells with a recombinant dimeric PD-L1, but not a monomeric PD-L1, enhanced PD-1: PD-1 bridging and inhibited proliferation and IFN-y production. The evidence disclosed herein reveal the mechanisms by which PD-1: SHP-2 interaction leads to SHP-2 activation. They also have implications for the design and development of PD-1-binding compounds to selectively suppress T cell responses by dimerizing PD-1 or to prevent the PD-1 inhibitory signal by disrupting PD-1 homodimerization.

Programmed death-ligand (PD-L1 and PD-L2)

PD-L1 has also been shown to bind to B7-1 (CD80), an interaction that also suppresses T-cell proliferation and cytokine production. Cancer cells drive high expression levels of PD-L1 on their surface, allowing activation of the inhibitory PD-1 receptor on any T cells that infiltrate the tumor microenvironment, effectively switching those cells off. Indeed, upregulation of PD-L1 expression levels has been demonstrated in many different cancer types, and high levels of PD-L1 expression have been linked to poor clinical outcomes. In some embodiments, the subject is undergoing treatment with an immune checkpoint immunotherapy selected from an agent that modulates PD-L1. In some embodiments, the subject is undergoing treatment with an immune checkpoint immunotherapy selected from an agent that modulates PD-L2.

In some embodiments, the immune-modulating agent targets one or more immune checkpoint genes including, for example, PD-1, PD-L1, and PD-L2. In some embodiments, the immune-modulating agent is a PD-1 inhibitor. In some embodiments, the immune-modulating agent is an antibody or antigen binding fragment thereof, specific for one or more of PD-1, PD-L1, and PD-L2.

For instance, in some embodiments, the immune-modulating agent is an antibody or antigen binding fragment thereof such as, by way of non-limitation, nivolumab, (ONO-4538/BMS-936558, MDX1106, OPDIVO, BRISTOL MYERS SQUIBB), pembrolizumab (KEYTRUDA, MERCK), pidilizumab (CT-011, CURE TECH), MK-3475 (MERCK), BMS 936559 (BRISTOL MYERS SQUIBB), MPDL328OA (ROCHE). In some embodiments, the immune-modulating agent targets one or more of CD137 or CD137L. In some embodiments, the immune-modulating agent is an antibody or antigen binding fragment thereof specific for one or more of CD137 or CD137L. For instance, in some embodiments, the immune-modulating agent is an antibody or antigen binding fragment thereof such as, by way of non-limitation, urelumab (also known as BMS-663513 and anti-4-1BB antibody). In some embodiments, the present chimeric protein is combined with urelumab (optionally with one or more of nivolumab, lirilumab, and urelumab) for the treatment of solid tumors and/or B-cell non-Hodgkin's lymphoma and/or head and neck cancer and/or multiple myeloma.

Autoimmune and Inflammatory Diseases

In some embodiments, the compositions and methods described herein are useful for treating autoimmune diseases, alloimmune responses, or any other disease, disorder or condition that involves a T cell response in a patient in need thereof. Generally, these are conditions in which the immune system of an individual (*e.g.,* activated T cells) attacks the individual's own tissues and cells, or implanted tissues, cells, or molecules (as in a graft or transplant). Non-limiting examples of diseases and disorders that can be treated according to the methods described herein, include, *e.g.,* autoimmune disease or disorder (*e.g.,* IBD and rheumatoid arthritis), transplant rejection, graft-versus-host disease (GVHD), inflammation, asthma, allergies, and chronic infection.

In one aspect, the present invention provides a method for treating an autoimmune disease or disorder or for treating inflammation in a patient in need thereof, comprising: (a) selecting an agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity; and (b) administering the agent to the patient.

In another aspect, the present invention provides a method of making an agent effective for the treatment of an autoimmune disease or disorder of for the treatment of inflammation, comprising: (a) identifying the agent by screening for a stimulation or increase of SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity; and (b) formulating the agent for administration to a patient having a autoimmune disease or disorder or inflammation.

In some embodiments, compounds that promote PD-1 homodimerization will promote the inhibitory function of PD-1 and will be useful for the treatment of autoimmune diseases, inflammation and for prevention of graft rejection after organ or hematopoietic stem cell transplantation.

In some embodiments, the agent which increases SHP-2-mediated PD-1 dimerization is a small molecule or peptide agent. In some embodiments, the small molecule or peptide agent stimulates an interaction between PD-1 and SHP-

2. In some embodiments, the small molecule or peptide agent stimulates an interaction between PD-1 and one or two of the SH2 domains of SHP-2.

In some embodiments, the small molecule or peptide agent stimulates an interaction between PD-1 and one or two of the SH2 domains of SHP-2 mediated by interactions at the immunoreceptor tyrosine-based switch motif (ITSM) of PD-1. In some embodiments, the small molecule or peptide agent stimulates an interaction between PD-1 and one or two of the SH2 domains of SHP-2 mediated by interactions at tyrosine 248 of PD-1. In some embodiments, the small molecule or peptide agent stimulates an interaction between PD-1 and one or two of the SH2 domains of SHP-2 mediated by interactions at arginine 32 (located in the N terminal SH2 domain of SHP-2) and/or arginine 138 (located in the C terminal SH2 domain of SHP-2). In some embodiments, the small molecule or peptide agent stimulates an interaction between PD-1 and two SH2 domains of SHP-2 mediated by interactions at (a) arginine 32 of the N terminal SH2 domain of SHP-2 and tyrosine 248 of PD-1 and (b) arginine 138 of the C terminal SH2 domain of SHP-2 and tyrosine 248 of PD-1. The arginine 32 and arginine 138 positions of the wild-type, human SHP-2 polypeptide sequence (Accession Number: Q06124; SEQ ID NO: 18) correspond to the arginine at amino acid position 32 in SEQ ID NO: 19 and the arginine at amino acid position 28 in SEQ ID NO: 20, respectively.

In some embodiments, the peptide agent has two domains which interact with tyrosine 248 of PD-1 and are separated by at least about 35 Angstroms. In some embodiments, the peptide agent two SH2 domains of SHP-2 which interact with tyrosine 248 of PD-1 and are separated by at least about 35 Angstroms.

In some embodiments, the peptide agent has two domains which interact with tyrosine 248 of PD-1 and are separated by at least about 35 Angstroms. In some embodiments, the peptide agent two SH2 domains of SHP-2 which interact with tyrosine 248 of PD-1 and are separated by at least about 35 Angstroms.

In some embodiments, the autoimmune disease or disorder is selected from multiple sclerosis,^p diabetes mellitus, lupus, celiac disease, Crohn's disease, ulcerative colitis, Guillain-Barre syndrome, scleroderms, Goodpasture's syndrome, Wegener's granulomatosis, autoimmune epilepsy, Rasmussen's encephalitis, Primary biliary sclerosis, Sclerosing cholangitis, Autoimmune hepatitis, Addison's disease, Hashimoto's thyroiditis, Fibromyalgia, Menier's syndrome; transplantation rejection (*e.g.*, prevention of allograft rejection) pernicious anemia, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, Sjogren's syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, and Grave's disease.

Other exemplary autoimmune diseases that can be treated with the methods of the present disclosure include, *e.g.*, type I diabetes, multiple sclerosis, thyroiditis (such as Hashimoto's thyroiditis and Ord's thyroiditis), systemic lupus erythematosus, scleroderma, psoriasis, arthritis, rheumatoid arthritis, alopecia greata, ankylosing spondylitis,

autoimmune hemolytic anemia, autoimmune hepatitis, Behcet's disease, Crohn's disease, dermatomyositis, glomerulonephritis, Guillain-Barre syndrome, IBD, lupus nephritis, myasthenia gravis, myocarditis, pemphigus/pemphigoid, pernicious anemia, polyarteritis nodosa, polymyositis, primary biliary cirrhosis, rheumatic fever, sarcoidosis, Sjogren's syndrome, ulcerative colitis, uveitis, vitiligo, and Wegener's granulomatosis.

In embodiments, the inflammation is acute inflammation, chronic inflammation, respiratory disease, atherosclerosis, restenosis, asthma, allergic rhinitis, atopic dermatitis, septic shock, rheumatoid arthritis, inflammatory bowel disease, inflammatory pelvic disease, pain, ocular inflammatory disease, celiac disease, Leigh Syndrome, Glycerol Kinase Deficiency, Familial eosinophilia (FE), autosomal recessive spastic ataxia, laryngeal inflammatory disease; Tuberculosis, Chronic cholecystitis, Bronchiectasis, Silicosis and other pneumoconioses.

Exemplary alloimmune responses that can be treated with the methods of the present disclosure include GVHD and transplant rejection. Thus, for example, the compositions disclosed herein can be administered as an “induction therapy” in preparation for a solid organ or stem cell transplant, or as “maintenance therapy” in solid organ or stem cell transplant recipients, and can also be administered to a solid organ or stem cell transplant recipient in order to facilitate early withdrawal of maintenance immunosuppressive therapy.

In some embodiments, the patient is undergoing treatment with an immunosuppressive agent. In embodiments, wherein the method further comprises administering an immunosuppressive agent. In embodiments, the administering of the agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity and the administering of the immunosuppressive agent is sequential or simultaneous.

In some embodiments, the immunosuppressive agent is a steroidal anti-inflammatory agent or a non-steroidal anti-inflammatory agent (NSAID), selected from salicylic acid, acetyl salicylic acid, methyl salicylate, glycol salicylate, salicylmides, benzyl-2,5-diacetoxybenzoic acid, ibuprofen, fulindac, naproxen, ketoprofen, etofenamate, phenylbutazone, and indomethacin.

In some embodiments, the immunosuppressive agent is a steroid, such as a corticosteroids selected from hydroxyltriamcinolone, alpha-methyl dexamethasone, beta-methyl betamethasone, beclomethasone dipropionate, betamethasone benzoate, betamethasone dipropionate, betamethasone valerate, clobetasol valerate, desonide, desoxymethasone, dexamethasone, diflorasone diacetate, diflucortolone valerate, fluadrenolone, fluclorolone acetonide, flumethasone pivalate, fluosinolone acetonide, fluocinonide, flucortine butylester, fluocortolone, fluprednidene (fluprednylidene) acetate, flurandrenolone, halcinonide, hydrocortisone acetate, hydrocortisone butyrate, methylprednisolone, triamcinolone acetonide, cortisone, cortodoxone, flucetonide, fludrocortisone, difluorosone diacetate, fluradrenolone acetonide, medrysone, amcinafel, amcinafide, betamethasone and the balance of its esters,

chloroprednisone, clocortelone, clescinolone, dichlorisone, difluprednate, flucloronide, flunisolide, fluoromethalone, fluperolone, fluprednisolone, hydrocortisone, meprednisone, paramethasone, prednisolone, prednisone, beclomethasone dipropionate.

In some embodiments, the immunosuppressive agent is a cytostatics such as alkylating agents, antimetabolites (*e.g.,* azathioprine, methotrexate), cytotoxic antibiotics, antibodies (*e.g.,* basiliximab, daclizumab, and muromonab), anti-immunophilins (*e.g.,* cyclosporine, tacrolimus, sirolimus), inteferons, opioids, TNF binding proteins, mycophenolates, and small biological agents *(e.g.,* fingolimod, myriocin).

Viral infections

A viral disease (or viral infection) occurs when an organism's body is invaded by pathogenic viruses, and infectious virus particles (virions) attach to and enter. In some embodiments compounds that disrupt PD-1 homodimerization will prevent interaction of SHP-2 with PD-1 and block PD-1-mediated inhibitory function. Such compounds will be useful for the enhancement of immune responses against chronic viral infections such as HIV, Hepatitis C, Cytomegalovirus (CMV) or Epstein Barr Virus (EBV).

In some embodiments, the disclosure provides a method for enhancement of immune responses against chronic viral infections. Exemplary examples of viral infections include, but are not limited to: infections caused by DNA Viruses (*e.g.,* Herpes Viruses such as Herpes Simplex viruses, Epstein-Barr virus, Cytomegalovirus; Pox viruses such as Variola (small pox) virus; Hepadnaviruses (e.g, Hepatitis B virus); Papilloma viruses; Adenoviruses); RNA Viruses (*e.g.,* HIV I, II; HTLV I, II; Poliovirus; Hepatitis A; Orthomyxoviruses (*e.g.,* Influenza viruses); Paramyxoviruses (*e.g.,* Measles virus); Rabies virus; Hepatitis C); Rhinovirus, Respiratory Syncytial Virus, West Nile Virus, Yellow Fever, Rift Valley Virus, Lassa Fever Virus, Ebola Virus, Lymphocytic Choriomeningitis Virus, which replicates in tissues including liver, and the like, Acquired immunodeficiency; Hepatitis; Gastroenteritis; Hemorrhagic diseases; Enteritis; Carditis; Encephalitis; Paralysis; Brochiolitis; Upper and lower respiratory disease; Respiratory Papillomatosis; Arthritis; Disseminated disease, hepatocellular carcinoma resulting from chronic Hepatitis C infection, viral meningitis, and HIV-related disease.

Cancer

Cancer is a group of diseases characterized by uncontrolled cell division which can lead to abnormal tissue and, in turn, disruption of normal physiologic processes and, possibly, death. Cancers have various etiologies and may be responsive to agents that affect aspects of these etiologies. For example, a reduction or loss of nucleic acids that are linked to cancer development may prove fruitful in the treatment of various cancers, including blood-based cancers and breast cancers. Such treatments may replace or supplement existing treatments.

In one aspect, the present invention provides a method for treating cancer in a patient in need thereof, comprising: (a) selecting an agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity; and (b) administering the agent to the patient.

In another aspect, the present invention provides a method of making an agent effective for the treatment of a cancer, comprising: (a) identifying the agent by screening for a disruption or decrease of SHP-2-mediated programmed cell death protein-1 (PD-1) dimerization or of PD-1 dimer activity; and (b) formulating the agent for administration to a patient having a cancer.

In yet another aspect, the present invention provides a method for predicting a cancer patient response to an immune checkpoint immunotherapy, comprising determining the presence of SHP-2-mediated PD-1 dimerization in a biological sample from the patient, wherein the presence of SHP-2-mediated PD-1 dimerization is indicative of an inhibitory immune signal and a likelihood of responding to the immune checkpoint immunotherapy.

In an aspect, the present invention provides method for treating cancer, comprising: (a) evaluating a subject's likelihood of response to an immune checkpoint immunotherapy, comprising evaluating a level of SHP-2-mediated PD-1 dimerization in a biological sample from the patient, wherein a presence or high level of SHP-2-mediated PD-1 dimerization is indicative of a cancer that is suitable for immune checkpoint immunotherapy; and (b) administering an immune checkpoint immunotherapy to the patient.

In some embodiments, the present disclosure encompasses methods of treating or preventing cancer and/or a metastasis in a subject in need thereof. In some embodiments, representative cancers and/or tumors and/or metastases of the present invention include a basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL;

mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), and Meigs' syndrome. See, *e, g.,* Weinberg, The Biology of Cancer, Garland Science: London 2006, the contents of which are hereby incorporated by reference. In some embodiments, the cancer to be treated or prevented is a blood-based cancer or related disease including, for example, a lymphoma, leukemia, myeloma or myelodysplastic/myeloproliferative neoplasm (MDS/MPN).

As used herein, the term subject or patient refers to any vertebrate including, without limitation, humans and other primates (*e.g.,* chimpanzees and other apes and monkey species), farm animals (*e.g.,* cattle, sheep, pigs, goats, and horses), domestic mammals (*e.g.,* dogs and cats), laboratory animals (*e.g.,* rodents such as mice, rats, and guinea pigs), and birds (*e.g.,* domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like). In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

Agents of the Disclosure

In one aspect, the present invention provides a method for treating cancer in a patient in need thereof, comprising: (a) selecting an agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity; and (b) administering the agent to the patient.

In embodiments, the agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity is a small molecule or peptide agent.

In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SHP-2 polypeptide, *e.g.,* is capable of disrupting an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2. In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 by preventing binding of the SH2 domain with a motif of the PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, *e.g.,* a motif comprising an immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 by preventing binding of an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) with the PD-1 polypeptide and/or by preventing binding of a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20) with the PD-1 polypeptide. In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and is capable of disrupting an interaction between a second

PD-1 polypeptide and a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20). In embodiments, the small molecule or peptide agent is capable of disrupting an interaction between the first PD-1 polypeptide and the N-SH2 domain by preventing binding of the N-SH2 domain with a motif of the first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and is capable of disrupting an interaction between the second PD-1 polypeptide and the C-SH2 domain by preventing binding of the C-SH2 domain with a motif of the second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248. In embodiments, the motif of the first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and the motif of the second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, each comprises a portion of immunoreceptor tyrosine-based switch motif (ITSM). As used herein, the term "portion" includes a full-length ITSM or a fraction thereof in which the fraction retains the ability to bind a SH2 domain. The tyrosine 248 position is relative to the wild-type, human PD-1 polypeptide sequence: Accession Number: Q15116; SEQ ID NO: 21.

In embodiments, the small molecule or peptide agent is capable of binding to a SH2 domain of SHP-2. In embodiments, the small molecule or peptide agent is capable of binding to an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) or to a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20). In embodiments, the peptide agent is capable of binding to an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and to a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20). In embodiments, the peptide agent comprises at least one motif of a PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248. In embodiments, the motif comprises a portion of an immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent comprises at least two motifs of a PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, wherein the at least two motifs are separated by a linker. In embodiments, each of the at least two motifs of a PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, comprises a portion of an immunoreceptor tyrosine-based switch motif (ITSM). As used herein, the term "portion of an ITSM" includes a full-length ITSM or a fraction thereof in which the fraction retains the ability to bind a SH2 domain. In embodiments, the linker comprises at least 3 amino acids and/or the linker is at least about 35 Angstroms long.

In embodiments, the peptide agent, *e.g.,* that is capable of binding two SH2 domains, comprises: (a) a first amino acid sequence of SEQ ID NO: 1, 22, or 23, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, (b) a second amino acid sequence of SEQ ID NO: 1, 22, or 23, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, and (c) a linker comprising at least 3 amino acids between the first amino acid sequence and the second amino acid sequence, optionally, the linker separates the two amino acid sequences by at least about 35 Angstroms. In embodiments, the linker separates the two amino acid sequences by at least about 50 Angstroms. In embodiments, the peptide agent comprises the amino acid sequence of SEQ ID NO: 3, 9, 10, or 11, optionally comprising a mutation of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids. In embodiments, the mutation is not of the tyrosine 248, with reference to

SEQ ID NO: 21. In embodiments, the peptide agent comprises or consists of SEQ ID NO: 3, 9, 10, or 11. In embodiments, the peptide agent comprises the amino acid sequence of SEQ ID NO: 3, 9, 10, 11, or 24, optionally comprising a mutation of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids. In embodiments, the mutation is not of the tyrosine 248, with reference to SEQ ID NO: 21. In embodiments, the peptide agent comprises or consists of SEQ ID NO: 3, 9, 10, 11, or 24.

In embodiments, the peptide agent which reduces PD-1 dimer activity is capable of binding to at least a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248; in this embodiment, the peptide agent lacks a phosphatase domain or comprises a non-functional phosphatase domain. In embodiments, the small molecule or peptide agent is capable of binding to the at least first PD-1 polypeptide at or near its immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent is capable of binding to the first PD-1 polypeptide and to an at least second PD-1 polypeptide. In embodiments, the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, or the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20. In embodiments, the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20. In embodiments, the first amino acid sequence and the second amino acid sequence are separated by a linker, *e.g.,* a linker comprising at least 3 amino acids and/or a linker that is at least about 35 Angstroms long. The arginine at amino acid position 32 in SEQ ID NO: 19 and the arginine at amino acid position 28 in SEQ ID NO: 20, respectively correspond to arginine 32 and arginine 138 of the wild-type, human SHP-2 polypeptide sequence: Accession Number: Q06124; SEQ ID NO: 18.

In another aspect, the present invention provides a method for treating an autoimmune disease or disorder or for treating inflammation in a patient in need thereof, comprising: (a) selecting an agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity; and (b) administering the agent to the patient.

In embodiments, the agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity is a small molecule or peptide agent. In embodiments, the small molecule or peptide agent is capable of stimulating an

interaction between a PD-1 polypeptide and a SHP-2 polypeptide. In embodiments, the small molecule or peptide agent is capable of stimulating an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and an interaction between a second PD-1 polypeptide and a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20).

In embodiments, the peptide agent is capable of binding to a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and to an at least second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248. In embodiments, the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM). In embodiments, the peptide agent comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20. In embodiments, the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20. In embodiments, the first amino acid sequence and the second amino acid sequence are separated by a linker, *e.g.,* a linker comprising at least 3 amino acids and/or a linker that is at least about 35 Angstroms long.

In embodiments, the peptide agent comprises: (a) a first amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, (b) a second amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, and (c) a linker comprising at least 3 amino acids between the first amino acid sequence and the second amino acid sequence, optionally, the linker separates the two amino acid sequences by at least about 35 Angstroms.

In embodiments, the peptide agent further comprises a functional phosphatase domain. In embodiments, the functional phosphatase domain comprises a protein tyrosine phosphatase (PTP) domain. As used herein, a phosphatase domain is any portion, *e.g.,* a catalytic domain, of a phosphatase enzyme that is capable of removing a phosphate group from a phosphorylated amino acid, *e.g.,* a phosphorylated tyrosine. In embodiments, the peptide agent comprises the amino acid sequence of SEQ ID NO: 18. In embodiments, the peptide agent does not comprise of the amino acid sequence of SEQ ID NO: 18.

The terms nucleic acid and polynucleotide are used interchangeably herein to refer to single- or double-stranded RNA, DNA, or mixed polymers. Polynucleotides may include genomic sequences, extra-genomic and plasmid sequences, and smaller engineered gene segments that express, or may be adapted to express polypeptides.

An isolated nucleic acid is a nucleic acid that is substantially separated from other genome DNA sequences as well as proteins or complexes such as ribosomes and polymerases, which naturally accompany a native sequence. The term embraces a nucleic acid sequence that has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems. A substantially pure nucleic acid includes isolated forms of the nucleic acid. Of course, this refers to the nucleic acid as originally isolated and does not exclude genes or sequences later added to the isolated nucleic acid by the hand of man.

The term polypeptide is used in its conventional meaning, *i.e.,* as a sequence of amino acids. The polypeptides are not limited to a specific length of the product. Peptides, oligopeptides, and proteins are included within the definition of polypeptide, and such terms may be used interchangeably herein unless specifically indicated otherwise. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. A polypeptide may be an entire protein, or a subsequence thereof.

An isolated polypeptide is one that has been identified and separated and/or recovered from a component of its natural environment. In some embodiments, the isolated polypeptide will be purified (1) to greater than 95% by weight of polypeptide as determined by the Lowry method, and more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, silver stain. Isolated polypeptide includes the polypeptide in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

When comparing polynucleotide and polypeptide sequences, two sequences are said to be “identical” if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are aligned.

As applied to polypeptides, the term substantial identity means that two peptide sequences, when aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity. In some embodiments, the two peptide sequences share at least 90 percent sequence identity. In some embodiments, the two

peptide sequences share at least 95 percent sequence identity. In some embodiments, the two peptide sequences share at least 99 percent sequence identity.

In some embodiments, residue positions that are not identical differ by conservative amino acid substitutions. Minor variations in the amino acid sequences of polypeptides are contemplated as being encompassed by the present disclosure, providing that the variations in the amino acid sequence maintain at least 60% amino acid sequence identity to a reference sequence (*e.g.,* the wild-type sequence). In some embodiments, the variations in the amino acid sequence maintain at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% amino acid identity to the reference sequence. In particular, conservative amino acid replacements are contemplated.

Linkers (or spacer elements of linkers) may be of any desired length, one end of which can be covalently attached to specific sites of the small molecule or peptide agent. The other end of the linker or spacer element may be attached to an amino acid or peptide linker. In embodiments, the linker is at least about 1 angstrom, 5 angstroms, 10 angstroms, 15 angstroms, 20 angstroms, 25 angstroms, 30 angstroms, 35 angstroms, 40 angstroms, 45 angstroms, 50 angstroms, 55 angstroms, 60 angstroms, 65 angstroms, 70 angstroms, 75 angstroms, 80 angstroms, 85 angstroms, 90 angstroms, 95 angstroms, or 100 angstroms long, inclusive of all endpoints.

In embodiments, the linker may be derived from naturally-occurring multi-domain proteins or are empirical linkers as described, for example, in Chichili *et al.*, (2013), Protein Sci. 22(2):153-167, Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369, the entire contents of which are hereby incorporated by reference. In some embodiments, the linker may be designed using linker designing databases and computer programs such as those described in Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369 and Crasto *et. al.*, (2000), Protein Eng. 13(5):309-312, the entire contents of which are hereby incorporated by reference.

In embodiments, the linker or spacer is not peptide-based. In embodiments, the linker is a synthetic linker such as PEG. In embodiments, the linker is 4 amino hexanoic acid (Ahx). In embodiments, the linker is 10 amino hexanoic acid (Ahx).

Mutations contemplated include substitutions, additions, and deletions, or any combination thereof. In some embodiments, the mutation converts the mutated amino acid to alanine. In some embodiments, the mutation converts the mutated amino acid to another amino acid (*e.g.,* glycine, serine, threonine, cysteine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tyrosine, tryptophan, aspartic acid, glutamic acid, asparagines, glutamine, histidine, lysine, or arginine). In some embodiments, the mutation converts the mutated amino acid to a non-natural amino acid (*e.g.,* selenomethionine). In some embodiments, the mutation converts the mutated amino acid to amino acid mimics (*e.g.,* phosphomimics). In some embodiments, the mutation is a conservative mutation. For example, the mutation

converts the mutated amino acid to amino acids that resemble the size, shape, charge, polarity, conformation, and/or rotamers of the mutated amino acids (*e.g.,* cysteine/serine mutation, lysine/asparagine mutation, histidine/phenylalanine mutation). In some embodiments, the mutation causes a shift in reading frame and/or the creation of a premature stop codon. In some embodiments, mutations cause changes to regulatory regions of genes or loci that affect expression of one or more genes.

In some embodiments, the mutation can be a change in 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acid residues.

Pharmaceutical Compositions

Another embodiment of the present disclosure is a pharmaceutical composition, or use of pharmaceutical composition, comprising an agent which decreases SHP-2-mediated PD-1 dimerization. Where clinical applications are contemplated, pharmaceutical compositions may be prepared in a form appropriate for the intended application. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

In some embodiments, a pharmaceutical composition comprises, an agent which decreases SHP-2-mediated PD-1 dimerization and a pharmaceutically acceptable carrier. An effective dose is an amount sufficient to affect a beneficial or desired clinical result.

In one aspect, the disclosure provides a method of making an agent effective for the treatment of a cancer, comprising: (a) identifying the agent by screening for a disruption or decrease of SHP-2-mediated PD-1 dimerization and (b) formulating the agent for administration to a cancer patient.

In one aspect, the disclosure provides a method of making an agent effective for the treatment of an autoimmune disease or disorder, comprising: (a) identifying the agent by screening for a stimulation or increase of SHP-2-mediated PD-1 dimerization and (b) formulating the agent for administration to an autoimmune disease or disorder patient.

A beneficial or desired clinical result may include, *inter alia*, a reduction in tumor size and/or tumor growth and/or a reduction of a cancer marker that is associated with the presence of cancer as compared to what is observed without administration of the small molecule or peptide agent. A beneficial or desired clinical result may also include, *inter alia*, an increased presence of a marker that is associated with a reduction of cancer as compared to what is observed without administration of the small molecule or peptide agent. Also included in a beneficial or desired clinical result is,

inter alia, an increased amount of a gene comprising a marker linked to cancer etiology as compared to what is observed without administration of the inhibitor. The gene comprising a marker linked to cancer etiology may include, for example, an immune checkpoint gene, such as PD-1, PD-L1, or PD-L2.

Combination Therapies

As described herein, the present invention relates to, in various embodiments, agents that may be used in the context of various combination therapies encompassed by the present invention.

Additional therapeutic agents may be administered simultaneously or sequentially with the disclosed agents, compounds, or compositions. Sequential administration includes administration before or after the disclosed agent, compound, or composition. In some embodiments, the additional therapeutic agent or agents may be administered in the same composition as the disclosed agent, compound, or composition. In other embodiments, there may be an interval of time between administration of the additional therapeutic agent and the disclosed agent, compound, or composition. In some embodiments, administration of an additional therapeutic agent with a disclosed agent, compound, or composition may allow lower doses of the other therapeutic agents and/or administration at less frequent intervals. When used in combination with one or more other active ingredients, the agents, compounds, or compositions of the present invention and the other active ingredients may be used in lower doses than when each is used singly. Accordingly, the pharmaceutical compositions of the present invention include those that contain one or more other active ingredients, in addition to an agent, compound, or composition of the present disclosure. The above combinations include combinations of an agent, compound, or composition of the present disclosure not only with one other active compound, but also with two or more other active compounds. For example, the agent, compound, or composition of the disclosure can be combined with a variety of anti-cancer drugs and chemotherapeutics.

In some embodiments, the method makes the cancer responsive or more responsive to a combination therapy of the immune checkpoint immunotherapy and one or more chemotherapeutic agents and/or radiotherapy.

In some embodiments, the present disclosure includes various cancer biologics, therapeutics, chemotherapeutics, or drugs known in the art. For exemplary purposes only, and not intending to be limiting, the following drugs may be used in the present invention: daunorubicin, doxorubicin, epirubicin, idarubicin, adriamycin, vincristine, carmustine, cisplatin, 5-fluorouracil, tamoxifen, prodasone, sandostatine, mitomycin C, foscarnet, paclitaxel, docetaxel, gemcitabine, fludarabine, carboplatin, leucovorin, tamoxifen, goserelin, ketoconazole, leuprolide flutamide, vinblastine, vindesine, vinorelbine, camptothecin, topotecan, irinotecan hydrochloride, etoposide, mitoxantrone, teniposide, amsacrine, merbarone, piroxantrone hydrochloride, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine (Ara-C), trimetrexate, acivicin, alanosine, pyrazofurin, pentostatin, 5-azacitidine, 5-azacitidine, 5-Aza-5-Aza-2'-deoxycytidine,

adenosine arabinoside (Ara-A), cladribine, ftorafur, UFT (combination of uracil and ftorafur), 5-fluoro-2'-deoxyuridine, 5-fluorouridine, 5'-deoxy-5-fluorouridine, hydroxyurea, dihydrolenchiorambucil, tiazofurin, oxaliplatin, melphalan, thiotepa, busulfan, chlorambucil, plicamycin, dacarbazine, ifosfamide phosphate, cyclophosphamide, pipobroman, 4-ipomeanol, dihydrolenperone, spiromustine, geldenamycin, cytochalasins, depsipeptide, 4'-cyano-3-(4-(*e.g.,* ZOLADEX) and 4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-3-methyl-3'-(trifluorometh- yl)propionanilide.

In some embodiments, the present compositions and methods find use in combination with checkpoint inhibitors – *e.g.,* in the treatment of various cancers. For instance, the present compositions and methods may supplement checkpoint inhibitor-based cancer therapies, *e.g.,* by improving patient response to the same (*e.g.,* by converting non-responders to responders, and/or increasing the magnitude of therapeutic response, and/or reducing the does or regimen needed for therapeutic response, and/or reducing one or more side effects of the checkpoint inhibitor-based cancer therapies).

In some embodiments, the immune checkpoint immunotherapy agent modulates PD-1. In some embodiments, the agent that modulates PD-1 is an antibody or antibody format specific for PD-1. In some embodiments, the antibody or antibody format specific for PD-1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

In some embodiments, the antibody or antibody format specific for PD-1 is selected from Nivolumab, Pembrolizumab, and Pidilizumab. In some embodiments, an antibody or antibody format specific for PD-1 is Nivolumab and can be administered at 240 mg every 2 weeks. In some embodiments, an antibody or antibody format specific for PD-1 is Pembrolizumab and can be administered at 200 mg every 3 weeks. In some embodiments, an antibody or antibody format specific for PD-1 is Pidilizumab and can be administered at 200 mg every 3 weeks.

In some embodiments, the immune checkpoint immunotherapy agent modulates PD-L1. In some embodiments, the agent that modulates PD-L1 is an antibody or antibody format specific for PD-L1. In some embodiments, the antibody or antibody format specific for PD-L1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody. In some embodiments, the antibody or antibody format specific for PD-L1 is selected from Atezolizumab, Avelumab Durvalumab and BMS-936559. In some embodiments, the antibody or antibody format specific for PD-L1 is BMS-936559 and can be administered at 0.1 mg/kg every 2 weeks. In some embodiments, the antibody or antibody format specific for PD-L1 is Atezolizumab and can be administered at 1200 mg every 3 weeks. In some embodiments, the antibody or antibody format specific for PD-L1 is Avelumab and can be administered at 10

mg/kg every 2 weeks. In some embodiments, the antibody or antibody format specific for PD-L1 is Durvalumab and can be administered at 10 mg/kg every 2 weeks.

In some embodiments, the agent that modulates PD-L2 is an antibody or antibody format specific for PD-L2. In some embodiments, the antibody or antibody format specific for PD-L2 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

In one aspect, the present invention provides a method for predicting a cancer patient response to an immune checkpoint immunotherapy, comprising determining the presence of SHP-2-mediated PD-1 dimerization in a biological sample from the patient, wherein the presence of SHP-2-mediated PD-1 dimerization is indicative of an inhibitory immune signal and a likelihood of responding to the immune checkpoint immunotherapy.

In yet another aspect, the present invention provides method for treating cancer, comprising: (a) evaluating a subject's likelihood of response to an immune checkpoint immunotherapy, comprising evaluating a level of SHP-2-mediated PD-1 dimerization in a biological sample from the patient, wherein a presence or high level of SHP-2-mediated PD-1 dimerization is indicative of a cancer that is suitable for immune checkpoint immunotherapy; and (b) administering an immune checkpoint immunotherapy to the patient.

In some embodiments, the administration is by intratumoral, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or direct injection into a cancer tissue.

In some embodiments, the patient is predicted to be poorly responsive or non-responsive to the immune checkpoint immunotherapy or has presented as poorly responsive or non-responsive to the immune checkpoint immunotherapy.

In some embodiments, the method reduces and/or mitigates one or more side effects of the immune checkpoint immunotherapy.

In some embodiments, the side effect is selected from decreased appetite, rashes, fatigue, pneumonia, pleural effusion, pneumonitis, pyrexia, nausea, dyspnea, cough, constipation, diarrhea, immune-mediated pneumonitis, colitis, hepatitis, endocrinopathies, hypophysitis, iridocyclitis, and nephritis.

In some embodiments, the method reduces the dose of the immune checkpoint immunotherapy. In some embodiments, the method reduces number of administrations of the immune checkpoint immunotherapy. In some embodiments, the method increases a therapeutic window of the immune checkpoint immunotherapy.

In some embodiments, the method elicits a potent immune response in less-immunogenic tumors. In some embodiments, the method converts a tumor with reduced inflammation ("cold tumor") to a responsive, inflamed tumor ("hot tumor"). In some embodiments, the method makes the cancer responsive or more responsive to a combination therapy of the immune checkpoint immunotherapy and one or more chemotherapeutic agents and/or radiotherapy.

Dosing and Administration

One will generally desire to employ appropriate salts and buffers to render delivery vehicles stable and allow for uptake by target cells. Aqueous compositions of the present invention comprise an effective amount of the delivery vehicle comprising an agent or compound of the present disclosure, (*e.g.,* liposomes or other complexes or expression vectors) dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

The terms pharmaceutically acceptable or pharmacologically acceptable refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, pharmaceutically acceptable carrier includes solvents, buffers, solutions, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like acceptable for use in formulating pharmaceuticals, such as pharmaceuticals suitable for administration to humans. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions, provided they do not inactivate the vectors or polynucleotides of the compositions.

The active compositions of the present disclosure may include classic pharmaceutical preparations. Administration of these compositions according to the present invention may be *via* any common route so long as the target tissue is available *via* that route. This includes oral, nasal, or buccal. Alternatively, administration may be by intratumoral, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or by direct injection into cancer tissue. The agents disclosed herein may also be administered by catheter systems. Such compositions would normally be administered as pharmaceutically acceptable compositions as described herein.

Upon formulation, solutions may be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations may easily be administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution generally is suitably buffered and the liquid diluent first rendered isotonic with, for example, sufficient saline or glucose. Such aqueous solutions may be used, for example, for intratumoral, intravenous, intramuscular, subcutaneous and intraperitoneal administration. Preferably, sterile aqueous media are employed as is

known to those of skill in the art, particularly in light of the present disclosure. By way of illustration, a single dose may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion (*see, e.g.,* Remington's Pharmaceutical Sciences, 15th Edition, pages 1035-1038 and 1570-1580, the contents of which are hereby incorporated by reference). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by the FDA Office of Biologics standards.

In some embodiments, the present disclosure includes an agent of the present disclosure and a second agent that is or comprises at least one other cancer biologic, therapeutic, chemotherapeutic or drug or an anti-inflammatory drug.

In some embodiments, the first and second agents may be administered in either order (*e.g.,* first then second or second then first) or concurrently.

In some embodiments, the agent of the present invention can be administered over any suitable period of time, such as a period from about 1 day to about 12 months. In some embodiments, for example, the period of administration can be from about 1 day to 90 days; from about 1 day to 60 days; from about 1 day to 30 days; from about 1 day to 20 days; from about 1 day to 10 days; from about 1 day to 7 days. In some embodiments, the period of administration can be from about 1 week to 50 weeks; from about 1 week to 50 weeks; from about 1 week to 40 weeks; from about 1 week to 30 weeks; from about 1 week to 24 weeks; from about 1 week to 20 weeks; from about 1 week to 16 weeks; from about 1 week to 12 weeks; from about 1 week to 8 weeks; from about 1 week to 4 weeks; from about 1 week to 3 weeks; from about 1 week to 2 weeks; from about 2 weeks to 3 weeks; from about 2 weeks to 4 weeks; from about 2 weeks to 6 weeks; from about 2 weeks to 8 weeks; from about 3 weeks to 8 weeks; from about 3 weeks to 12 weeks; or from about 4 weeks to 20 weeks.

In some embodiments, the agent which decreases SHP-2-mediated PD-1 dimerization can be administered every day, every other day, every week, every 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, or every 20 weeks, or every month.

In some embodiments, a therapeutically effective amount of a composition or agent of the present disclosure may be about 0.01 mg/kg per day to about 10 mg/kg per day. In some embodiments, dosages can range from about 0.1 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 3 mg/kg, 5 mg/kg, 6 mg/kg, 7.5 mg/kg, or about 10 mg/kg. In some embodiments, the dose will be in the range of about 0.1 mg/day to about 5 mg/kg; about 0.1 mg/day to about 10 mg/kg; about 0.1 mg/day to about 20 mg/kg; about 0.1 mg to about 30 mg/kg; or about 0.1 mg to about 40 mg/kg.

In some embodiments, a therapeutically effective amount of a composition or agent of the present disclosure may be about 0.1 mg to about 50 mg/kg or in single, divided, or continuous doses (which dose may be adjusted for the patient's weight in kg, body surface area in m^2, and age in years).

In some embodiments, a therapeutically effective amount of the composition or agent of the present disclosure, may be about 1 mg/kg to about 1000 mg/kg, about 5 mg/kg to about 950 mg/kg, about 10 mg/kg to about 900 mg/kg, about 15 mg/kg to about 850 mg/kg, about 20 mg/kg to about 800 mg/kg, about 25 mg/kg to about 750 mg/kg, about 30 mg/kg to about 700 mg/kg, about 35 mg/kg to about 650 mg/kg, about 40 mg/kg to about 600 mg/kg, about 45 mg/kg to about 550 mg/kg, about 50 mg/kg to about 500 mg/kg, about 55 mg/kg to about 450 mg/kg, about 60 mg/kg to about 400 mg/kg, about 65 mg/kg to about 350 mg/kg, about 70 mg/kg to about 300 mg/kg, about 75 mg/kg to about 250 mg/kg, about 80 mg/kg to about 200 mg/kg, about 85 mg/kg to about 150 mg/kg, and about 90 mg/kg to about 100 mg/kg.

In some embodiments, a therapeutically effective amount of a composition or agent of the present disclosure 0.01 mg/kg to about 500 mg/kg, for example, about 0.1 mg/kg to about 200 mg/kg (such as about 100 mg/kg), or about 0.1 mg/kg to about 10 mg/kg (such as about 0.1 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 3 mg/kg, 5 mg/kg, 6 mg/kg, 7.5 mg/kg, or about 10 mg/kg).

Kits of the Disclosure

The invention also provides kits that can simplify the administration of any agent disclosed herein. An exemplary kit of the invention comprises any composition described herein in unit dosage form. In some embodiments, the unit dosage form is a container, such as a pre-filled syringe, which can be sterile, containing any agent described herein and a pharmaceutically acceptable carrier, diluent, excipient, or vehicle. The kit can further comprise a label or printed instructions instructing the use of any agent described herein. The kit may also include a lid speculum, topical anesthetic, and a cleaning agent for the administration location. The kit can further comprise one or more additional agent, such as a biologic, therapeutic, chemotherapeutic or drug described herein. In some embodiments, the kit comprises a container containing an effective amount of a composition of the invention and an effective amount of another composition, such those described herein.

Although the inhibitory function of PD-1 has been attributed to its interaction with SHP-2 *via* PD-1 the immunoreceptor tyrosine-based switch motif (ITSM)-pY248, it has remained poorly understood whether PD-1 can induce SHP-2 enzymatic activation and how such effect might be mechanistically regulated. The below-disclosed data shows that PD-1 can induce SHP-2 catalytic activity and this requires the presence of two phosphorylated ITSM-Y248 motifs precisely spaced to meet the distance between the phosphotyrosine binding sites of the SHP-2 SH2 domains. In

contrast, similarly-designed bisphosphorylated peptides generated by covalent joining of two PD-1 immunoreceptor tyrosine-based inhibitory motif (ITIM)-pY223 peptides or one PD-1 ITIM-pY223 and one ITSM-pY248 does not induce SHP-2 catalytic activation. The ability of PD-1 ITSM-pY248 - but not PD-1 ITIM-pY223 - to induce SHP-2 activation was correlated with inhibition of antigen-mediated IL-2 production, which was abrogated when PD-1 ITSM-pY248 was mutagenized to phenylalanine.

The below-disclosed data also shows that an additional requirement for PD-1-mediated SHP-2 interaction and SHP-2 catalytic activation is the presence of two intact SH2 domains in SHP-2; loss of the active site of either SH2 domain disrupts the interaction with PD-1 and the induction of PD-1-mediated SHP-2 activation. Without wishing to be bound by theory, the requirement for two precisely-spaced PD-1 ITSM-pY248 and the requirement of both intact SH2 domains of SHP-2 for PD-1: SHP-2 interaction and PD-1-mediated activation of SHP-2 is consistent with a model in which SHP-2 binds phosphorylated ITSM-Y248 residues in two PD-1 polypeptides using its N-SH2 domain for one PD-1 and its C-SH2 domain for the second PD-1 to form a PD-1 dimer (**FIG. 2D**). Indeed, the below-disclosed data demonstrates that, in live cells, after PD-1 phosphorylation by the TCR-proximal Src family tyrosine kinase Fyn, PD-1 dimers are formed. Such dimers are disrupted by the loss of the active site of either SHP-2 SH2 domain or the absence of Fyn kinase activity. Without wishing to be bound by theory, SHP-2 activation by PD-1 may be due to the interaction of the tandem SH2 domains with the phosphorylatedtyrosines in PD-1 ITIM-pY223 and PD-1 ITSM-pY248, similarly to that proposed for IRS, the below-disclosed data shows that the binding preference of SHP-2 tandem SH2 domains is incompatible with such model of SHP-2 activation. The two SH2 domains of SHP-2 have a roughly antiparallel or roughly perpendicular orientation relative to one another with the phosphopeptide binding sites widely separated (**FIG. 6C**). The distance between the phosphopeptide binding sites of the SH2 domains is 41 Å in the crystal structure of the tandem SH2 domains and appears to be critical for phosphoprotein recognition and for enzymatic activation of SHP-2. The below-disclosed data shows that a bisphosphorylated peptide (which meets the spacing requirements for dual SHP-2 SH2 domain binding by covalent joining of one pITIM-Y223 and one pITSM-Y248 phosphopeptide with a 4 amino hexanoic acid (Ahx) spacer) did not induce SHP-2 activity, whereas a similarly-designed bisphosphorylated peptide generated by covalently joining two PD-1 pITSM-Y248 peptides induced robust SHP-2 phosphatase activity.

PD-1 together with CTLA-4 comprise the inhibitory receptors of the CD28/CTLA-4/B7-1/B7-2 superfamily. CTLA-4 is a covalent dimer. Its higher avidity for the B7 ligands than for CD28 results from the binding of each CTLA-4 dimer to two divalent B7 polypeptides. The crystal structure of CTLA-4: B7 complexes suggests that CTLA-4 covalent dimer can bind to noncovalent dimers of B7-1 to form a lattice of CTLA-4-B7 interactions. Such a lattice can function to form a stable signaling complex at the T cell: APC interface. In contrast to CTLA-4, PD-1 does not have the conserved cysteine located proximal to the transmembrane domain; thus, it does not appear to be structurally-equipped to form a covalent dimer. Indeed, crystallography studies of the PD-1: PD-L1 binding regions have shown that PD-1 stays as a

monomer in solution. The below-disclosed data shows that PD-1 can form a PD-1: PD-1 noncovalent dimeric complex *via* a previously-unidentified mechanism. Formation of this complex is guided by an "inside-out" signaling sequence induced by TCR-mediated phosphorylation of PD-1 by Src family kinases. Without wising to be bound by theory, the below-disclosed data strongly suggests that formation of this PD-1: PD-1 dimeric complex is essential for inhibitory function in live cells because only mutagenesis of the PD-1 phosphotyrosine, which is required both for PD-1 dimer formation and SHP-2 enzymatic activation, abrogated PD-1-mediated inhibition during antigen-specific stimulation.

The below-disclosed data unexpectedly shows that phosphorylation of PD-1 cytoplasmic tail also has an active role in PD-L1-mediated PD-1 oligomerization. The data shows that PD-1: PD-1 dimer formation induced by dimeric PD-L1 was impaired when phosphorylation of PD-1 cytoplasmic tail was compromised by the expression of a kinase dominant negative Fyn. pITSM-Y248 appears to be required for SHP-2 enzymatic activation; this data explains why TCR-mediated signaling is required concomitantly with PD-1 ligation by its natural ligands to induce PD-1 inhibitory function. Without wishing to be bound by theory, it is expected that the altered signaling state in anergic and exhausted T cells might favor phosphorylation of PD-1-Y248, PD-1: PD-1 complex formation and sustained activation of SHP-2-independent manner, leading to T cell immune dysfunction. Notably, early studies had reported that activation of the Src family kinase Fyn, which the below-disclosed data shows is required for PD-1 phosphorylation and interaction with SHP-2, has an active role in inducing and maintaining the T cell anergic state.

PD-1 partitions in the TCR microclusters which consist of TCR and proximal signaling molecules. Using a planar lipid bilayer system, it was previously observed that recruitment of PD-1 to the TCR microclusters is induced during antigen recognition and inhibits T cell activation by bringing SHP-2 to the TCR proximal signaling molecules. Recruitment of PD-1 in the TCR microclusters can occur in the absence of ITSM-Y248 phosphorylation but, under these conditions, fails to inhibit T cell activation. The below-disclosed data suggests that not only ITSM-Y248 phosphorylation but also spatial distribution of phosphorylated PD-1 polypeptides in proximity to TCR signaling substrates in the TCR microclusters will affect the inhibitory function of PD-1 because only PD-1 polypeptides bridged through pITSM-Y248 by the two SH2 domains of SHP-2 at such a distance that can induce SHP-2 conformational change allowing activation of SHP-2 phosphatase will be responsible for inhibition of T cell responses.

Without wishing to be bound by theory, the below-disclosed data reveals why TCR signaling is required for the activation of the inhibitory effect of PD-1 and the mechanistic role of PD-1 pITSM-Y248 in PD-1-mediated inhibition. It is further expected that PD-1 dimers can bind to covalent or noncovalent dimers of PD-L1 and PD-L2 to form a lattice of PD-1: PD-L1 interactions; thereby forming a stable signaling complex at the T cell: APC interface. The below-disclosed data reveals the geometry of PD-1: SHP-2 interaction that leads to SHP-2 activation; exploiting this interaction will reveal avenues for the development of PD-1-binding compounds which selectively suppress T cell

responses by dimerizing PD-1 or to prevent the PD-1 inhibitory signal by disrupting PD-1 dimerization.

In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner.

EXAMPLES

Example 1: Materials and Methods

Cultures of Jurkat, Raji and COS cells were performed in 37°C/5% CO_2 incubator in RPMI 1640 supplemented with 2 mM L-glutamine (Cellgro/Mediatech, Manassas, VA), 10% heat-inactivated fetal bovine serum (FBS) (Atlanta Biologicals, Flowery Branch, GA), 10 mM HEPES, 1 mM sodium pyruvate, 50 U/ml Pen/Strep (from Cellgro/Mediatech, Manassas, VA), and 15 µg/ml gentamycin (from Gibco/Invitrogen, Grand Island, NY). Gibco 293H cells (Fisher Scientific) were cultured in DMEM supplemented with 10% heat-inactivated FBS, 10 mM HEPES, 1% glutamax, 1% Pen/Strep, 15 µg/ml gentamycin. Jurkat cells were stably transfected with PD-1 and cultured in presence of 5 µg/ml selection antibiotic blasticidin. Before stimulation experiments, Jurkat cells were rested overnight at 37 °C in RPMI-1640 containing 2% FBS. Raji cells were stably transfected with PD-L1 and cultured in presence of 10 µg/ml blasticidin). For short-term activation, Jurkat cells were re-suspended at 100 x 10^6 cells/ml in pre-warmed RPMI 1640 containing 10 mM HEPES and mixed with an equal volume of RPMI/HEPES containing equal numbers of tosylactivated magnetic beads (conjugated either with anti-CD3, anti-CD28 mAbs and IgG or with anti-CD3, anti-CD28 and PD-L1-Ig). The cells-beads mixture was then centrifuged for 1 minute at 300 *g* at room temperature and placed immediately at 37 °C. At the indicated time points, the reaction was stopped by adding cold PBS, cell-bead pellet disruption by pipetting and placement on ice. Preparation of Dynabeads M-450 (Thermo Scientific) tosylactivated magnetic beads was done as previously described with slight modifications. Briefly, the following mAbs were used: anti-CD3 (UCHT1, Biolegend) and anti-CD28 (CD28.2, Biolegend). 2 x 10^8 magnetic beads were coated with anti-CD3 (8%), anti-CD28 (6%) and either PD-L1-Ig fusion protein or control IgG comprised the remaining 86% of the total protein (100 µg total protein). All incubations were performed in 0.1 M sodium phosphate buffer for 18 hr at 37 °C with constant rotation. The beads were then washed 3 times and stored at 4 °C. For Raji mediated stimulation Raji cells were resuspended at 1 x 10^6 cells/ml in RPMI complete medium and loaded with 0.5 ng/ml SEE (Toxin Technologies) by 30 min rotation at 37 °C followed by three washes to remove excess SEE. Jurkat cells were cultured in 96-well tissue culture plates, at 10^5 cells/well with equal numbers of Raji cells (with or without SEE loading) in a final volume of 100 µl. When indicated, a PD-1 blocking antibody (clone EH12) or an isotype control IgG was added in the cultures.

COS cell transfection

COS cells were plated 24 hours before transfection on 10 cm dishes at a density of 0.8 x 10^6 cells/10 cm/dish. Next day the cells were transfected by GeneJuice transfection reagent (EMD Millipore Corp., Billerica, MA) according to the manufacturer's instructions. Briefly, for each plate, 0.6 ml plain RPMI was mixed with 18 µl GeneJuice reagent and incubated 10 min at room temperature (RT). 12 µg total plasmid DNA was added in the mixture followed by 30 min incubation at RT. The mixture was added dropwise to the plate and cells were incubated 24 hours at 37 °C, medium was replaced and cells were harvested at 48 hr post-transfection, trypsinized and lysed for subsequent western blot analysis.

Immunoprecipitation and immunoblotting

To prepare lysates, cells were washed in PBS and lysed in lysis buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM MgCl2, 10% glycerol and 1% NP-40 supplemented with 2 mM sodium orthovanadate, 1 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride (PMSF), and protease Inhibitor Cocktail (Thermo Scientific). Cell lysates were resolved by SDS-PAGE and then analyzed by Western blotting with the indicated antibodies. The mAb against FLAG (clone M2) was from Sigma, St. Louis, MO. The mouse monoclonal anti-PD-1 antibodies clones EH12 and EH33 have been previously described. The rabbit polyclonal anti-363 phospho-Y248 (ITSM) PD-1 antibody was developed. Immunoprecipitations were performed with PD-1 mAb clone EH12 covalently conjugated to Dynabeads protein G (Thermo Scientific). Briefly, 40 µl of beads/sample were incubated for 15 min at RT with gentle rotation with 10 µg PD-1 antibody in PBS containing 0.02% Tween-20 in a final volume of 200 µl. Beads were then washed in 200 µl conjugation buffer (20 mM NaHPO4, 150 mM NaCl, pH 7.5) followed by 30 min gentle rotation at RT with 250 µl 5 mM Bis(Sulfosuccinimidyl) substrate (BS3) (Thermo Scientific) in conjugation buffer. The reaction was stopped by adding 12.5 µl quenching buffer (1M Tris, pH 7.5) followed by 15 min gentle rotation at RT. Beads were washed in IP buffer (lysis buffer without NP-40) and subsequently incubated with 500 µg of cell lysates overnight at 4 °C with gentle rotation. One sample of antibody conjugated beads without lysate was used as a negative control. Beads were washed and boiled 5 min in western blot denaturing sample buffer followed by quick spin, magnetic bead removal. The supernatant was then analyzed by SDS-PAGE, transferred to a nitrocellulose membrane, western blotted with the indicated antibodies and exposed to digital imager FluorChem E (Proteinsimple, San Jose, CA).

DNA constructs, cloning and mutagenesis

SHP-2 cDNA (Addgene, Cambridge, MA) was used to generate Glutathione-S-transferase (GST) fusions to SHP-2 wild type full length (SHP-2-WT-FL) and deletion mutants (**FIG. 1A**) using the pGEX 4T-3 vector (GE Healthcare Life Sciences, Marlborough, MA) and Flag-tagged mutants using the p3xFLAG-CMV10 vector (Addgene, Cambridge, MA).

Human PD-1 and PD-L1 cDNAs were expressed in pEF6 vector. For mutagenizing the human PD-1 tyrosine residues Y223 (within the ITIM motif) and Y248 (within the ITSM motif) and the arginine residues of SHP-2 R32 (within the N-SH2 domain) and R138 (within the C-SH2 domain) the QuickChange Lightning Site-Directed Mutagenesis kit from Agilent Technologies was used. All mutations were HD cloning kit from Clontech Laboratories, Inc., Mountain View, CA, following the manufacturer's instructions45. For NanoBiT experiments in HEK-293 cells, human PD-1 cDNA was cloned in Large BiT pBit1.1C [TK/LgBit] and in Small BiT pBit2.1C[TK/SmBit] vectors (Promega). For NanoBiT experiments in Jurkat T cells, human PD-1 cDNA was cloned in Large BiT pBiT1.3-C [CMV/LgBiT/Hyg] and in Small BiT pBiT2.3-C [CMV/SmBiT/Blast] vectors (Promega). The cDNAs for kinase active and kinase inactive forms of Lck, Fyn, ZAP-70, were inserted in pcDNA1.1/Amp vectors (Invitrogen).

GST-SHP-2 protein production, purification and pull-down assay

For pull-down assays the GST-fusion constructs of SHP-2 were expressed in E. coli BL21(D3) LysS. 100 µl bacteria were inoculated in 50 ml LB medium containing 100 µg/ml ampicillin and cultured overnight at 37°C on a shaker at 225 rpm. Next day the culture was added in 300 ml LB containing ampicillin and grown up to OD=0.6. Then SHP-2 expression was induced using 200 µM IPTG for 3 hr at 37 °C with shaking at 225 rpm. Proteins were extracted by B-PER bacterial protein extraction kit (Thermo Scientific, Rockford, IL) and purified on glutathione-sepharose (GE Healthcare Life Sciences, Marlborough, MA). GST and GST fusion proteins (10 µg each) were incubated for 1 hour at 4°C with 50 µl of glutathione-Sepharose in GST-buffer and then incubated with cell extracts (500 µg per sample) overnight followed by SDS-PAGE and Western blotting analysis with the indicated antibodies. For preparation of purified recombinant proteins for Biacore surface plasmon resonance analysis, the GST tag was removed by incubating 10-15 ml of bacterial lysate (containing the GST-SHP-2 proteins) with 2 ml glutathione-sepharose (1 ml bed volume) for 2 hours and gentle rotation at 4 °C. The approximately 1 ml bed volume of glutathione-sepharose was washed 3 x 10 ml PBS and was mixed with 80 units of thrombin (GE Healthcare Life Sciences, Marlborough, MA) and PBS up to 2 ml followed by overnight on-column digestion at RT with gentle rotation. The cleaved untagged SHP-2 proteins were then collected by 3 x 1 ml PBS washes, concentrated by Amicon Ultra-4, 10K centrifugal filters and subjected to FPLC purification. The protein was subsequently purified as previously described with small modifications. Specifically, 1 mg protein was diluted in a buffer with final concentration 50 mM NaCl, 20 mM Tris-HCl (pH 8.5), 1 mM dithiothreitol (DTT) and applied to a Bio-Rad Q1 Anion exchange column UNO Q1 equilibrated with 20 mM Tris (pH 8.5), 50 mM NaCl, 1 mM DTT. The protein was eluted with 80 ml gradient from 50–250 mM NaCl (in 20 mM Tris pH 8.5). 2-ml fractions were collected at a flow rate of 2 ml/min and analyzed for SHP-2 enrichment and purity by SDS-PAGE, Coomassie staining and immunoblotting. Enriched fractions were pooled and buffer was exchanged to PBS by PD-10 desalting columns and concentrated by Amicon Ultra-4, 10K centrifugal filters. Protein concentration was measured by a standard Bradford assay (Bio-Rad Laboratories).

Assessment of SHP-2 interaction with PD-1 ITIM-pY223 and PD-1 ITSM-pY248 by electrophoretic mobility shift with Native PAGE.

For assessment of immunoreceptor tyrosine-based inhibitory motif (ITIM)-pY223 and the immunoreceptor tyrosine-based switch motif (ITSM)-pY248 binding on SHP-2 SH2 domains, a SHP-2 peptide comprising amino acids 1-225, which only contains the SHP-2 N-SH2 and C-SH2 domains in their natural tandem sequence (referred to as SHP-2 tandem-SH2), was produced and purified. Binding with monophosphotyrosyl ITIM-pY223 (pITIM, KEDPSAVPVFSVD(pY)GELDFQWRE; SEQ ID NO: 2) or monophosphotyrosyl ITSM-pY248 (pITSM, KTPEPPVPCVPEQTE(pY)ATIVFPS; SEQ ID NO: 1) peptide was assessed. 20 µM of SHP-2 tandem-SH2 were mixed with either pITIM or pITSM peptide at various molar ratios in 50 mM HEPES, 100 mM NaCl, pH 7.4, in a final volume of 20 µl and incubated for one hour at room temperature. 5 µl 5X Native Sample Buffer were added and Native PAGE was performed at 4oC with 8-16% gradient Tris-Glycine gel (Bio-Rad) followed by Coomassie stain.

Surface Plasmon Resonance

Measurements were made using a Biacore 3000 (GE Healthcare) at the Dana-Farber Cancer Institute proteomics core facility. Phosphotyrosyl peptide pITSM (KTPEPPVPCVPEQTE(pY)ATIVFPS) (SEQ ID NO: 1) was synthesized at the Tufts University Protein Synthesis Core Facility. For immobilization, presentation of the peptide at high concentration was used to overcome the difficulties in immobilizing the negatively charged phosphopeptides (pI≤3) to the negatively charged sensor chip. 2 mg/ml of phosphopeptide in mM HEPES, 1 M NaCl, pH 7.5, was presented to the sensor chip surface, previously activated with N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3- dimethylaminopropyl)-carbodiimide (EDC), for 7 min (up to 250 resonance units of phosphopeptides were immobilized) followed by inactivation of the surfaces with ethanolamine. Removal of non-covalently bound peptides was achieved using a 3-min pulse of 2 M guanidine hydrochloride followed by re-equilibration in running buffer HBS-EP (GE Healthcare Life Sciences). Efficient immobilization of the phosphopeptides was confirmed by binding of phosphotyrosine-specific 4G10 monoclonal antibody (Upstate Biotechnology, Inc. Cat. Number 05-321). An average of RU responses of 365 + 82.4 was obtained corresponding to an estimated immobilized ligand concentration of 1.2 mM ($Conc_{ligand}$ = $Response_{ligand}$ / 100 x Mr_{ligand} (mol liter $^{-1}$), which is about 3,750 x higher than the maximum SHP-2 concentration (320 nM), thus potentiating bridging. Negative control surfaces were prepared by performing the immobilization under identical conditions as above in the absence of both peptide and protein and used to correct for bulk refractive index signals introduced by the protein storage buffers. Experimental binding measurements were performed using HBS-P running buffer supplemented with 6.6 mM phosphate, to maintain identical PO43+ concentration across all protein concentrations, and further supplemented with 10 mM DTT and adjusted to pH 7.4. After each cycle, the chip was regenerated with a 1 min pulse of 3 M NaCl and 1 min pulse of 6 M guanidine hydrochloride, pH 7.0 as previously described. Neither loss of peptide

or tyrosine phosphorylation (as assessed by anti-phosphotyrosine binding) nor change in baseline RU was apparent during the course of an assay. Sensograms of SHP-2 WT, SHP-2 mutagenized proteins SHP-2-R32A, SHP-2-R138A at 0, 20, 40, 80, 160, 320 nM and of the single SH2 domains, N-SH2 and C-SH2, at 0, 1, 3, 10, μM and at 900 second (15 min) association and 900 sec dissociation time were analyzed by the BIAevaluation software (GE Healthcare Life Sciences) in each instance determining separately the *k*d (dissociation rate constant) followed by the *k*a (association rate constant) and calculation of KD=*kd/ka*.

NanoBiT assay for receptor dimerization

For experiments with HEK-293 cells, one day before transfection, HEK-293 was plated as 20,000 cells in 0.1 ml DMEM complete medium per well, in 6 replicates per condition, on a white 96-well tissue culture plate. The following day cells were transfected by Fugene HD (Promega Corporation, Madison, WI) according to the manufacturer's instructions. Briefly, plasmid DNA was diluted to 12.5 μg/ml in 0.1 ml Opti-MEM I (Fisher Scientific). For 4 plasmids, 3.125 μg/ml of each one in the above mixture was used. For consistency, in the case of fewer plasmids (such as in the case of the 2-plasmid positive control pair NanoBiT constructs), the remaining plasmid DNA was supplemented with appropriate amount of empty vector (up to 12.5 μg/ml). 3.75 μl of Fugene were added to each DNA-Opti-MEM I mix and after 10 min incubation at room temperature 8 μl of each mixture were added per well and cells were placed back in the incubator. 30 hours after transfection, 25 μl of 1X Nano-Glo Live Cell Assay Substrate (Promega) was added per well and luminescence was read on a SpectraMax M3 plate reader (Molecular Devices). For experiments in Jurkat cells, transfection was performed by electroporation. Briefly, for each transfection 10 x 10^6 cells were resuspended in 400 μl pre warmed RPMI complete medium. For 2 plasmids co-transfection we used 25 μg of each (total 50 μg). For 3 plasmids co-transfection we used 20 μg of each (total 60 μg). Cells were transfected in a 4 mm electroporation cuvette with a Bio-Rad electroporator (250V, 975 μF, 400 Ohm) and cells were immediately transferred to 50 ml medium and placed in the incubator for 36h. Then cells were harvested and co-cultured in a white 96-well tissue culture plate with Raji cells (with or without SEE loading) for 6h followed by addition of 25 μl of 1X Nano-Glo Live Cell Assay Substrate (Promega) and luminescence measurement on a SpectraMax M5 plate reader (Molecular Devices). Where indicated, the allosteric SHP-2 inhibitor SHP099 (Medchem Express) was added in the cultures in increasing concentrations; vehicle (DMSO) was used as control.

Measurement of SHP-2 activity

Catalytic activity of SHP-2 was monitored by a fluorescent assay using the substrate 6,8- difluoro-4-methylumbelliferone (DiFMUP) as previously with slight modifications. Specifically, the phosphatase reactions were performed in 50 mM HEPES, pH 7.4, 100 mM NaCl and 10 mM DTT in 96-well black polystyrene plate, flat bottom, non-binding surface, using a final reaction volume of 100 μl. 1.6 μg/ml of SHP-2-WT, SHP-2-R32A or SHP-2-R138A

mutant proteins were incubated with 20 μM DiFMUP with the indicated concentrations of phosphotyrosyl peptides or without peptide at 37 °C. Fluorescence signal was monitored every 1 min for 3 min by a SpectraMax5 microplate reader and reaction rate was calculated by the change of fluorescence signal with time using excitation and emission wavelengths of 340 nm and 450 nm, respectively. Under these conditions product formation was linear with respect to the time of incubation. Results were normalized against the basal activity determined for each condition in the absence of peptide and activity was calculated. All peptides were synthesized at the Tufts University Core Facility. The sequence of the phosphopeptides used were as follows, with pY indicating the phosphorylated tyrosine.

MMonophosphoryl ITSM peptide: KTPEPPVPCVPEQTE(pY)ATIVFPS (SEQ ID NO: 1).

MMonophosphoryl ITIM peptide: KEDPSAVPVFSVD(pY)GELDFQWRE (SEQ ID NO: 2).

DBisphosphoryl ITSM peptide (bpITSM): ITSM-Ahx4-ITSM: TE(pY)ATIVFP-Ahx4-QTE(pY)ATIVFPS (SEQ ID NO: 3; comprising SEQ ID NO: 22-Ahx4-SEQ ID NO: 23).

DBisphosphoryl ITIM peptide (bpITIM): ITIM-Ahx4-ITIM: VD(pY)GELDFQ-Ahx4-SVD(pY)GELDFQW (SEQ ID NO: 4).

DBisphosphoryl ITIM-ITSM peptide (pITIM-pITSM): ITIM-Ahx4-ITSM: VD(pY)GELDFQ-Ahx4- QTE(pY)ATIVFPS (SEQ ID NO: 5).

Monophosphoryl IRS-Y727 peptide: TGD(pY)MNMSPVG (SEQ ID NO: 6).

Monophosphoryl IRS-Y1172: SLN(pY)IDLDLVK (SEQ ID NO: 7).

Bisphosphoryl IRS peptide (bpIRS), 1172-Ahx4-1222: LN(pY)IDLDLV-Ahx4-LST(pY)ASINFQK (SEQ ID NO: 8).

KTPEPPVPCVPEQTE(pY)ATIVFPS-Ahx4-KTPEPPVPCVPEQTE(pY)ATIVFPS (SEQ ID NO: 9; comprising SEQ ID NO: 1-Ahx4-SEQ ID NO: 1).

KTPEPPVPCVPEQTE(pY)ATIVFPS-Ahx4-TE(pY)ATIVFP (SEQ ID NO: 10; comprising SEQ ID NO: 1-Ahx4-SEQ ID NO: 22).

KTPEPPVPCVPEQTE(pY)ATIVFPS-Ahx4-QTE(pY)ATIVFPS (SEQ ID NO: 11; comprising SEQ ID NO: 1-Ahx4-SEQ ID NO: 23).

KEDPSAVPVFSVD(pY)GELDFQWRE-Ahx4-KEDPSAVPVFSVD(pY)GELDFQWRE (SEQ ID NO: 12).

KEDPSAVPVFSVD(pY)GELDFQWRE-Ahx4-VD(pY)GELDFQ (SEQ ID NO: 13).

KEDPSAVPVFSVD(pY)GELDFQWRE-Ahx4-SVD(pY)GELDFQW (SEQ ID NO: 14).

KTPEPPVPCVPEQTE(pY)ATIVFPS-Ahx4-KEDPSAVPVFSVD(pY)GELDFQWRE (SEQ ID NO: 15).

KTPEPPVPCVPEQTE(pY)ATIVFPS-Ahx4-VD(pY)GELDFQ (SEQ ID NO: 16).

KTPEPPVPCVPEQTE(pY)ATIVFPS-Ahx4-SVD(pY)GELDFQW (SEQ ID NO: 17).

TE(pY)ATIVFP (SEQ ID NO: 22).

QTE(pY)ATIVFPS (SEQ ID NO: 23).

Bisphosphoryl ITSM peptide pITSM-Ahx10-pITSM (bpITSM-Ahx10): TE(pY)ATIVFP-Ahx10-QTE(pY)ATIVFPS (SEQ ID NO: 24).

Cytokine production

Culture supernatants were harvested at the indicated time points and the concentration of IL-2 was assessed by ELISA assay kits (Biolegend or R&D Systems) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed by Student's t-test or ANOVA and Tukey's multiple comparisons test. A p value of <0.05 was considered statistically significant.

Example 2: PD-1: SHP-2 Interaction Results in Activation of SHP-2

The cytoplasmic tail of PD-1 contains two tyrosine-based structural motifs, an immunoreceptor tyrosine-based inhibitory motif (ITIM) (V/L/I/XpYXX/L/V) and an immunoreceptor tyrosine-based switch motif (ITSM) (TXpYXXV/I). Mutational studies have shown that PD-1 inhibitory function is mainly dependent on the ITSM phosphotyrosine, which preferentially recruits SHP-2 phosphatase, resulting in dephosphorylation and downregulation of downstream effector molecules. Mass spectrometry studies have shown that PD-1 phospho-ITSM peptide can act as a docking site in vitro for both SHP-2 and SHP-1, whereas phospho-ITIM peptide can associate with SHP-212. Although both SHP-1 and SHP-2 were found to interact with the PD-1 ITSM in these systems, live cell imaging to visualize events that occur during T-cell activation in real time showed that only SHP-2 interacts with PD-1 in live cells. Notably, the ability of PD-1 to inhibit T cell activation first requires TCR and CD28 co-stimulation, suggesting that T cell activation signals may be necessary for PD-1 to obtain the ability to manifest its inhibitory function.

SHP-2 has two tandem SH2 domains, N-terminal (N-SH2) and C-terminal SH2 (C-SH2), followed by a single protein tyrosine phosphatase (PTP) domain, and a C-terminal hydrophobic tail with two tyrosine phosphorylation sites (**FIG. 6A**). Exposure of cells to a variety of extracellular stimuli triggers the binding of SHP-2 *via* its SH2 domains to tyrosine phosphorylated receptors for growth factors such as platelet-derived growth factor (PDGF) as well as to tyrosine-phosphorylated docking proteins including insulin receptor substrates (IRSs), signal regulatory protein a (SIRPa; also known as SHP substrate-1 (SHPS-1), Grb2- associated binder proteins (Gabs), and fibroblast growth factor receptor substrate (FRS). Such interactions are important not only for activation of the phosphatase activity but also for the recruitment of SHP-2 to sites near the plasma membrane where potential substrate proteins may be located. The mechanisms that regulate SHP-2 enzymatic activity have been broadly studied. At the basal state, the N-SH2 domain of SHP-2 binds the phosphatase domain in an auto-inhibitory closed conformation and directly blocks the active phosphatase site. Interaction of the N-SH2 domain with phosphotyrosine peptide disrupts the interaction of N-SH2 with the phosphatase active site and activates the enzyme (**FIG. 6B**). The C-SH2 domain contributes binding energy and specificity but does not have a direct role in enzymatic activation. The crystal structure of SHP-2 has shown that the two SH2 domains of SHP-2 have a roughly antiparallel or roughly perpendicular orientation relative to one another, with the phosphopeptide binding sites lying fully exposed on the surface of the polypeptide and widely spaced (**FIG. 6C**). In this example, the mechanisms by which PD-1: SHP-2 interaction results in activation of SHP-2 phosphatase activity was examined.

Phosphorylation of PD-1 ITSM Y248 by TCR proximal Src family kinases Fyn and Lck is required for interaction with SHP-2

To examine how TCR signaling regulates the ability of PD-1 to interact with SHP-2 and identify the mechanism by which PD-1: SHP-2 interaction activates SHP-2 phosphatase activity. Since PD-1 is expressed after activation of human or mouse T cells either pre-activation of T cells or lentiviral transduction of PD-1 have been used to induce PD-1 expression in primary T cells. To avoid changes that can be induced by such approaches in the tyrosine phosphorylation state of key signaling components of the TCR pathway, Jurkat T cells stably expressing human PD-1 (J-PD1 cells) were generated (**FIG. 7A**). Incubation of J-PD-1 cells with magnetic beads conjugated with aCD3/aCD28/IgG or aCD3/aCD28/PDL1-Ig5, followed by PD-1 immunoprecipitation showed that PD-1: SHP-2 interaction was not induced by CD3 and CD28 ligation alone but was strongly induced upon simultaneous CD3, CD28 and PD-1 co-ligation (**FIG. 1A**, **FIG. 8A**, and **FIG. 9**).

To determine whether phosphorylation of PD-1 ITSM Y248 has an essential role in the PD-1: SHP-2 interaction, the role of Y248 phosphorylation was examined using a phospho-ITSM pY248-specific antibody pPD1(Y248). J-PD-1 cells and J-PD-1-Y248F Jurkat T cells (which expressed human PD-1 with tyrosine 248 mutated to phenylalanine) was used

(**FIG. 7B**) and were incubated with magnetic beads conjugated with aCD3/aCD28/IgG or aCD3/aCD28/PDL1-Ig. PD-1 immunoprecipitation showed that PD-1 ITSM-Y248 phosphorylation and SHP-2 co-precipitation were detected after aCD3/aCD28/PDL1-Ig incubation in J-PD-1 cells but was abrogated in J-PD-1-Y248F cells (**FIG. 1B** and **FIG. 8B**), indicating that Y248 in PD-1 ITSM has an essential role in PD-1: SHP-2 interaction.

Because Y248 phosphorylation requires TCR signaling simultaneously with PD-1 ligation (**FIG. 1A**) and PD-1 lacks intrinsic kinase activity, which TCR-proximal kinases might be able to mediate PD-1 Y248 phosphorylation was examined. COS cells were co-transfected with PD-1 cells, SHP-2, and either kinase-active or dominant negative forms of Fyn, Lck or ZAP-70 cDNAs. PD-1 immunoprecipitation showed that the Src family kinase Fyn and to a lesser extent Lck, but not ZAP-70, mediated phosphorylation of PD-1 ITSM Y248 and PD-1: SHP-2 interaction (**FIG. 1C** and **FIG. 8C**). Using Fyn-deficient primary mouse T cells (**FIG. 10A**) and the Lck-deficient Jurkat T cell line JCaM1.6 stably transfected with human PD-1 (**FIG. 11A**), it was determined that activation of Fyn and Lck during stimulation with αCD3/αCD28/PDL1-Ig coated beads is required for phosphorylation of PD-1 ITSM Y248 and interaction with SHP-2 (**FIG. 10B**, **FIG. 10C**, **FIG. 11B**, and **FIG. 11C**).

To determine whether phosphorylation of PD-1 by Src family kinases at Y248 is necessary and sufficient for interaction with SHP-2, constructs having single mutations of PD-1 ITIM (Y223F), ITSM (Y248F) or double mutations of ITIM and ITSM (Y223F/Y248F) were generated and co-transfected into COS cells with each PD-1 mutant or PD-1 wild type and cDNAs of Fyn and SHP-2. PD-1 immunoprecipitation showed that SHP-2 interacted with PD-1-WT, and that mutation of ITIM (Y223F) did not affect this interaction (**FIG. 1D** and **FIG. 8D**). In contrast, mutation of ITSM (Y248F) as well as the double mutation Y223F/Y248F abrogated SHP-2 co-precipitation (**FIG. 1D** and **FIG. 8D**), indicating that Y248 in the ITSM is necessary and sufficient to mediate interaction of PD-1 with SHP-2 after phosphorylation by Src family kinases.

Interaction of both SH2 domains of SHP-2 is required for SHP-2 binding to PD-1

To determine how SHP-2 interacts with PD-1, GST-fusion proteins (**FIG. 2A**) of SHP-2 full-length (GST-SHP-2-FL) and four SHP-2 deletion mutants were generated, GST-SHP-2-PTP, which contains only the protein tyrosine phosphatase (PTP) domain, GST-SHP-2-DNSH2, lacking the N-terminal SH2 domain, and GST-SHP-2-N-SH2 and GST-SHP-2-C-SH2, which contain only SHP-2 N134 SH2 or C-SH2 single domains. In lysates of J-PD-1 cells stimulated with aCD3/aCD28/PDL1-Ig, binding of PD-1 was detected in pulldown experiments with GST-SHP-2 FL and GST-SHP-2-DNSH2, whereas no interaction of PD-1 was detected with GST-SHP-2-PTP (**FIG. 2B** and **FIG. 12A**). Notably, either single SH2 domain-containing GST-fusion protein, GST-SHP-2-N-SH2 or GST SHP-2-C-SH2, could pulldown PD-1 (**FIG. 2B** and **FIG. 12A**). Similar results were obtained using primary human T cells (**FIG. 13A** and **FIG. 13B**). These results show that both N- and C-SH2 domains of SHP-2 are able to bind to PD-1 in vitro.

Because each of the two SH2 domains of SHP-2 could interact with PD-1 when GST-SHP-2 fusion proteins were inclubaed with cell lysates in vitro, it was examined whether SHP-2 binding to PD-1 ITSM-Y248 might be mediated by selective interaction with one of the SHP-2 SH2 domains when co-expressed in cells. For this purpose, COS cells were transfected with SHP-2 constructs in which contained inactivating mutations in the active site of each SH2 domain: specifically SHP-2's arginine 32 residue (which is in the N-SH2 domain and which corresponds to the amino acid at position 32 of SEQ ID NO: 19) and SHP-2's arginine 138 (which is in the C-SH2 domain and which corresponds to the amino acid at position 28 of SEQ ID NO: 20); these residues are critical for mediating binding of each SH2 domain of SHP-2 with phosphotyrosine). Mutagenesis of arginine to alanine at the active site of either SH2 domain (R32A or R138A) abrogated SHP-2 interaction with PD-1 wild type (**FIG. 2C** and **FIG. 12B**, upper panels). Similarly, mutagenesis of either SH2 domain equally disrupted the interaction of SHP-2 with endogenous PD-1 in primary human T cells (**FIG. 12C** and **FIG. 12D**). Wild type SHP-2 was still able to interact with PD-1 ITIM-Y223F mutant containing intact Y248 but the inactivating mutations of each SH2 domain (R32A and R138A) abrogated interaction of SHP-2 with PD-1 ITIM-Y223F mutant (**FIG. 2C**, and **FIG. 12B**, middle panels). As expected, PD-1 ITSM-Y248F mutant abrogated interaction of PD-1 with wild type SHP-2 (**FIG. 2C** and **FIG. 12B**, bottom panels), consistent with the findings that PD-1 ITSM-Y248 is essential for interaction with SHP-2 (**FIG. 1B** and **FIG. 1D**). Importantly, PD-1 ITSM154 Y248F mutant abrogated interaction of PD-1 with either SHP-2 mutant (**FIG. 2C** and **FIG. 12C**, bottom panel), indicating that each SH2 domain of SHP-2 interacts with PD-1 ITSM-Y248 when co-expressed in live cells. Assessment of ITIM-pY223 or ITSM-pY248 phosphopeptide binding to SHP-2 SH2 domains by Native PAGE binding assay showed that electrophoretic mobility shift of SHP-2, as a consequence of phosphopeptide binding, is induced by ITSM-pY248 but not by ITIM-pY223 (**FIG. 2D**) confirming that ITIM-pY223 is not a binding partner of SHP-2 SH2 domains. Because both SH2 domains are involved in the interaction with ITSM-pY248 (**FIG. 2C**) and the PD-1 polypeptide has only one ITSM Y248, these cellular and biochemical studies suggest that after T cell activation and PD-1 phosphorylation, SHP-2 binds phosphorylated ITSM-Y248 residues in two PD-1 polypeptides using its N-SH2 domain for one PD-1 and its C159 SH2 domain for a second PD-1 to form a PD-1 dimeric complex (**FIG. 2E**). Consistent with this model, the above-described binding studies (**FIG. 2D**) showed that complete shift of SHP-2 from the unbound to the SHP-2: ITSM-pY248 bound form occurs at an ITSM-pY248 to SHP-2 ratio of greater than two.

The results in FIG. 2D also indicated that when ITIM-pY223 and ITSM- pY248 phosphopeptides were incubated with SHP-2 SH2 domains at the same molar ratio, ITSM- pY248 is the preferred binding partner of SHP-2. Consistently, compared with a peptide containing both phosphorylated tyrosines of the native PD-1 cytoplasmic tail (FIG. 2F, top panel), electrophoretic mobility shift of t-SHP-2 was preserved by a peptide containing phosphorylation of Y248 (PD-1cyto-ITIM-pITSM), but not by a peptide containing phosphorylation of Y223 (PD-1cyto-pITIM-ITSM) (FIG. 2F middle and bottom panel).

The results of the experiments of this example are consistent with two models disclosed herein of PD-1: SHP-2 interaction when co-expressed in cells. One possible model is that PD-1 ITSM-pY248 serves as the high affinity binding site for one of the SHP-2 SH2 domains thereby being a pre-requisite for the binding of the second SH2 domain on PD-1 ITIM Y223 that serves the low affinity interaction site (FIG. 2G). This model can provide an explanation as to why PD-1 ITSM-Y248F disrupted the interaction of PD-1 with either SHP-2 WT or each SHP-2 SH2 active site mutant (FIG. 2C, bottom panel) and why ITIM-Y223F did not affect interaction with SHP-2 WT. A second model is that both SH2 domains interact with ITSM- pY248 and because the PD-1 molecule has only one ITSM-Y248, SHP-2 binds phosphorylated ITSM-pY248 residues in two PD-1 molecules using its N-SH2 domain for one PD-1 and its C-SH2 domain for a second PD-1 to form a PD-1 dimer (FIG. 2H). The latter can explain why PD-1 ITSM-Y248F disrupted the interaction of PD-1 with either SHP-2 WT or each SHP-2 SH2 active site mutant (FIG. 2C, bottom panel). This model can also explain why in cells expressing PD-1 ITIM-Y223F the interaction of PD-1 with SHP-2 WT was not affected but the interaction of PD-1 with each SH2 active site mutant was disrupted (FIG. 2C, middle panel).

Surface plasmon resonance (SPR) analysis of SHP-2 interaction with PD-1 ITSM-pY248

Since the mutational and single SH2 domain analysis revealed the importance of both SH2 domains in interaction of WT SHP-2 with phosphorylated PD-1 ITSM-Y248, this interaction was examined using surface plasmon resonance (SPR) to analyze the SHP-2 interaction with immobilized PD-1 ITSM-pY248 phosphopeptide in more detail. Tyrosine phosphorylation of the immobilized PD-1 ITSM-pY248 phosphopeptide was confirmed by 4G10 mAb binding (**FIG. 14A**). Three recombinant proteins, SHP-2-WT, SHP-2-R32A and SHP-2- R138A were produced, purified (**FIG. 14B** to **FIG. 14D**), and assessed for binding to PD-1 ITSM-pY248 phosphopeptide by SPR. Loss of the N-SH2 active site in SHP-2-R32A, reduced association kinetics with PD-1 ITSM-pY248 phosphopeptide, decreasing the affinity approximately 240-fold compared to SHP-2-WT (KD=7.11 μM vs. KD=0.0293 μM, respectively). Loss of the C-SH2 active site in SHP-2-R138A decreased the affinity approximately 150-fold compared to SHP-2-WT (KD=4.53 μM vs. KD=0.0293 μM, respectively) (**FIG. 3A**). Purified proteins comprising each single SHP-2 SH2 domain were assessed for their binding affinity for PD-1 ITSM-pY248 (**FIG. 14B**). The data showed that the affinity of the N-SH2 domain is approximately 5-fold higher than the affinity of the C-SH2 domain for ITSM-pY248 (KD=0.33 μM vs. KD=1.79 μM, respectively) (**FIG. 3B**). The higher affinity of the N-SH2 than C-SH2 for PD-1 ITSM-pY248 phosphopeptide, may explain why SHP-2-R32A, which retains an intact C-SH2 but has a mutagenized N-SH2 domain, had 240-fold lower affinity than SHP-2-WT while SHP-2-R138A, which has a mutagenized C-SH2 but retains an intact N178 SH2 domain had 150-fold lower affinity for PD-1 ITSM-pY248 phosphopeptide than SHP-2-WT (**FIG. 3A**). These results show that each of the SH2 domains of SHP-2 is involved in the interaction of SHP-2 with PD-1 ITSM-pY248. Importantly, the affinity of each individual SH2 domain is negligible compared to the affinity of SHP-2-WT (N-SH2 KD=0.33 μM, C-SH2 KD=1.79 μM, SHP-2WT KD=0.0293 μM) (**FIG. 3A** and **FIG. 3B**). SHP-2-full length interacted with PD-1 ITSM-pY248

coated surface in a dose-dependent manner (KD=0.0197 μM) (**FIG. 3C**). Because ITSM-pY248 was immobilized at concentrations that permit bridging, these findings indicate that binding of both SH2 domains of SHP-2 full length protein to PD-1 ITSM-pY248 is responsible for the higher binding affinity of SHP-2-WT compared to the binding of each individual SH2 domain. No specific interaction of SHP-2 protein was detected with PD-1 ITIM-pY223-coated surface (**FIG. 3D**). Together these cellular and biochemical findings strongly suggest that SHP-2 bridges two phosphorylated ITSM-Y248 residues in a PD-1 receptor dimer using both its SH2 domains (**FIG. 2E**).

In addition, the calculated affinity of the N-SH2 domain is approximately 75-fold higher than that of the C-SH2 domain for ITSM-pY248 (K_D=2.2 μM vs. K_D=161 μM, respectively) (**FIG. 20B** and **FIG. 20C**). Importantly, the affinity of each individual SH2 domain is negligible compared with the calculated affinity of SHP-2-full length (N-SH2 K_D=2.2 μM, C-SH-2 K_D=161 μM, SHP-2 K_D=0.0197 μM) (**FIG. 20A, FIG. 20B**, **FIG. 20C**). These findings suggest that interaction of both SH2 domains of SHP-2-full length protein to PD-1 ITSM-pY248 might be responsible for the higher binding of SHP- 2-full length compared with the binding of each individual SH2 domain.

To examine further the nature of PD-1: SHP-2 interaction, stoichiometry was assessed by isothermal titration calorimetry (ITC). The SHP-2 protein construct that contains only the two tandem SH2 domains (t-SHP-2) and a phosphopeptide corresponding to the native PD-1 cytoplasmic tail in which both ITIM-Y223 and ITSM-Y248 tyrosines were phosphorylated (PD-201 1cyto-pITIM-pITSM) were used to assess how PD-1: SHP-2 interaction might occur in the presence of both phosphotyrosines. PD-1cyto-pITIM-pITSM: t-SHP-2 interaction occurred at 1:1 stoichiometry (**FIG. 3E**). In addition, a phosphopeptide was used corresponding to the native PD-1 cytoplasmic tail in which ITSM-Y248 but not ITIM-Y223, which was phosphorylated (PD-1cyto-ITIM-pITSM). PD-1cyto-ITIM-pITSM: t-SHP-2 interaction occurred at 2:1 stoichiometry (**FIG. 3F**) providing biophysical evidence that interaction of SHP-2 SH2 domains with ITSM-pY248 from two PD-1 molecules to form a PD-1 dimer is feasible. Together these results show that when purified proteins are used in a cell-free system, PD-1: SHP-2 interaction can occur in two different ways: 1) One PD-1 molecule can bind with SHP-2 tandem SH2 domains likely using both phosphotyrosines, each of which interacts with one SH2 domain; 2) PD-1: SHP-2 interaction can also occur by binding of SHP-2 SH2 domains solely with ITSM-pY248 phosphotyrosine. In the latter binding mode, ITSM- pY248 on two PD-1 molecules interact with each of the tandem SH2 domains of SHP-2 indicating that SHP-2 can bridge two PD-1 molecules via its N-SH2 and C-SH2 domains, to form a PD-1 dimer.

SHP-2 bridges two PD-1 polypeptides via PD-1 ITSM pY248

To test this hypothesis, the NanoBiT proximity assay (Promega) was used, which permits detection of protein: protein interaction in living cells by employing a split luciferase enzyme. Large BiT (LgBiT; 17.6 kDa) and Small BiT (SmBiT; 11 amino acids) luciferase subunits, expressed in separate plasmids, are fused to proteins of interest and expressed

in live cells. Interaction of protein: protein molecules, brings the LgBiT and SmBiT subunits into close proximity to form a functional luciferase enzyme that generates a bright luminescent signal. Such protein: protein interactions can be followed in real time inside the living cells by using a non-lytic cell permeable detection reagent.

To examine whether two PD-1 polypeptides on the same cell surface are brought into proximity by SHP-2, a pair of PD-1 NanoBiT plasmids were generated: PD-1-LgBiT, in which the PD-1 cytoplasmic domain was linked to the LgBiT peptide sequence, and PD-1-SmBiT, in which the PD-1 cytoplasmic domain was linked to the SmBiT peptide sequence. Because luminescence is generated only when the LgBiT and SmBiT peptides come together and form an active luciferase enzyme, this would be achieved only if two PD-1 polypeptides stably interact (**FIG. 4A**).

HEK-293 cells were transfected with PD-1-LgBiT and PD-1-SmBiT together with SHP-2 and Fyn kinase, to recapitulate the effect of TCR-mediated signaling and interaction with SHP-2 required for PD-1 phosphorylation. Assessment of complex formation between PD-1-SmBiT and PD-1-LgBiT by measuring luminescent signal showed that in the presence of kinase-active Fyn but not kinase inactive Fyn, SHP-2 induced PD-1: PD-1 interaction (**FIG. 4A**, **FIG. 15A**, and **FIG. 15B**). Expression level of PD-1 induced by PD-1-LgBiT and PD-1-SmBiT transfection was comparable to that induced in activated primary human T cells (**FIG. 16A** and **FIG. 16B**), indicating that this result was not a consequence of artificial increase of PD-1 copy numbers on the cell surface beyond physiologic levels due to transfection. Co-transfection of PD-1-SmBiT and PD-1-LgBiT with kinase active Fyn together with either SHP-2-R32A or SHP-2-R138A, which are mutagenized in the active site of N-SH2 or C-SH2 domain, respectively, showed that loss of the active site of either SH2 domain of SHP-2 abrogated PD-1: PD-1 interaction (**FIG. 4B)**. Loss of the active sites of both SH2 domains in SHP-2- R32A/R138A double mutant induced the greatest decline. Similarly, mutation of the PD-1 ITSM-Y248, which serves as the binding site for the SH2 domains of SHP-2, abrogated PD1: PD-1 interaction (**FIG. 4C**). Notably, ligation of PD-1 with dimeric recombinant PD-L1 but not monomeric PD-L1 induced a modest increase of PD-1 dimerization and this effect was significantly diminished in the presence of kinase-inactive Fyn (**FIG. 4D**). Together these results indicate that formation of a PD-1 dimer requires intact SHP-2 SH2 domains and phosphorylation of PD-1 ITSM-pY248.

To further investigate the role of SHP-2 SH2 domains in the formation of the PD-1: PD-1 dimer, the allosteric SHP-2 inhibitor SHP099 (which prevents the conformation change of SHP-2 and the release of the N-SH2 domain from the PTP site that is induced upon interaction of N-SH2 with phosphorylated substrates) was used. Addition of increasing amounts of SHP099, but not vehicle control, in HEK-293 cells, transfected with PD-1-SmBiT and PD-1-LgBiT together with kinase active Fyn and SHP-2, showed that preventing the conformational change of SHP-2 SH2 domains induced a dose-dependent inhibition of PD-1: PD-1 interaction (**FIG. 4E**). Thus, a PD-1: PD-1 complex can be formed in live cells and requires PD-1 phosphorylation and interaction with SH2 domains of SHP-2.

To examine whether PD-1 dimers bridged by SHP-2 are formed in T cells during antigen encounter and PD-1 ligation, primary human T cells or Jurkat T cells were transfected with PD-1-LgBiT and PD-1-SmBiT. The expression level of PD-1 induced by PD-1-LgBiT and PD-1-SmBiT transfection was comparable to that induced in activated primary human T cells (**FIG. 16A** and **FIG. 16B**), confirming that PD-1-LgBiT and PD-1-SmBiT transfection did not induce artificial overexpression of PD-1. The transfected primary human T cells or Jurkat T cells were stimulated with Raji cells which stably expressed human PD-L1 with or without prior loading with SEE (**FIG. 17A** and **FIG. 17B**). Co-culture with Raji-PD-L1 loaded with SEE but not without SEE loading resulted in an elevated luminescent signal. This was significantly diminished by expression of SHP-2-R32A or SHP-2- R138A that were mutagenized in the active site of N-SH2 or C-SH2 domain, respectively, or the SHP-2-DM that was mutagenized in the active sites of both N-SH2 and C-SH2 domains (**FIG. 4F** and **FIG. 4G**). Thus, similarly to the pathway complementation approach employed in HEK-293 cells, antigen encounter and PD-1 ligation in T cells results in PD-1: PD-1 interaction mediated by the SH2 domains of SHP-2. Notably, although in primary T cells, loss of the active site of either SH2 domain of SHP-2 abrogated luciferase activity induced by PD-1: PD-1 interaction (**FIG. 4F**), a degree of luciferase activity remained detectable in Jurkat T cells under the same conditions (**FIG. 4G**). This residual activity likely represents clustering of PD-1 polypeptides not dependent on the interactions of PD-1 phosphotyrosines with SHP-2, as previously observed in a different experimental system. Mechanisms such as integrin-mediated and cytoskeletal actin-regulated microcluster formation, might be involved in this process and might have higher degree of activity in Jurkat than in primary T cells, accounting for this difference.

Interaction of SHP-2 with two PD-1 ITSM pY248 residues is required for activation of SHP-2 phosphatase activity

Tyrosine phosphorylated motifs that interact with SHP-2 SH2 domains have a decisive role in the activation of SHP-2 PTP enzymatic activity. To examine how phospho-PD-1 binding affects SHP-2 phosphatase activation, SHP-2 activity measurements were performed using DiFMUP (6,8-difluoro-4-methylumbelliferyl phosphate) substrate. PD-1 ITSM224 pY248 (pITSM) and PD-1 ITIM-pY223 (pITIM) monophosphorylated peptides were used as experimental groups, monophosphorylated peptide IRS-1-pY1172 (pIRSY1172) was used as positive control, and IRS-1-pY727 (pIRSY727) was used as a negative control. Assessment of SHP-2 phosphatase enzymatic activity showed that the effect of PD-1 pITIM was comparable to the established negative control pIRSY727 (**FIG. 5A**). In contrast, at peptide concentrations above 1 μM, PD-1 pITSM induced approximately 5-fold increase of SHP-2 activity, which was higher than the activity induced by pIRSY1172 (**FIG. 5A**), previously established as a monophosphorylated peptide that can induce SHP-2 phosphatase activation.

These studies indicated that binding of both SH2 domains of SHP-2 to PD-1 ITSM-Y248 bridges two PD-1 polypeptides to form a PD-1: PD-1 dimer (**FIG. 4A** to **FIG. 4G**). For this reason, how the interaction of SHP-2 with two phosphorylated

PD-1 ITSM-Y248 would affect SHP-2 phosphatase activity was examined. The two SH2 domains of SHP-2 have a roughly antiparallel or roughly perpendicular orientation relative to one another with the phosphopeptide binding sites widely separated (**FIG. 6C**). The distance between the phosphopeptide binding sites of the SH2 domains is 41A in the crystal structure of the tandem SH2 domains and is critical for phosphoprotein recognition and for enzymatic activation of SHP-2. A bisphosphoryl pITSM-Y248 peptide (bpITSM) that matches the spacing between the phosphopeptide binding sites of the SHP-2 SH2 domains was generated, by covalent joining of two pITSM242 Y248 phosphopeptides with a 4 amino hexanoic acid (Ahx) spacer (SEQ ID NO: 3). For comparison, a similarly designed peptide was generated with two pITIM-Y223 phosphopeptide sequences (bisphosphoryl ITIM: "bpITIM") by covalent attachment of two pITIM-Y223 with a 4-Ahx spacer to determine whether SHP-2 would interact with two phosphorylated PD-1 ITIM-Y223 (SEQ ID NO: 4). In addition, a similarly designed peptide was generated with one pITIM and one pITSM-Y248 covalently attached with a 4-Ahx spacer (bisphosphorylated ITIM-ITSM: "pITIM-pITSM"; SEQ ID NO: 5) to examine whether interaction of SHP-2 with one pITIM-Y223 and one pITSM-Y248 might activate SHP-2 phosphatase activity. Measurements of SHP-2 catalytic activity showed that bpITIM (SEQ ID NO: 4) or pITIM-pITSM (SEQ ID NO: 5) did not induce an increase in the catalytic activity of SHP-2 compared to monophosphoryl pITIM or monophosphoryl pITSM, respectively (**FIG. 5A**). Strikingly, bpITSM (SEQ ID NO: 3) caused a robust increase in SHP-2 activity at 1,000- fold lower phosphopeptide concentrations than monomeric pITSM (**FIG. 5A**). Progressive shortening of the linker length led to a pronounced drop-off in catalytic activity (**FIG. 5B**), confirming that two PD-1 pITSM-Y248 (SEQ ID NO: 3) can activate SHP-2 phosphatase only when engaged under conditions that meet the spatial requirements of the tandem SH2 256 domains of SHP-2. Compared with bpIRS, an established bisphosphoryl peptide activator of SHP-2 catalytic activity, PD-1 bpITSM was approximately three times more potent in inducing SHP-2 activation (**FIG. 18**). Loss of the active site of either N-SH2 or C-SH2 domain in SHP-2-R32A and SHP-2-R138A, respectively, abrogated SHP-2 activity caused by bpITSM at peptide concentrations below 1 μM (**FIG. 5C**). A small induction of SHP-2 activity in the SHP-2-R138A was observed at peptide concentrations above 1 μM. Most likely, at high peptide concentrations, high affinity binding of the bpITSM to the intact N-SH2 domain of SHP-2-R138A mediates some SHP-2 activity. However, maximum activation of SHP-2 phosphatase activity is induced by binding of both its SH2 domains with bisphosphorylated PD-1 ITSM-pY248 phosphopeptide (**FIG. 5A** to **FIG. 5C**), which recapitulates the interaction of two spaced PD-1 polypeptides with the two SH2 domains of SHP-2.

To further investigate the role of pITIM-Y223 and pITSM-Y248 in the induction of SHP-2 phosphatase activity, bpITSM (the phosphopeptide containing two pITSM regions that could induce SHP-2 activation) was tested with the reaction supplemented with increasing amounts of monophosphorylated pITIM or monophosphorylated pITSM peptide to assess if they could compete with bpITSM phophopeptide for SHP-2 binding and phosphatase activation. Although pITIM did not disrupt SHP-2 activation induced by the bpITSM phosphopeptide, addition of pITSM decreased SHP-2 phosphatase activity (**FIG. 5D**). These data indicate that SHP-2 binding to ITSM is preferred and for this reason, pITIM

did not affect interaction of SHP-2 with bpITSM and SHP-2 catalytic activation. In contrast, pITSM that can only bind one SH2 domain, competes with bpITSM for dual SH2 domain binding and compromises phosphatase activation. These results show that SHP-2 activation requires simultaneous binding of both SH2 domains to two pITSM-Y248 residues.

To recapitulate binding of SHP-2 with two pITSM-Y248 spaced similarly to the phosphotyrosines of PD-1 cytoplasmic tail, a bisphosphorylated peptide was generated by covalently joining two pITSM-Y248 phosphopeptides with a 10-Ahx spacer (bpITSM-Ahx10; SEQ ID NO: 24), which corresponds to the distance of PD-1 phosphotyrosines. Although PD-1cyto-pITIM-pITSM induced catalytic activity of SHP-2 compared to the negative control pIRSY727, bpITSM-Ahx10 (SEQ ID NO: 24) caused a higher increase in SHP-2 activation (FIG. 5G) that was measurable only when using 3x lower enzyme concentrations than in all other phosphatase assays.

The superior ability of the bisphosphorylated bpITSM-Y248 peptide to induce SHP-2 activation *in vitro* was correlated with PD-1-mediated inhibition of antigen-induced IL-2 production in T cells, which was abrogated when PD-1 ITSM-pY248 but not when ITIM-pY223 was mutagenized to phenylalanine (**FIG. 19**).

Based on these results, it was examined whether the ability of PD1 ITSM-pY248 to induce SHP-2 phosphatase activation was correlated with PD-1-mediated functional inhibition of T cell activation. Jurkat T cells stably transfected with PD-1 WT (J-PD1) or with PD-1 bearing mutations of either PD-1 ITIM-Y223F (J-PD-1-Y223F) or ITSM-Y248F (J-PD-1-Y248F) were cultured with Raji-control or PD-L1-expressing Raji cells loaded with SEE (**FIG. 7A** to **FIG. 7C**). Stimulation in the presence of Raji-PD-L1 cells abrogated IL-2 production in J-PD-1 and J-PD-1- Y223F T cells, which express an intact ITSM-Y248, and this inhibitory effect was significantly reversed by PD-1 blocking antibody (**FIG. 5E** and **FIG. 19**). In contrast, J-PD-1-Y248F T cells, which express an intact ITIM-Y223, which is unable to induce SHP-2 phosphatase activation, but a mutagenized ITSM-Y248, which is essential for induction of SHP-2 phosphatase activation (**FIG. 5A**), were resistant to PD-L1-mediated inhibition of IL-2 production (**FIG. 5E**). Together these biochemical, SPR, NanoBiT, phosphatase activation and functional results are consistent with PD-1: PD-1 dimer complex formation, which is initiated by TCR-mediated activation of kinases that lead to phosphorylation of PD-1-ITSM Y248 and binding of both SHP-2 SH2 domains to form a PD-1 dimer resulting in SHP-2 phosphatase activation and inhibition of activated T cell responses (**FIG. 5F**).

Further, the experiments disclosed herein demonstrate ITSM-Y248 phosphorylation and also how spatial distribution of phosphorylated PD-1 molecules in proximity to TCR signaling substrates in the TCR microclusters will affect the inhibitory function of PD-1, because only PD-1 molecules bridged through pITSM-Y248 by the two SH2 domains of SHP-2 at such a distance that can induce SHP-2 conformational change allowing activation of SHP-2 phosphatase will be responsible for inhibition of T cell responses. Moreover, in contrast to the recruitment of PD-1 to the TCR

microclusters that is independent of ITSM-pY24811, SHP-2- mediated bridging of PD-1 molecules depends on ITSM-pY248, as determined by the experiments disclosed herein. The experiments disclosed herein explain why ITSM-Y248 has an indispensable role in PD-1-mediated inhibitory function and unravel a mechanism of how SHP-2 enzymatic activation can be induced by one PD-1 phosphotyrosine.

OTHER EMBODIMENTS

It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

INCORPORATION BY REFERENCE

All patents and publications referenced herein are hereby incorporated by reference in their entireties.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

As used herein, all headings are simply for organization and are not intended to limit the disclosure in anyway.

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CLAIMS

What is claimed is:

1. A method for treating cancer in a patient in need thereof, comprising:

(a) selecting an agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity; and

(b) administering the agent to the patient.

2. The method of claim 1, wherein the agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity is a small molecule or peptide agent.

3. The method of claim 2, wherein the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SHP-2 polypeptide.

4. The method of claim 2 or 3, wherein the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2.

5. The method of any one of claims 2-4, wherein the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 by preventing binding of the SH2 domain with a motif of the PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248.

6. The method of claim 5, wherein the motif of the PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, comprises an immunoreceptor tyrosine-based switch motif (ITSM).

7. The method of any one of claims 2-6, wherein the small molecule or peptide agent is capable of disrupting an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 by preventing binding of an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) with the PD-1 polypeptide and/or by preventing binding of a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20) with the PD-1 polypeptide.

8. The method of any one of claims 2-7, wherein the small molecule or peptide agent is capable of disrupting an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and is capable of disrupting an interaction between a second PD-1 polypeptide and an carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20).

9. The method of claim 8, wherein the small molecule or peptide agent is capable of disrupting an interaction between the first PD-1 polypeptide and the N-SH2 domain by preventing binding of the N-SH2 domain with a motif of the first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and is capable of disrupting an interaction between the second PD-1 polypeptide and the C-SH2 domain by preventing binding of

the C-SH2 domain with a motif of the second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248.

10. The method of claim 9, wherein the motif of the first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and the motif of the second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, each comprises a portion of immunoreceptor tyrosine-based switch motif (ITSM).

11. The method of any one of claims 2-4, wherein the small molecule or peptide agent is capable of binding to a SH2 domain of SHP-2.

12. The method of claim 11, wherein the small molecule or peptide agent is capable of binding to an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) or to a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20).

13. The method of claim 11 or claim 12, wherein the peptide agent is capable of binding to an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and to a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20).

14. The method of any one of claims 11-13, wherein the peptide agent comprises at least one motif of a PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248.

15. The method of claim 14, wherein the motif comprises a portion of an immunoreceptor tyrosine-based switch motif (ITSM).

16. The method of claim 14 or claim 15, wherein the peptide agent comprises at least two motifs of a PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, wherein the at least two motifs are separated by a linker.

17. The method of claims 16, wherein each of the at least two motifs of a PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, comprises a portion of an immunoreceptor tyrosine-based switch motif (ITSM).

18. The method of claim 16 or claim 17, wherein the linker comprises at least 3 amino acids and/or is at least about 35 Angstroms long.

19. The method of any one of claims 16-18, wherein the linker separates the at least two motifs by at least about 35 Angstroms.

20. The method of any one of claims 11-19, wherein the peptide agent comprises:

(a) a first amino acid sequence of SEQ ID NO: 1, 22, or 23, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids,

(b) a second amino acid sequence of SEQ ID NO: 1, 22, or 23, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, and

(c) a linker comprising at least 3 amino acids between the first amino acid sequence and the second amino acid sequence, optionally, the linker separates the at least two motifs by at least about 35 Angstroms.

21. The method of any one of claims 11-20, wherein the peptide agent comprises the amino acid sequence of SEQ ID NO: 3, 9, 10, 11, or 24, optionally comprising a mutation of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids.

22. The method of claim 20 or claim 21, wherein the mutation is not of the tyrosine 248, with reference to SEQ ID NO: 21.

23. The method of claim 21, wherein the peptide agent comprises SEQ ID NO: 3, 9, 10, 11, or 24.

24. The method of claim 21, wherein the peptide agent consists of SEQ ID NO: 3, 9, 10, 11, or 24.

25. The method of claim 2, wherein the peptide agent which reduces PD-1 dimer activity is capable of binding to at least a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248; wherein the peptide agent lacks a phosphatase domain or comprises a non-functional phosphatase domain.

26. The method of claim 25, wherein the small molecule or peptide agent is capable of binding to the at least first PD-1 polypeptide at or near its immunoreceptor tyrosine-based switch motif (ITSM).

27. The method of claim 25 or claim 26, wherein the peptide agent is capable of binding to the first PD-1 polypeptide and to an at least second PD-1 polypeptide.

28. The method of claim 27, wherein the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM).

29. The method of any one of claims 25 to 28, wherein the peptide agent comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20.

30. The method of claim 29, wherein the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, or the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20.

31. The method of claim 29 or claim 30, wherein the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20.

32. The method of claim 31, wherein the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20.

33. The method of claim 31 or claim 32, wherein the first amino acid sequence and the second amino acid sequence are separated by a linker.

34. The method of claim 33, wherein the linker comprises at least 3 amino acids and/or is at least about 35 Angstroms long.

35. The method of any one of the above claims, wherein the patient is undergoing treatment with an immune checkpoint immunotherapy selected from an agent that modulates one or more PD-1, programmed death-ligand 1 (PD-L1), or programmed death-ligand 2 (PD-L2).

36. The method of any one of the above claims, wherein the method further comprises administering an agent that modulates one or more of PD-1, PD-L1, or PD-L2.

37. The method of claim 36, wherein the administering of the agent that modulates one or more of PD-1, PD-L1, or PD-L2 and the administering of the agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity is sequential or simultaneous.

38. The method of claim 36 or claim 37, wherein the agent that modulates PD-1 is an antibody or antibody format specific for PD-1.

39. The method of claim 38, wherein the antibody or antibody format specific for PD-1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

40. The method of claim 38, wherein the antibody or antibody format specific for PD-1 is selected from nivolumab, pembrolizumab, and pidilizumab.

41. The method of claim 36 or claim 37, wherein the agent that modulates PD-L1 is an antibody or antibody format specific for PD-L1.

42. The method of claim 41, wherein the antibody or antibody format specific for PD-L1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

43. The method of claim 41, wherein the antibody or antibody format specific for PD-L1 is selected from atezolizumab, avelumab, durvalumab, and BMS-936559.

44. The method of claim 36 or claim 37, wherein the agent that modulates PD-L2 is an antibody or antibody format specific for PD-L2.

45. The method of claim 44, wherein the antibody or antibody format specific for PD-L2 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

46. The method of any one of the above claims, wherein the administering of the agent which decreases SHP-2-mediated PD-1 dimerization and/or reduces PD-1 dimer activity is by intratumoral, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or direct injection into cancer tissue.

47. The method of any one of claims 36-46, wherein the administering of the agent that modulates one or more of PD-1, PD-L1, or PD-L2 is by intratumoral, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or direct injection into cancer tissue.

48. The method of any one of the above claims, wherein the patient is predicted to be poorly responsive or non-responsive to the immune checkpoint immunotherapy or has presented as poorly responsive or non-responsive to the immune checkpoint immunotherapy.

49. The method of any one of the above claims, wherein the method reduces and/or mitigates one or more side effects of the immune checkpoint immunotherapy.

50. The method of claim 49, wherein the side effect is selected from decreased appetite, rashes, fatigue, pneumonia, pleural effusion, pneumonitis, pyrexia, nausea, dyspnea, cough, constipation, diarrhea, immune-mediated pneumonitis, colitis, hepatitis, endocrinopathies, hypophysitis, iridocyclitis, and nephritis.

51. The method of any one of claims 35-50, wherein the method reduces the dose of the immune checkpoint immunotherapy.

52. The method of any one of claims 35-51, wherein the method reduces number of administrations of the immune checkpoint immunotherapy.

53. The method of any one of claims 35-52, wherein the method increases a therapeutic window of the immune checkpoint immunotherapy.

54. The method of any one of the above claims, wherein the method elicits a potent immune response in less-immunogenic tumors.

55. The method of any one of the above claims, wherein the method converts a tumor with reduced inflammation ("cold tumor") to a responsive, inflamed tumor ("hot tumor").

56. The method of any one of claims 35-55, wherein the method makes the cancer responsive or more responsive to a combination therapy of the immune checkpoint immunotherapy and one or more chemotherapeutic agents and/or radiotherapy.

57. The method of claim 56, wherein the chemotherapeutic agent is selected from one or more of daunorubicin, doxorubicin, epirubicin, idarubicin, adriamycin, vincristine, carmustine, cisplatin, 5-fluorouracil, tamoxifen, prodasone, sandostatine, mitomycin C, foscarnet, paclitaxel, docetaxel, gemcitabine, fludarabine, carboplatin, leucovorin, tamoxifen, goserelin, ketoconazole, leuprolide flutamide, vinblastine, vindesine, vinorelbine, camptothecin, topotecan, irinotecan hydrochloride, etoposide, mitoxantrone, teniposide, amsacrine, merbarone, piroxantrone hydrochloride, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine (Ara-C), trimetrexate, acivicin, alanosine, pyrazofurin, pentostatin, 5-azacitidine, 5-azacitidine, 5-Aza-5-Aza-2'-deoxycytidine, adenosine arabinoside (Ara-A), cladribine, ftorafur, UFT (combination of uracil and florafur), 5-fluoro-2'-deoxyuridine, 5-fluorouridine, 5'-deoxy-5-fluorouridine, hydroxyurea, dihydrolenchiorambucil, tiazofurin, oxaliplatin, melphalan, thiotepa, busulfan, chlorambucil, plicamycin, dacarbazine, ifosfamide phosphate, cyclophosphamide, pipobroman, 4-ipomeanol, dihydrolenperone, spiromustine, geldenamycin, cytochalasins, depsipeptide, 4'-cyano-3-(4-(*e.g.,* ZOLADEX), and 4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-3-methyl-3'-(trifluorometh- yl)propionanilide.

58. The method of any one of claims 35-57, wherein the patient is predicted to be poorly responsive or non-responsive to the immune checkpoint immunotherapy based on expression of one or more of PD-1, PD-L1, or PD-L2, in the patient's biological specimen.

59. The method of any one of claims 35-58, wherein the patient is predicted to be poorly responsive or non-responsive to an agent that modulates one or more of PD-1, PD-L1, and PD-L2 based on low on expression of PD-1, PD-L1, and PD-L2 in a tumor specimen from the patient.

60. The method of any one of claims 35-59, wherein the patient is predicted to be poorly responsive or non-responsive to an agent that modulates one or more of PD-1, PD-L1, and PD-L2 based on a tumor proportion score (TPS) of less than about 49% for PD-L1 staining.

61. The method of any one of the above claims, wherein the cancer is selected from one or more of basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (*e.g.,* small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (*e.g.,* that associated with brain tumors), and Meigs' syndrome.

62. A method for treating an autoimmune disease or disorder or for treating inflammation in a patient in need thereof, comprising:

(a) selecting an agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity; and

(b) administering the agent to the patient.

63. The method of claim 62, wherein the agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity is a small molecule or peptide agent.

64. The method of claim 63, wherein the small molecule or peptide agent is capable of stimulating an interaction between a PD-1 polypeptide and a SHP-2 polypeptide.

65. The method of claim 62 or 63, wherein the small molecule or peptide agent is capable of stimulating an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and an interaction between a second PD-1 polypeptide and a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20).

66. The method of claim 62, wherein the peptide agent is capable of binding to a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and to an at least second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248.

67. The method of claim 66, wherein the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM).

68. The method of claim 66 or claim 67, wherein the peptide agent comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20.

69. The method of claim 68, wherein the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20.

70. The method of claim 69, wherein the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20.

71. The method of claim 69 or claim 70, wherein the first amino acid sequence and the second amino acid sequence are separated by a linker.

72. The method of claim 71, wherein the linker comprises at least 3 amino acids and/or is at least about 35 Angstroms long.

73. The method of any one of claims 66-72, wherein the peptide agent comprises:

(a) a first amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids,

(b) a second amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, and

(c) a linker comprising at least 3 amino acids between the first amino acid sequence and the second amino acid sequence, optionally, the linker separates the two amino acid sequences by at least about 35 Angstroms.

74. The method of any one of claims 66-73, wherein the peptide agent further comprises a functional phosphatase domain.

75. The method of claim 74, wherein the functional phosphatase domain comprises a protein tyrosine phosphatase (PTP) domain.

76. The method of any one of claims 66-75, wherein the peptide agent comprises the amino acid sequence of SEQ ID NO: 18.

77. The method of any one of claims 66-75, wherein the peptide agent does not comprise of the amino acid sequence of SEQ ID NO: 18.

78. The method of any one of claims 62-77, wherein the autoimmune disease or disorder is selected from multiple sclerosis, diabetes mellitus, lupus, celiac disease, Crohn's disease, ulcerative colitis, Guillain-Barre syndrome, scleroderms, Goodpasture's syndrome, Wegener's granulomatosis, autoimmune epilepsy, Rasmussen's encephalitis, Primary biliary sclerosis, Sclerosing cholangitis, Autoimmune hepatitis, Addison's disease, Hashimoto's thyroiditis, Fibromyalgia, Menier's syndrome; transplantation rejection (*e.g.*, prevention of allograft rejection) pernicious anemia, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, Sjogren's syndrome, lupus erythematosus, multiple sclerosis, myasthenia gravis, Reiter's syndrome, and Grave's disease and/or the inflammation is selected from inflammation is acute inflammation, chronic inflammation, respiratory disease, atherosclerosis, restenosis, asthma, allergic rhinitis, atopic dermatitis, septic shock, rheumatoid arthritis, inflammatory bowel disease, inflammatory pelvic disease, pain, ocular inflammatory disease, celiac disease, Leigh Syndrome, Glycerol Kinase Deficiency, Familial eosinophilia (FE), autosomal recessive spastic ataxia, laryngeal inflammatory disease; Tuberculosis, Chronic cholecystitis, Bronchiectasis, Silicosis and other pneumoconioses.

79. The method of any one of claims 62-78, wherein the patient is undergoing treatment or has undergone treatment with an immunosuppressive agent.

80. The method of any one of claims 62-79, wherein the method further comprises administering an immunosuppressive agent.

81. The method of claim 56, wherein the administering of the agent which increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity and the administering of the immunosuppressive agent is sequential or simultaneous.

82. The method of any one of claims 55-57, wherein the immunosuppressive agent is a steroidal anti-inflammatory agent or a non-steroidal anti-inflammatory agent (NSAID), selected from salicylic acid, acetyl salicylic acid, methyl salicylate, glycol salicylate, salicylmides, benzyl-2,5-diacetoxybenzoic acid, ibuprofen, fulindac, naproxen, ketoprofen, etofenamate, phenylbutazone, and indomethacin.

83. The method of any one of claims 55-57, wherein the immunosuppressive agent is a steroid, such as a corticosteroids selected from hydroxyltriamcinolone, alpha-methyl dexamethasone, beta-methyl betamethasone, beclomethasone dipropionate, betamethasone benzoate, betamethasone dipropionate, betamethasone valerate, clobetasol valerate, desonide, desoxymethasone, dexamethasone, diflorasone diacetate, diflucortolone valerate, fluadrenolone, fluclorolone acetonide, flumethasone pivalate, fluosinolone acetonide, fluocinonide, flucortine

butylester, fluocortolone, fluprednidene (fluprednylidene) acetate, flurandrenolone, halcinonide, hydrocortisone acetate, hydrocortisone butyrate, methylprednisolone, triamcinolone acetonide, cortisone, cortodoxone, flucetonide, fludrocortisone, difluorosone diacetate, fluradrenolone acetonide, medrysone, amcinafel, amcinafide, betamethasone and the balance of its esters, chloroprednisone, clocortelone, clescinolone, dichlorisone, difluprednate, flucloronide, flunisolide, fluoromethalone, fluperolone, fluprednisolone, hydrocortisone, meprednisone, paramethasone, prednisolone, prednisone, beclomethasone dipropionate.

84. The method of any one of claims 55-57, wherein the immunosuppressive agent is a cytostatic such as alkylating agents, antimetabolites (*e.g.*, azathioprine, methotrexate), cytotoxic antibiotics, antibodies (*e.g.*, basiliximab, daclizumab, and muromonab), anti-immunophilins (*e.g.*, cyclosporine, tacrolimus, sirolimus), inteferons, opioids, TNF binding proteins, mycophenolates, and small biological agents (*e.g.*, fingolimod, myriocin).

85. A method of making an agent effective for the treatment of a cancer, comprising:

(a) identifying the agent by screening for a disruption or decrease of SHP-2-mediated programmed cell death protein-1 (PD-1) dimerization or of PD-1 dimer activity; and

(b) formulating the agent for administration to a patient having a cancer.

86. The method of claim 85, wherein the agent is a small molecule or peptide agent.

87. The method of claim 86, wherein the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and a SHP-2 polypeptide.

88. The method of claims 86 or 87, wherein the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2.

89. The method of any one of claims 86-88, wherein the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 which prevents their interaction at the immunoreceptor tyrosine-based switch motif (ITSM) of the PD-1 polypeptide.

90. The method of any one of claims 86-89, wherein the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and a SH2 domain of SHP-2 which prevents their interaction at tyrosine 248 of PD-1.

91. The method of any one of claim 86-90, wherein the small molecule or peptide agent disrupts or decreases an interaction between a PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19), *e.g.,* at the arginine at amino acid position 32 of SEQ ID NO: 19, or disrupts or decreases an interaction between the PD-1 polypeptide and a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20), *e.g.,* at the arginine at amino acid position 28 of SEQ ID NO: 20.

92. The method of any one of claim 86-91, wherein the small molecule or peptide agent disrupts or decreases an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19), *e.g.,* at the arginine at amino acid position 32 of SEQ ID NO: 19, and disrupts or decreases an interaction between a second PD-1 polypeptide and an carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20), *e.g.,* at the arginine at amino acid position 28 of SEQ ID NO: 20.

93. The method of any one of claim 86-92, wherein the peptide agent that disrupts or decreases PD-1 dimer activity is capable of binding to at least a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248; wherein the peptide agent lacks a phosphatase domain or comprises a non-functional phosphatase domain.

94. The method of claim 93, wherein the peptide agent is capable of binding to the at least first PD-1 polypeptide at or near its immunoreceptor tyrosine-based switch motif (ITSM).

95. The method of claim 93 or claim 94, wherein the peptide agent is capable of binding to the first PD-1 polypeptide and to an at least second PD-1 polypeptide.

96. The method of claim 95, wherein the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM).

97. The method of any one of claims 93 to 96, wherein the peptide agent comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20.

98. The method of claim 97, wherein the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, or the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20.

99. The method of claim 97 or claim 98, wherein the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20.

100. The method of claim 99, wherein the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20.

101. The method of claim 99 or claim 100, wherein the first amino acid sequence and the second amino acid sequence are separated by a linker.

102. The method of claim 101, wherein the linker comprises at least 3 amino acids and/or is at least about 35 Angstroms long.

103. A method of making an agent effective for the treatment of an autoimmune disease or disorder or for treatment of inflammation, comprising:

(a) identifying the agent by screening for a stimulation or increase of SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity; and

(b) formulating the agent for administration to a patient having an autoimmune disease or disorder or inflammation.

104. The method of claim 103, wherein the agent which stimulates or increases SHP-2-mediated PD-1 dimerization and/or increases PD-1 dimer activity is a small molecule or peptide agent.

105. The method of claim 104, wherein the small molecule or peptide agent is capable of stimulating an interaction between a PD-1 polypeptide and a SHP-2 polypeptide.

106. The method of claim 104 or 105, wherein the small molecule or peptide agent is capable of stimulating an interaction between a first PD-1 polypeptide and an amino terminal SH2 domain of SHP-2 (N-SH2; SEQ ID NO: 19) and an interaction between a second PD-1 polypeptide and a carboxy terminal SH2 domain of SHP-2 (C-SH2; SEQ ID NO: 20).

107. The method of claim 104, wherein the peptide agent is capable of binding to a first PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248, and to an at least second PD-1 polypeptide comprising a tyrosine 248, *e.g.,* a phosphorylated tyrosine 248.

108. The method of claim 107, wherein the peptide agent is capable of binding to the first PD-1 polypeptide and to the at least second PD-1 polypeptide at or near each polypeptide's immunoreceptor tyrosine-based switch motif (ITSM).

109. The method of claim 107 or claim 108, wherein the peptide agent comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 19 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 20.

110. The method of claim 109, wherein the peptide agent comprises a first amino acid sequence that is at least 95% identical to SEQ ID NO: 19 and a second amino acid sequence that is at least 95% identical to SEQ ID NO: 20.

111. The method of claim 110, wherein the amino acid sequence that is at least 95% identical to SEQ ID NO: 19 comprises an arginine at its amino acid position 32, relative to SEQ ID NO: 19, and the amino acid sequence that is at least 95% identical to SEQ ID NO: 20 comprises an arginine at its amino acid position 28, relative to SEQ ID NO: 20.

112. The method of claim 109 or claim 111, wherein the first amino acid sequence and the second amino acid sequence are separated by a linker.

113. The method of claim 112, wherein the linker comprises at least 3 amino acids and/or is at least about 35 Angstroms long.

114. The method of any one of claims 107-113, wherein the peptide agent comprises:

(a) a first amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids,

(b) a second amino acid sequence of SEQ ID NO: 19 or 20, optionally comprising a mutation of 1, 2, 3, 4, or 5 amino acids, and

(c) a linker comprising at least 3 amino acids between the first amino acid sequence and the second amino acid sequence, optionally, the linker separates the two amino acid sequences by at least about 35 Angstroms.

115. The method of any one of claims 107-114, wherein the peptide agent further comprises a functional phosphatase domain.

116. The method of claim 115, wherein the functional phosphatase domain comprises a protein tyrosine phosphatase (PTP) domain.

117. The method of any one of claims 107-116, wherein the peptide agent comprises the amino acid sequence of SEQ ID NO: 18.

118. The method of any one of claims 107-116, wherein the peptide agent does not comprise the amino acid sequence of SEQ ID NO: 18.

119. A method for predicting a cancer patient response to an immune checkpoint immunotherapy, comprising determining the presence of SHP-2-mediated PD-1 dimerization in a biological sample from the patient, wherein the presence of SHP-2-mediated PD-1 dimerization is indicative of an inhibitory immune signal and a likelihood of responding to the immune checkpoint immunotherapy.

120. The method of claim 119, wherein the immune checkpoint immunotherapy is an agent that modulates one or more of PD-1, PD-L1, or PD-L2.

121. The method of claim 120, wherein the agent that modulates PD-1 is an antibody or antibody format specific for PD-1.

122. The method of claim 121, wherein the antibody or antibody format specific for PD-1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

123. The method of claim 121, wherein the antibody or antibody format specific for PD-1 is selected from nivolumab, pembrolizumab, and pidilizumab.

124. The method of claim 120, wherein the agent that modulates PD-L1 is an antibody or antibody format specific for PD-L1.

125. The method of claim 124, wherein the antibody or antibody format specific for PD-L1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

126. The method of claim 124, wherein the antibody or antibody format specific for PD-L1 is selected from atezolizumab, avelumab, durvalumab, and BMS-936559.

127. The method of claim 120, wherein the agent that modulates PD-L2 is an antibody or antibody format specific for PD-L2.

128. The method of claim 127, wherein the antibody or antibody format specific for PD-L2 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

129. A method for treating cancer, comprising:

(a) evaluating a subject's likelihood of response to an immune checkpoint immunotherapy, comprising evaluating a level of SHP-2-mediated PD-1 dimerization in a biological sample from the patient, wherein a presence or high level of SHP-2-mediated PD-1 dimerization is indicative of a cancer that is suitable for immune checkpoint immunotherapy; and

(b) administering an immune checkpoint immunotherapy to the patient.

130. The method of claim 129, wherein the immune checkpoint immunotherapy is an agent that modulates one or more of PD-1, PD-L1, or PD-L2.

131. The method of claim 130, wherein the agent that modulates PD-1 is an antibody or antibody format specific for PD-1.

132. The method of claim 131, wherein the antibody or antibody format specific for PD-1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

133. The method of claim 131, wherein the antibody or antibody format specific for PD-1 is selected from nivolumab, pembrolizumab, and pidilizumab.

134. The method of claim 130, wherein the agent that modulates PD-L1 is an antibody or antibody format specific for PD-L1.

135. The method of claim 134, wherein the antibody or antibody format specific for PD-L1 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

136. The method of claim 134, wherein the antibody or antibody format specific for PD-L1 is selected from atezolizumab, avelumab, durvalumab, and BMS-936559.

137. The method of claim 130, wherein the agent that modulates PD-L2 is an antibody or antibody format specific for PD-L2.

138. The method of claim 137, wherein the antibody or antibody format specific for PD-L2 is selected from one or more of a monoclonal antibody, polyclonal antibody, antibody fragment, Fab, Fab', Fab'-SH, F(ab')2, Fv, single chain Fv, diabody, linear antibody, bispecific antibody, multispecific antibody, chimeric antibody, humanized antibody, human antibody, and fusion protein comprising the antigen-binding portion of an antibody.

FIG. 1A

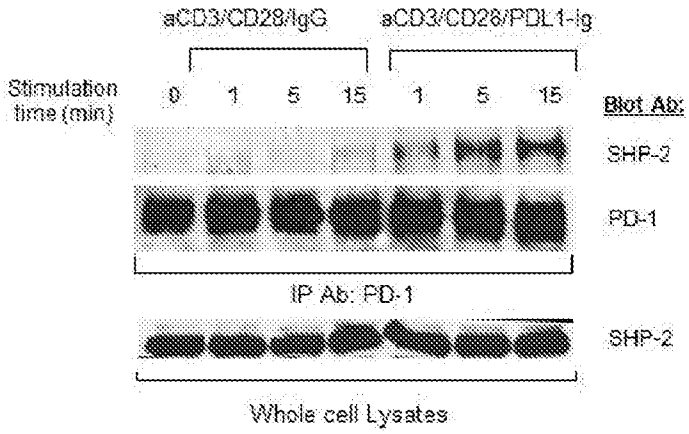

aCD3/CD28/IgG
aCD3/CD28/PDL1-Ig
Stimulation time (min)
0 1 5 15 1 5 15
Blot Ab:
SHP-2
PD-1
IP Ab: PD-1
SHP-2
Whole cell Lysates

FIG. 1B

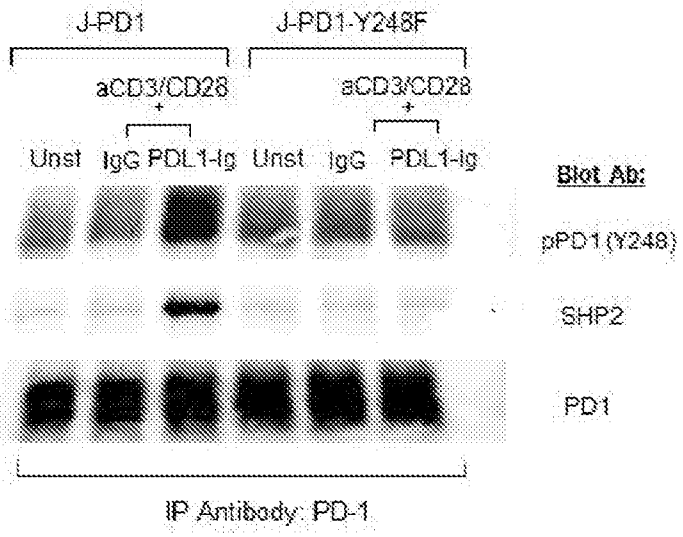

J-PD1
J-PD1-Y248F
aCD3/CD28 + IgG
aCD3/CD28 + PDL1-Ig
Unst IgG PDL1-Ig Unst IgG PDL1-Ig
Blot Ab:
pPD1 (Y248)
SHP2
PD1
IP Antibody: PD-1

FIG. 1C

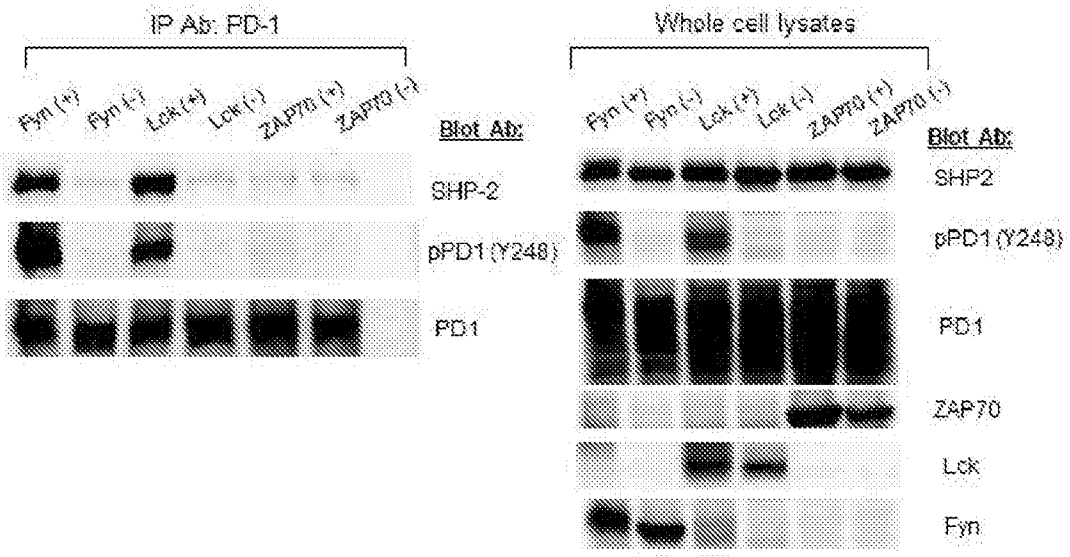

IP Ab: PD-1
Whole cell lysates
Fyn (+)
Fyn (-)
Lck (+)
Lck (-)
ZAP70 (+)
ZAP70 (-)
Blot Ab:
SHP-2
pPD1 (Y248)
PD1
SHP2
ZAP70
Lck
Fyn

FIG. 1D

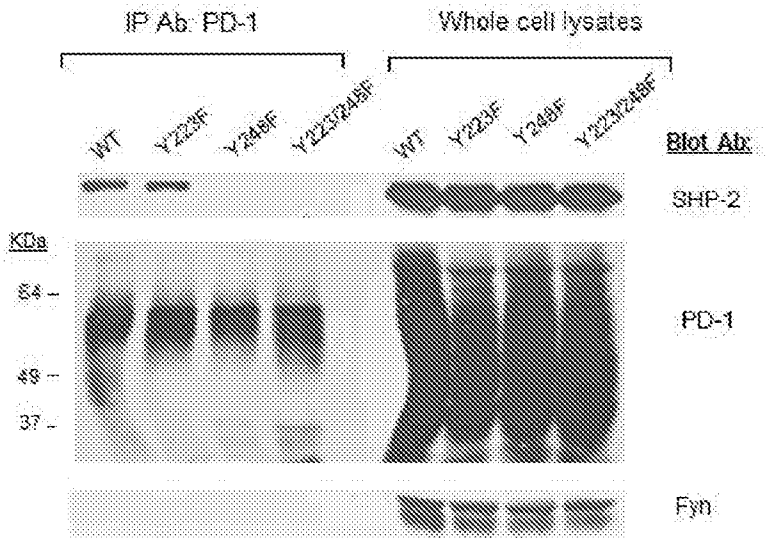

IP Ab: PD-1
Whole cell lysates
WT
Y223F
Y248F
Y223/248F
Blot Ab:
SHP-2
KDa
54 –
48 –
37 –
PD-1
Fyn

FIG. 2A

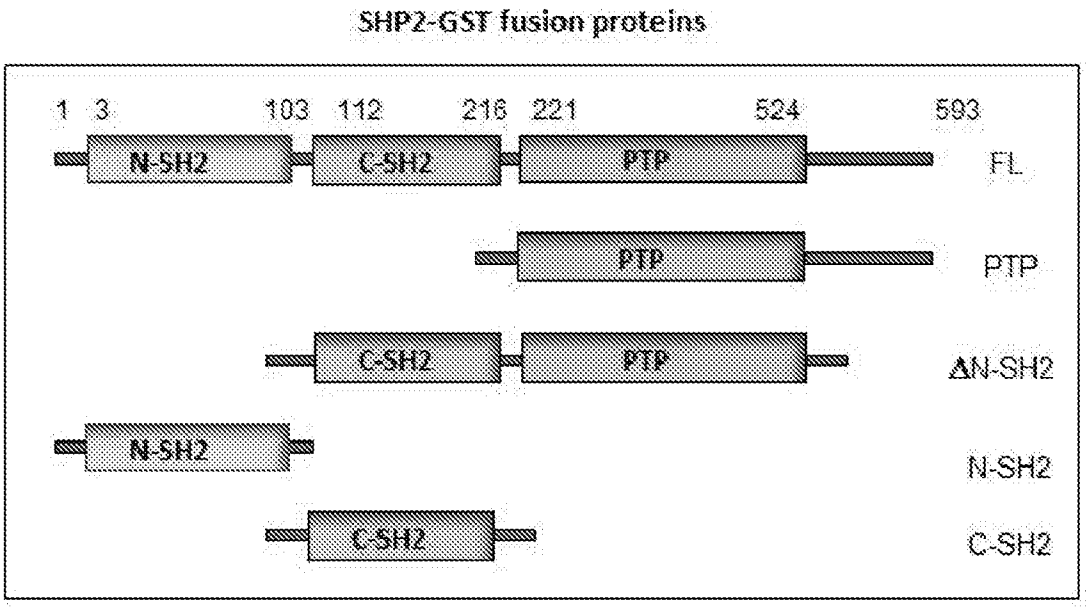

SHP2-GST fusion proteins
1
3
103
112
216
221
524
593
N-SH2
C-SH2
PTP
FL
PTP
ΔN-SH2
N-SH2
C-SH2

FIG. 2B

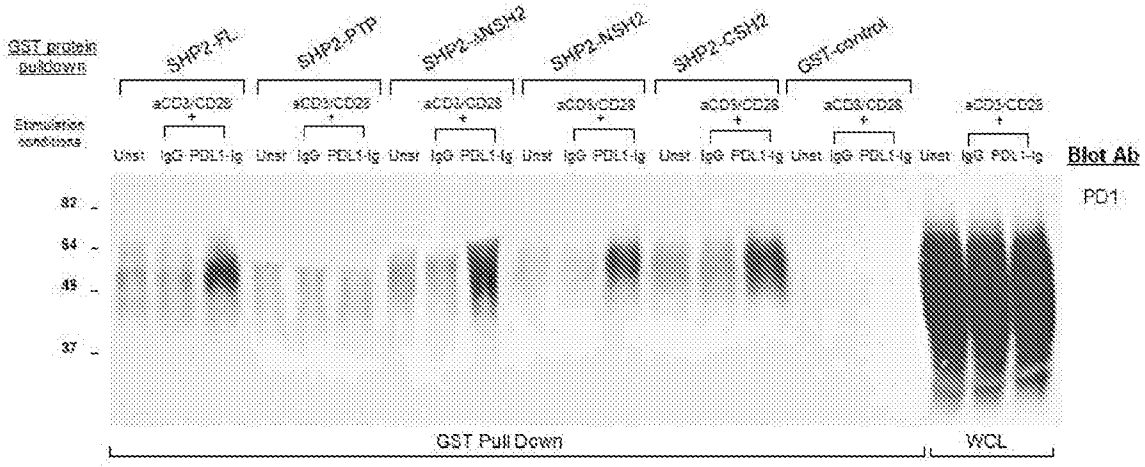

GST protein pulldown
SHP2-FL
SHP2-PTP
SHP2-ΔNSH2
SHP2-NSH2
SHP2-CSH2
GST-control
Stimulation conditions
aCD3/CD28
Unst
IgG
PDL1-Ig
Blot Ab
PD1
82
64
49
37
GST Pull Down
WCL

FIG. 2C

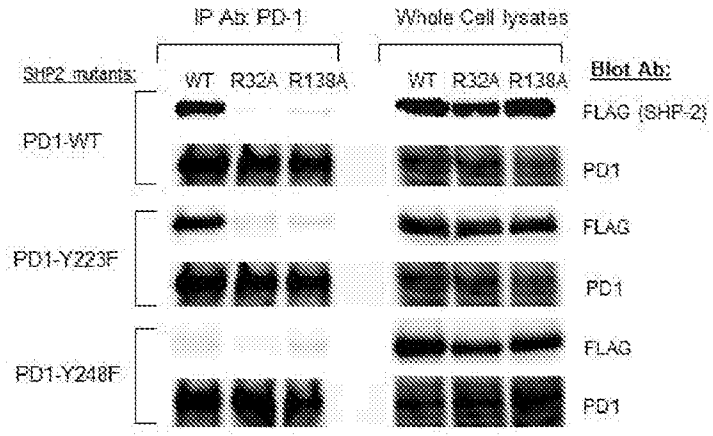

IP Ab: PD-1
Whole Cell lysates
SHP2 mutants:
WT R32A R138A
WT R32A R138A
Blot Ab:
PD1-WT
FLAG (SHP-2)
PD1
PD1-Y223F
FLAG
PD1
PD1-Y248F
FLAG
PD1

FIG. 2D

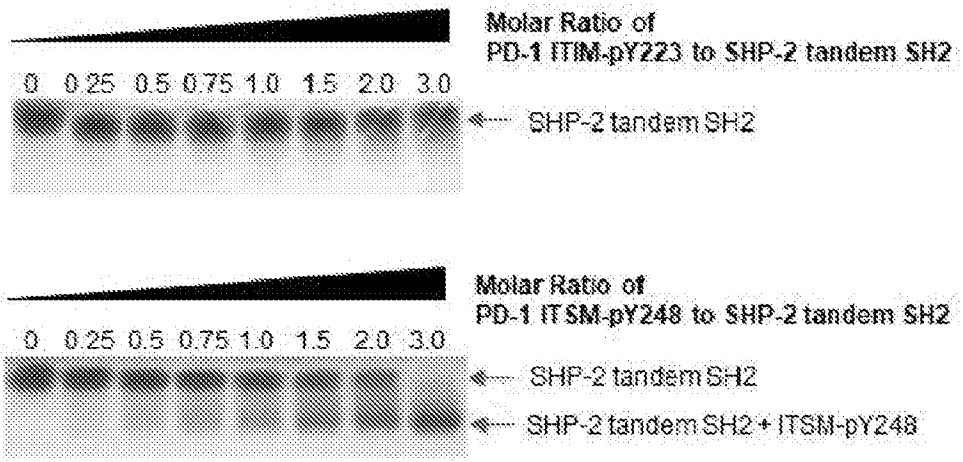

Molar Ratio of
PD-1 ITIM-pY223 to SHP-2 tandem SH2
0 0.25 0.5 0.75 1.0 1.5 2.0 3.0
SHP-2 tandem SH2
Molar Ratio of
PD-1 ITSM-pY248 to SHP-2 tandem SH2
0 0.25 0.5 0.75 1.0 1.5 2.0 3.0
SHP-2 tandem SH2
SHP-2 tandem SH2 + ITSM-pY248

FIG. 2E

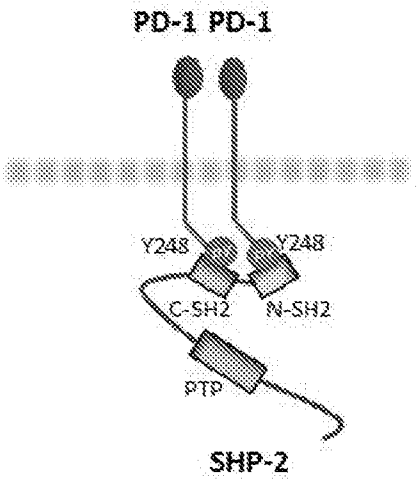

PD-1 PD-1
Y248
Y248
C-SH2
N-SH2
PTP
SHP-2

FIG. 2F

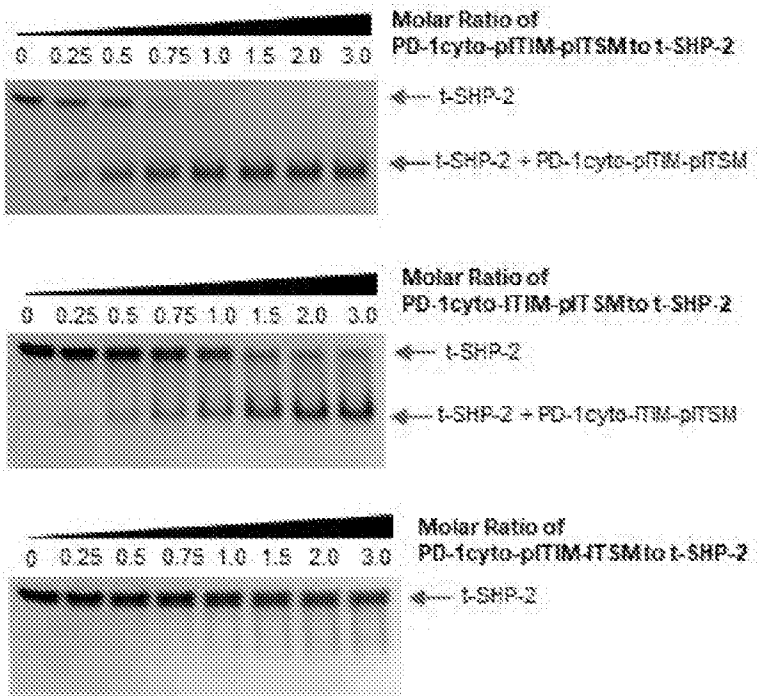

Molar Ratio of
PD-1cyto-pITIM-pITSM to t-SHP-2
0 0.25 0.5 0.75 1.0 1.5 2.0 3.0
t-SHP-2
t-SHP-2 + PD-1cyto-pITIM-pITSM
Molar Ratio of
PD-1cyto-ITIM-pITSM to t-SHP-2
0 0.25 0.5 0.75 1.0 1.5 2.0 3.0
t-SHP-2
t-SHP-2 + PD-1cyto-ITIM-pITSM
Molar Ratio of
PD-1cyto-pITIM-ITSM to t-SHP-2
0 0.25 0.5 0.75 1.0 1.5 2.0 3.0
t-SHP-2

FIG. 2G

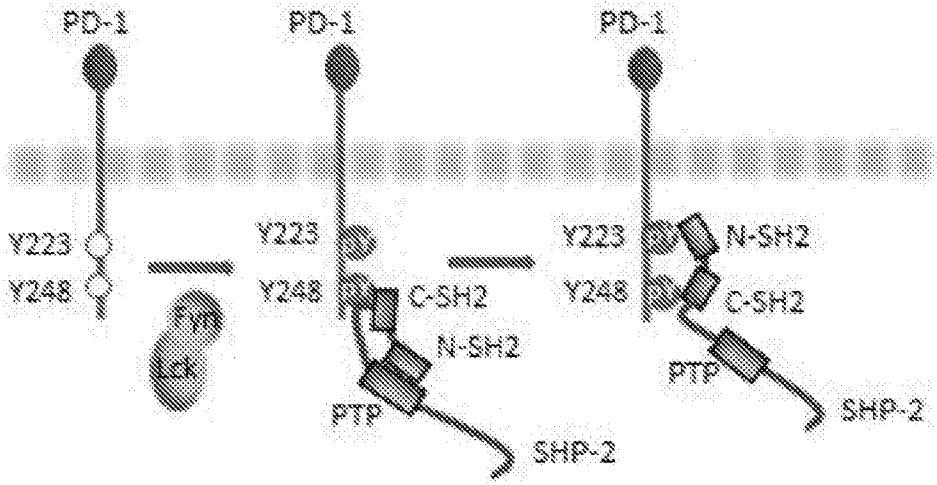

PD-1
PD-1
PD-1
Y223
Y248
Fyn
Lck
Y223
Y248
C-SH2
N-SH2
PTP
SHP-2
Y223
Y248
N-SH2
C-SH2
PTP
SHP-2

FIG. 2H

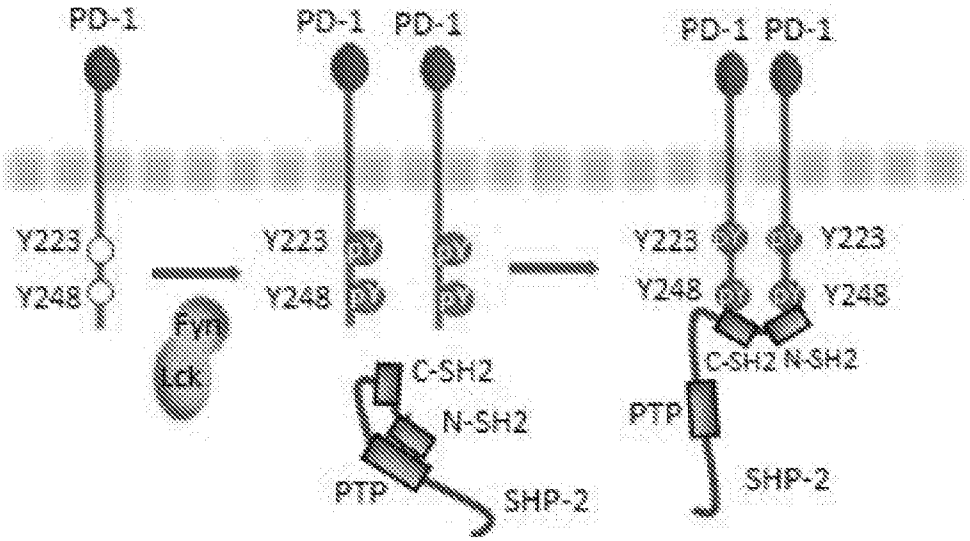

PD-1
PD-1
PD-1
PD-1
PD-1
Y223
Y248
Fyn
Lck
Y223
Y248
C-SH2
N-SH2
PTP
SHP-2
Y223
Y248
Y223
Y248
C-SH2
N-SH2
PTP
SHP-2

FIG. 3A

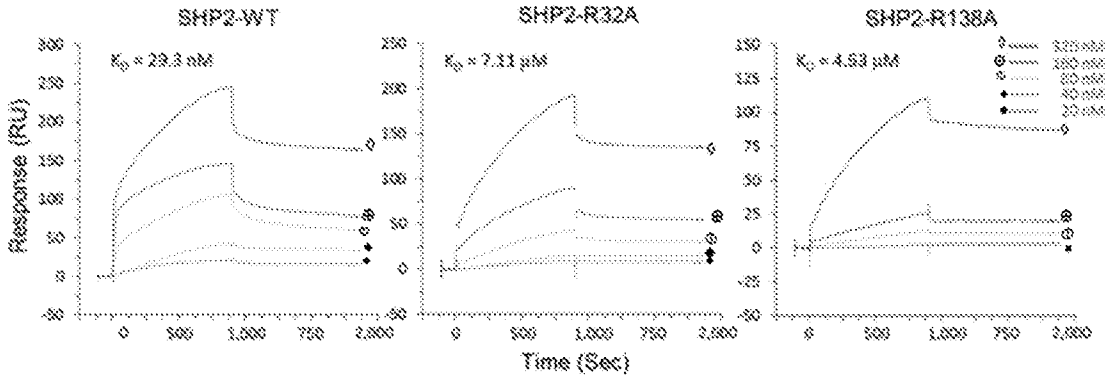

SHP2-WT
SHP2-R32A
SHP2-R138A
Response (RU)
Time (Sec)

FIG. 3B

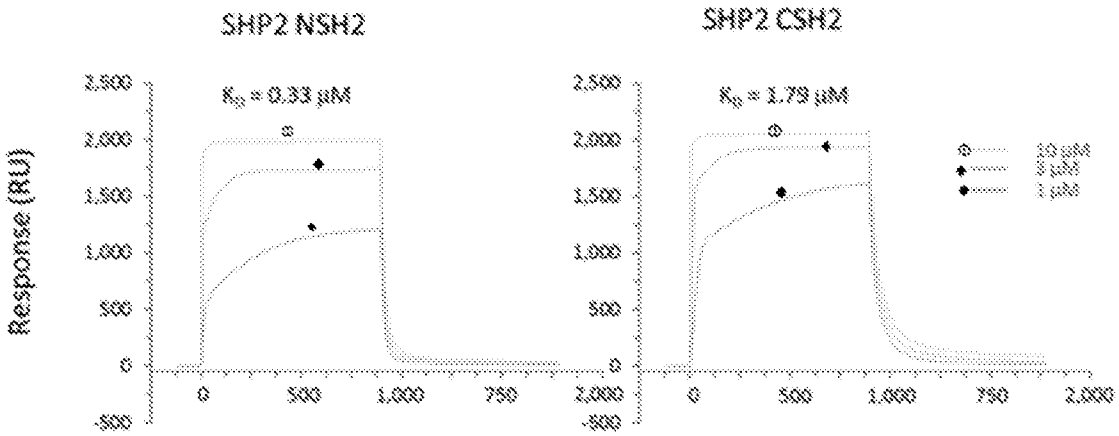

SHP2 NSH2
SHP2 CSH2
Response (RU)

FIG. 3C

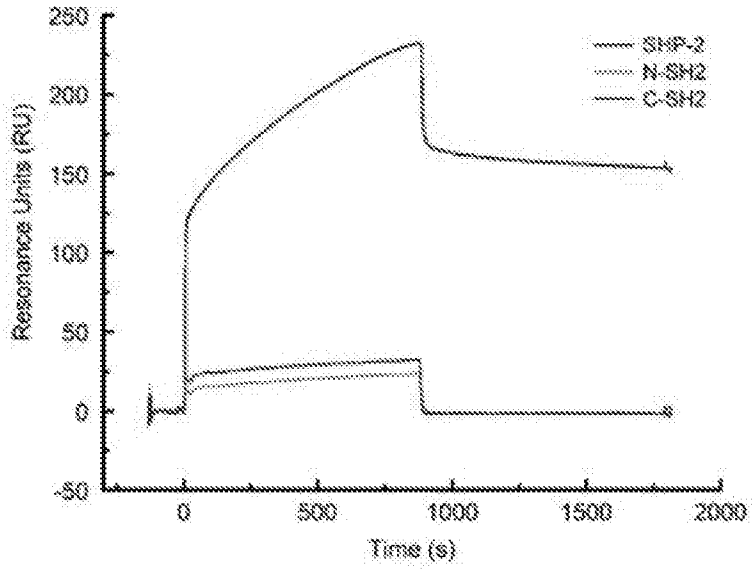

SHP-2
N-SH2
C-SH2
Resonance Units (RU)
250
200
150
100
50
0
-50
0
500
1000
1500
2000
Time (s)

FIG. 3D

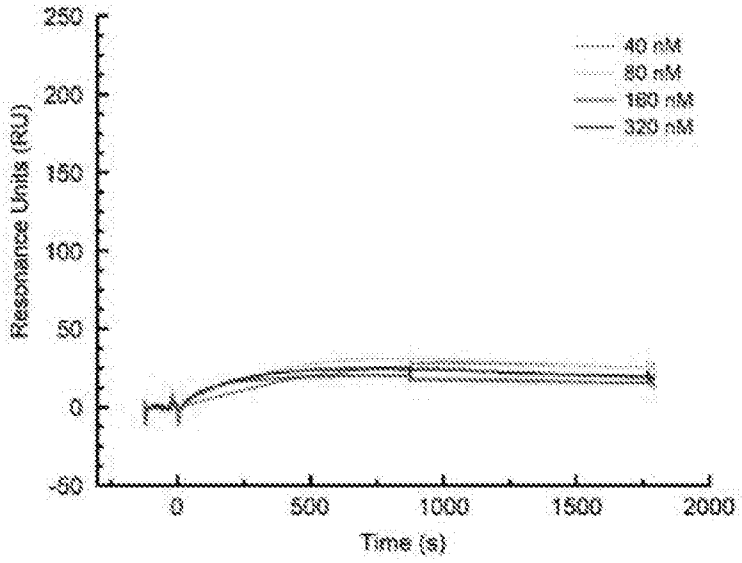

40 nM
80 nM
160 nM
320 nM
Resonance Units (RU)
250
200
150
100
50
0
-50
0
500
1000
1500
2000
Time (s)

FIG. 3E

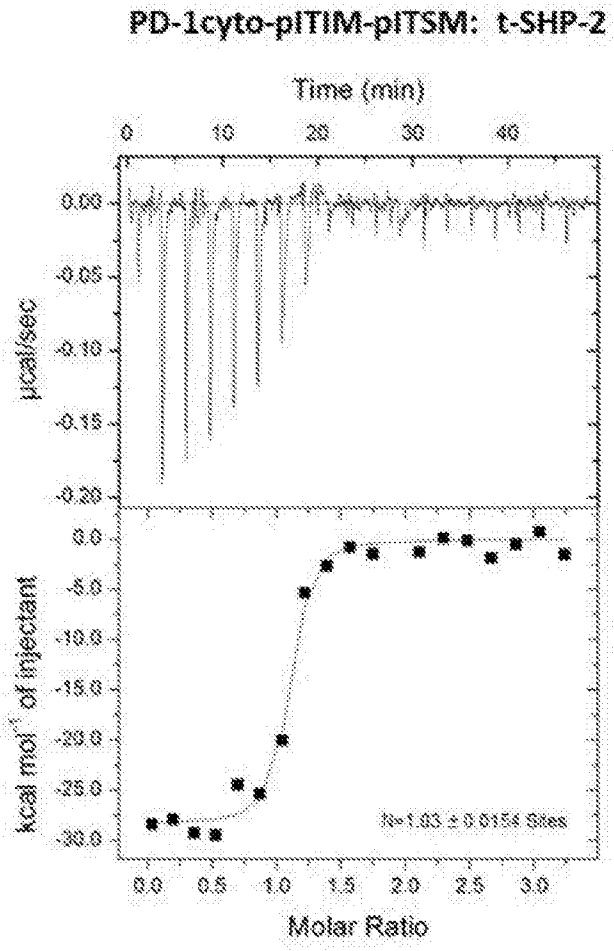

PD-1cyto-pITIM-pITSM: t-SHP-2
Time (min)
0
10
20
30
40
0.00
-0.05
-0.10
-0.15
-0.20
µcal/sec
0.0
-5.0
-10.0
-15.0
-20.0
-25.0
-30.0
kcal mol-1 of injectant
N=1.03 ± 0.0154 Sites
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Molar Ratio

FIG. 3F

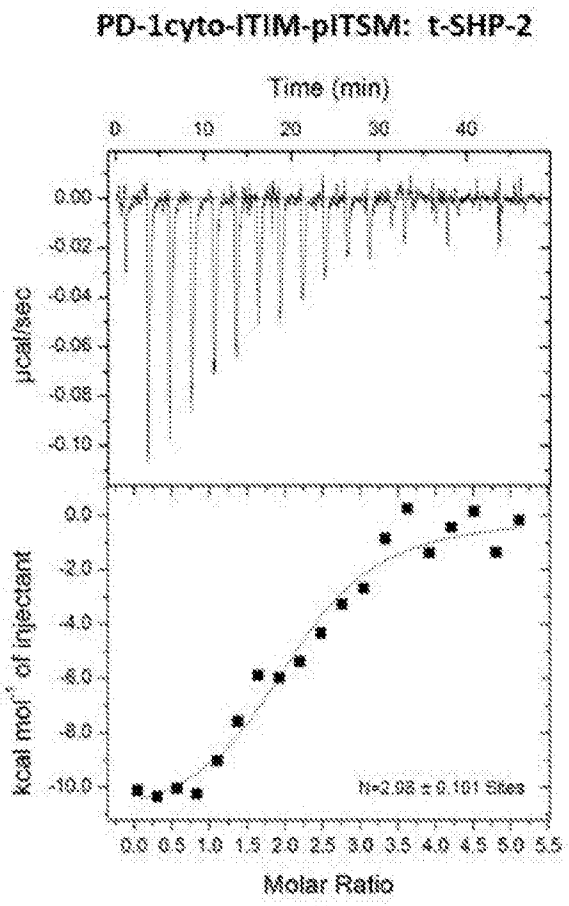

PD-1cyto-ITIM-pITSM: t-SHP-2
Time (min)
0
10
20
30
40
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
µcal/sec
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
kcal mol-1 of injectant
N=2.32 ± 0.101 Sites
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5
Molar Ratio

FIG. 4A

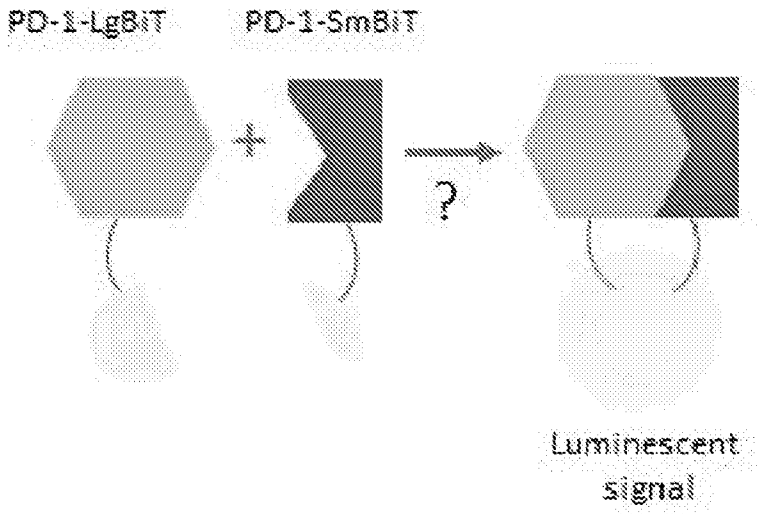

PD-1-LgBiT
PD-1-SmBiT
?
Luminescent signal

FIG. 4B

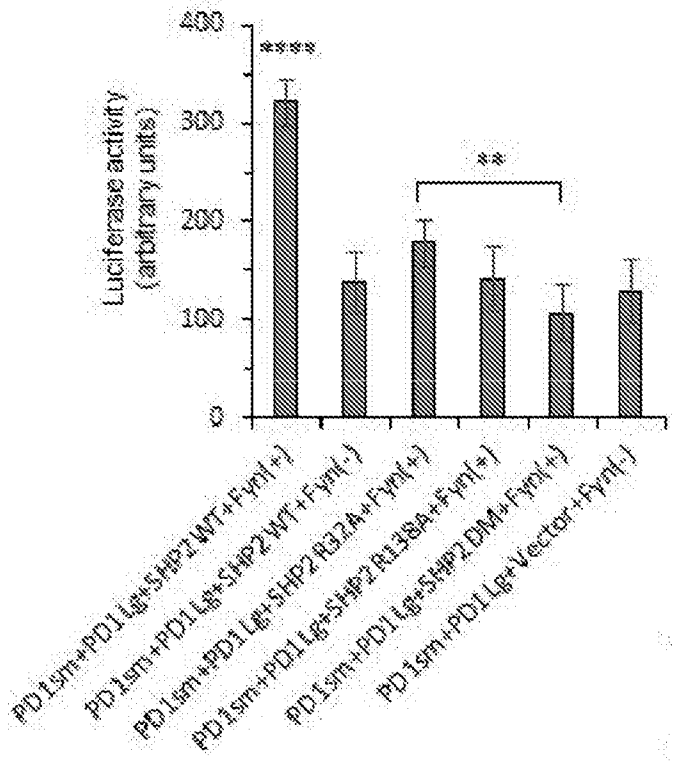

Luciferase activity (arbitrary units)
400
300
200
100
0
****
**
PD1sm+PD1Lg+SHP1WT+Fyn(+)
PD1sm+PD1Lg+SHP2WT+Fyn(+)
PD1sm+PD1Lg+SHP2R32A+Fyn(+)
PD1sm+PD1Lg+SHP2R138A+Fyn(+)
PD1sm+PD1Lg+SHP2DM+Fyn(+)
PD1sm+PD1Lg+Vector+Fyn(-)

FIG. 4C

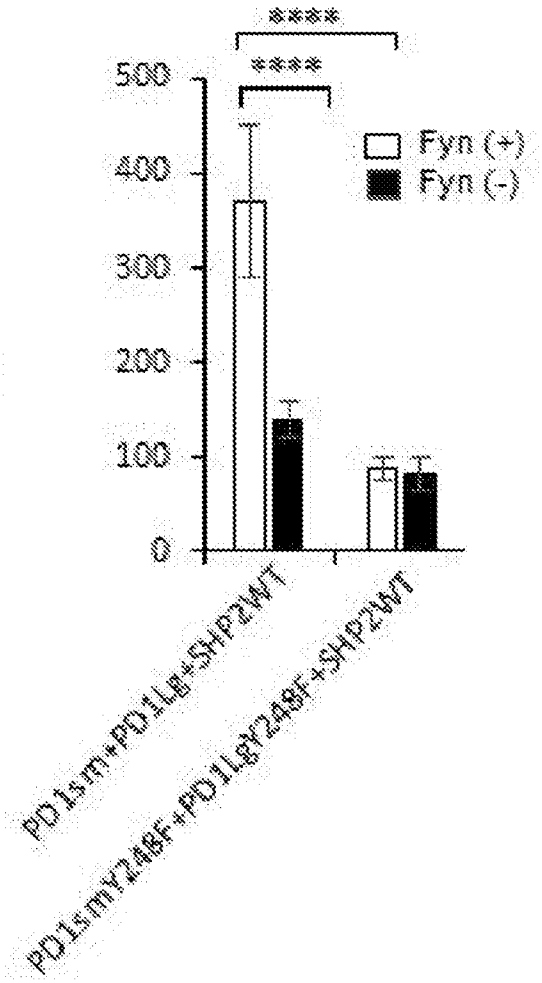

****
****
500
400
300
200
100
0
Fyn (+)
Fyn (-)
PD1smY248F+PD1LGY248F+SHP2WT
PD1smY248F+PD1LGY248F+SHP2MT

FIG. 4D

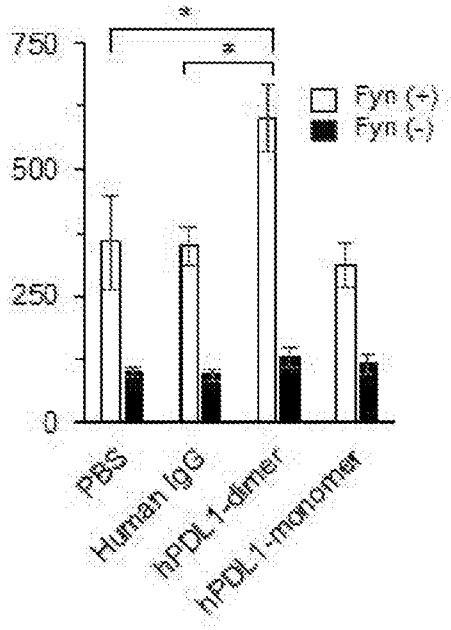


FIG. 4E

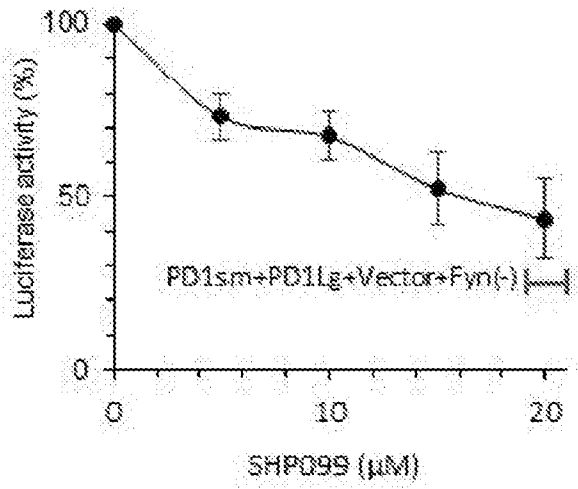


FIG. 4F

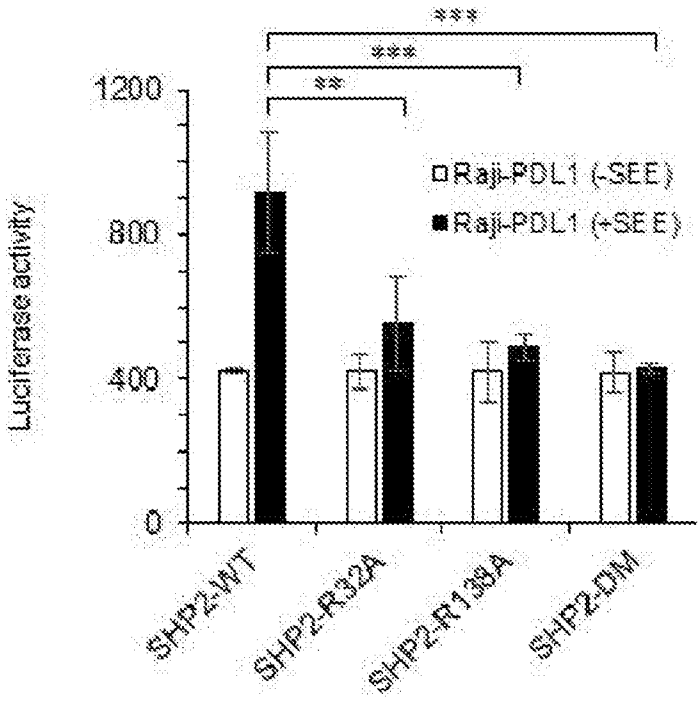


FIG. 4G

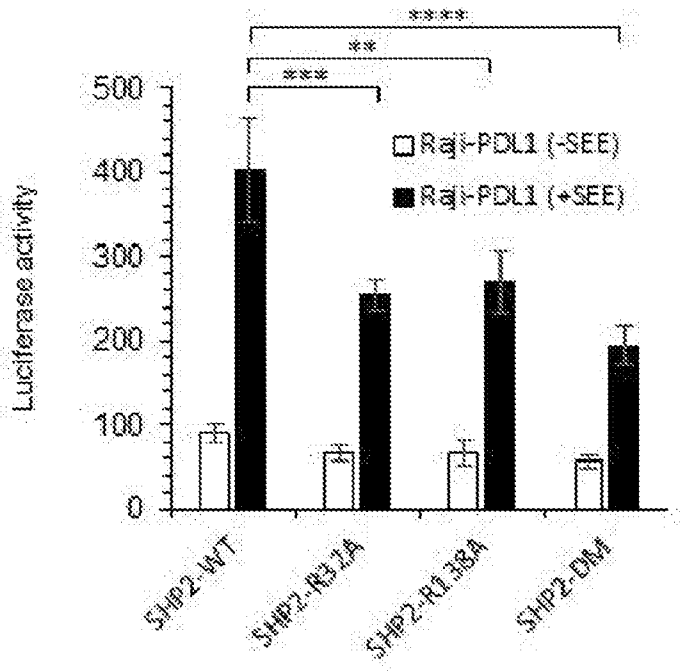

****
**
***
500
400
300
200
100
0
Luciferase activity
Raji-PDL1 (-SEE)
Raji-PDL1 (+SEE)
SHP2-WT
SHP2-R31A
SHP2-R138A
SHP2-DM

FIG. 5A

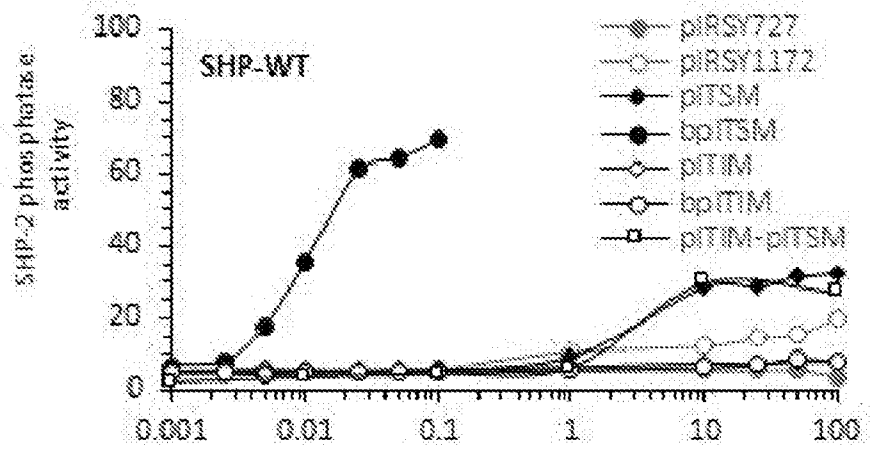

100
80
60
40
20
0
SHP-2 phosphatase activity
SHP-WT
pIRSY727
pIRSY1172
pITSM
bpITSM
pITIM
bpITIM
pITIM-pITSM
0.001
0.01
0.1
1
10
100

FIG. 5B

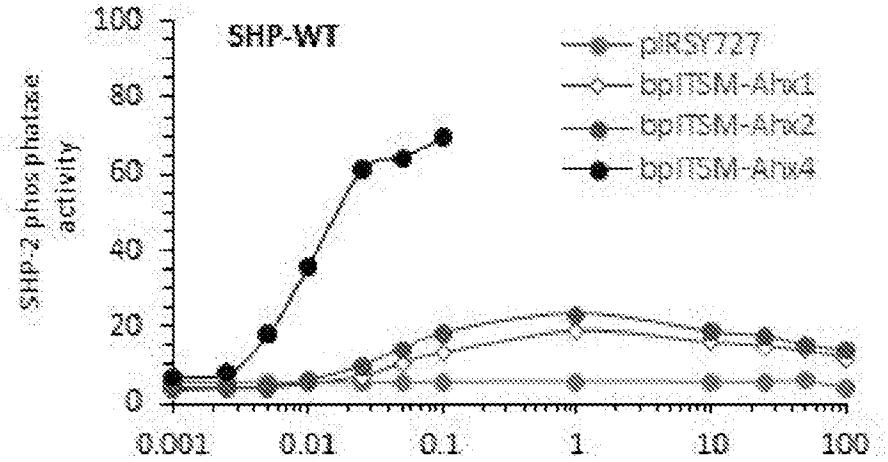

SHP-WT
pIRSI727
bpITSM-Ahx1
bpITSM-Ahx2
bpITSM-Ahx4
SHP-2 phosphatase activity
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100

FIG. 5C

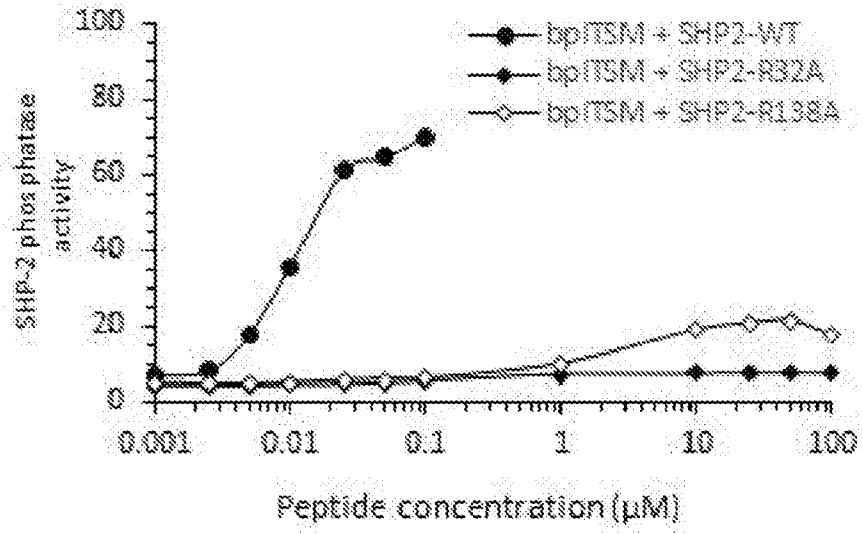

bpITSM + SHP2-WT
bpITSM + SHP2-R32A
bpITSM + SHP2-R138A
SHP-2 phosphatase activity
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
Peptide concentration (μM)

FIG. 5D

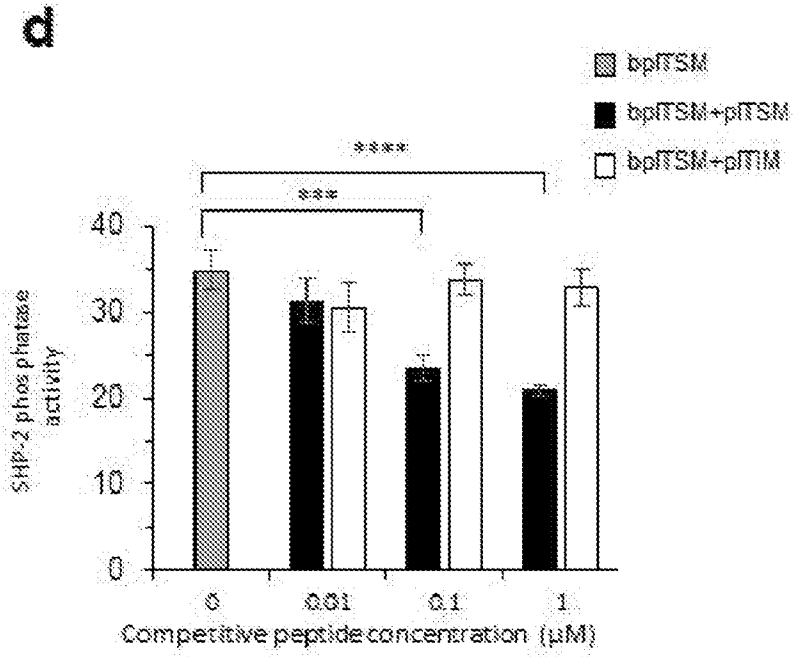

d
bpITSM
bpITSM+pITSM
bpITSM+pITIM
****
***
SHP-2 phosphatase activity
40
30
20
10
0
0
0.01
0.1
1
Competitive peptide concentration (μM)

FIG. 5E

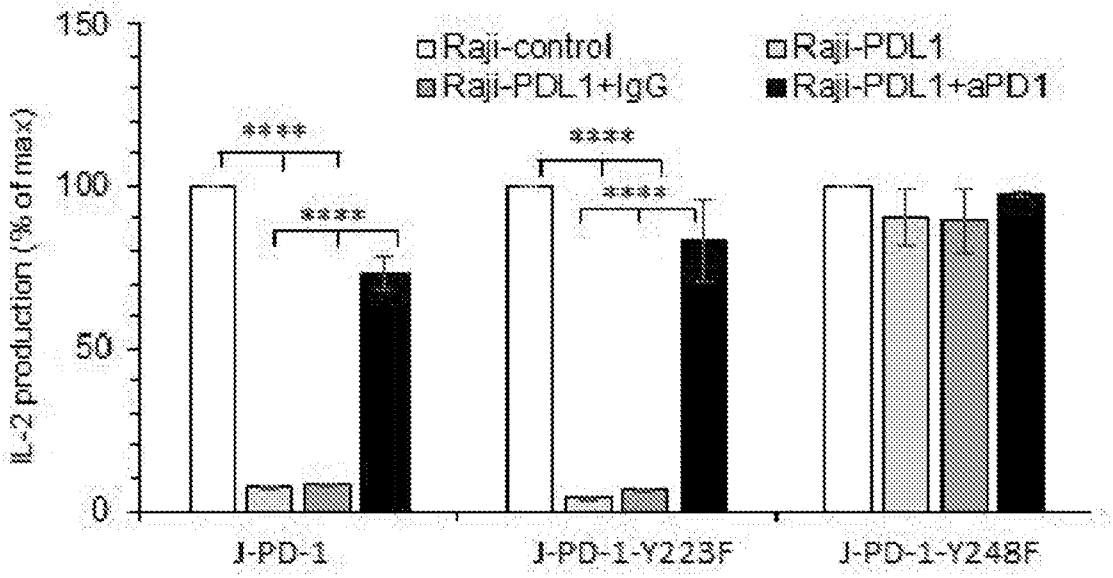

Raji-control
Raji-PDL1
Raji-PDL1+IgG
Raji-PDL1+aPD1
IL-2 production (% of max)
150
100
50
0
****
****
****
****
J-PD-1
J-PD-1-Y223F
J-PD-1-Y248F

FIG. 5F

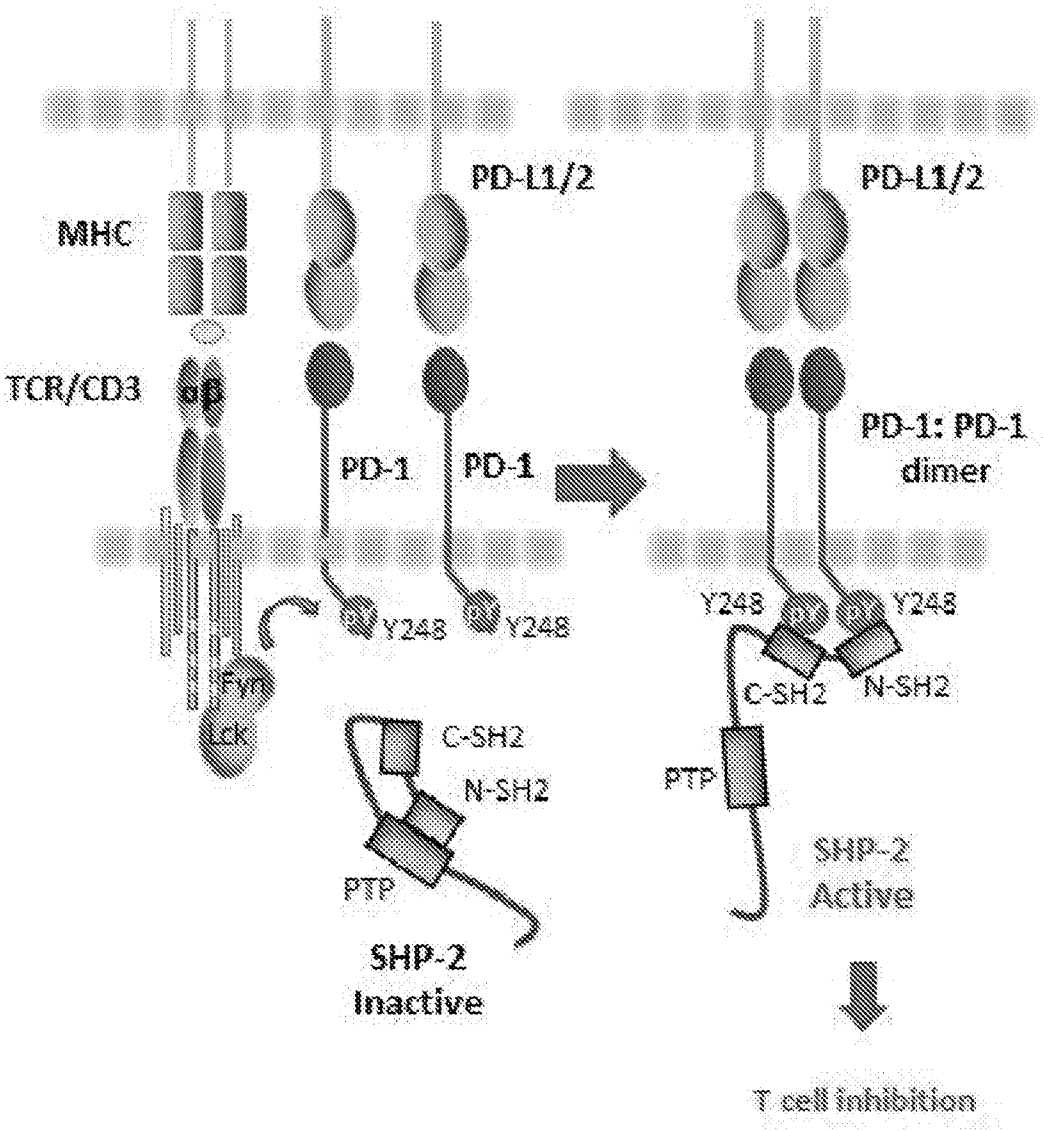

PD-L1/2
MHC
TCR/CD3
αβ
PD-1
PD-1
Y248
Y248
Fyn
Lck
C-SH2
N-SH2
PTP
SHP-2
Inactive
PD-L1/2
PD-1: PD-1
dimer
Y248
Y248
C-SH2
N-SH2
PTP
SHP-2
Active
T cell inhibition

FIG. 5G

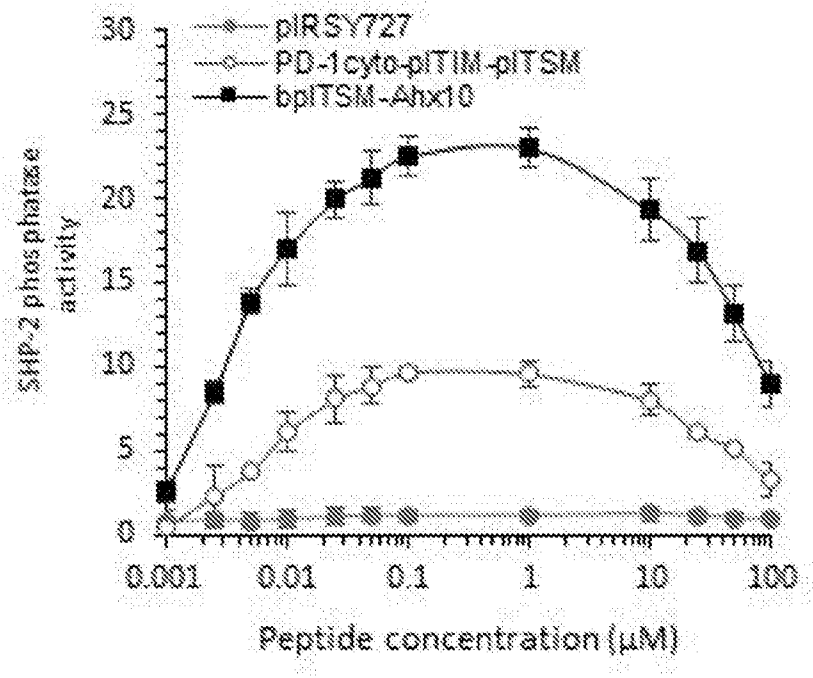

pIRSY727
PD-1cyto-pITIM-pITSM
bpITSM-Ahx10
SHP-2 phosphatase activity
30
25
20
15
10
5
0
0.001
0.01
0.1
1
10
100
Peptide concentration (μM)

FIG. 6A

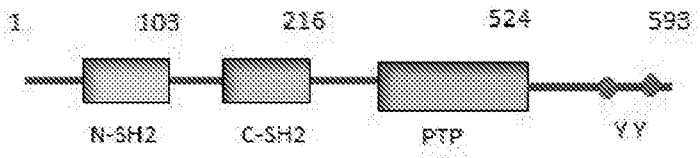

1
103
216
524
593
N-SH2
C-SH2
PTP
YY

FIG. 6B

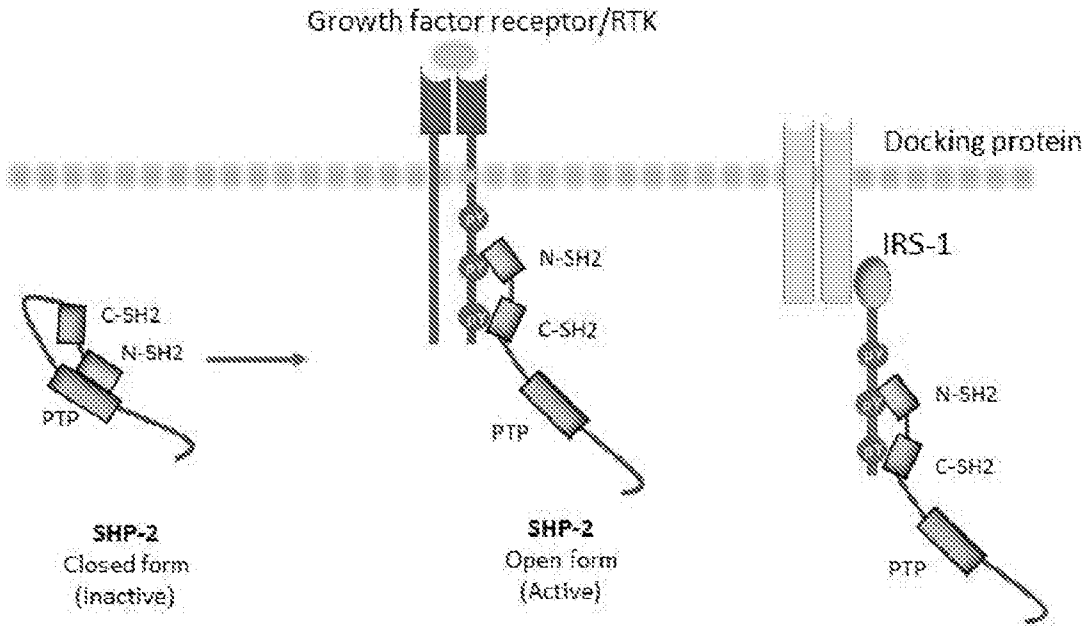

Growth factor receptor/RTK
Docking protein
IRS-1
N-SH2
C-SH2
PTP
SHP-2
Closed form
(inactive)
SHP-2
Open form
(Active)

FIG. 6C

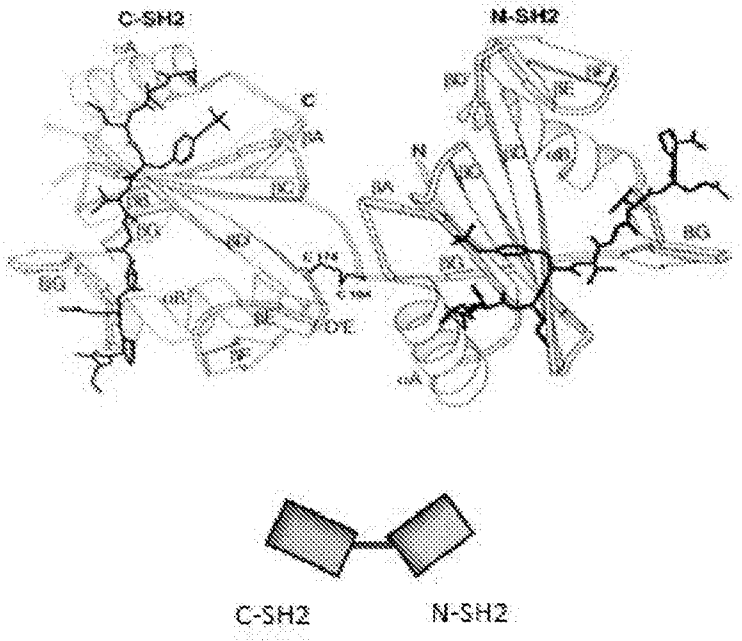


FIG. 7A

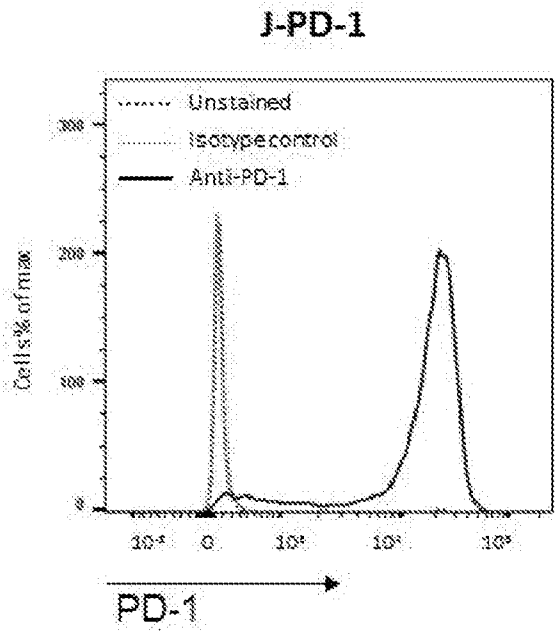

J-PD-1
Unstained
Isotype control
Anti-PD-1
Cells % of max
PD-1

FIG. 7B

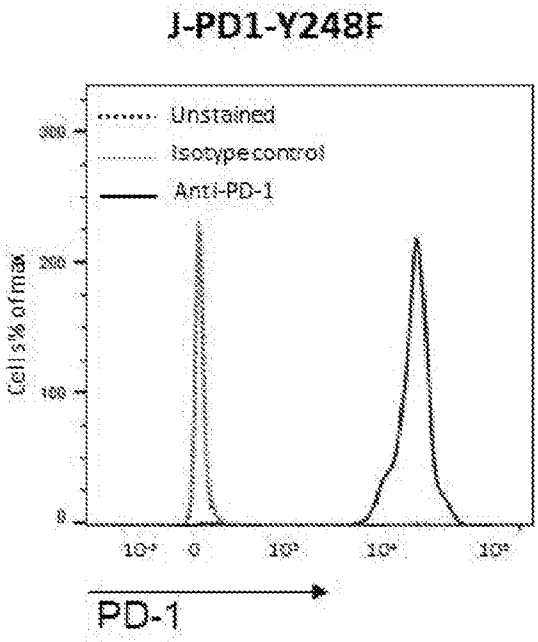

J-PD1-Y248F
Unstained
Isotype control
Anti-PD-1
Cells % of max
PD-1

FIG. 7C

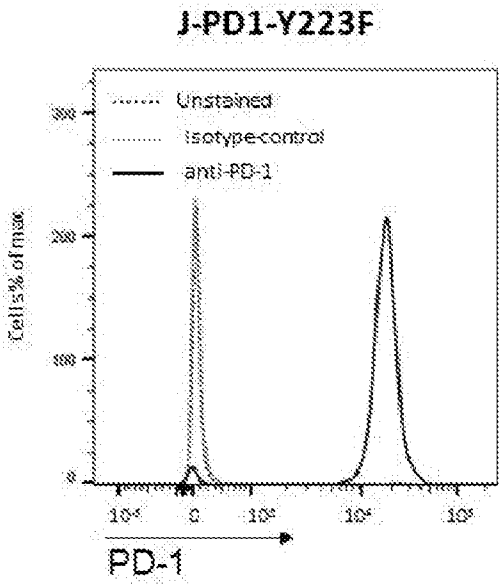

J-PD1-Y223F
Unstained
Isotype-control
anti-PD-1
Cells% of max
PD-1

FIG. 8A

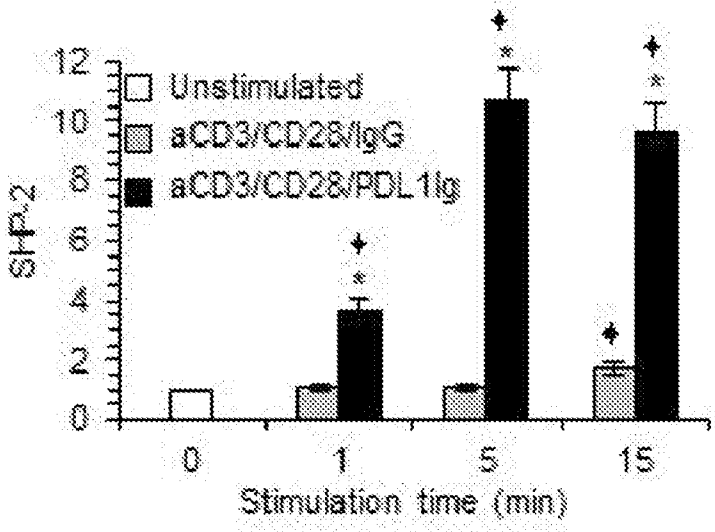

Unstimulated
aCD3/CD28/IgG
aCD3/CD28/PDL1Ig
SHP-2
Stimulation time (min)

FIG. 8B

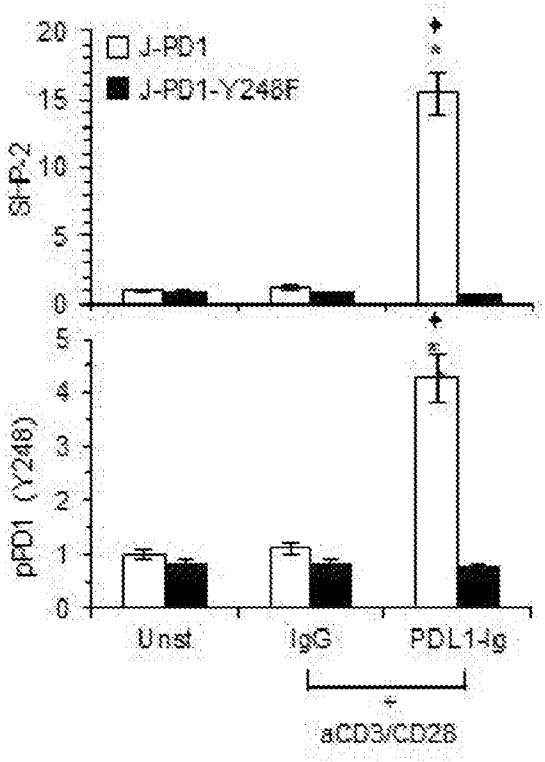

J-PD1
J-PD1-Y248F
SHP-2
pPD1 (Y248)
Unst
IgG
PDL1-Ig
aCD3/CD28

FIG. 8C

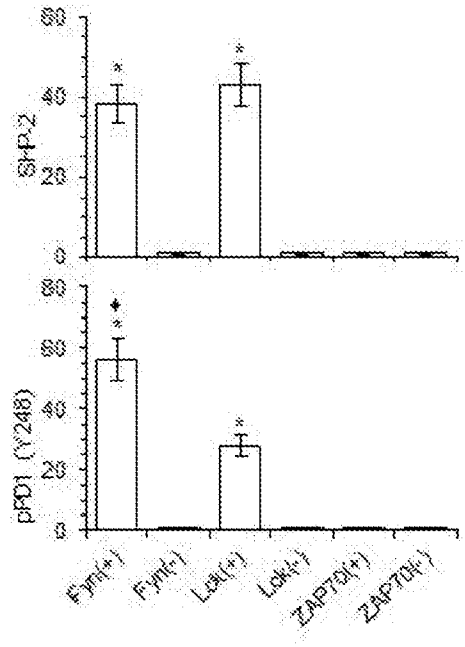

SHP-2
pPD1 (Y248)
Fyn(+)
Fyn(-)
Lck(+)
Lck(-)
ZAP70(+)
ZAP70(-)

FIG. 8D

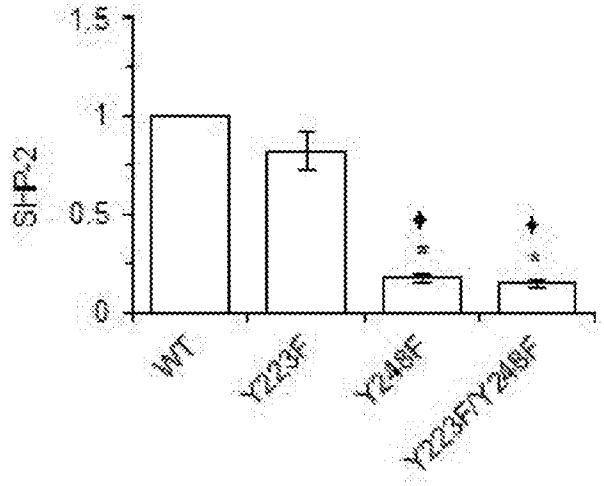

1.5
1
0.5
0
SHP-2
WT
Y223F
Y248F
Y223FY248F

FIG. 9

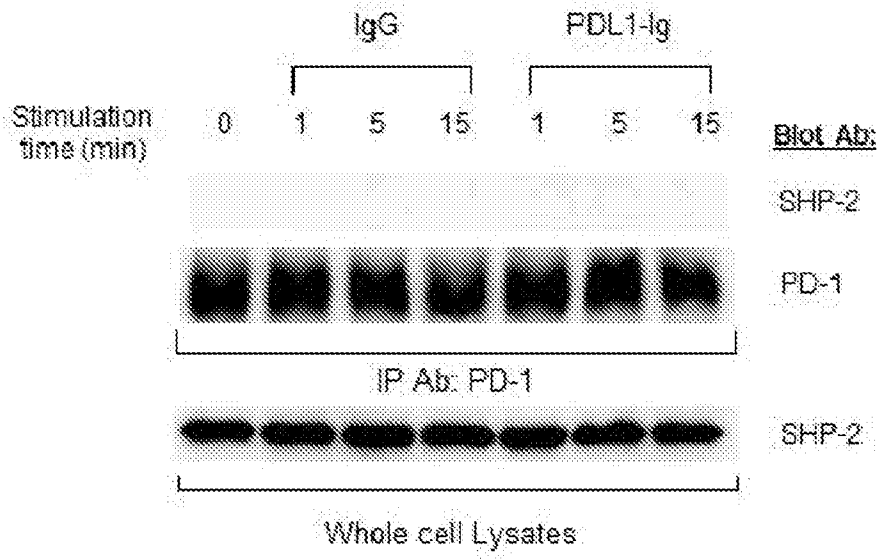

IgG
PDL1-Ig
Stimulation time (min)
0
1
5
15
1
5
15
Blot Ab:
SHP-2
PD-1
IP Ab: PD-1
SHP-2
Whole cell Lysates

FIG. 10A

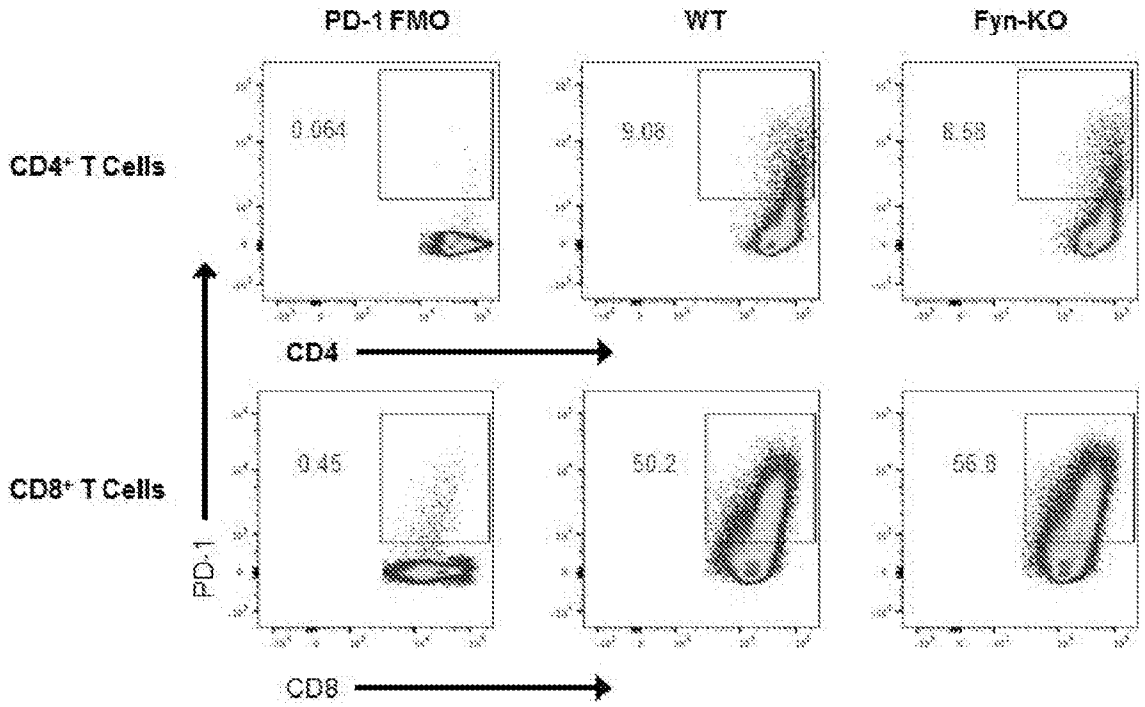


FIG. 10B

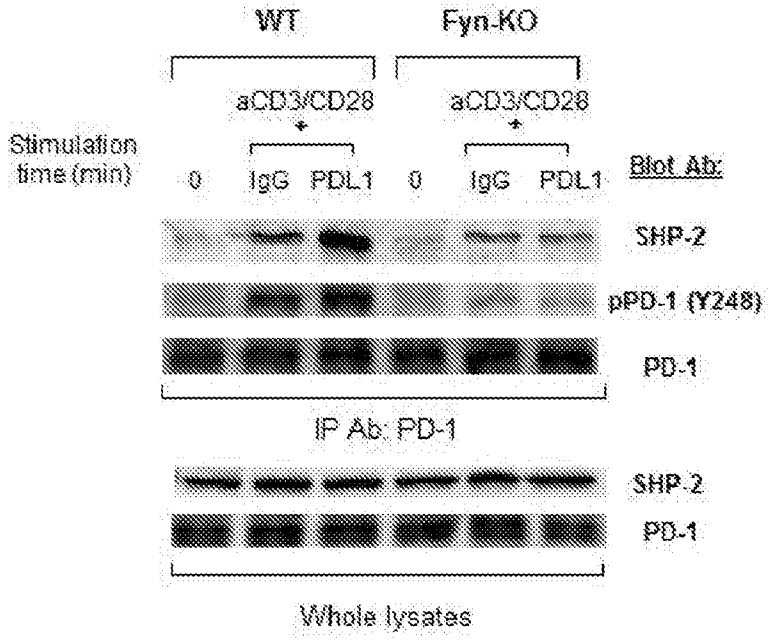


FIG. 10C

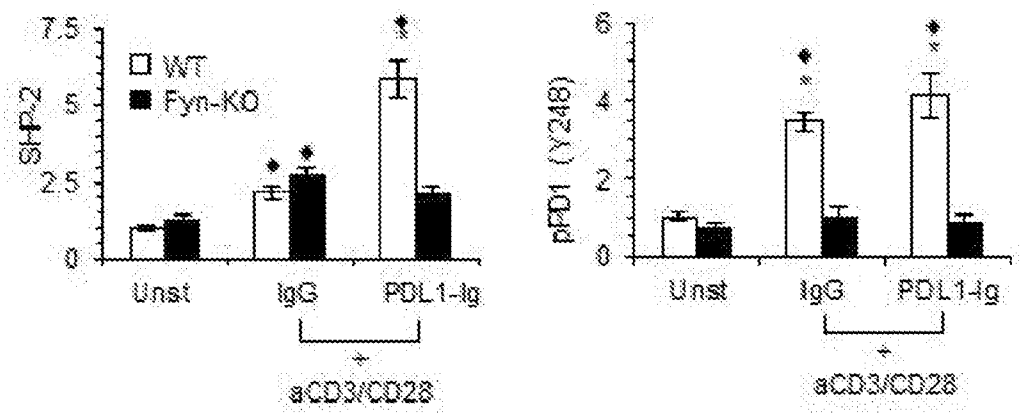


FIG. 11A

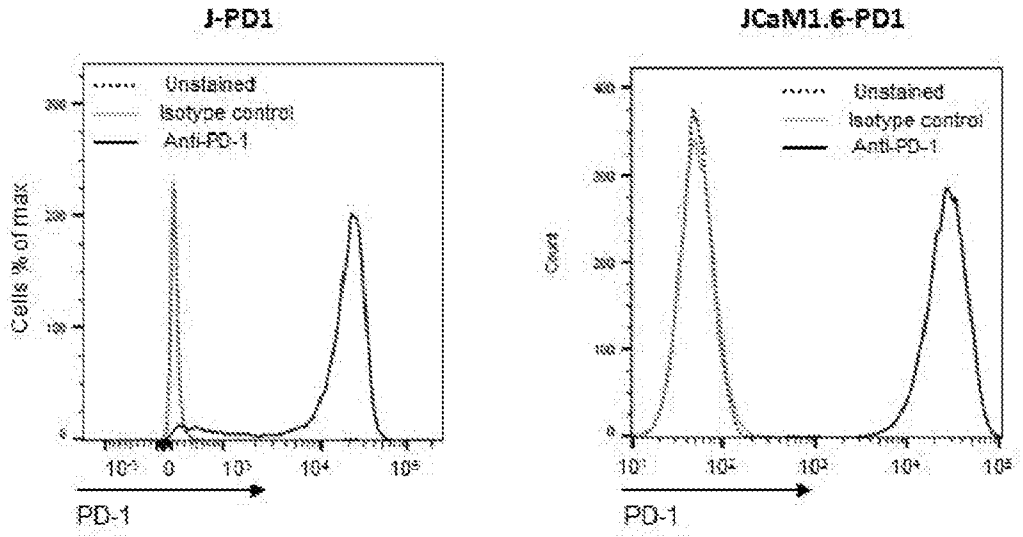


FIG. 11B

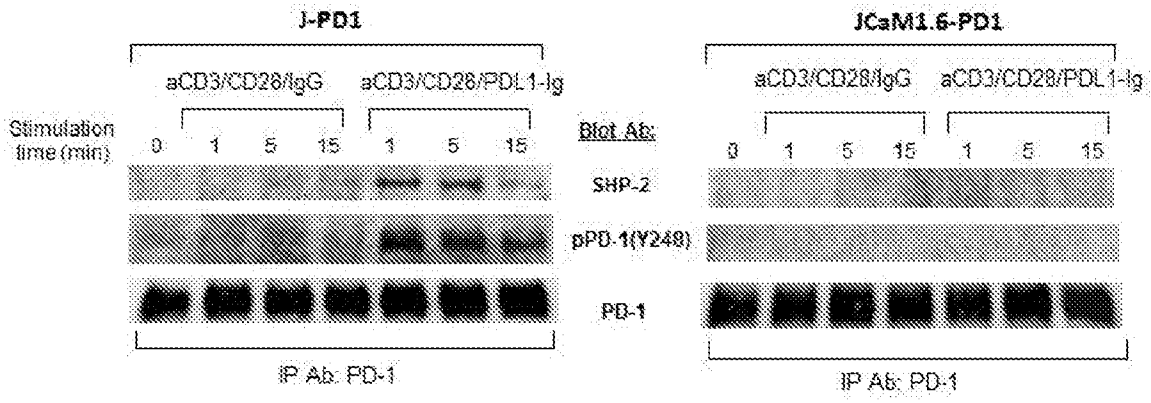


FIG. 11C

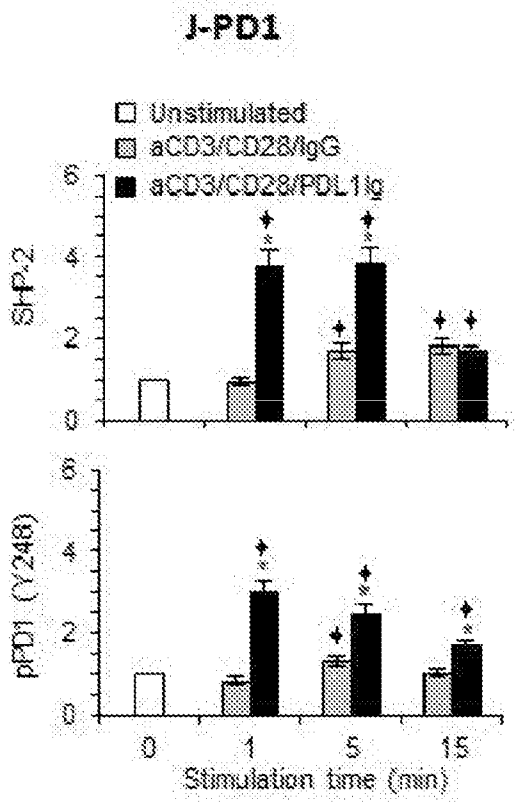


FIG. 12A

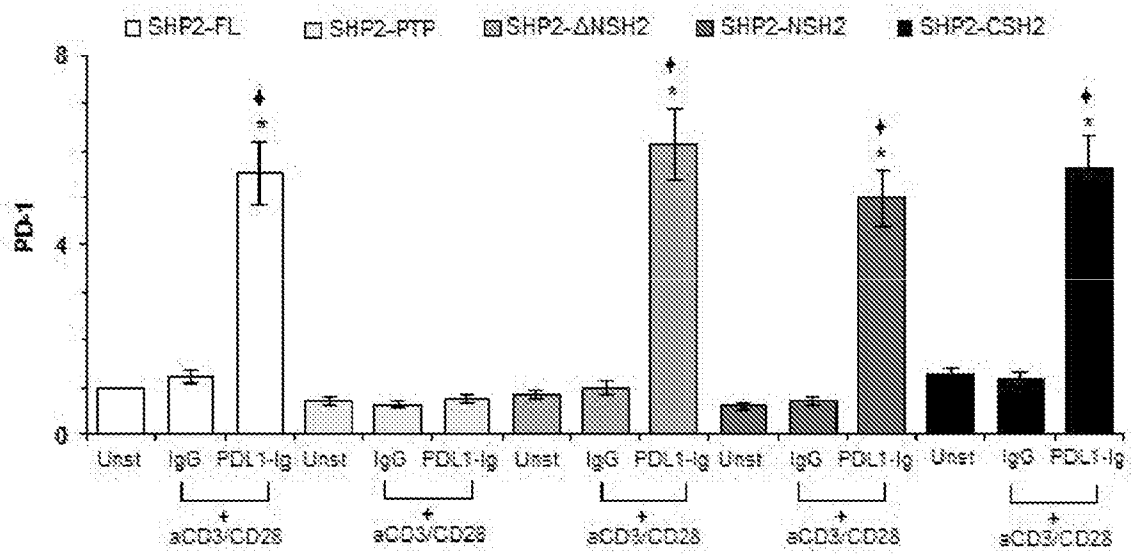


FIG. 12B

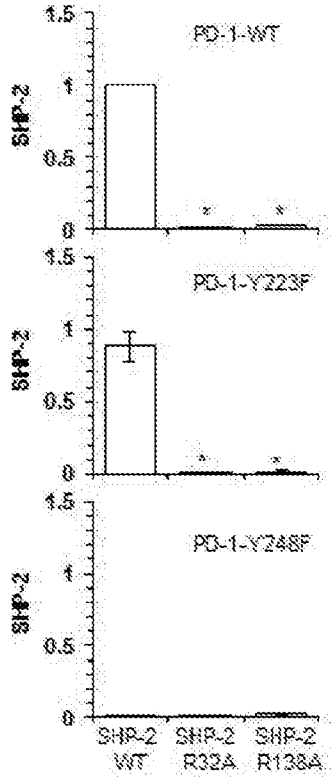


FIG. 12C

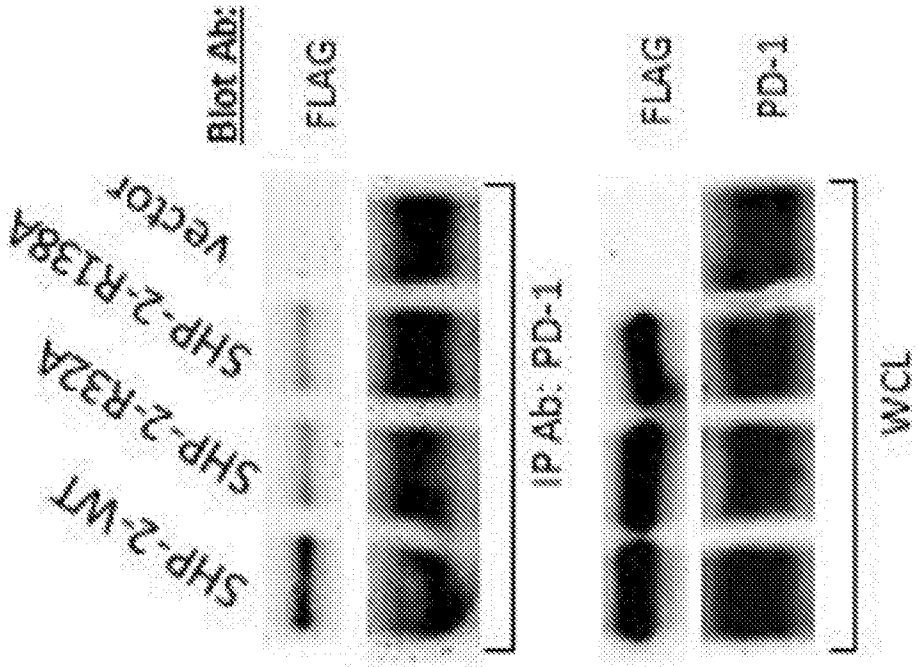

Blot Ab:
FLAG
FLAG
PD-1
SHP-2-WT
SHP-2-R32A
SHP-2-R138A
Vector
IP Ab: PD-1
WCL

FIG. 12D

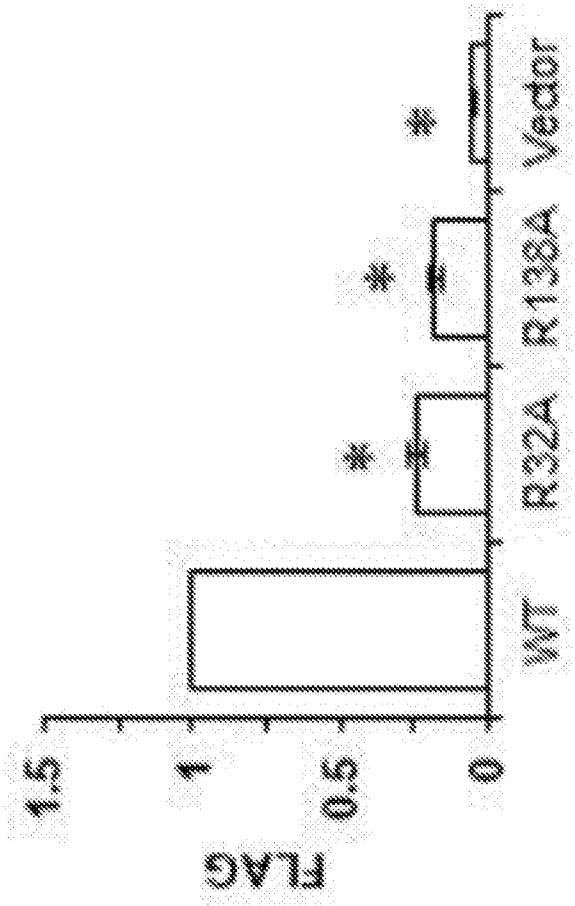


FIG. 13A

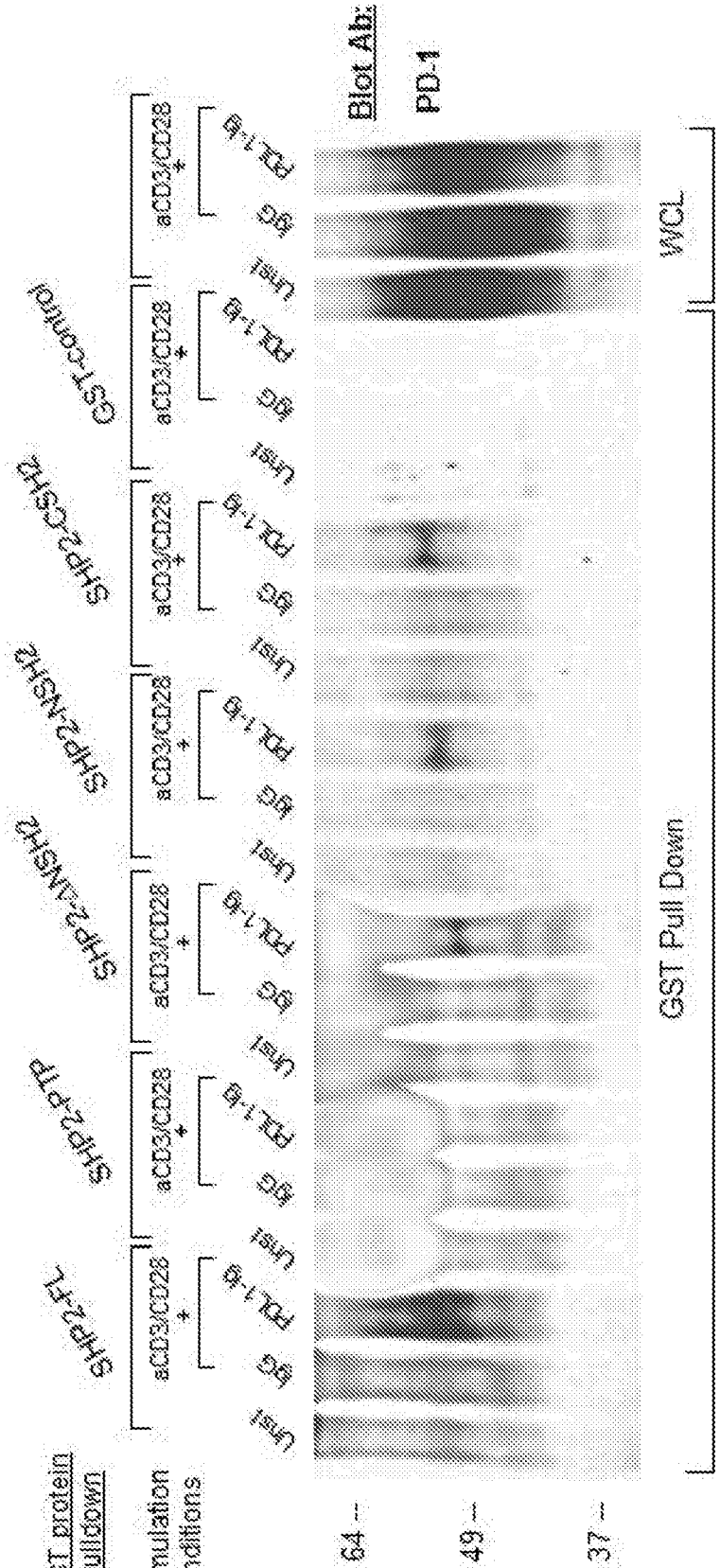

GST protein pulldown
Stimulation conditions
SHP2-FL
SHP2-PTP
SHP2-ΔNSH2
SHP2-ΔNSH2
SHP2-CSH2
GST-control
Unst
IgG
PDL1-Ig
aCD3/CD28
Blot Ab:
PD-1
64 --
49 --
37 --
GST Pull Down
WCL

FIG. 13B

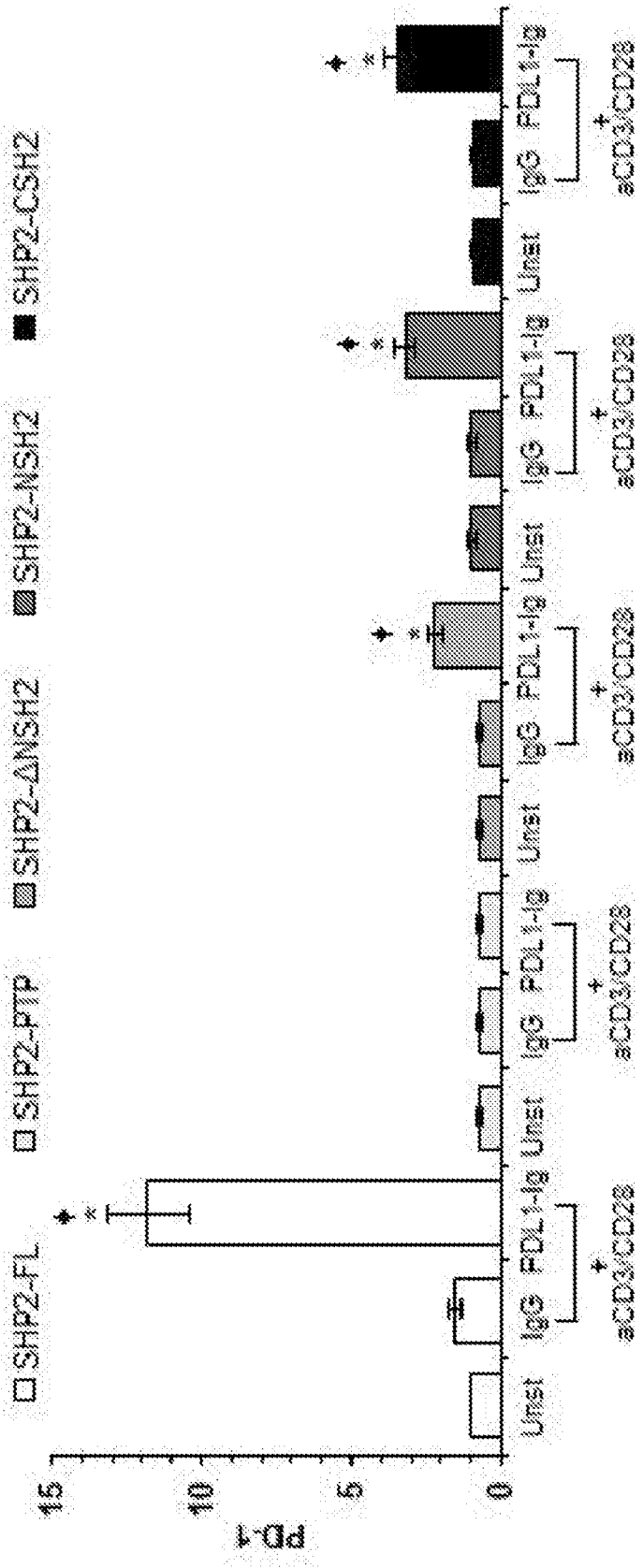

SHP2-FL
SHP2-PTP
SHP2-ΔNSH2
SHP2-NSH2
SHP2-CSH2
PD-1
15
10
5
0
Unst
IgG
PDL1-Ig
αCD3/CD28

FIG. 14A

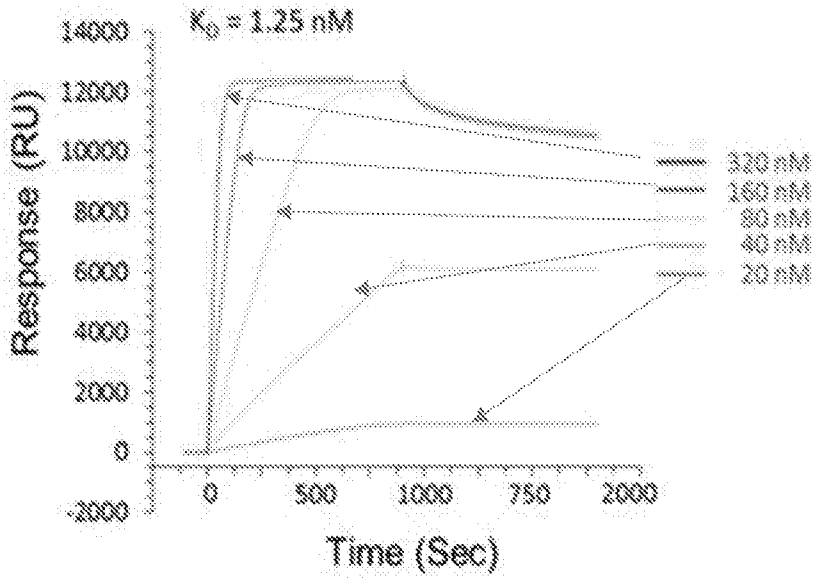


FIG. 14B

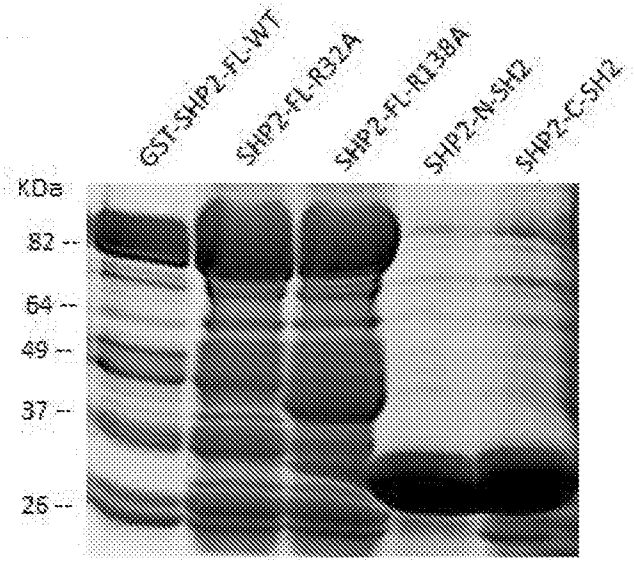


FIG. 14C

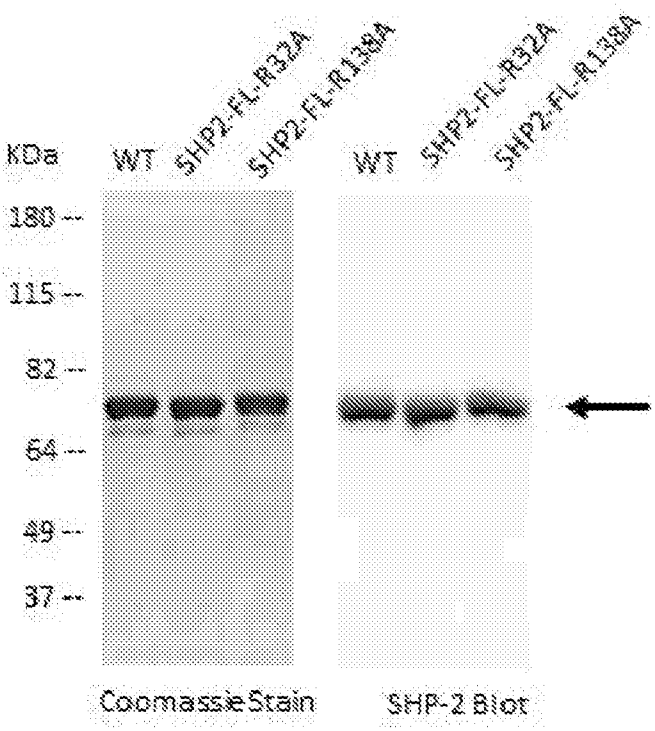


FIG. 14D

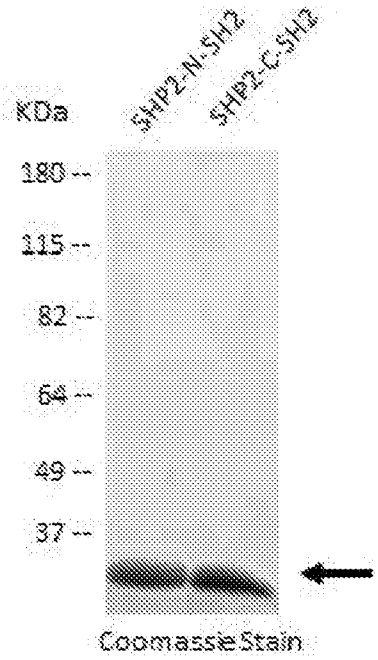


FIG. 15A

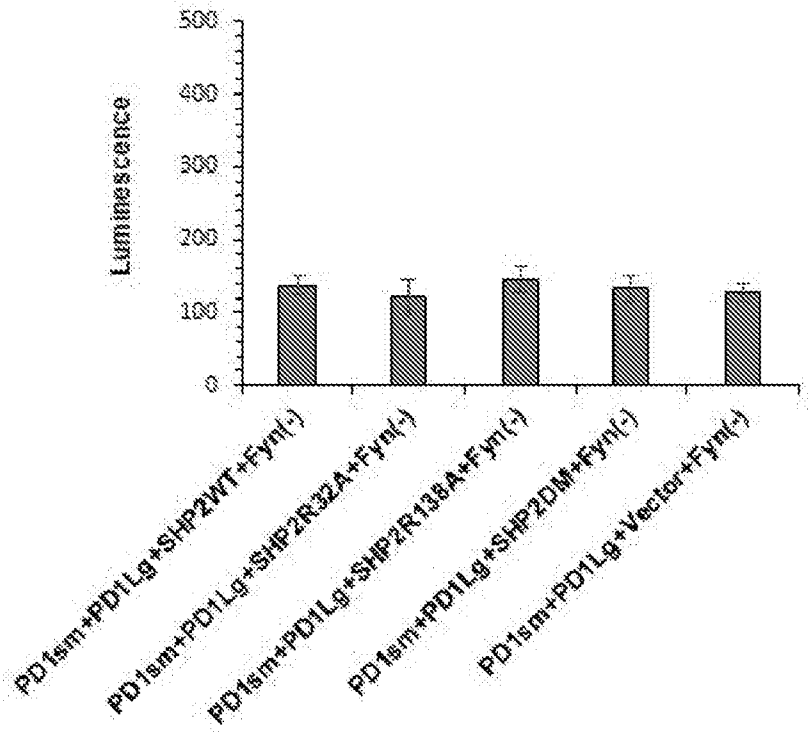


FIG. 15B

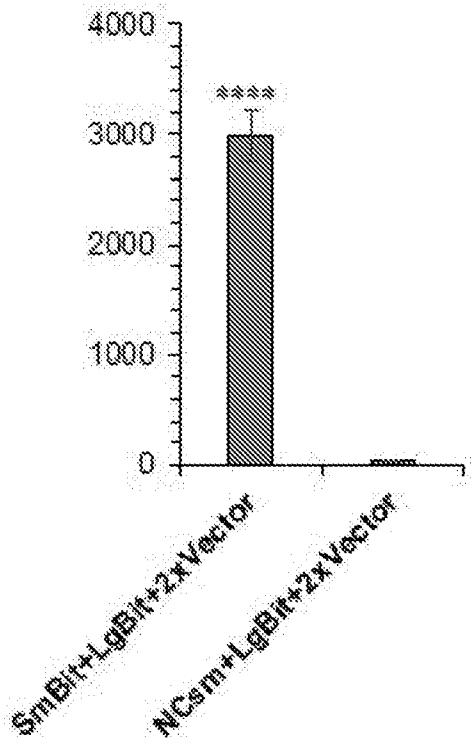


FIG. 16A

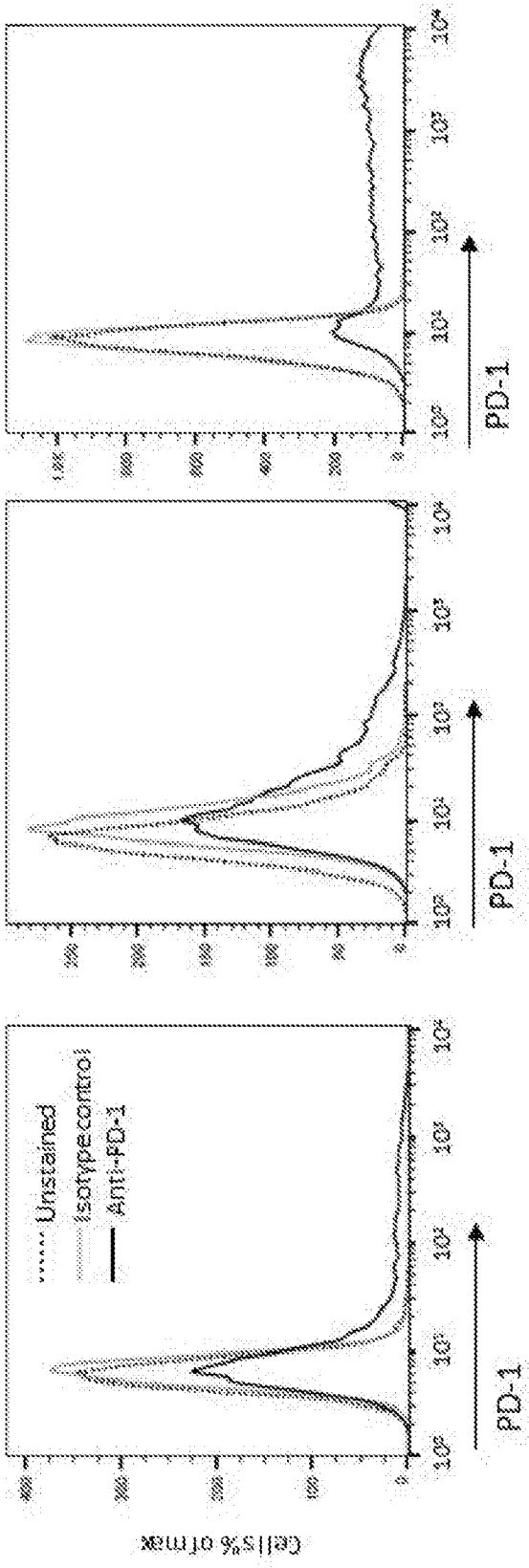

HEK-293
Jurkat T cells
Primary T cells
Unstained
Isotype control
Anti-PD-1
PD-1
PD-1
PD-1
Cell Count
Cell Count
Cell Count

FIG. 16B

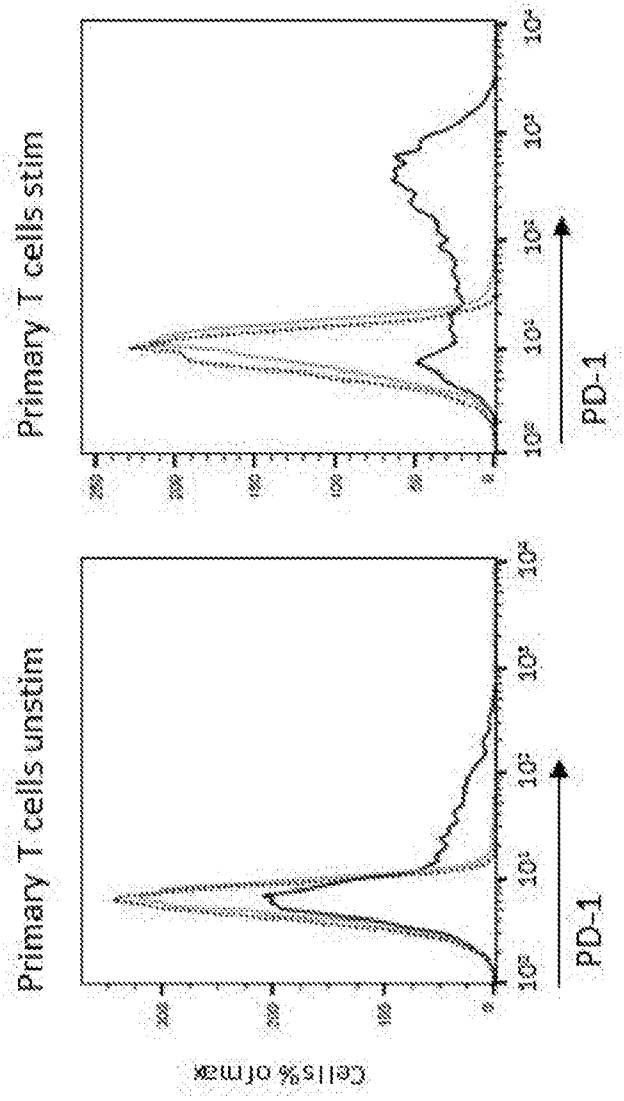

Primary T cells unstim
Primary T cells stim
Cells % of max
PD-1
PD-1

FIG. 17A

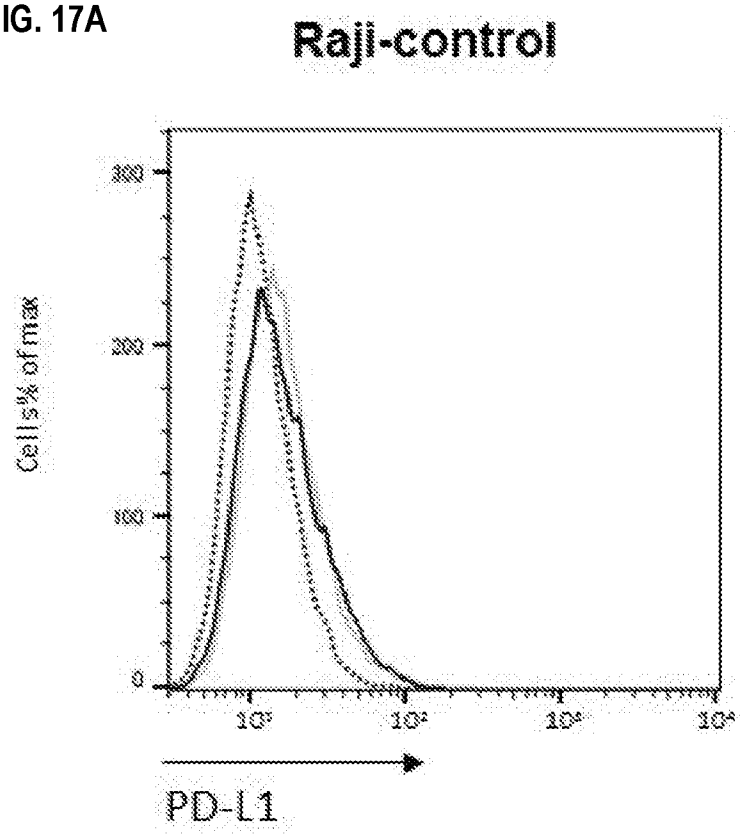


FIG. 17B

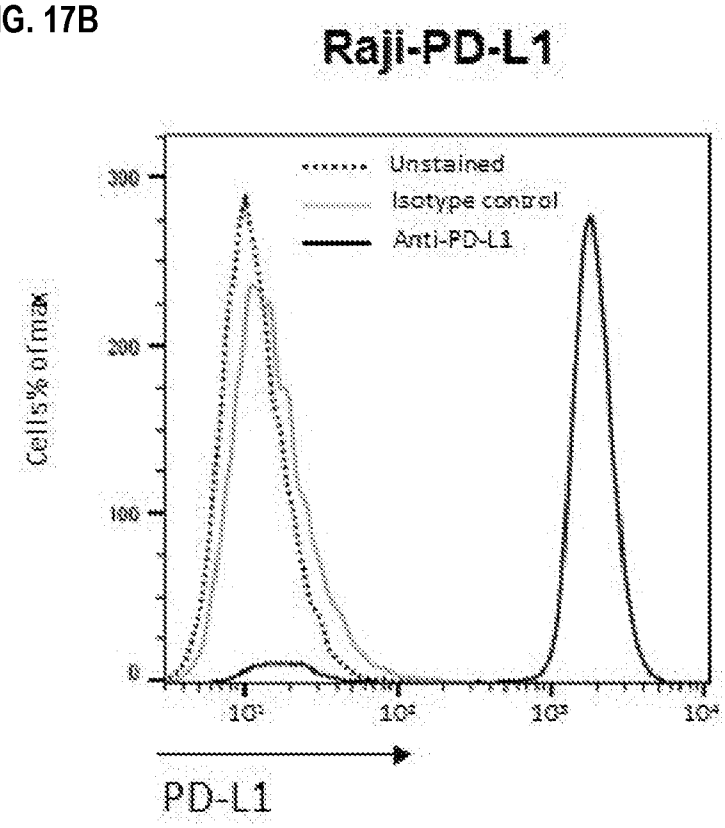


FIG. 18

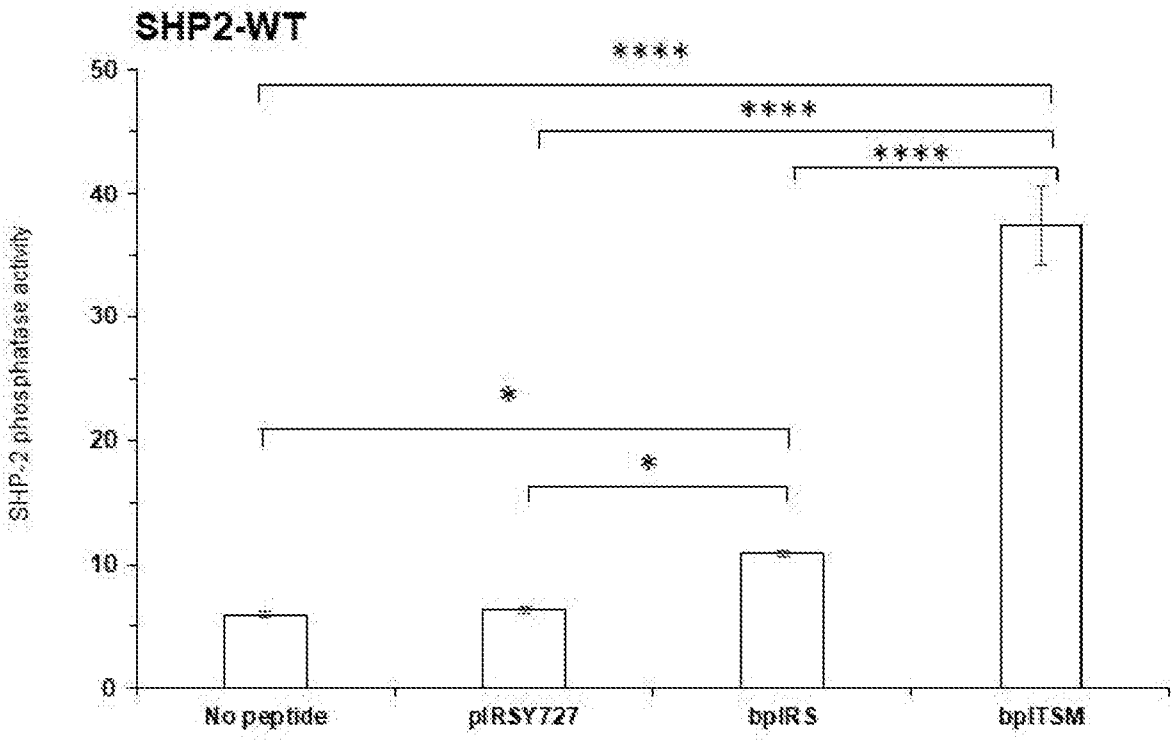

SHP2-WT
SHP-2 phosphatase activity
50
40
30
20
10
0
****
****
****
*
*
No peptide
pIRSY727
bpIRS
bpITSM

FIG. 19

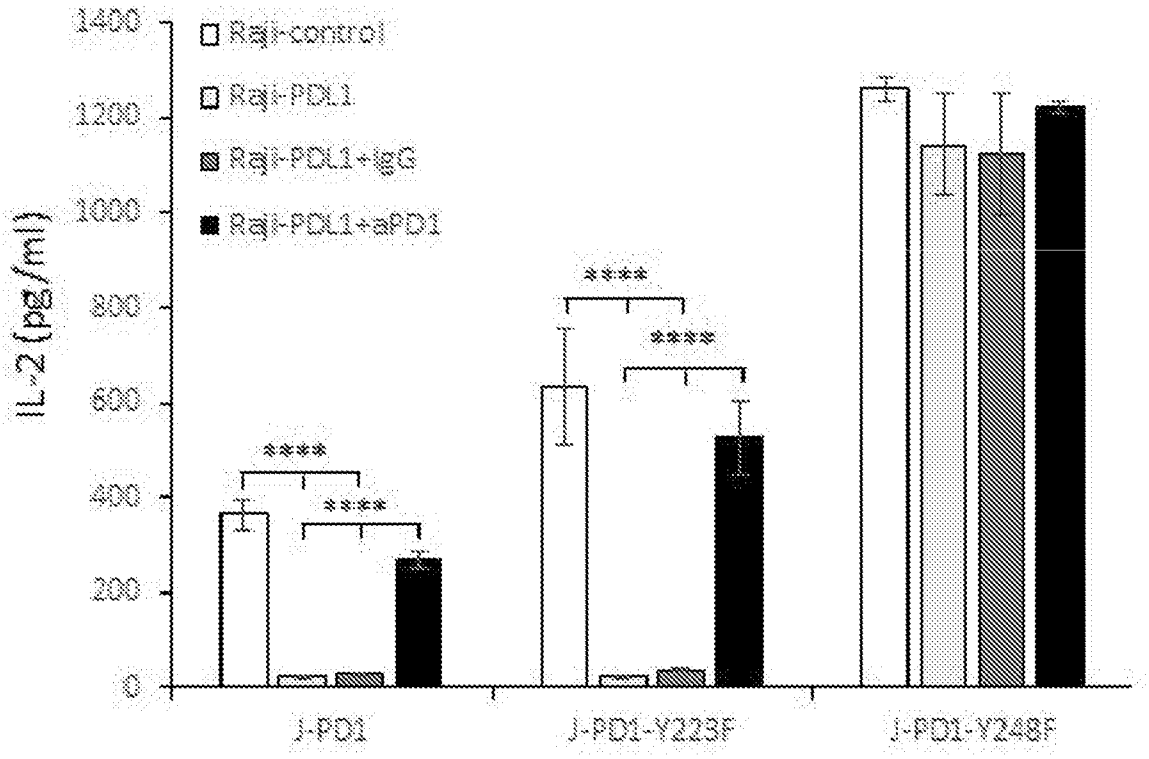

Raji-control
Raji-PDL1
Raji-PDL1+IgG
Raji-PDL1+aPD1
IL-2 (pg/ml)
1400
1200
1000
800
600
400
200
0
****
****
****
****
J-PD1
J-PD1-Y223F
J-PD1-Y248F

FIG. 20A

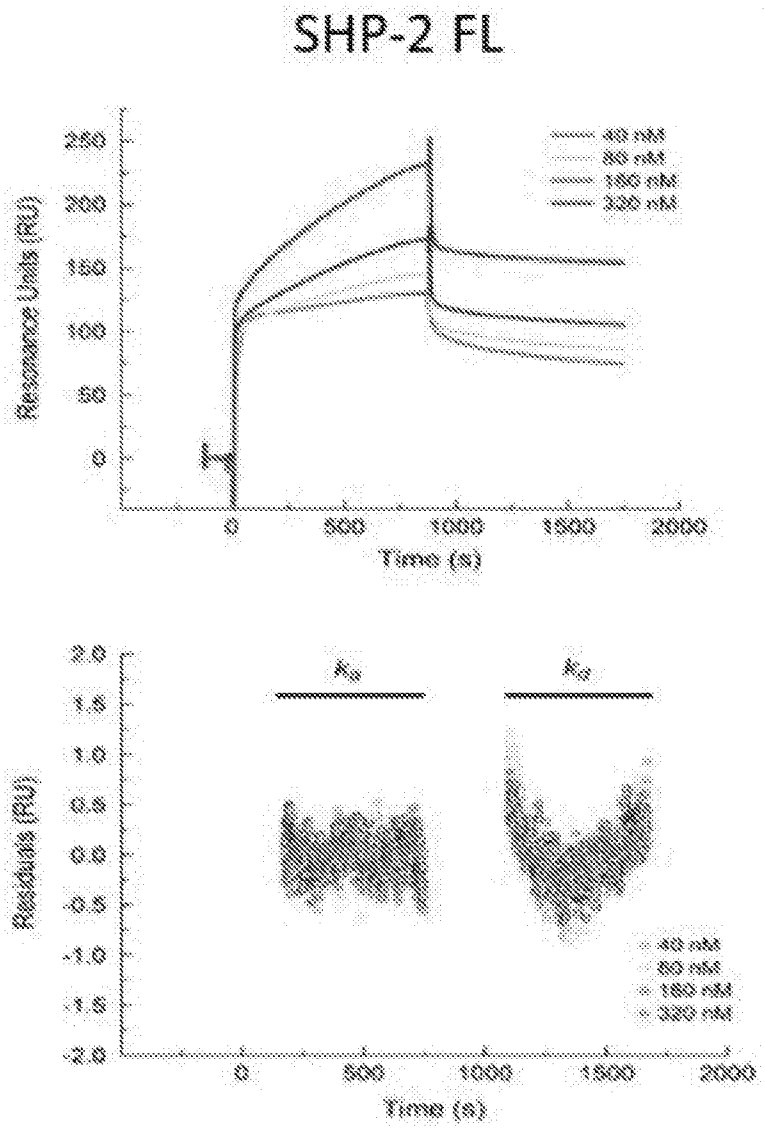

SHP-2 FL
Response Units (RU)
250
200
150
100
50
0
40 nM
80 nM
160 nM
320 nM
0
500
1000
1500
2000
Time (s)
Residuals (RU)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
K_D
K_d
40 nM
80 nM
160 nM
320 nM
0
500
1000
1500
2000
Time (s)

FIG. 20B

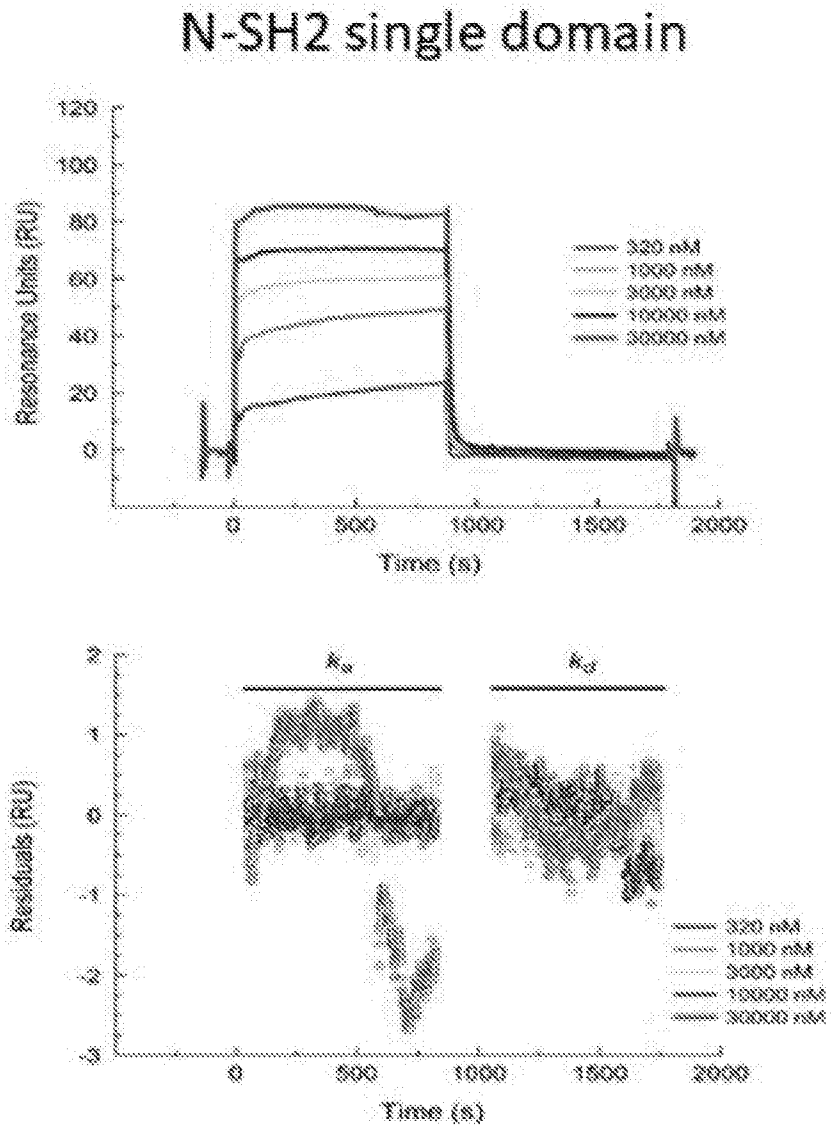
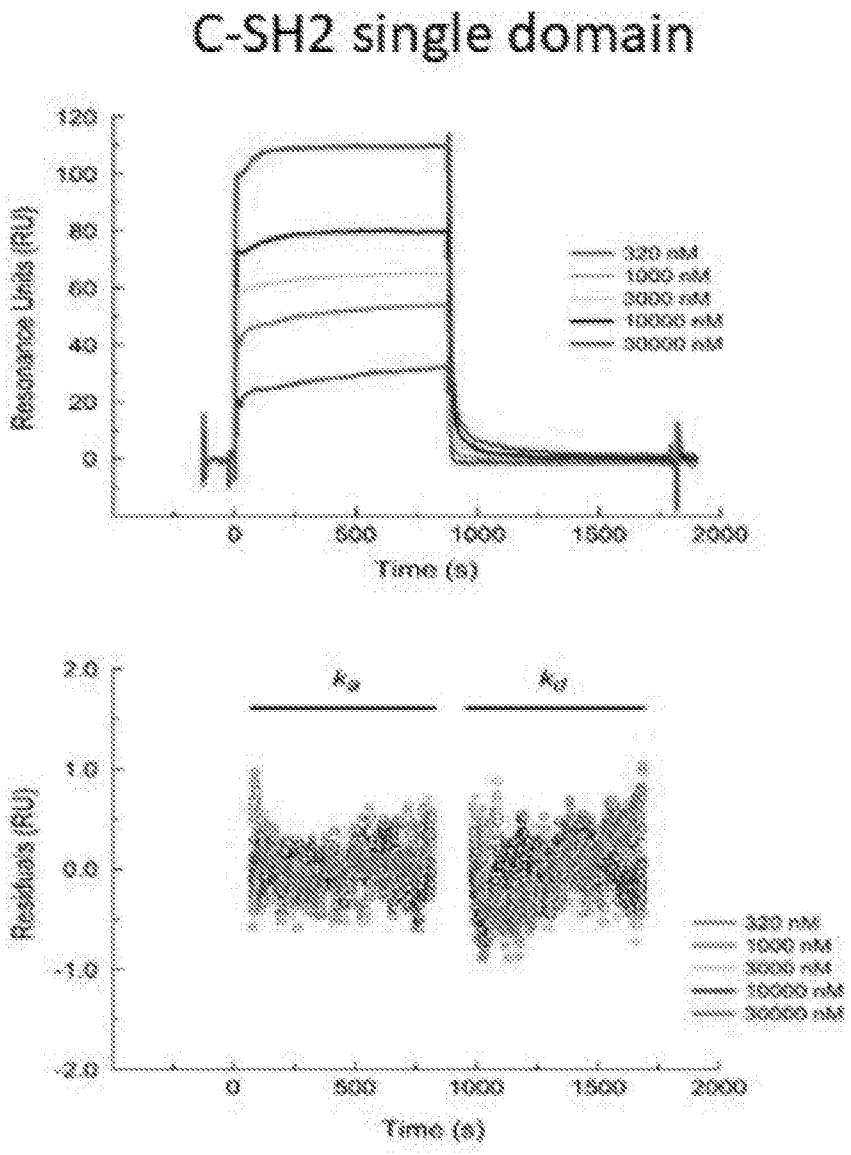

N-SH2 single domain
Resonance Units (RU)
120
100
80
60
40
20
0
0
500
1000
1500
2000
Time (s)
320 nM
1000 nM
3000 nM
10000 nM
30000 nM
Residuals (RU)
3
1
0
-1
-2
-3
k_a
k_d
0
500
1000
1500
2000
Time (s)
320 nM
1000 nM
3000 nM
10000 nM
30000 nM

FIG. 20C


C-SH2 single domain
Resonance Units (RU)
120
100
80
60
40
20
0
0
500
1000
1500
2000
Time (s)
320 nM
1000 nM
3000 nM
10000 nM
30000 nM
Residuals (RU)
2.0
1.0
0.0
-1.0
-2.0
k_a
k_d
Time (s)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/65983

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/395, 38/46; C12N 9/16; C07K 16/30, 14/47; A61P 35/00; G01N 33/68, 33/574 (2020.01)

CPC - A61K 39/395, 38/465, 38/46; C12N 9/16; C07K 16/30, 14/47; A61P 35/00; G01N 33/68, 33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2018/160731 A1 (NOVARTIS AG, et al.) 20 December 2018; page 7, lines 10-13; page 8, line 3; page 18, line 33 to page 19, line 2; page 27, lines 5-31; page 35, line 32 to page 36, line 25; page 59, lines 28-31; page 397, line 25 to page 398, line 21	1-3, 4/2-3, 25-26, 27/25-26, 28/27/25-26 --- 123, 126, 133, 136
X --- Y	WO 2018/231339 A2 (DANA-FARBER CANCER INSTITUTE, INC. et al.) 07 September 2018; Table 1; page 2, lines 15-30; page 3, line 1 to page 5, line 11; page 5, line 30 to page 6, line 3; page 8, lines 1-8; page 11, lines 5-6; page 14, line 19 to page 15, line 16; page 28, lines 22-33; page 40, lines 1-14; page 44, line 13 to page 45, line 8; page 63, line 3 to page 64, line 12; page 70, line 25 to page 71, line 5; page 73, lines 13-28; page 76, lines 4-9; page 76, line 26 to page 77 line 3; page 79, lines 13-33; page 80, line 14 to page 81, line 6; page 81, lines 8-15; page 83, lines 28-31; page 84, lines 18-26; page 86, lines 27-33; page 87, lines 19-28; page 89, lines 10-20; page 90, lines 21-27; page 95, line 2 to page 96, line 13; page 101, line 21 to page 102, line 32; page 103, lines 24-29	1-3, 4/2-3, 25-26 --- 85-87, 88/86-87, 119-138
Y	(PATSOUKIS, N. et al.) The Two SH2 Domains Of SHP-2 Bridge Two PD-1 Molecules Resulting In SHP-2 Activation And PD-1-Mediated Inhibition. Blood. 29 November 2018, Vol. 132, Supp. 1, page 862; abstract; doi: 10.1182/blood-2018-99-117486	4/2-3, 85-87, 88/86-87, 119-138
A	WO 2018/084204 A1 (KYOTO UNIVERSITY) 11 May 2018; entire document	1-3, 4/2-3, 25-26, 27/25-26, 28/27/25-26, 85-87, 88/86-87, 119-138

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2020 (17.04.2020)	21 MAY 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/65983

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,156,551 A (NEEL, B. et al.) 5 December 2000; entire document	1-3, 4/2-3, 25-26, 27/25-26, 28/27/25-26, 85-87, 88/86-87, 119-138

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/65983

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

3. ☒ Claims Nos.: 5-24, 29-61, 71-84, 89-102, 112-118
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

-***-Please See Supplemental Page-***-

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

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

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

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

1-3, 4/2-3, 25-26, 27/25-26, 28/27/25-26, 85-87, 88/86-87, and 119-138

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/65983

-***-Continued from Box No. III Observations where unity of invention is lacking: -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-4, 25-28, 85-88, and 119-138 are directed toward methods for treating cancer, making an agent effective for the treatment of cancer, and predicting a cancer patient response to the treatment.

Group II, Claims 62-70, 103-111 are directed toward methods for treating an autoimmune disease, and making an agent effective for the treatment of an autoimmune disease.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include cancer, not present in Group II; the special technical features of Group II include an autoimmune disease, not present in Group I.

Groups I and II share the technical features including: a method for treating a disease or disorder, comprising: (a) selecting an agent which modulates SHP-2-mediated PD-1 dimerization and/or PD-1 dimer activity; and (b) administering the agent to the patient; and a method of making an agent effective for the treatment of a disease or disorder, comprising: (a) identifying the agent by screening for modulation of SHP-2-mediated PD-1 dimerization and/or PD-1 dimer activity; and (b) formulating the agent for administration to a patient having a disease or disorder.

However, these shared technical features are previously disclosed by WO 2018/084204 A1 to Kyoto University (hereinafter 'Kyoto'), in view of the abstract 'The Two SH2 Domains of SHP-2 Bridge Two PD-1 Molecules Resulting in SHP-2 Activation and PD-1 Mediated Inhibition' by Patsoukis et al. (hereinafter 'Patsoukis').

Kyoto discloses a method for treating a disease or disorder (a method for determining the effectiveness of treating a disease or disorder; Claim 8), comprising: (a) selecting an agent which modulates PD-1 activity (selecting an anti-PD-1 antibody which inhibits PD-1 signaling (selecting an agent which modulates PD-1 activity); Claims 5, 6); and (b) administering the agent to the patient (administering the agent to the patient; Claim 8); and a method of making an agent effective for the treatment of a disease or disorder (a method of formulating (making) an agent effective for the treatment of a disease or disorder; Claim 8, page 13, second paragraph-third paragraph), comprising: (a) identifying the agent by screening for modulation of PD-1 activity (identifying the agent by screening for modulation of PD-1 activity; Claims 3, 5); and (b) formulating the agent for administration to a patient having a disease or disorder (formulating the agent for administration to a patient having a disease or disorder; Claim 8, page 13, second paragraph-third paragraph).

Kyoto does not disclose an agent which modulates SHP-2-mediated PD-1 dimerization and/or PD-1 dimer activity; and a method of making an agent effective for the treatment of a disease or disorder, comprising: (a) identifying the agent by screening for modulation of SHP-2-mediated PD-1 dimerization and/or PD-1 dimer activity; and (b) formulating the agent for administration to a patient having a disease or disorder

Patsoukis discloses wherein SHP-2 interacts with two PD-1 molecules, forming a PD-1:PD-1 dimer (wherein SHP-2 interacts with two PD-1 molecules, forming a PD-1:PD-1 dimer; page 2).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to have modified the disclosure of Kyoto to have determined whether the antibodies effective to inhibit PD-1 signaling, as disclosed by Kyoto, functioned via modulation of SHP-2 mediated PD-1 dimerization, as disclosed by Patsoukis, in order to better determine the means of functioning of the PD-1 antibodies as an effective treatment for cancer and to aid in the selection of patients to be treated therewith.

Since none of the special technical features of the Groups I and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Kyoto and Patsoukis references, unity of invention is lacking.