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(54) Title: PD-1 AND CTLA-4 DUAL INHIBITOR PEPTIDES

(57) Abstract: This disclosure provides peptides which have a strong affinity for the checkpoint receptor "programmed death 1" (PD-1). These peptides block the interaction of PD-1 with its ligand PD-L1 as well as the interaction of CTLA4 with CD86 and can therefore be used for various therapeutic purposes, such as inhibiting the progression of a hyperproliferative disorder, including cancer; treating infectious diseases; enhancing a response to vaccination; treating sepsis; and promoting hair re-pigmentation or lightening of pigmented skin lesions.

PD-1 AND CTLA-4 DUAL INHIBITOR PEPTIDES

[01] This application claims priority to and incorporates by reference U.S. provisional application Serial No. 62/510,900 filed on May 25, 2017.

[02] This application incorporates by reference the contents of a 9.97 kb text filed created on May 25, 2018 and named "00047900258sequencelisting.txt," which is the sequence listing for this application.

[03] Each scientific reference, patent, and published patent application cited in this disclosure is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[04] This disclosure relates generally to immunomodulatory peptides.

BACKGROUND

[05] Programmed cell death-1 (PD-1) and its ligands, PD-L1 and PD-L2, are widely expressed and exert a number of immunoregulatory roles in T cell activation, including attenuation of immunity against tumor cells and infectious agents. PD-1 is therefore an attractive target for a variety of therapeutic applications. Cytotoxic T-lymphocyte-associated antigen (CTLA-4) provides a negative signal to T cells and is also an attractive therapeutic target. There is a continuing need for useful modulators of immune checkpoint pathways.

BRIEF DESCRIPTION OF THE FIGURES

[06] **Figure 1** is a graph providing single concentration BIACORE® data for peptide LD01 binding to human PD-1.

[07] **Figure 2** is a graph providing single concentration BIACORE® data for peptide LD01 binding to mouse PD-1.

[08] **Figure 3** is a graph showing kinetics of peptide LD01 binding to mouse PD-1.

[09] **Figures 4A-C** are graphs showing the effects of peptides on the binding of PD-L1-Fc fusion protein to cell surface-expressed human PD-1. **Figure 4A**, low concentration range of peptide LD01. **Figure 4B**, high concentration range of peptide LD01. **Figure 4C**, peptide LD01.

[10] **Figure 5** is a graph showing effect of peptide LD01 on the binding of PD-L1-Fc fusion protein to PD-1 in a cell-free assay.

[11] **Figure 6** is a graph showing splenocyte proliferation as a function of anti-CD3 antibody concentration.

[12] **Figure 7** is a graph showing that peptide LD01 enhances proliferation of anti-CD3-stimulated splenocyte proliferation in the presence and absence of exogenously added PD-L1-Fc fusion protein.

[13] **Figure 8** is a graph showing the effect of peptide LD01 on the number of pulmonary tumor nodules in a mouse melanoma model. * $p < 0.05$.

[14] **Figure 9** is a graph showing the percent reduction in pulmonary tumor nodules by peptide LD01 in a mouse melanoma model.

[15] **Figure 10** compares the effect of peptide LD01 on pulmonary tumor nodules in a mouse melanoma model as described in Example 5. Left (Cohort 4), treatment with 200 μg peptide LD01. Right (Cohort 1), no treatment.

[16] **Figure 11** is a graph showing the effect of peptides LD12, LD01, LD10da, and LD16da on the number of pulmonary tumor nodules in a mouse melanoma model. * $p < 0.05$.

[17] **Figure 12** is a graph showing the effect of peptide LD01 on the immunogenicity of a malaria vaccine (Example 6).

[18] **Figure 13** is a graph showing the effect of peptides LD12, LD01, LD10da, and LD16da on the immunogenicity of a malaria vaccine (Example 6).

[19] **Figure 14** is a graph showing the effect of peptide LD01 on the number of IFNγ-secreting T cells per 2×10^5 splenocytes in a mouse sepsis model.

[20] **Figure 15** is a graph showing that peptide LD01 increases IFN-γ secretion by human PBMCs 4-fold relative to Staphylococcal enterotoxin B (SEB) alone.

DETAILED DESCRIPTION

[21] This disclosure provides peptides that block the interaction of the checkpoint receptor "programmed death 1" (PD-1) with its ligand PD-L1 and which also block the binding of CD86 to CTLA-4. In some embodiments, a disclosed peptide is modified using chemical or recombinant methods to enhance its stability or other pharmacokinetic properties. See, *e.g.*, US 2017/0020956. Modifications include, but are not limited to, replacement of one or more L-amino acid with its corresponding D-form, acetylation on a C- and/or N-terminal residue, amidation on a C- and/or N-terminal residue, cyclization, esterification, glycosylation, acylation, attachment of myristic or palmitic acid, addition of an N-terminal glycine, addition of lipophilic moieties such as long fatty acid chains, and PEGylation.

[22] Non-limiting examples of peptides and modified versions thereof are included in the table below, in which "NH_2" indicates C-terminal amidation, "CH_3CO" indicates N-terminal acetylation, and a lower case letter indicates the D form of the amino acid.

peptide	amino acid sequence	SEQ ID NO:
LD01	CRRTSTGQISTLRVNITAPLSQ-NH_2	1
LD11	CHHTSTGQISTLRVNITAPLSQ	5
LD04	STLRVNITAPLSQRYRVRIR	7
LD40	QISTLRVNITA	8
LD01r	RTSTGDITSLRVITA	11
LD10	STGQISTLRVNITAPLSQ	12
LD10 R9A	STGQISTLAVNITAPLSQ	15
LD10 P15A	STGQISTLRVNITAALSQ	17
LD17	STGQISTLRVNITAPLSQ-NH_2	21
LD17m	STGQISTARVNITAPLSQ-NH_2	22
LD10da	sTGQISTLRVNITAPLSQ-NH_2	24
LD01 TQ19	TSTGQISTLRVNITAPLSQ-NH_2	27
LD01 TQ19aa	CH_3CO-TSTGQISTLRVNITAPLSQ-NH_2	29
LD01 TQ19 da / LD16da	tSTGQISTLRVNITAPLSQ-NH_2	30
LD01(u)	CRRTSTGQISTLRVNITAPLSQ	36
LD17m(u)	STGQISTARVNITAPLSQ	37
LD01 TQ19(u)	TSTGQISTLRVNITAPLSQ	38

[23] Peptides can be made by any method known in the art, including synthetic methods, recombinant methods, or both. Synthetic methods include solid-phase and solution methods, and may include the use of protective groups. See, *e.g.*, Bodanszky et al. (1976), McOmie (1973), Merrifield (1963), Neurath et al. (1976), Stuart & Young (1984).

[24] Recombinant production of peptides can be carried out using any nucleotide sequence(s) encoding the peptides in any suitable expression system. Nucleic acid molecules encoding one or more of the disclosed peptides can be incorporated into an expression cassette that includes control elements operably linked to the coding sequences. Control elements include, but are not limited to, initiators, promoters (including inducible, repressible, and constitutive promoters), enhancers, and polyadenylation signals. Signal sequences can be included. The expression cassette can be provided in a vector that can be introduced into an appropriate host cell for production of the peptide(s). Methods of constructing expression cassettes and expression vectors are well known. Expression vectors can include one or more expression cassettes encoding one or more peptides comprising, consisting essentially or, or consisting of SEQ ID NO:5, 7, 8, 11, 12, 15, 17, 36, 37, 38, or 39.

[25] In some embodiments, one or more peptides are expressed as a component of a fusion protein. Other components of the fusion protein can be, for example, a cytokine or an engineered T cell receptor (TCR). A fusion protein can comprise one or more linkers between its components. In some embodiments, a linker between a peptide and another component of the fusion protein can comprise a proteolytic cleavage site to release the peptide after expression of the fusion protein. *See, e.g.*, US 2016/0138066; US 2018/0135060; US 2014/0343251; US 2012/0142891; Rodríguez et al., 2014.

[26] In some embodiments, a component of a fusion protein is a moiety, such as albumin or transthyretin, which can enhance the plasma half-life of the peptide. In other embodiments, a peptide or a modified version of a peptide is conjugated to the moiety. Methods of preparing such conjugates are well known in the art (*e.g.*, Penchala *et al.*, 2015; Kontermann, 2016; Zorzi *et al.*, 2017).

[27] In some embodiments, a component of a fusion protein is a partner molecule, such as a peptide or protein such as an antibody intended to increase the half-life of a peptide or modified peptide *in vivo* and/or to provide specific delivery to a target tissue or cell. Alternatively, a peptide or modified version thereof can be conjugated to the partner molecule. Conjugation may

be direct or can be via a linker. In some of these embodiments, a peptide or a modified version thereof can be altered to substitute one or more amino acids with amino acids used to attach partner molecules, such as lysine, or by N-terminal extension of the peptide with, *e.g.*, 1, 2, 3, or 4 glycine spacer molecules.

[28] This disclosure also provides CAR-T cells that express one or more of the disclosed peptides. Methods of preparing CAR-T cells are disclosed, for example, in U.S. Patent 9,328,156; U.S. Patent 9,845,362; and U.S. Patent 9,101,584.

[29] This disclosure also provides oncolytic viruses containing a nucleic acid molecule encoding one or more of the disclosed peptides. See US 2017/0157188; Lawler et al., 2017; US 2015/0250837. Oncolytic viruses include, but are not limited to, reovirus, Seneca Valley virus, vesicular stomatitis virus, Newcastle disease virus, herpes simplex virus, morbillivirus virus, retrovirus, influenza virus, Sindbis virus, poxvirus, and adenovirus.

[30] Examples of oncolytic reovirus include REOLYSIN® (pelareorep) and reoviruses disclosed in US 2017/0049829.

[31] Examples of oncolytic Seneca Valley virus include NTX-101 (Rudin et al., 2011).

[32] Examples of oncolytic vesicular stomatitis virus are disclosed in Stojdl et al., 2000; and Stojdl et al., 2003.

[33] Examples of oncolytic Newcastle disease virus include 73-T PV701 and HDV-HUJ strains (see also Phuangsab et al., 2001; Lorence et al., 2007; and Freeman et al., 2006).

[34] Examples of oncolytic herpes simplex virus include NV1020 (Geevarghese et al., 2010) and T-VEC (Andtbacka et al., 2013).

[35] Examples of oncolytic morbillivirus virus include oncolytic measles viruses such as MV-Edm (McDonald et al., 2006) and HMWMAA (Kaufmann et al., 2013).

[36] Examples of oncolytic retrovirus are disclosed in Lu et al., 2012.

[37] Examples of oncolytic influenza virus are disclosed, for example, in US 2018/0057594.

[38] Examples of oncolytic Sindbis virus are disclosed, for example, in Lundstrom, 2017.

[39] Examples of oncolytic poxvirus are disclosed, for example, in Chan & McFadden, 2014.

[40] Examples of oncolytic adenovirus include ONYX-015 (Khuri et al., 2000) and H101 or Oncorine (Liang, 2018).

Therapeutic Uses

[41] The peptides and modified versions thereof disclosed herein have a number of therapeutic applications, including treating hyperproliferative disorders, including cancer, treating infectious diseases, enhancing a response to vaccination, treating sepsis, promoting hair re-pigmentation, and promoting lightening of a pigmented skin lesion. "Treat," as used herein, includes reducing or inhibiting the progression of one or more symptoms of the condition for which a peptide or modified version thereof is administered.

[42] "Administer" as used herein includes direct administration of a disclosed peptide or modified version thereof as well as indirect administration.

[43] In some embodiments, one or more of the disclosed peptides and/or modified versions thereof, are directly administered. In some of these embodiments, a peptide carrier system is used. A number of peptide carrier systems are known in the art, including microparticles, polymeric nanoparticles, liposomes, solid lipid nanoparticles, hydrophilic mucoadhesive polymers, thiolated polymers, polymer matrices, nanoemulsions, and hydrogels. *See* Patel et al. (2014), Bruno et al. (2013), Feridooni et al. (2016). Any suitable system can be used.

[44] In some embodiments, an engineered T cell that expresses and secretes one or more disclosed peptides can be used to deliver PD-1 inhibition at the site of engagement of the T cell receptor with an antigen. The T cell-based therapy can be, for example, a CAR-T cell that expresses one or more of the disclosed peptides. Either inducible or constitutive expression can be used.

[45] In some embodiments, an oncolytic virus can be used to deliver one or more of the disclosed peptides. Either inducible or constitutive expression can be used.

[46] In other embodiments one or more of the disclosed peptides are delivered using one or more nucleic acids encoding the peptide(s) (*e.g.*, DNA, cDNA, PNA, RNA or a combination thereof); see, *e.g.*, US 2017/0165335. Nucleic acids encoding one or more peptides can be delivered using a variety of delivery systems known in the art. Nucleic acid delivery systems include, but are not limited to, gene-gun; cationic lipids and cationic polymers; encapsulation in liposomes, microparticles, or microcapsules; electroporation; virus-based, and bacterial-based

delivery systems. Virus-based systems include, but are not limited to, modified viruses such as adenovirus, adeno-associated virus, herpes virus, retroviruses, vaccinia virus, or hybrid viruses containing elements of one or more viruses. US 2002/0111323 describes use of "naked DNA," *i.e.*, a "non-infectious, non-immunogenic, non-integrating DNA sequence," free from "transfection-facilitating proteins, viral particles, liposomal formulations, charged lipids and calcium phosphate precipitating agents," to administer a peptide. Bacterial-based delivery systems are disclosed, *e.g.*, in Van Dessel et al. (2015) and Yang et al. (2007).

[47] In some embodiments, a peptide is administered via an RNA molecule encoding the peptide. In some embodiments, the RNA molecule is encapsulated in a nanoparticle. In some embodiments, the nanoparticle comprises a cationic polymer (*e.g.*, poly-L-lysine, polyamidoamine, polyethyleneimine, chitosan, poly(β-amino esters). In some embodiments, the nanoparticle comprises a cationic lipid or an ionizable lipid. In some embodiments, the RNA molecule is conjugated to a bioactive ligand (*e.g.*, N-acetylgalactosamine (GalNAc), cholesterol, vitamin E, antibodies, cell-penetrating peptides). See, *e.g.*, Akinc et al. (2008), Akinc et al. (2009), Anderson et al. (2003), Behr (1997), Boussif et al. (1995), Chen et al. (2012), Dahlman et al. (2014), Desigaux et al. (2007), Dong et al. (2014), Dosta et al. (2015), Fenton et al. (2016), Guo et al. (2012), Howard et al. (2006), Kaczmarek et al. (2016), Kanasty et al. (2013), Kauffman et al. (2015), Kozielski et al. (2013), Leus et al. (2014), Lorenz et al. (2004), Love et al. (2010), Lynn & Langer (2000), Moschos et al. (2007), Nair et al. (2014), Nishina et al. (2008), Pack et al. (2005), Rehman et al. (2013), Schroeder et al. (2010), Tsutsumi et al. (2007), Tzeng et al. (2012), Won et al. (2009), Xia et al. (2009), Yu et al. (2016).

[48] In some embodiments, an RNA molecule can be modified to reduce its chances of degradation or recognition by the immune system. The ribose sugar, the phosphate linkage, and/or individual bases can be modified. See, *e.g.*, Behlke (2008), Bramsen (2009), Chiu (2003), Judge & MacLachlan (2008), Kauffman (2016), Li (2016), Morrissey (2005), Prakash (2005), Pratt & MacRae (2009), Sahin (2014), Soutschek (2004), Wittrup & Lieberman (2015). In some embodiments, the modification is one or more of a ribo-difluorotoluyl nucleotide, a 4′-thio modified RNA, a boranophosphate linkage, a phosphorothioate linkage, a 2′-O-methyl (2'-OMe) sugar substitution, a 2′-fluoro (2′-F), a 2′-O-methoxyethyl (2′-MOE) sugar substitution, a locked nucleic acid (LNA), and an L-RNA.

[49] In some embodiments, administration is carried out in conjunction with one or more other therapies. "In conjunction with" includes administration together with, before, or after administration of the one or more other therapies.

Pharmaceutical Compositions, Routes of Administration, and Devices

[50] One or more peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses, as discussed above, are typically administered in a pharmaceutical composition comprising a pharmaceutically acceptable vehicle. The "pharmaceutically acceptable vehicle" may comprise one or more substances which do not affect the biological activity of the peptides or modified versions thereof and, when administered to a patient, does not cause an adverse reaction. Pharmaceutical compositions may be liquid or may be lyophilized. Lyophilized compositions may be provided in a kit with a suitable liquid, typically water for injection (WFI) for use in reconstituting the composition. Other suitable forms of pharmaceutical compositions include suspensions, emulsions, and tablets.

[51] Pharmaceutical compositions can be administered by any suitable route, including, but not limited to, intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, epidural, intratumoral, transdermal (*e.g.*, US 2017/0281672), mucosal (*e.g.*, intranasal or oral), pulmonary, and topical (*e.g.*, US 2017/0274010) routes. See, *e.g.*, US 2017/0101474.

[52] Administration can be systemic or local. In addition to local infusions and injections, implants can be used to achieve a local administration. Examples of suitable materials include, but are not limited to, sialastic membranes, polymers, fibrous matrices, and collagen matrices.

[53] Topical administration can be by way of a cream, ointment, lotion, transdermal patch (such as a microneedle patch), or other suitable forms well known in the art.

[54] Administration can also be by controlled release, for example, using a microneedle patch, pump and/or suitable polymeric materials. Examples of suitable materials include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters.

[55] Devices comprising any of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above include, but are not limited to, syringes, pumps, transdermal patches, spray devices, vaginal rings, and pessaries.

Treatment of Hyperproliferative Disorders, Including Cancer

[56] In some embodiments, one or more of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above are administered to a patient to inhibit the progression of a hyperproliferative disorder, including cancer. Such inhibition may include, for example, reducing proliferation of neoplastic or pre-neoplastic cells; destroying neoplastic or pre-neoplastic cells; and inhibiting metastasis or decreasing the size of a tumor.

[57] Examples of cancers include, but are not limited to, melanoma (including cutaneous or intraocular malignant melanoma), renal cancer, prostate cancer, breast cancer, colon cancer, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, and T-cell lymphoma.

Combination Cancer Therapies

[58] In some embodiments, one or more of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above are administered in conjunction with one or more other cancer therapies or immunotherapies, such as those described below.

[59] In some embodiments, the second therapy comprises a second agent that reduces or blocks the activity of PD-1 (*e.g.*, nivolumab, pembrolizumab, durvalumab) or CTLA-4 (*e.g.*, ipilimumab, tremelimumab).

[60] In some embodiments, the second therapy comprises an agent that reduces or blocks the activity of PD-L1 (*e.g.*, atezolizumab).

[61] In some embodiments, the second therapy comprises an agent that reduces or blocks the activity of other inhibitory checkpoint molecules and/or molecules that suppress the immune system. These molecules include, but are not limited to:

1. Lymphocyte-activation gene-3 (LAG-3; see He et al., 2016; Triebel et al., 1990);
2. V-domain Immunoglobulin Suppressor of T cell Activation (VISTA, also known as c10orf54, PD-1H, DD1α, Gi24, Dies1, and SISP1; see US 2017/0334990, US 2017/0112929, Gao et al., 2017, Wang et al., 2011; Liu et al., 2015);
3. T-cell Immunoglobulin domain and Mucin domain 3 (TIM-3; see US 2017/0198041, US 2017/0029485, US 2014/0348842, Sakuishi et al., 2010);
4. killer immunoglobulin-like receptors (KIRs; see US 2015/0290316);
5. agents that inhibit indoleamine (2,3)-dioxygenase (IDO; see Mellemgaard et al., 2017);
6. B and T Lymphocyte Attenuator (BTLA; see US 2016/09222114); and
7. A2A adenosine receptor (A2AR; see Beavis et al., 2015; US 2013/0267515; US 2017/0166878; Leone et al., 2015; Mediavilla-Varela et al., 2017; Young et al., 2016).

[62] Agents that reduce or block the activity of LAG-3 include, but are not limited to, BMS-986016, IMP321, and GSK2831781 (He et al., 2016).

[63] Agents that reduce or block the activity of VISTA include, but are not limited to, small molecules, such as CA-170, and antibodies (*e.g.*, Le Mercier et al., 2014).

[64] Agents that reduce or block the activity of TIM-3 include, but are not limited to, antibodies such as MBG453 and TSR-022; see Dempke et al., 2017.

[65] Agents that reduce or block the activity of KIRs include, but are not limited to, monoclonal antibodies such as IPH2101 and Lirilumab (BMS-986015, formerly IPH2102); see Benson & Caligiuri, 2014.

[66] Agents that reduce or block the activity of IDO include, but are not limited to, epacadostat and agents disclosed in US 2017/0037125.

[67] Agents that reduce or block the activity of BTLA include, but are not limited to, peptides (*e.g.*, Spodzieja et al., 2017).

[68] Agents that reduce or block the activity of A2AR include, but are not limited to, small molecules such as CPI-444 and vipadenant.

[69] In some embodiments, the second therapy comprises a cytokine (*e.g.*, interleukin 7).

[70] In some embodiments, the second therapy comprises an agonist of a stimulatory checkpoint molecule. These molecules include, but are not limited to:

1. CD40;
2. OX40;
3. glucocorticoid-induced tumor necrosis factor-related protein (GITR); and
4. Inducible T-cell COStimulator (ICOS).

[71] Agonists of CD40 include, but are not limited to, CD40 agonist monoclonal antibodies such as cp-870,893, ChiLob7/4, dacetuzumab, and lucatumumab. See, *e.g.*, Vonderheide et al., 2007; Khubchandani et al., 2009; Johnson et al., 2010; Bensinger et al., 2012; Vonderheide and Glennie, 2013; Johnson et al., 2015.

[72] Agonists of OX40 include, but are not limited to, OX40 agonist antibodies such as MOXR0916, MED16469, MED10562, PF-045618600, GSK3174998, and INCCAGN01949, and OX40L-Fc fusion proteins, such as MEDI6383. See, *e.g.*, Huseni et al., 2014; Linch et al., 2015; Messenheimer et al., 2017. See also Shrimali et al., 2017.

[73] Agonists of GITR include, but are not limited to, MEDI1873. See, *e.g.*, Schaer et al., 2012; Tigue et al., 2017.

[74] Agonists of ICOS include, but are not limited to, ICOS agonist antibodies JTX-2011 and GSK3359609. See, *e.g.*, Harvey et al., 2015; Michaelson et al., 2016.

[75] In other embodiments, the second therapy comprises a 4-1BB agonist (Shindo et al., 2015), such as urelumab; a 4-1BB antagonist (see US 2017/0174773); an inhibitor of anaplastic lymphoma kinase (ALK; Wang et al., 2014; US 2017/0274074), such as crizotinib, ceritinib, alectinib, PF-06463922, NVP-TAE684, AP26113, TSR-011, X-396, CEP-37440, RXDX-101; an inhibitor of histone deacetylase (HDAC; see US 2017/0327582); a VEGFR inhibitor, such as axitinib, sunitinib, sorafenib, tivozanib, bevacizumab; and/or an anti-CD27 antibody, such as varlilumab.

[76] In some embodiments, the second therapy comprises a cancer vaccine (*e.g.*, Duraiswamy et al., 2013). A "cancer vaccine" is an immunogenic composition intended to elicit an immune response against a particular antigen in the individual to which the cancer vaccine is administered. A cancer vaccine typically contains a tumor antigen which is able to induce or stimulate an immune response against the tumor antigen. A "tumor antigen" is an antigen that is present on the surface of a target tumor. A tumor antigen may be a molecule which is not expressed by a non-tumor cell or may be, for example, an altered version of a molecule expressed by a non-tumor cell (*e.g.*, a protein that is misfolded, truncated, or otherwise mutated).

[77] In some embodiments, the second therapy comprises a chimeric antigen receptor (CAR) T cell therapy. See, *e.g.*, John et al., 2013; Chong et al., 2016.

[78] In some embodiments, one or more of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above are administered in conjunction with a CAR-T cell cancer therapy to increase the efficacy of the CAR-T cell cancer therapy.

[79] In some embodiments, one or more of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above are administered in conjunction with an oncolytic virus as disclosed, for example, in US 2017/0143780.Non-limiting examples of oncolytic viruses are described above.

Additional Therapeutic Uses

[80] In some embodiments, one or more of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above are administered to a patient

to treat infectious diseases, including chronic infections, caused, *e.g.*, by viruses, fungi, bacteria, and protozoa, and helminths.

[81] Examples of viral agents include human immunodeficiency virus (HIV), Epstein Barr Virus (EBV), *Herpes simplex* (HSV, including HSV1 and HSV2), Human Papillomavirus (HPV), *Varicella zoster* (VSV) *Cytomegalovirus* (CMV), and hepatitis A, B, and C viruses.

[82] Examples of fungal agents include *Aspergillus*, *Candida*, *Coccidioides*, *Cryptococcus*, *and Histoplasma capsulatum*.

[83] Examples of bacterial agents include *Streptococcal* bacteria (*e.g.*, *pyogenes*, *agalactiae*, *pneumoniae*), *Chlamydia pneumoniae*, *Listeria monocytogenes*, and *Mycobacterium tuberculosis*.

[84] Examples of protozoa include *Sarcodina* (*e.g.*, *Entamoeba*), *Mastigophora* (*e.g.*, *Giardia*), *Ciliophora* (*e.g.*, *Balantidium*), and *Sporozoa* (*e.g.*, *Plasmodium falciparum*, *Cryptosporidium*).

[85] Examples of helminths include *Platyhelminths* (*e.g.*, trematodes, cestodes), *Acanthocephalins*, and *Nematodes*.

[86] In some embodiments, one or more of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above are administered as a vaccine adjuvant, to enhance a response to vaccination (*e.g.*, by increasing effector T cells and/or reducing T cell exhaustion). The vaccine can be, for example, an RNA vaccine (*e.g.*, US 2016/0130345, US 2017/0182150), a DNA vaccine, a recombinant vector, a protein vaccine, or a peptide vaccine. Such vaccines can be delivered, for example, using virus-like particles, as is well known in the art.

[87] In some embodiments, one or more of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above are administered to treat sepsis.

[88] In some embodiments, one or more of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above are administered to promote hair color re-pigmentation. In some embodiments, one or more of the peptides, modified

peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described herein are administered to promote lightening of pigmented skin lesions.

EXAMPLE 1. Peptide LD01 Binds to Human and Mouse PD-1

[89] BIACORE® assays were carried out using a BIACORE® T-200 at 25°C. The assay and regeneration buffers contained 10 mM HEPES (pH 7.4), 150 mM NaCl, 3mM EDTA, and 0.05% P20. The immobilization buffer was 10mM sodium acetate, pH 5.0. The flow rate used for immobilizing the ligand was 5 µl/min. The flow rate for kinetics analysis was 30 µl/min.

[90] **Scouting**. 4400 RU of peptide LD01 was directly immobilized on flow cell 2 of the CM5 chip by amine coupling method (EDC/NHS). The un-occupied sites were blocked with 1M ethanol amine. Scouting was performed at a single analyte concentration of 290nM to confirm yes/no binding. Flow cell1 was kept blank and used for reference subtraction. Binding of analyte to the ligand was monitored in real time.

[91] **Full Kinetics**. Based on the scouting results, full kinetics was performed at analyte concentration of 100nM, followed by serial dilution to 50, 25, 12.5, 6.25, 3.125, 1.562 and 0 nM concentration or as indicated. K_D was determined from the observed k_{on} (on rate) and k_{off} (off rate) or by steady state equilibrium kinetics for the interactions with fast off rate.

[92] Chi square (χ^2) analysis was carried out between the actual Sensorgram and the sensorgram generated from the BIAnalysis software to determine the accuracy of the analysis. χ^2 value within 1- 2 is considered significant (accurate) and below 1 is highly significant (highly accurate).

[93] The single concentration binding data are shown in Figure 1 and Figure 2 for the human and mouse PD1, respectively. The kinetic data for binding to mouse PD-1 are shown in Figure 3. These data demonstrate that peptide LD01 binds to both human and mouse PD-1 and support the use of mouse models in the examples below.

EXAMPLE 2. Peptide LD01 Enhances Binding of a Human PD-L1-Fc Fusion Protein to Human PD-1 Expressed on the Surface of Jurkat cells

[94] A human T cell line (Jurkat) stably expressing human PD-1 on its surface was purchased from Promega. This cell line was cultured using sterile techniques and maintained in log-phase

growth. PD-1 protein expression levels were measured using an anti-human PD-1 antibody labeled with allophycocyanin (APC) for quality assessment.

[95] For the experiment, 200 µL of 2.5*10^5 cells were plated in staining buffer into a microtiter plate. These cells were incubated with varying concentrations of peptide LD01 in staining buffer for 1 hour, washed, and then incubated with PD-L1-Fc fusion protein for 1 hour. After another wash step, the cells were incubated with anti-human Fc labeled with AF647 for 30 minutes. After several washes, the mean fluorescence intensity due to PD-L1-Fc binding to PD-1 was measured by flow cytometry.

[96] The results are shown in Figures 4A-C. These results demonstrate that peptide LD01 enhances binding of the human PD-L1-Fc fusion protein to human PD-1-expressing Jurkat cells with an increasing dose-response trend.

EXAMPLE 3. Peptide LD01 Enhances Binding of PD-L1 Fc to PD-1 in a Cell-Free Assay

[97] Microtiter plates were coated with peptide LD01 at 4 °C overnight. The plates were washed and blocked with 1% BSA for 1 hour at room temperature. The plates were then incubated with 100 µL of 1 µg/mL PD-1 at room temperature for 1 hour. The plates were again washed and incubated with PD-L1 at room temperature for 1 hour. After another round of washes, the plates were incubated with anti-human IgG-HRP. After incubation for 1 hour and washes, the HRP was detected by incubating with TMB substrate, and the OD were read on a microplate reader.

[98] The results are shown in Figure 5. These results confirm that peptide LD01 enhances binding of the human PD-L1-Fc fusion protein to human PD-1 in a cell-free assay.

EXAMPLE 4. Effect of Peptide LD01 on Mouse Splenocyte Proliferation

[99] PD-L1-Fc fusion protein is hypothesized to reduce anti-CD3 induced splenocyte proliferation. An experiment was carried out to determine whether the effect of peptide LD01 on PD-L1-Fc-mediated proliferation.

[100] Carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled mouse splenocytes (400,000 cells, 5 µM CFSE) were stimulated using 1 µg/mL anti-CD3 antibody and varying levels of PD-L1-Fc or an irrelevant control Fc. To monitor the effect of peptide LD01 on PD-1, varying concentrations of peptide LD01 (1 µM, 5 µM, and 25 µM) were tested for their effect on

the proliferation of the splenocytes. The CFSE dilution profiles were analyzed by flow cytometry after 3 days of treatment.

[101] Titration results of cell numbers and anti-CD3 antibody concentration are shown in Figure 6, and the peptide LD01 data is shown in Figure 7. These results demonstrate that peptide LD01 enhances proliferation of anti-CD3 stimulated mouse splenocytes in the presence and absence of exogenously added PD-L1-Fc.

EXAMPLE 5. Effect of Peptide LD01 in a Mouse Melanoma Model

[102] To assess the *in vivo* functionality of peptide LD01, anti-metastasis activity was evaluated in a syngeneic model of B16-F-10 mouse melanoma in immunocompetent C57/BL6 mice. In brief, murine B16-F10-LacZ cells obtained from ATCC were cultured in RPMI-1640 media supplemented with 10% FBS. Once confluent, cells were collected, and 2 x 10^5 cells in 200 µl of PBS were injected into each mouse intravenously via the tail vein. After cells were delivered, animals were assigned randomly to the study cohorts (5 mice in each group). Test sample, route of treatment and treatment days are detailed in Table 1A. Because the *in vivo* half-life of peptides is relatively short (hours) compared to antibodies (weeks), peptides were delivered intravenously and given more frequently.

Table 1A

Cohort	Test sample	Route	Treatment days
1	No treatment		
2	MOG control peptide (200µg)	i.v.	1, 2, 4, 6, 8, 12
3	peptide LD01 (20µg)	i.v.	1, 2, 4, 6, 8, 12
4	peptide LD01 (200µg)	i.v.	1, 2, 4, 6, 8, 12
5	Anti-PD-L1 antibody (200µg)	i.p.	2, 5, 7, 9, 12
6	Anti-PD1 antibody (200µg)	i.p.	2, 5, 7, 9, 12

[103] The negative control peptide used in this study was the myelin oligodendrocyte glycoprotein (MOG) peptide (MEVGWYRSPFSRVVHLYRNGK, SEQ ID NO:33). 200µg of peptide or antibody was administered per treatment day. At day14 post cell injection, the mice were euthanized, lungs removed and fixed in Fekete's solution. The tumor nodules were counted.

[104] The number of tumor nodules for individual mice are shown in Figure 8, while percentage reduction of tumor nodules in each cohort relative to no treatment (Cohort 1) is shown in Figure 9. Significant differences between the no treatment (Cohort 1) and peptide

LD01 (Cohort 3) or anti-PD-1 antibody (Cohort 6) were detected using the unpaired t-test and denoted by * $p < 0.05$ in Figure 8. Examples of macroscopic lung images for the no treatment (Cohort 1) and peptide LD01 (Cohort 4) are shown in Figure 10. These results demonstrate that peptide LD01 (Cohorts 3 and 4) is functionally active *in vivo*, reducing lung metastasis by ~50% relative to no treatment (Cohort 1). Moreover, peptide LD01 (Cohorts 3 and 4) efficacy was similar to or slightly above treatment with anti-PD-L1 or anti-PD-1 antibodies (Cohorts 5 and 6).

[105] Peptides LD01, LD10da, and LD16da were tested as described above, this time using peptide LD12 (cohort 2) as the negative control. Test sample, route of treatment and treatment days are detailed in Table 1B.

Table 1B

Cohort	Test sample	Route	Treatment days
1	Saline; No treatment		
2	+ LD12	i.v.	1, 2, 4, 6, 8, 12
3	+ LD01	i.v.	1, 2, 4, 6, 8, 12
4	+ LD10da	i.v.	1, 2, 4, 6, 8, 12
5	+ LD16da	i.v.	1, 2, 4, 6, 8, 12
6	α-PD1 mAb	i.v.	1, 2, 4, 6, 8, 12

[106] The number of tumor nodules for individual mice are shown in Figure 11. Significant differences between the no treatment saline (Cohort 1) and LD01 (Cohort 3), LD16da (Cohort 5) or α-PD-1 mAb (Cohort 6) were detected using the one-way ANOVA test (* $p < 0.05$) in Figure 11. These results demonstrate that LD01 (Cohort 3) and LD16da (Cohort 5) are functionally active *in vivo*, reducing lung metastasis by ~30% relative to no treatment saline (Cohort 1).

EXAMPLE 6. Effect of Peptide LD01 on the Immunogenicity of a Malaria Vaccine

[107] The effect of peptide LD01 on the immunogenicity of a malaria vaccines was evaluated in immunocompetent Balb/c mice. Balb/c mice were immunized intramuscularly with 10^{10} virus particles of a recombinant replication defective adenovirus expressing the *Plasmodium yoelli* circumsporozoite protein (AdPyCSP). Test sample, number of mice, route of treatment, and treatment days are shown in Table 2A.

Table 2A

Cohort	Test Sample	# Mice	Route	Treatment days
1	AdPyCSP only	5	--	--
2	AdPyCSP + control OVA peptide (200 μg)	5	i.p.	0, 1, 3, 5, 7
3	AdPyCSP + peptide LD01 (200 μg)	5	i.p.	0, 1, 3, 5, 7
4	AdPyCSP + anti-PD1 antibody (200 μg)	5	i.p.	0, 1, 3, 5, 7
5	AdPyCSP + anti-PDL1 antibody (200 μg)	5	i.p.	0, 1, 3, 5, 7

[108] The negative control peptide used in this study was the ovalbumin (OVA) peptide (SIINFEKL, SEQ ID NO:34). At day 12 post immunization, mice were euthanized, individual spleens removed, and the number of splenic CSP-specific, IFNγ-secreting $CD8^+$ T cells were determined by ELISPOT assay. For the ELISPOT assay splenocytes were stimulated with the peptide SYVPSAEQI (SEQ ID NO:35), an H-2Kd-restricted $CD8^+$ T cell epitope of PyCSP.

[109] Shown in Figure 12 is the average number ± standard deviation of CSP-specific, IFNγ-secreting CD8 T cells per 0.5 x 10^6 splenocytes for each cohort. Significant differences between the AdPyCSP alone (Cohort 1) and peptide LD01 (Cohort 3), anti-PD-1 antibody (Cohort 4) or anti-PD-L1 antibody (Cohort 5) were detected using the one-way ANOVA test (**** $p < 0.0005$, *** $p < 0.001$, and * $p < 0.05$). These results further demonstrate that peptide LD01 (Cohort 3) is functionally active in vivo, increasing the number of CSP-specific, IFNγ-secreting CD8 T cells ~2-fold relative to AdPyCSP alone (Cohort 1). Moreover, peptide LD01 (Cohort 3) immunogenicity was slightly above treatment with anti-PD-1 or -PD-L1 antibody (Cohort 4 and 5).

[110] Peptides LD01, LD10da, and LD16da were tested as described above, this time using peptide LD12 (cohort 2) as the negative control. Test sample, route of treatment and treatment days are detailed in Table 2B.

Table 2B

Cohort	Test Sample	# Mice	Route	Treatment days
1	AdPyCSP only	5	--	--
2	AdPyCSP + LD12 (200 μg)	5	i.p.	0, 1, 3, 5, 7
3	AdPyCSP + LD01 (200 μg)	5	i.p.	0, 1, 3, 5, 7
4	AdPyCSP + LD10da (200 μg)	5	i.p.	0, 1, 3, 5, 7
5	AdPyCSP + LD16da (200 μg)	5	i.p.	0, 1, 3, 5, 7
6	AdPyCSP + α-PD1 mAb (200 μg)	5	i.p.	0, 1, 3, 5, 7

[111] At day 12 post immunization, mice were euthanized, individual spleens removed, and the number of splenic CSP-specific, IFNγ-secreting $CD8^+$ T cells were determined by ELISPOT assay. For the ELISPOT assay splenocytes were stimulated with the peptide SYVPSAEQI (SEQ ID NO:35), an H-2Kd-restricted $CD8^+$ T cell epitope of PyCSP.

[112] Shown in Figure 13 is the average number ± standard deviation of CSP-specific, IFNγ-secreting $CD8^+$ T cells per 5×10^5 splenocytes for each cohort. Significant differences relative to AdPyCSP alone were detected using the one-way ANOVA test (**** $p < 0.0001$, ** $p < 0.005$). These results further demonstrate that LD01 (Cohort 3) and LD10da (Cohort 4) are functionally active in vivo, increasing the number of CSP-specific, IFNγ-secreting CD8 T cells ~1.5 to 2.5-fold, respectively, relative to AdPyCSP alone (Cohort 1). Moreover, LD10da (Cohort 4) immunogenicity was slightly above treatment with α-PD-1 mAb (Cohort 6).

EXAMPLE 7. Effect of Peptide LD01 in a Mouse Sepsis Model

[113] The effect of peptide LD01 was studied in a representative, clinically relevant model of sepsis, *i.e.*, CD1 mice with intra-abdominal peritonitis induced by cecal ligation and puncture (CLP).

[114] CD1 mice were anesthetized, a midline abdominal incision made, the cecum was ligated and punctured, and then the incision closed. 200μg of peptide LD01 or a control peptide (SEQ ID NO:2) was administered intraperitoneally at 6, 24, and 48 hours after surgery. Administration of peptide LD01 conferred a survival advantage with the peptide LD01 cohort having a survival rate greater than double than the survival of the control peptide, 70% (7/10) versus 30% (3/10), respectively.

[115] In a separate study, the effect of peptide LD01 on augmenting IFNγ production by T cells during sepsis was assessed. Following CLP surgery, mice were treated at 6, 24, 48, 72 and 96 hours with peptide LD01 or the control peptide. Twenty-four hours later, mice were euthanized, individual spleens were removed, and the number of splenic IFNγ-secreting T cells were determined by ELISPOT assay. For the ELISPOT assay splenocytes were stimulated via anti-CD3 and anti-CD28. Shown in Figure 14 is the number of IFNγ-secreting T cells per 2×10^5 splenocytes. Peptide LD01 treatment caused an increase in the number of IFNγ-secreting T cells relative to the control peptide, which supports the utility of peptide LD01 in reversing the sepsis-induced defect in IFNγ secretion by T cells.

Example 8. PATHHUNTER® Checkpoint Signaling Assays

[116] Peptides were tested for their ability to inhibit the binding of PD-L1 to PD-1 or CD86 to CTLA-4 using PATHHUNTER® checkpoint signaling assays (DiscoverX).

[117] PATHHUNTER® PD-1 and PD-L1 and CTLA4 and CD86 cell lines were expanded from freezer stocks according to standard procedures. PD-1 cells were pre-incubated with peptides, followed by incubation with PD-L1 cells. PD-1 Jurkat cells (20,000 cells per well) were seeded in a total volume of 50 µL into white-walled, 96-well microplates in assay buffer. Serial dilution of peptide stocks was performed to generate 11X sample in assay buffer. 10 µL of 11X sample was added to PD-1 cells and incubated at 37°C for 60 minutes. 50 µL U-2 OS PD-L1 cells (30,000 cells per well in assay buffer) were added, and the cells were co-cultured at room temperature for 2 hours. Similarly, CTLA4 cells were pre-incubated with peptides, followed by incubation with CD86 cells. CTLA4 Jurkat cells (20,000 cells per well) were seeded in a total volume of 50 µL into white-walled, 96-well microplates in assay buffer. Serial dilution of peptide stocks was performed to generate 11X sample in assay buffer. 10 µL of 11X sample was added to CTLA4 cells and incubated at 37°C for 60 minutes. 50 µL U-2 OS CD86 cells (30,000 cells per well in assay buffer) were added, and the cells were co-cultured at room temperature for 4 hours.

[118] Compound activity was analyzed using CBIS data analysis suite (ChemInnovation, CA). The results are shown in Tables 3 and 4. For antagonist mode assays, percentage inhibition efficacy was calculated using the following formula, in which RLU means relative light units:

$$100 \times \left[1 - \left[\frac{(\text{mean RLU of test sample}) - (\text{mean RLU of vehicle control})}{(\text{mean RLU of } EC_{80} \text{ control}) - (\text{mean RLU of vehicle control})} \right] \right]$$

[119] The tested peptides exhibited a wide range of inhibition efficacy in the PD-1 as well as the CTLA-4 PATHHUNTER® assays as shown in Tables 3 and 4. Surprisingly, many of the peptides showed high efficacy in both assays, indicating their dual specificity for PD-1 and CTLA-4.

Table 3. Effect of Peptides on the Binding of PD-L1 to PD-1

SEQ ID NO:	peptide	conc. (μM)	% inhibition				
			Study 1	Study 2	Study 4	Study 5	Study 6
1	LD01	100	50.5	79	65	24	83
2	LD02	100	7	10			
3	LD12	100	14	5		18	4
4	LD12a	100				18.5	
5	LD11	100	44	59			
6	LD03	100				0	
7	LD04	100				87	
8	LD40	100				80	
9	LD41	100					1.4
10	LD42	100					0.8
11	LD01r	125		74	66		
12	LD10	106	79	80	92		86
13	LD10 Q4A	100					3
14	LD10 T7A	100					1
15	LD10 R9A	100					53
16	LD10 N11A	100					2
17	LD10 P15A	100					35
18	LD10 L8A	100					6
19	LD10 L16A	100					6
20	LD10 T13A	100					4
21	LD17	100		90		94.5	
22	LD17m	100				78	
23	LD10aa	50			10		
24	LD10da	100			91		26
25	LD10da m	100					2
26	LD16	100		90			0
27	LD01 TQ19	100			93	22	
28	LD16m	100				15	
29	LD01 TQ19 aa	100			91		
30	LD01 TQ19 da / LD16 da	100			96		1
31	LD01 TQ19 da m	100					6
32	LD05	100	18				

Table 4. Effect of Peptides on the Binding of CD86 to CTLA-4

SEQ ID NO:	peptide	conc. (μM)	% inhibition				
			Study 1	Study 2	Study 4	Study 5	Study 6
1	LD01	100		51	16	21	46
2	LD02	100		-1	0		
3	LD12	100		-10	-7	8	-10
4	LD12a	100				6	
5	LD11	100		28			
6	LD03	100				-9	
7	LD04	100				76.5	
8	LD40	100				81.5	
9	LD41	100					-9
10	LD42	100					-4
11	LD01r	125			77		
12	LD10	106		88	95		95
13	LD10 Q4A	100					-16.5
14	LD10 T7A	100					-17
15	LD10 R9A	100					49.5
16	LD10 N11A	100					-17
17	LD10 P15A	100					13
18	LD10 L8A	100					-11.5
19	LD10 L16A	100					-3
20	LD10 T13A	100					-7
21	LD17	100				102	
22	LD17m	100				82	
23	LD10aa	50			18		
24	LD10da	100			98		22
25	LD10da m	100					-12
26	LD16	100					0
27	LD01 TQ19	100			91	18	
28	LD16m	100				17	
29	LD01 TQ19 aa	100			96		
30	LD01 TQ19 da / LD16 da	100			99		-54
31	LD01 TQ19 da m	100					-2

Example 9. Effect of LD01 on the IFN-γ secretion by human PBMCs

[120] Human PBMCs from a healthy individual were stimulated with 5ng/ml of Staphylococcal enterotoxin B (SEB) +/- LD01 (100μM). At 72 hours, supernatants were collected, and IFN-γ was measured by cytometric bead arrays. The results, shown in Figure 15, demonstrate that LD01 increases IFN-γ secretion by human PBMCs 4-fold relative to SEB alone.

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CLAIMS

1. A peptide, which is

(a) selected from the group consisting of: LD01 (SEQ ID NO:1), LD11 (SEQ ID NO:5), LD10 (SEQ ID NO:12), LD10 R9A (SEQ ID NO:15), LD10 P15A (SEQ ID NO:17), LD17 (SEQ ID NO:21), LD17m (SEQ ID NO:22), LD10da (SEQ ID NO:24), LD01 TQ19 aa (SEQ ID NO:29), LD01 TQ19 da (SEQ ID NO:30), peptide LD01(u) (SEQ ID NO:36), peptide LD17m(u) (SEQ ID NO:37), and peptide LD01 TQ19(u) (SEQ ID NO:38); or

(b) selected from the group consisting of LD01 (SEQ ID NO:1), LD11 (SEQ ID NO:5), LD10 (SEQ ID NO:12), LD10 R9A (SEQ ID NO:15), LD10 P15A (SEQ ID NO:17), LD17 (SEQ ID NO:21), LD17m (SEQ ID NO:22), LD10da (SEQ ID NO:24), LD01 TQ19 aa (SEQ ID NO:29), and LD01 TQ19 da (SEQ ID NO:30), LD04 (SEQ ID NO:7), LD40 (SEQ ID NO:8), and LD01r (SEQ ID NO:11), peptide a (SEQ ID NO:36), peptide LD17m(u) (SEQ ID NO:37), and peptide LD01 TQ19(u) (SEQ ID NO:38), wherein the peptide comprises one or more modifications selected from the group consisting of C-terminal amidation, N-terminal acetylation, and replacement of at least one L-amino acid with the corresponding D-amino acid; or

(c) selected from the group consisting of LD01 (SEQ ID NO:1), LD11 (SEQ ID NO:5), LD10 (SEQ ID NO:12), LD10 R9A (SEQ ID NO:15), LD10 P15A (SEQ ID NO:17), LD17 (SEQ ID NO:21), LD17m (SEQ ID NO:22), LD01 TQ19 aa (SEQ ID NO:29), LD04 (SEQ ID NO:7), LD40 (SEQ ID NO:8), and LD01r (SEQ ID NO:11), peptide LD01(u) (SEQ ID NO:36), peptide LD17m(u) (SEQ ID NO:37), and peptide LD01 TQ19(u) (SEQ ID NO:38), wherein the N-terminal amino acid is a D amino acid.

2. An expression construct encoding a peptide selected from the group consisting of a peptide of claim 1, LD04, LD40, and LD01r.

3. The expression construct of claim 2, which is present in a CAR-T cell or an oncolytic virus.

4. A pharmaceutical composition comprising:

(a) an active agent selected from the group consisting of:

(i) a peptide selected from the group consisting of a peptide of claim 1, LD04, LD40, and LD01r;

(ii) a nucleic acid encoding the peptide;

(iii) a CAR-T cell expressing the peptide; and

(iv) an oncolytic virus expressing the peptide; and

(b) a pharmaceutically acceptable carrier.

5. The pharmaceutical composition of claim 4, wherein the active agent is the nucleic acid, wherein the nucleic acid is selected from the group consisting of DNA, cDNA, PNA, and RNA.

6. The pharmaceutical composition of claim 5, wherein the nucleic acid is RNA.

7. The pharmaceutical composition of claim 6, wherein the RNA comprises a modification selected from the group consisting of (i) modification of a ribose sugar, (ii) modification of a phosphate linkage, and (iii) modification of a base.

8. The pharmaceutical composition of claim 7, wherein the modification is selected from the group consisting of a ribo-difluorotoluyl nucleotide, a 4′-thio modified RNA, a boranophosphate linkage, a phosphorothioate linkage, a 2′-O-methyl (2’-OMe) sugar substitution, a 2′-fluoro (2′-F), a 2′-O-methoxyethyl (2′-MOE) sugar substitution, a locked nucleic acid (LNA), and an L-RNA.

9. The pharmaceutical composition of claim 4, wherein the active agent is the peptide, wherein the peptide is provided with a peptide carrier system selected from the group consisting of a microparticle, a polymeric nanoparticle, a liposome, a solid lipid nanoparticle, a hydrophilic mucoadhesive polymer, a thiolated polymer, a polymer matrix, a nanoemulsion, and a hydrogel.

10. A method of inhibiting the progression of a hyperproliferative disorder, treating an infectious disease, enhancing a response to vaccination, treating sepsis, promoting hair re-pigmentation, or promoting lightening of a pigmented skin lesion, comprising administering to an individual in need thereof an effective amount of the pharmaceutical composition of any of claims 4-9.

11. The method of claim 10, wherein the pharmaceutical composition is administered to inhibit progression of the hyperproliferative disorder.

12. The method of claim 11, wherein the hyperproliferative disorder is a cancer.

13. The method of claim 12, wherein the cancer is a melanoma.

14. The method of any of claims 10-13, further comprising administering a second therapy to the patient.

15. The method of claim 14, wherein the second therapy is selected from the group consisting of:

(i) a cancer vaccine;

(ii) a chimeric antigen receptor (CAR) T cell therapy;

(iii) a therapy that comprises reducing or blocking activity of a molecule selected from the group consisting of PD-1, PD-L1, lymphocyte-activation gene-3 (LAG-3), cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), V-domain Immunoglobulin Suppressor of T cell Activation (VISTA), T-cell Immunoglobulin domain and Mucin domain 3 (TIM-3), a killer immunoglobulin-

like receptor (KIR), indoleamine (2,3)-dioxygenase (IDO), B and T Lymphocyte Attenuator (BTLA), A2A adenosine receptor (A2AR);

(iv) a cytokine;

(v) an agonist of a molecule selected from the group consisting of CD40, OX40, glucocorticoid-induced tumor necrosis factor-related protein (GITR), and Inducible T-cell COStimulator (ICOS);

(vi) an oncolytic virus; and

(vii) a therapeutic agent selected from the group consisting of a 4-1BB agonist, a 4-1BB antagonist, an inhibitor of anaplastic lymphoma kinase (ALK), an inhibitor of histone deacetylase (HDAC), and an inhibitor of VEGFR.

16. The method of claim 10, wherein the pharmaceutical composition is administered to treat an infectious disease or as a vaccine adjuvant to a vaccine against the infectious disease.

17. The method of claim 16, wherein the infectious disease is malaria or hepatitis B.

18. The method of claim 10, wherein the at least one peptide is administered to treat sepsis.

19. The method of claim 10, wherein the at least one peptide is administered to promote hair re-pigmentation or to promote lightening of a pigmented skin lesion.

20. The composition of any of claims 4-9 for use in inhibiting the progression of a hyperproliferative disorder, treating an infectious disease, enhancing a response to vaccination, treating sepsis, promoting hair re-pigmentation, or promoting lightening of a pigmented skin lesion.

21. Use of the composition of any of claims 4-9 in the manufacture of a medicament for inhibiting the progression of a hyperproliferative disorder, treating an infectious disease, enhancing a response to vaccination, treating sepsis, promoting hair re-pigmentation, or promoting lightening of a pigmented skin lesion.

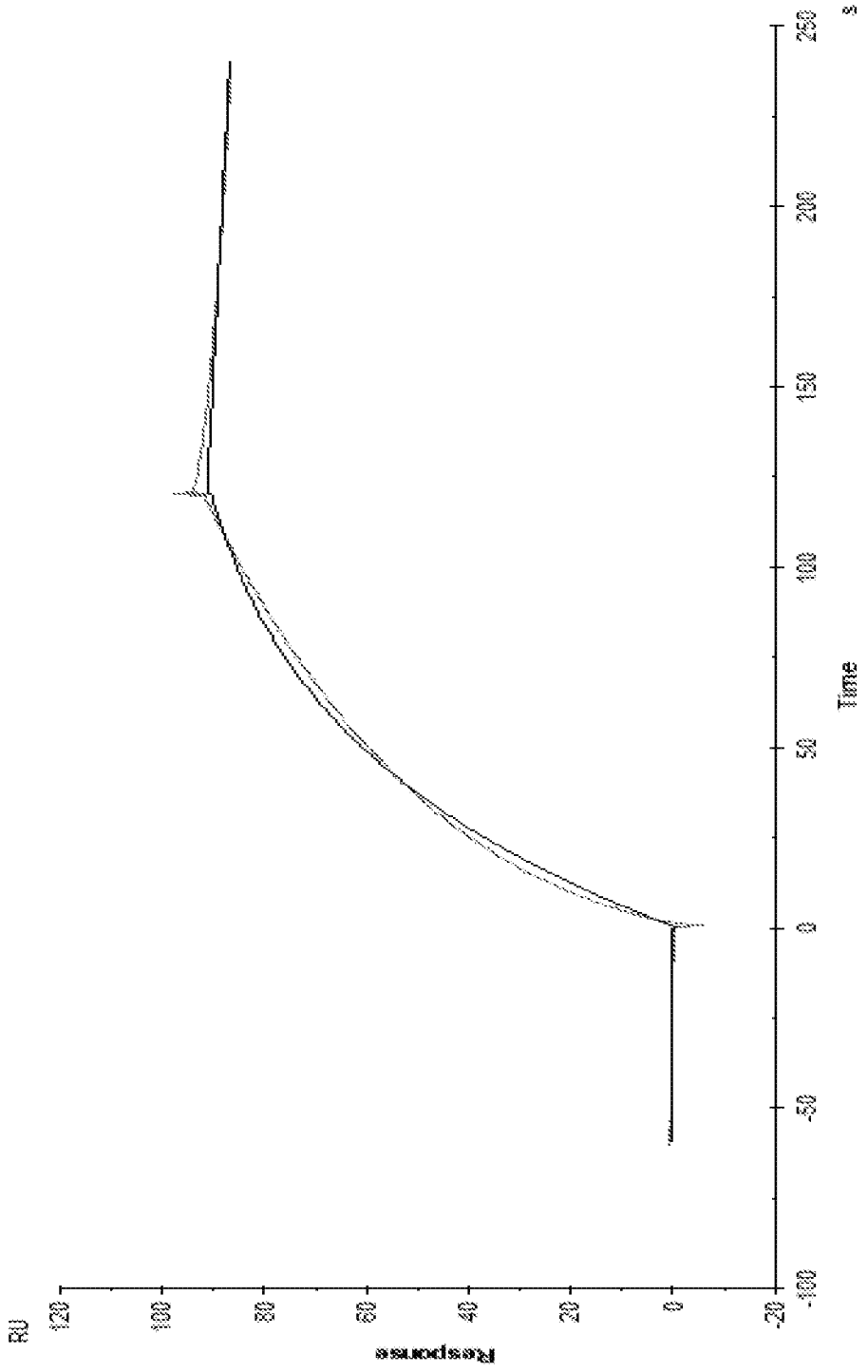
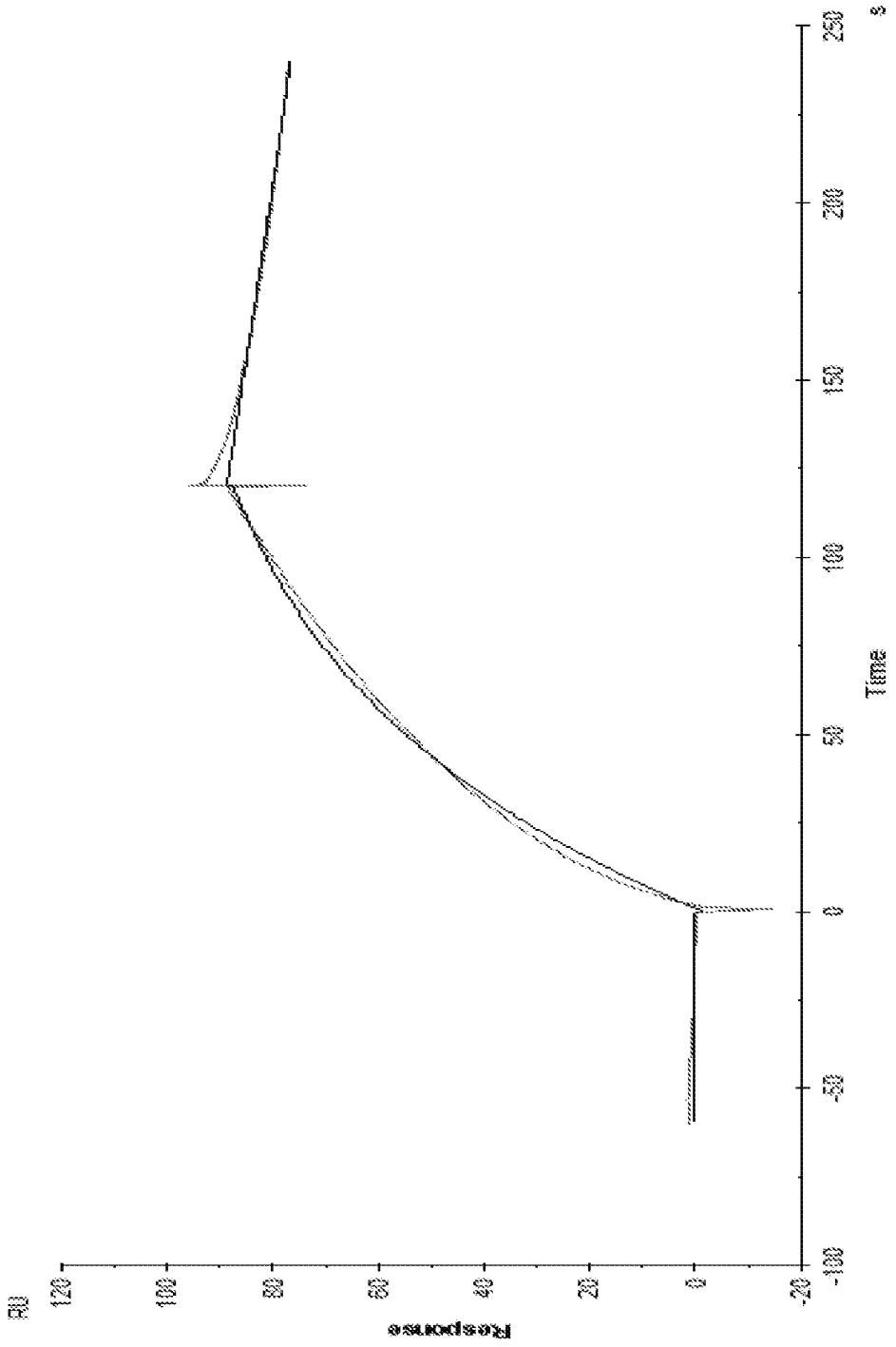

RU
120
100
80
60
40
20
0
-20
Response
-100
-50
0
50
100
150
200
250
s
Time

FIG. 1

FIG. 2


RU
120
100
80
60
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0
-20
Response
-100
-50
0
50
100
150
200
250
s
Time

FIG. 3

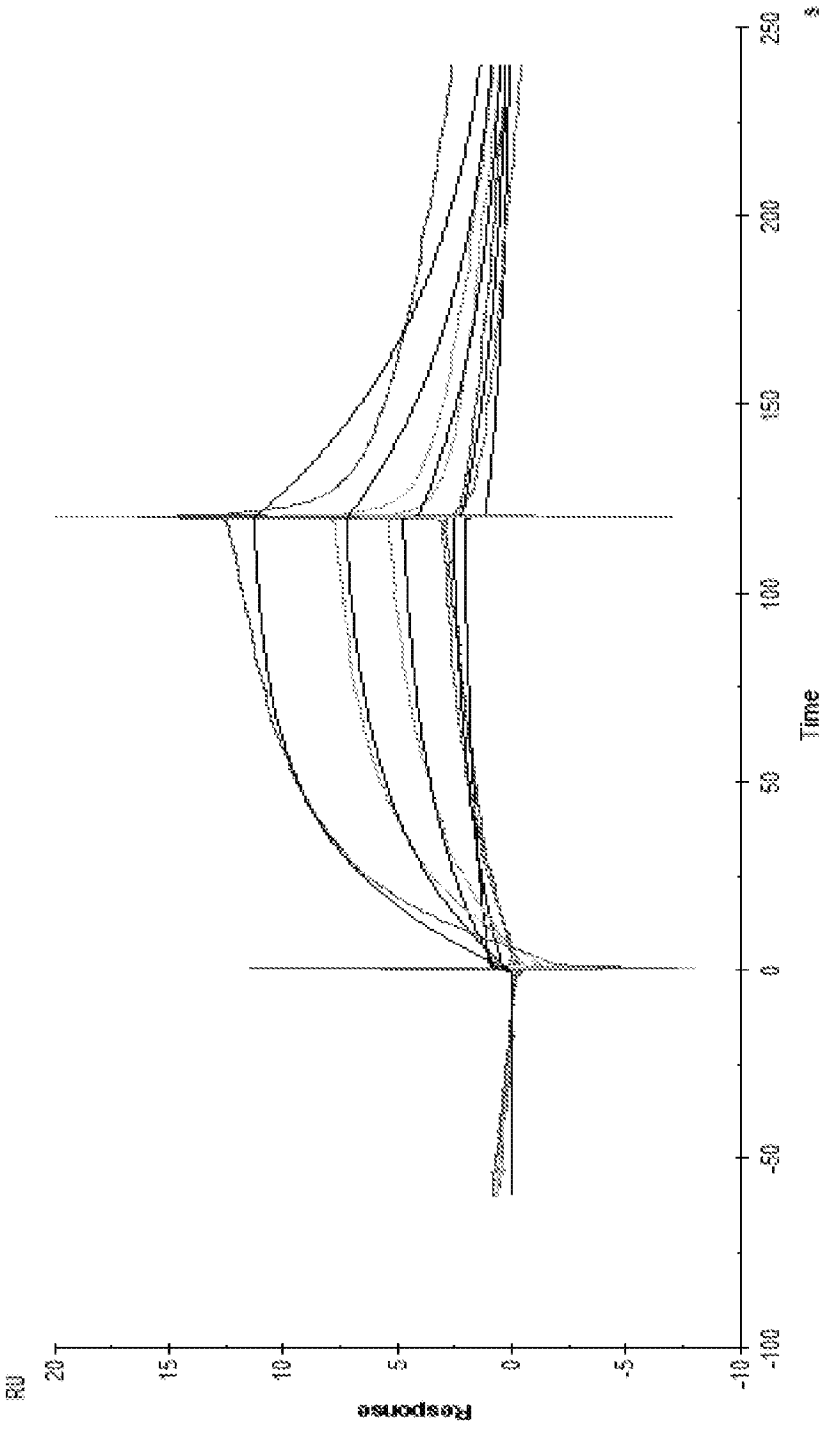


FIG. 4A

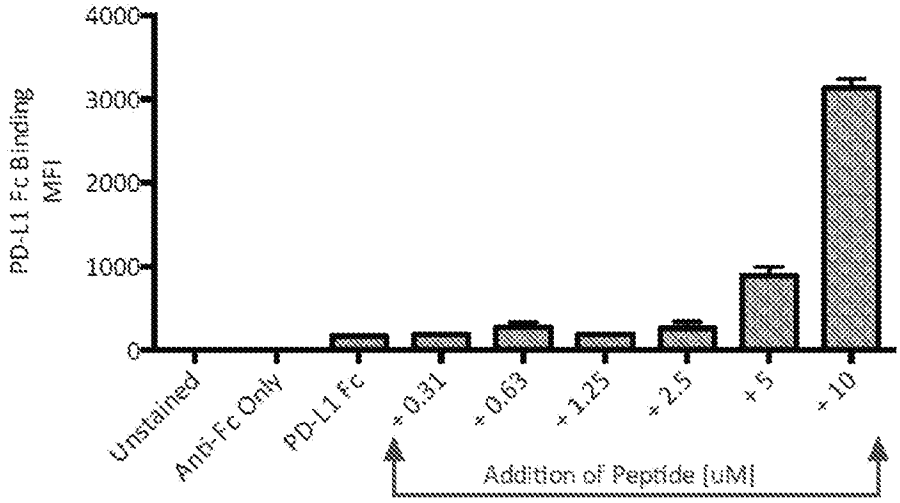

PD-L1 Fc Binding MFI
4000
3000
2000
1000
0
Unstained
Anti-Fc Only
PD-L1 Fc
+ 0.31
+ 0.63
+ 1.25
+ 2.5
+ 5
+ 10
Addition of Peptide [uM]

FIG. 4B

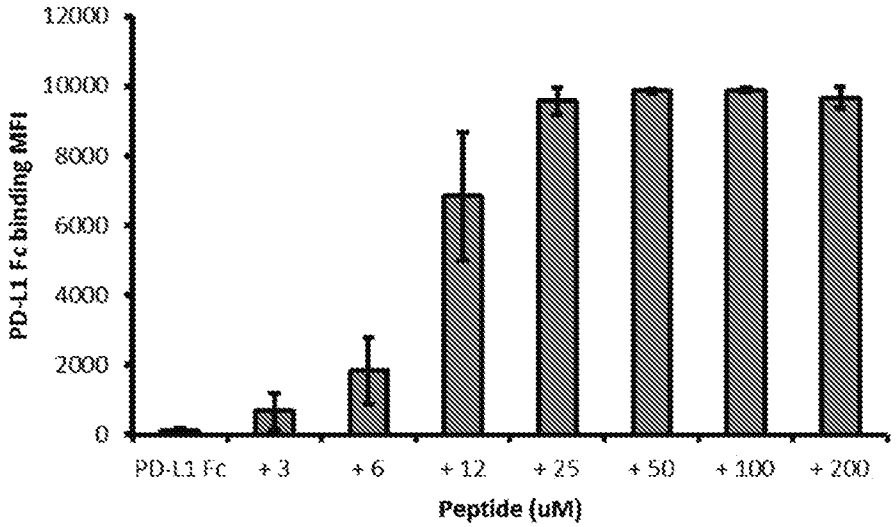

PD-L1 Fc binding MFI
12000
10000
8000
6000
4000
2000
0
PD-L1 Fc
+ 3
+ 6
+ 12
+ 25
+ 50
+ 100
+ 200
Peptide (uM)

FIG. 4C

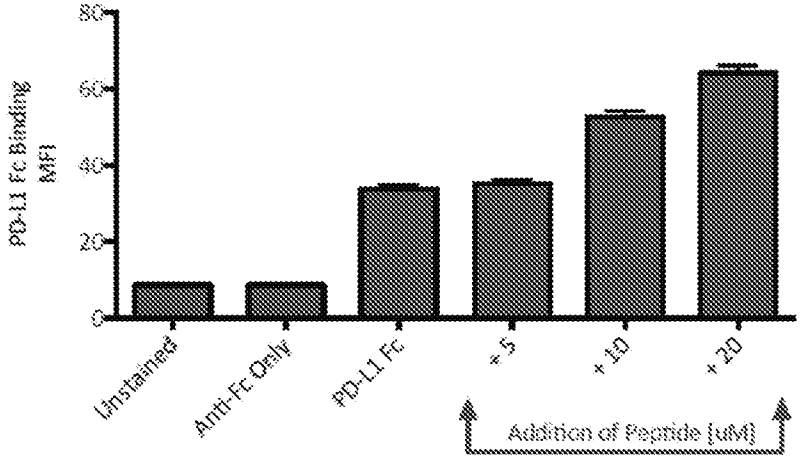

PD-L1 Fc Binding MFI
80
60
40
20
0
Unstained
Anti-Fc Only
PD-L1 Fc
+ 5
+ 10
+ 20
Addition of Peptide (uM)

FIG. 5

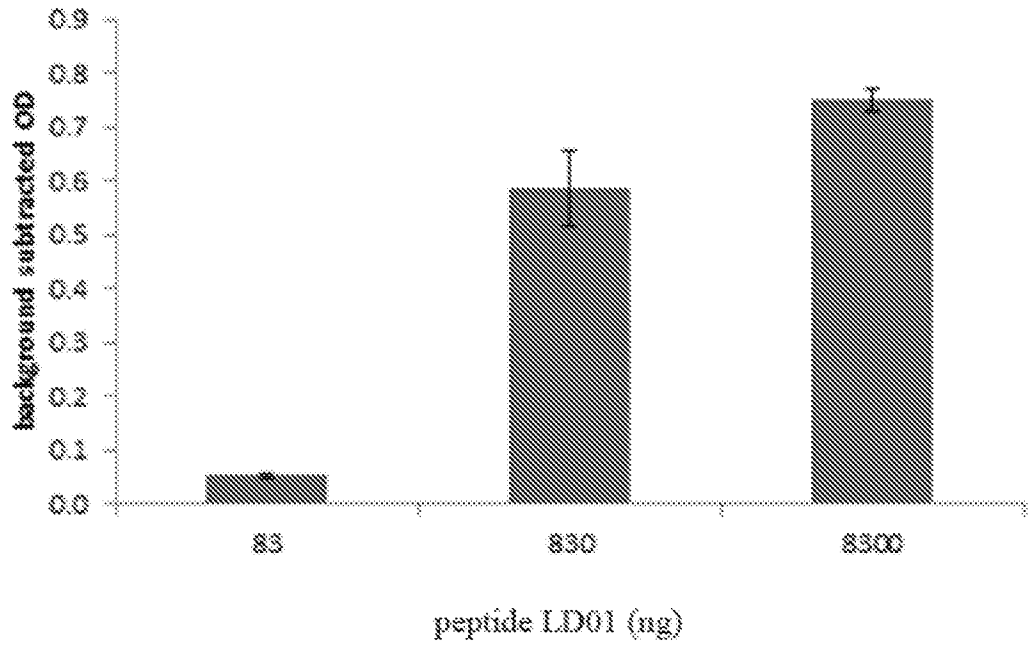

background subtracted OD
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
83
830
8300
peptide LD01 (ng)

FIG. 6

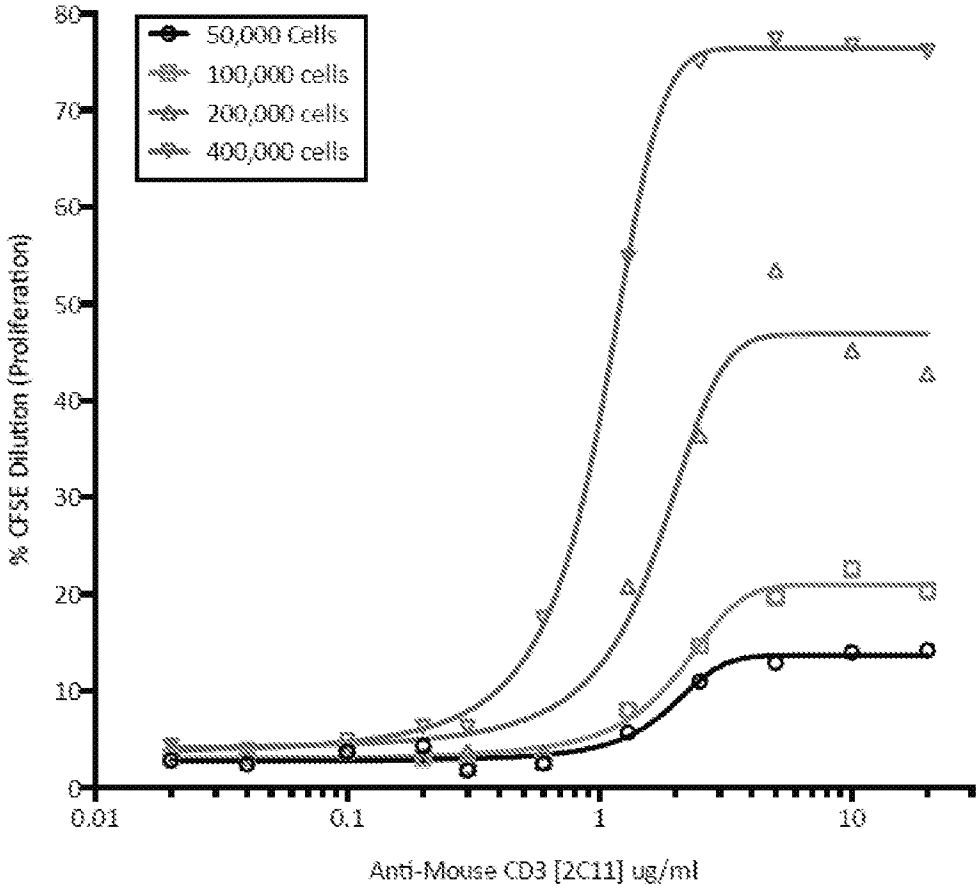

50,000 Cells
100,000 cells
200,000 cells
400,000 cells
% CFSE Dilution (Proliferation)
80
70
60
50
40
30
20
10
0
0.01
0.1
1
10
Anti-Mouse CD3 [2C11] ug/ml

FIG. 7

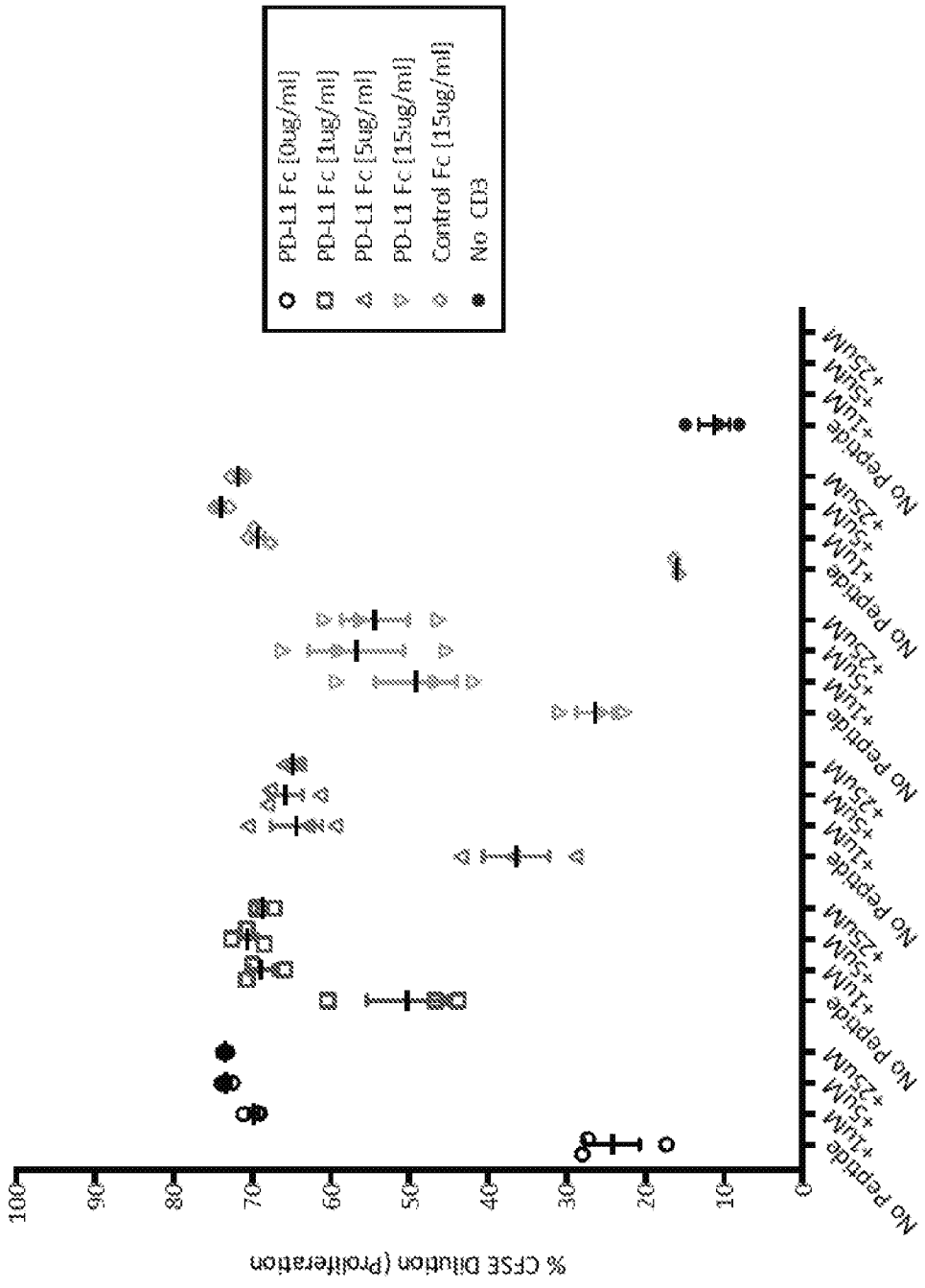

PD-L1 Fc [0ug/ml]
PD-L1 Fc [1ug/ml]
PD-L1 Fc [5ug/ml]
PD-L1 Fc [15ug/ml]
Control Fc [15ug/ml]
No CD3
% CFSE Dilution (Proliferation
100
90
80
70
60
50
40
30
20
10
0
No peptide
+1uM
+5uM
+25uM

FIG.8

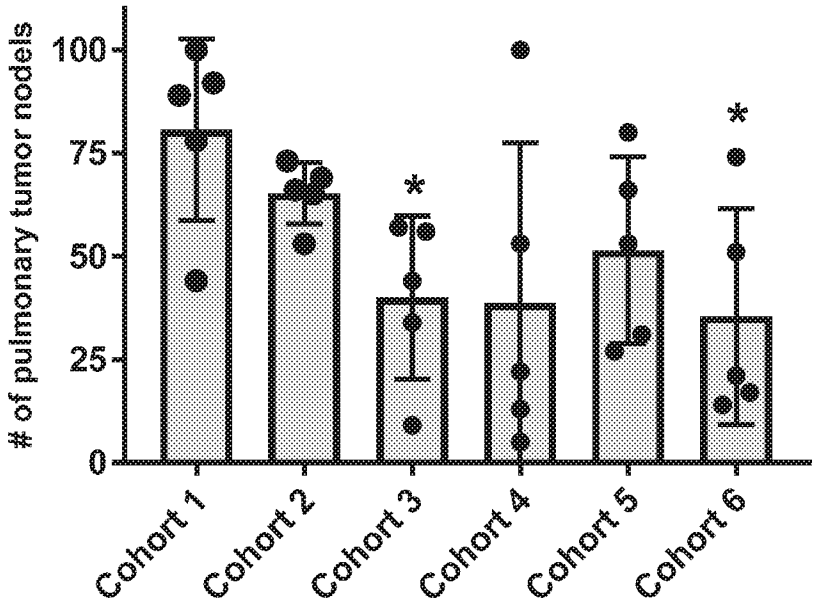

# of pulmonary tumor nodels
100
75
50
25
0
*
*
Cohort 1
Cohort 2
Cohort 3
Cohort 4
Cohort 5
Cohort 6

FIG. 9

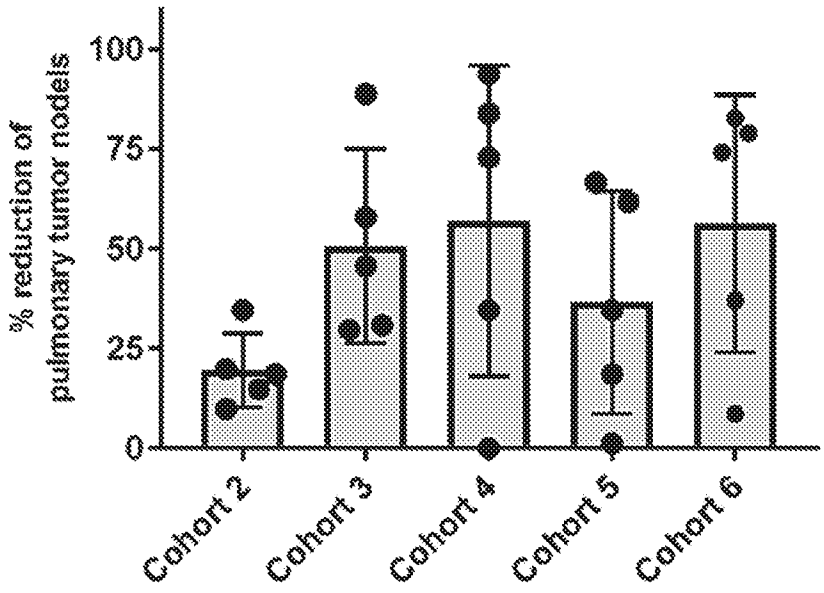

% reduction of pulmonary tumor nodels
100
75
50
25
0
Cohort 2
Cohort 3
Cohort 4
Cohort 5
Cohort 6

FIG. 10

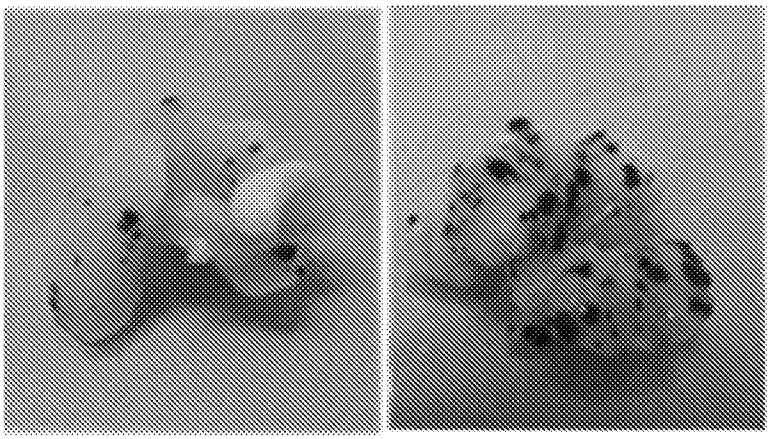

Cohort 4
Cohort 1

FIG. 11

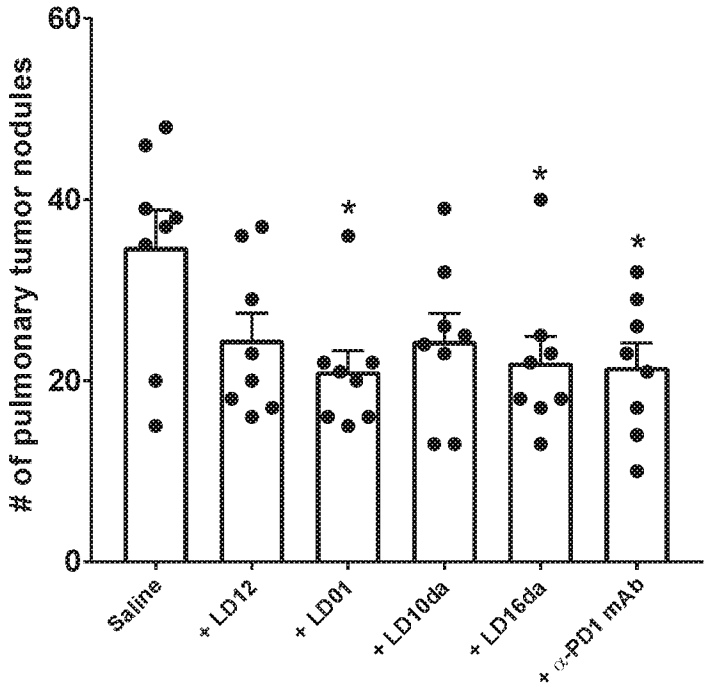

# of pulmonary tumor nodules
60
40
20
0
*
*
*
Saline
+ LD12
+ LD01
+ LD10da
+ LD16da
+ α-PD1 mAb

FIG. 12

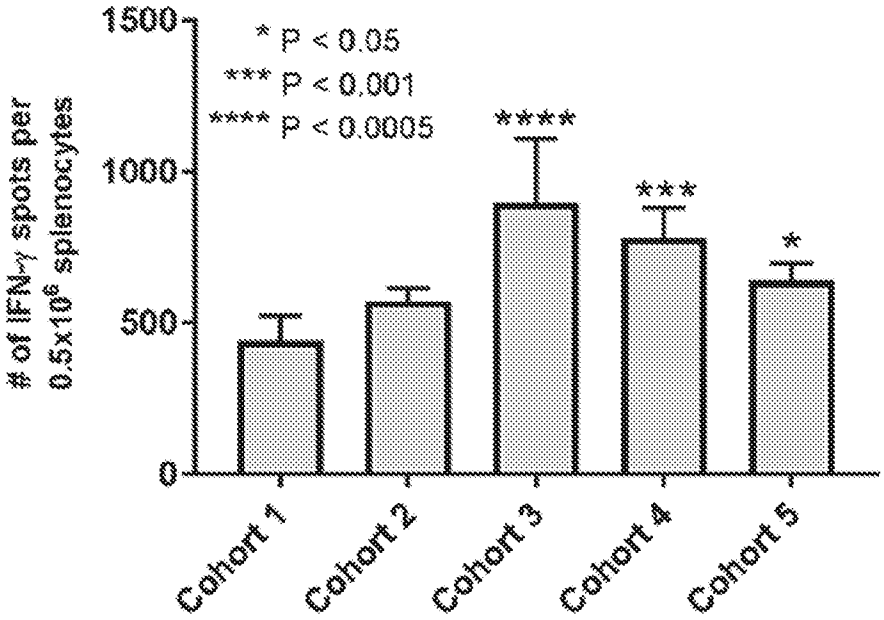

1500
1000
500
0
# of IFN-γ spots per 0.5x10^6 splenocytes
* P < 0.05
*** P < 0.001
**** P < 0.0005
****
***
*
Cohort 1
Cohort 2
Cohort 3
Cohort 4
Cohort 5

FIG. 13

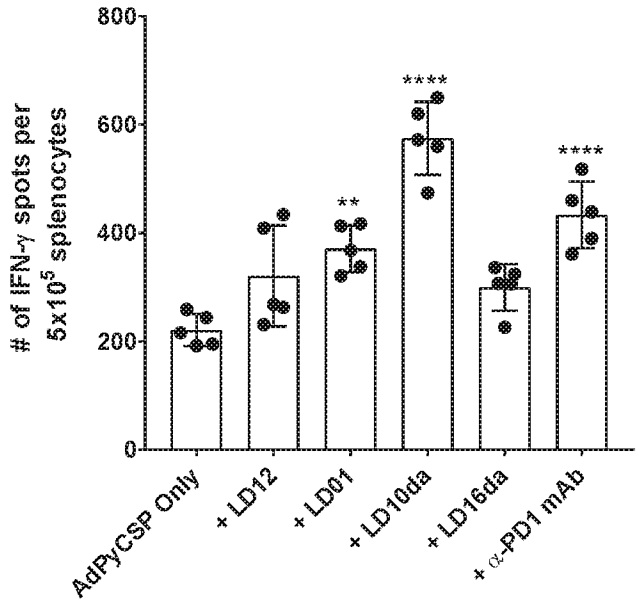

800
600
400
200
0
# of IFN-γ spots per 5x10^5 splenocytes
**
****
****
AdPyCSP Only
+ LD12
+ LD01
+ LD10da
+ LD16da
+ α-PD1 mAb

FIG. 14

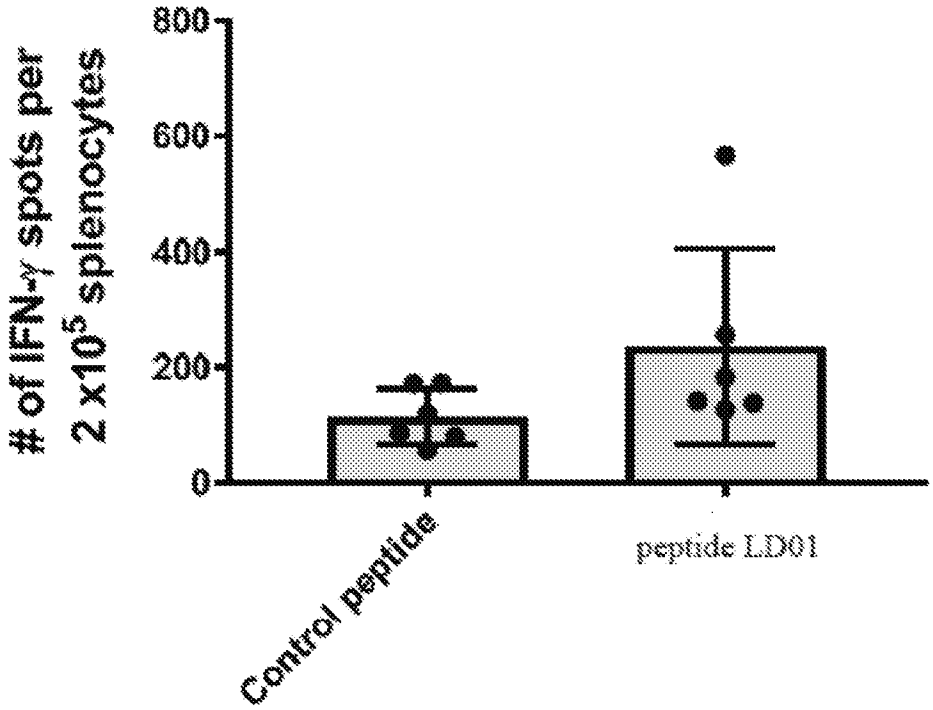
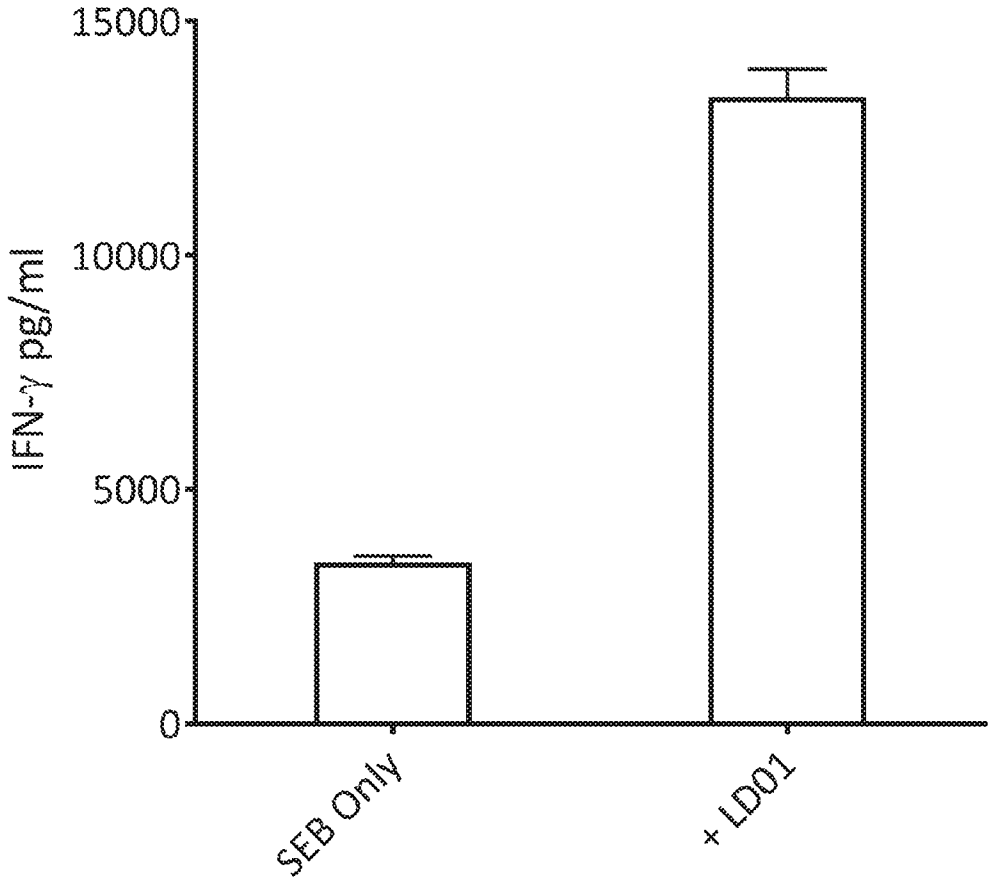

# of IFN-γ spots per
2 x10^5 splenocytes
800
600
400
200
0
Control peptide
peptide LD01

FIG. 15



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/034625

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

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2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/034625

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/705 A61K38/17
ADD.

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)
C07K A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2014/209804 A1 (BIOMED VALLEY DISCOVERIES INC [US]) 31 December 2014 (2014-12-31) Whole document, especially the claims.	1-21
A	US 2013/071403 A1 (ROLLAND ALAIN P [US] ET AL) 21 March 2013 (2013-03-21) Whole document, especially the claims.	1-21
A	WO 98/42752 A1 (BRIGHAM & WOMENS HOSPITAL [US]) 1 October 1998 (1998-10-01) Whole document, especially the claims.	1-21
A	WO 2017/023753 A1 (UNIV FLORIDA [US]) 9 February 2017 (2017-02-09) Whole document, especially the claims.	1-21

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

[X] See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

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"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
27 August 2018	06/09/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kools, Patrick

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DURAISWAMY ET AL: "Dual Blockade of PD-1 and CTLA-4 Combined with Tumor Vaccine Effectively Restores T-Cell Rejection Function in Tumors", CANCER RESEARCH, vol. 73, no. 12, 15 June 2013 (2013-06-15) , pages 3591-3603, XP055218335, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-12-4100 the whole document -----	1-21
A	HAO-NAN CHANG ET AL: "Blocking of the PD-1/PD-L 1 Interaction by a D-Peptide Antagonist for Cancer Immunotherapy", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, vol. 54, no. 40, 10 August 2015 (2015-08-10), pages 11760-11764, XP055247320, ISSN: 1433-7851, DOI: 10.1002/anie.201506225 the whole document -----	1-21

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2018/034625

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		WO 2017023753 A1	09-02-2017