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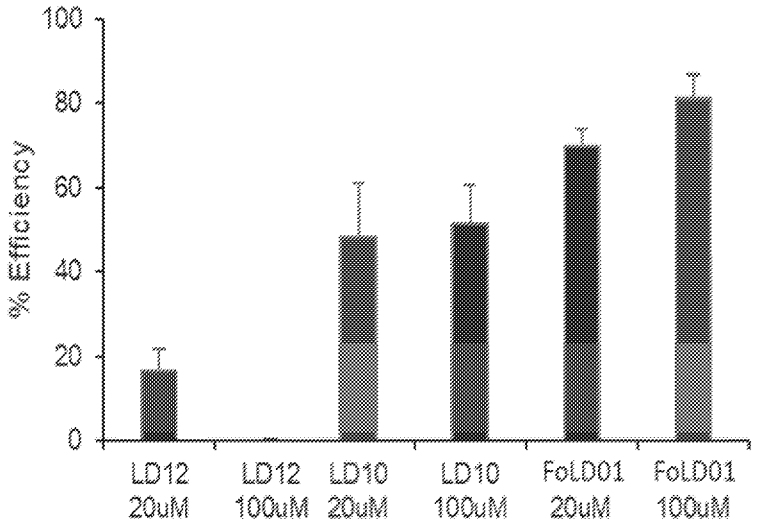
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FIG. 1



(57) Abstract: This disclosure provides peptides which 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.

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IMMUNOMODULATORY PEPTIDES

[01] This application incorporates by reference the contents of a 1.52 kb text filed created on October 7, 2020 and named "00047900276sequencelisting.txt," which is the sequence listing for this application.

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

TECHNICAL FIELD

[03] This disclosure relates generally to immunomodulatory peptides.

BACKGROUND

[04] Programmed cell death-1 (PD1) 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. PD1 is therefore an attractive target for a variety of therapeutic applications. There is a continuing need for useful modulators of immune checkpoint pathways.

BRIEF DESCRIPTION OF THE FIGURES

[05] Figure 1 is a graph showing the ability of various peptides to inhibit the activity of PD1 to recruit SHP1 using PATHHUNTER® checkpoint signaling assays (DiscoverX).

[06] Figure 2 is a graph showing how peptides increase the number of IFN$_\gamma$-positive CD8 T cells measured by IFN$_\gamma$-ELISPOT when combined with a vaccine antigen (AdPyCS).

[07] Figure 3A, graph comparing MC38 tumor volume in mice, normalized to day 0 values for each animal. Cohorts of mice received intra-tumoral (IT) injections of 50 μg FoLD01 peptide or intraperitoneal injections of antibodies against CTLA4 and/or PD1. The median value for control cohort (DMSO/PBS injected intra-tumorally) is shown as a heavier line.

[08] Figure 3B, graph comparing normalized tumor growth kinetics for the indicated groups. Error bars represent cohort mean and 95% confidence intervals.

[09] Figure 3C, graph comparing normalized tumor growth at day 16, p values determined using non-parametric Mann-Whitney t-test.

[10] Figure 4A, graph comparing Pan02 tumor volume in mice, normalized to day 0 values for each animal. Cohorts of mice received intra-tumoral injections of 50 μg FoLD04 or intraperitoneal injections of antibodies against CTLA4 and/or PD1. The median value for control cohort (DMSO/PBS injected intra-tumorally) is shown as a heavier line.

[11] Figure 4B, graph comparing normalized tumor growth kinetics for the indicated groups. Error bars represent cohort mean and 95% confidence intervals.

[12] Figure 4C, graph comparing normalized tumor growth at day 21, p values determined using non-parametric Mann-Whitney t-test.

[13] Figure 4D, graph comparing normalized tumor growth at day 27, p values determined using non-parametric Mann-Whitney t-test.

DETAILED DESCRIPTION

[14] This disclosure provides peptides that antagonize the activity of the checkpoint receptor "programmed death 1" (PD-1). The amino acid sequences of these peptides are shown below.

peptide	amino acid sequence	SEQ ID NO:
FoLD01	STNQVSALRVNILFPLSQ	1
FoLD02	STNQVSALKVNILFPLSQ	2
FoLD03	STGQVSTLRVNITAPLSQ	3
FoLD04	QVSALRVNILF	4

[15] In some embodiments, the disclosed peptides consist of the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4. In some embodiments, the disclosed peptides comprise the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4. In some embodiments, the disclosed peptides consist essentially of the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

[16] 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.

[17] 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).

[18] Recombinant production of unmodified 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 of, or consisting of SEQ ID NO:1, 2, 3, or 4.

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

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

[21] 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.

[22] 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.

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

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

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

[26] 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).

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

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

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

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

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

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

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

Therapeutic Uses

[34] The disclosed peptides 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.

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

[36] In some embodiments, one or more of the disclosed peptides 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.

[37] In some embodiments, an engineered T cell that expresses and secretes one or more disclosed peptides can be used to deliver PD1 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.

[38] 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.

[39] 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).

[40] 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).

[41] 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.

[42] 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

[43] One or more 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.

[44] 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.

[45] 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.

[46] 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.

[47] 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.

[48] Devices comprising any of the 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

[49] In some embodiments, one or more of the 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.

[50] 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

[51] In some embodiments, one or more of the 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.

[52] In some embodiments, the second therapy comprises a second agent that reduces or blocks the activity of PD1 (*e.g.*, nivolumab, pembrolizumab, durvalumab) or CTLA-4 (*e.g.*, ipilimumab, tremelimumab).

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

[54] 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, PD1H, 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).

[55] 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).

[56] 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).

[57] 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.

[58] 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.

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

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

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

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

[63] 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).

[64] 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.

[65] 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.

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

[67] 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.

[68] 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.

[69] 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).

[70] 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.

[71] In some embodiments, one or more of the 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.

[72] In some embodiments, one or more of the 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

[73] In some embodiments, one or more of the 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.

[74] 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.

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

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

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

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

[79] In some embodiments, one or more of the 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.

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

[81] In some embodiments, one or more of the 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, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described herein are administered to promote lightening of pigmented skin lesions.

Example 1. PATHHUNTER® Checkpoint Signaling Assays

[82] Peptides were tested for their ability to inhibit the binding of PDL1 to PD1 using PATHHUNTER® checkpoint signaling assays (DiscoverX).

[83] Jurkat cells expressing PD1 and SHP1 proteins, each fused to a fragment of enzyme fragment complementation (EFC) system, were co-incubated with PDL1-presenting U2OS cells. This results in PD1 activation and SHP1 recruitment to the PD1 receptors, bringing together the two EFC fragments and generating a light signal. Inhibitory peptides or antibodies added to the culture would reduce the light signal.

[84] Cells in co-culture were incubated at room temperature (RT) for 2 h (PD1 assay). The assay signal was generated using the PATHHUNTER® Bioassay Detection kit. FoLD01 (SEQ ID NO:1), LD10 (SEQ ID NO:5), and LD12 (SEQ ID NO:6) peptides were tested in duplicate at two concentrations, 20 μM and 100 μM. Peptides were dissolved in water or DMSO and diluted in assay buffer.

[85] Microplates were read following signal generation using a PerkinElmer ENVISION™ instrument for chemiluminescent signal detection. The percentage of inhibition efficacy was calculated using the following formula, in which "RLU" means relative light units:

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

[86] The results demonstrate that the FoLD01 peptide inhibited PD1-PDL1 interaction to a greater extent than peptides LD10 and LD12 (Figure 1).

Example 2. Evaluation of Peptides in an AdPyCS Mouse Model

[87] Groups of five mice were immunized intramuscularly with a recombinant replication defective adenovirus expressing the *Plasmodium yoelli* circumsporozoite protein (AdPyCSP) and then treated subcutaneously with peptides LD10da (SEQ ID NO:6, in which the first amino acid is a D-amino acid, 1 μg), FoLD01 (1 μg, 10μg), or an anti-PD1 monoclonal antibody (10 μg). Ten days post-injection, mice were euthanized and individual spleens removed. The number of IFNγ-secreting antigen-specific CD8+ T cells were determined using an ELISPOT assay (Figure 2).

[88] The results showed that peptide FoLD01 enhances the number of antigen-specific CD8+ T cells relative to stimulation with antigen only (one-way ANOVA, statistical differences are relative to AdPyCS-only stimulation).

Example 3. Assessment of Peptides in an MC38 Tumor Model

[89] Mice were injected subcutaneously with $1x10^6$ MC38 (colon carcinoma) cells and tumor volumes were measured on day 2, 5, 9, 12, and 16 (end of study) and normalized to day 0 values for each animal. When the mean tumor size reached approximately 80-120 mm^3, mice were randomized and treatment was started. Peptides were tested in an MC38 tumor model. FoLD01

peptide was administered intra-tumorally 2x a week (50 μg dose). Monoclonal antibodies were administered intraperitoneally 2x a week (5 mg/kg (anti-CTLA4) and 10 mg/kg (anti-PD1) dose). Statistical test used: Unpaired, non-parametric, two-tailed Mann-Whitney test. [85] Control used: intra-tumor injection of DMSO diluted in PBS (for FoLD01 injected intra-tumorally). The results are shown in Figure 3 (A-C). In general, administration of anti-CTLA4 mAb and anti-CTLA4 + anti-PD1 mAb resulted in significant decreases in tumor volume. Administration of anti-PD1 mAb resulted in a limited decrease in tumor volume, which may have been attributed to the 1 x 10^6 cells MC38 cells injected.

[90] Intra-tumoral injection of FoLD01 peptide significantly decreased tumor volume relative to the intra-tumoral DMSO/PBS control using the non-parametric Mann-Whitney t-test. The P value was 0.0499.

Example 4. Assessment of Peptides in a Pan02 Tumor Model

[91] Mice were injected subcutaneously with 3x10^6 Pan02 (pancreatic adenocarcinoma) cells in the right rear flank. Tumor volumes were measured at least twice weekly in two dimensions using a caliper. When the mean tumor size reached approximately 80-120 mm^3, mice were randomized and treatment was started. FoLD04 was administered intra-tumorally 2x a week (50 μg dose). Monoclonal antibodies were administered intraperitoneally 2x a week (5 mg/kg (anti-CTLA4) and 10 mg/kg (anti-PD1) dose).

[92] Tumor volumes were measured on day 4, 7, 11, 14, 18, 21, 25 and 27 (end of study) and normalized to day 0 values for each animal. Statistical test used: Unpaired, non-parametric, two-tailed Mann-Whitney test.

[93] Control used: intra-tumor injection of DMSO diluted in PBS (for FoLD04 injected intra-tumorally). The results are shown in Figure 4.

Summary and interpretation of Pan02 Model Results

[94] Treatment with anti-CTLA4 mAb and anti-CTLA4 + anti-PD1 mAb resulted in significant decreases in tumor volume regardless of time point assessed and statistical test used. The anti-PD1 mAb resulted in sporadic significant decreases in tumor volume depending on time point assessed and statistical test used.

[95] When administered intra-tumorally, the FoLD04 peptide demonstrated significantly decreased tumor volume relative to DMSO/PBS IT control using the non-parametric Mann-Whitney t-test.

[96] Based on the time points assessed and the statistical tests used, subcutaneous administration of the peptides trended toward control of tumor volume but did not approach statistical significance.

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CLAIMS

1. A peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

2. An expression construct encoding a peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

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 having an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4;

(ii) a nucleic acid encoding the peptide;

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

(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 claim 4.

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 pancreatic cancer or a colon carcinoma.

14. The method of claim 10, 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 PD1, 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.

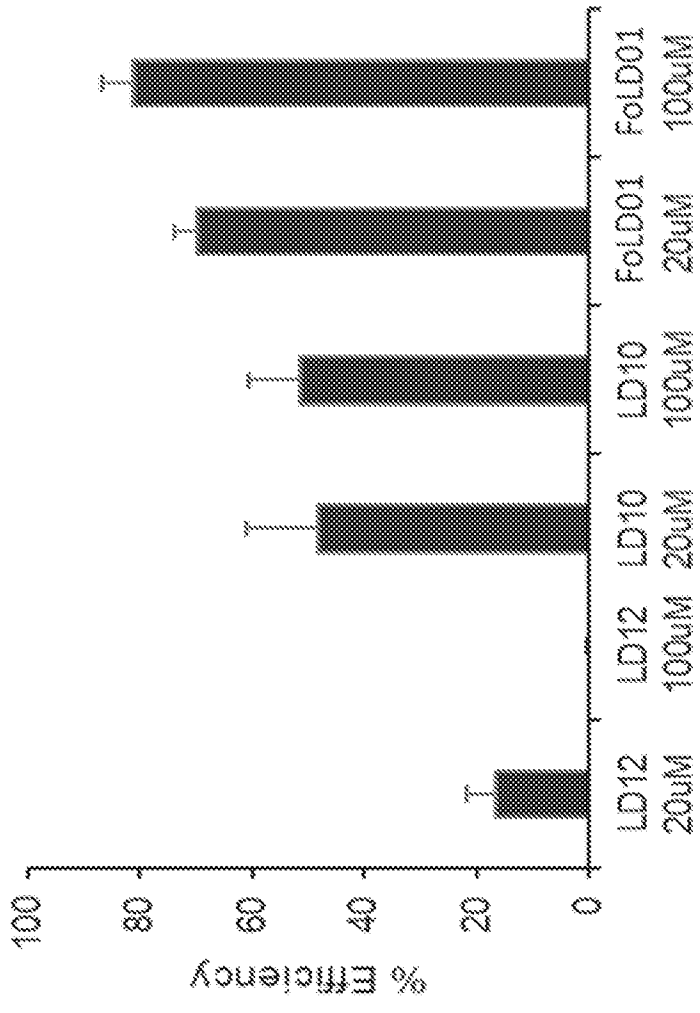

% Efficiency
100
80
60
40
20
0
LD12 20uM
LD12 100uM
LD10 20uM
LD10 100uM
FoLD01 20uM
FoLD01 100uM

FIG. 1

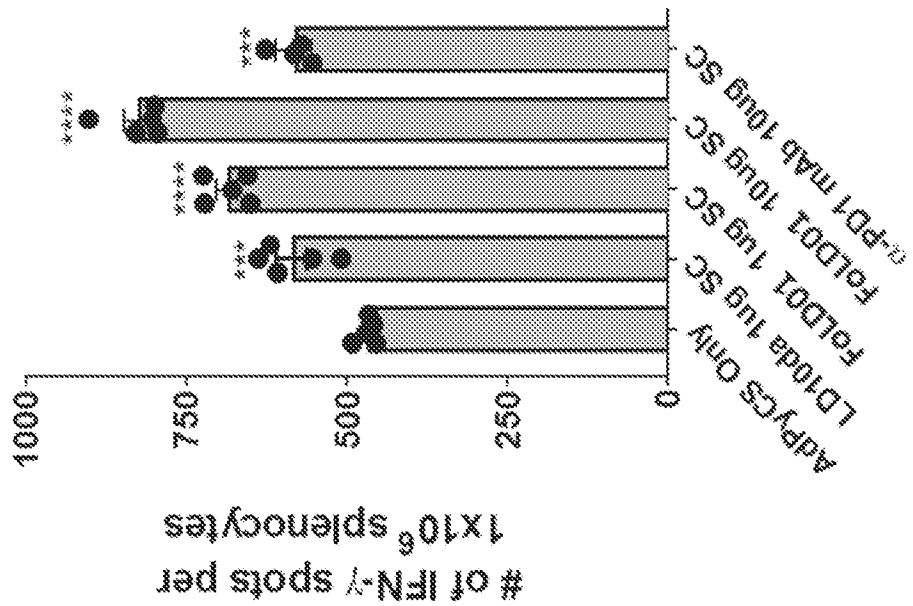

# of IFN-γ spots per
1x10^6 splenocytes
1000
750
500
250
0
AdPyCS Only
LD10da 1ug SC
foLD01 1ug SC
foLD01 10ug SC
foLD01 10ug SC +PD1 mAb 10ug SC
****
****
****
***
***

FIG. 2

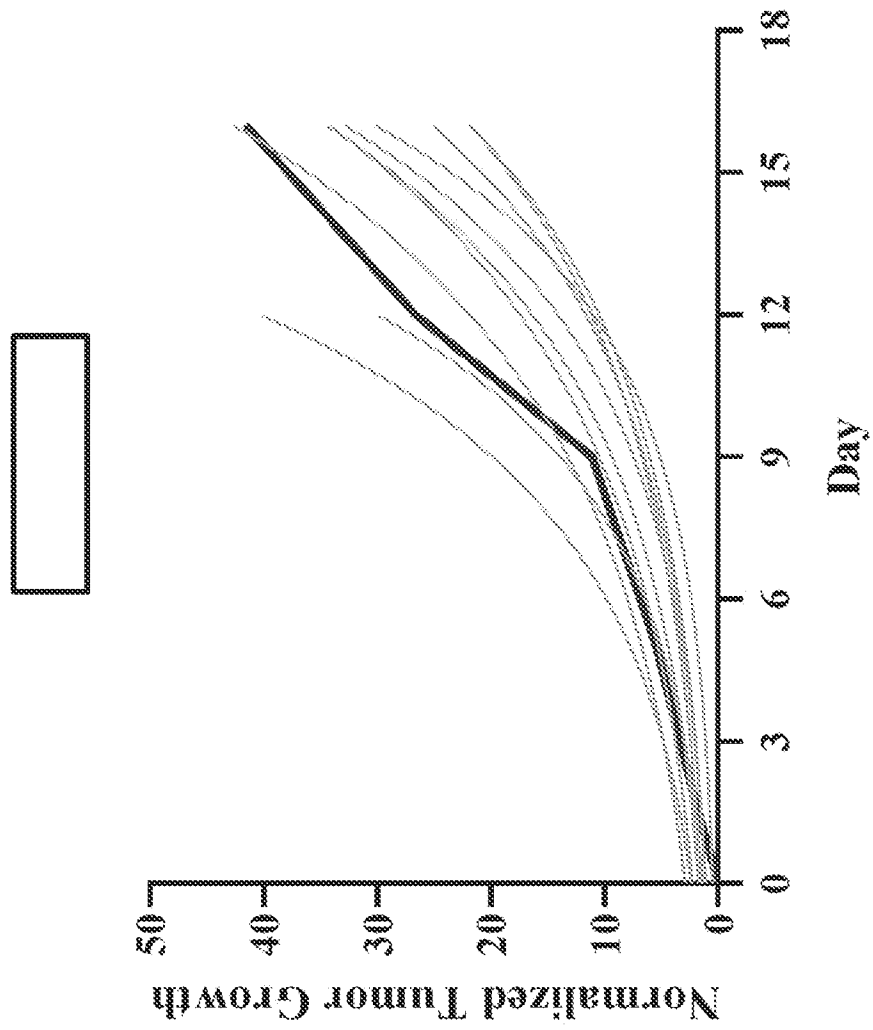
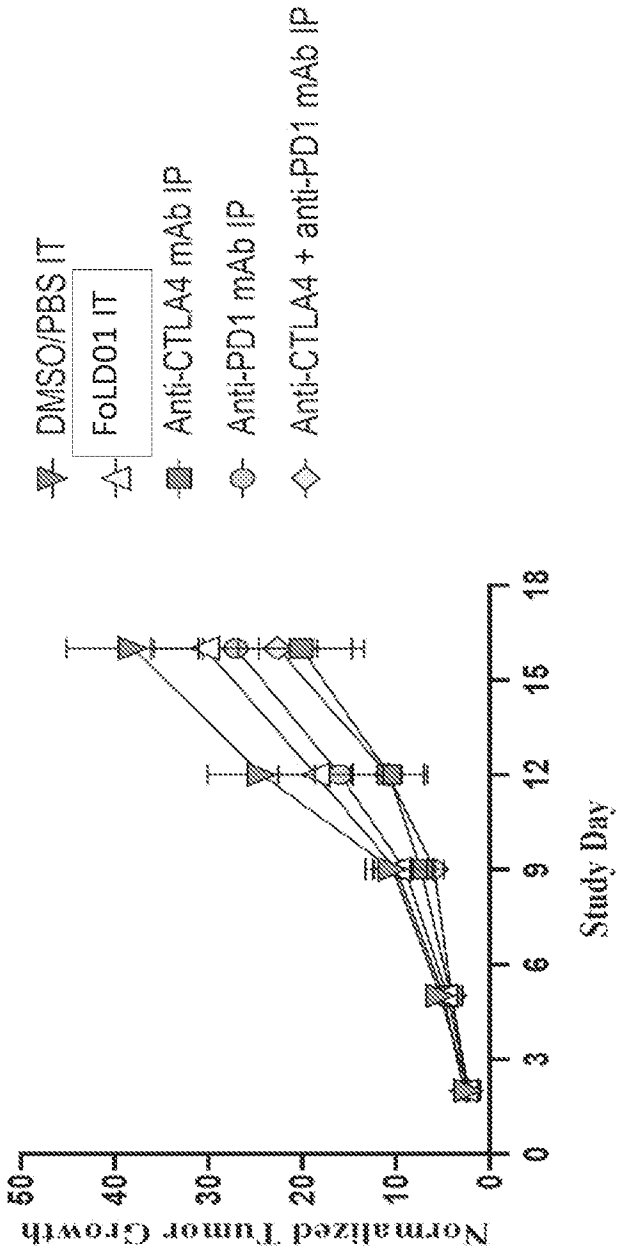


FIG. 3A

FIG. 3B


DMSO/PBS IT
FoLD01 IT
Anti-CTLA4 mAb IP
Anti-PD1 mAb IP
Anti-CTLA4 + anti-PD1 mAb IP
Normalized Tumor Growth
0
10
20
30
40
50
0
3
6
9
12
15
18
Study Day

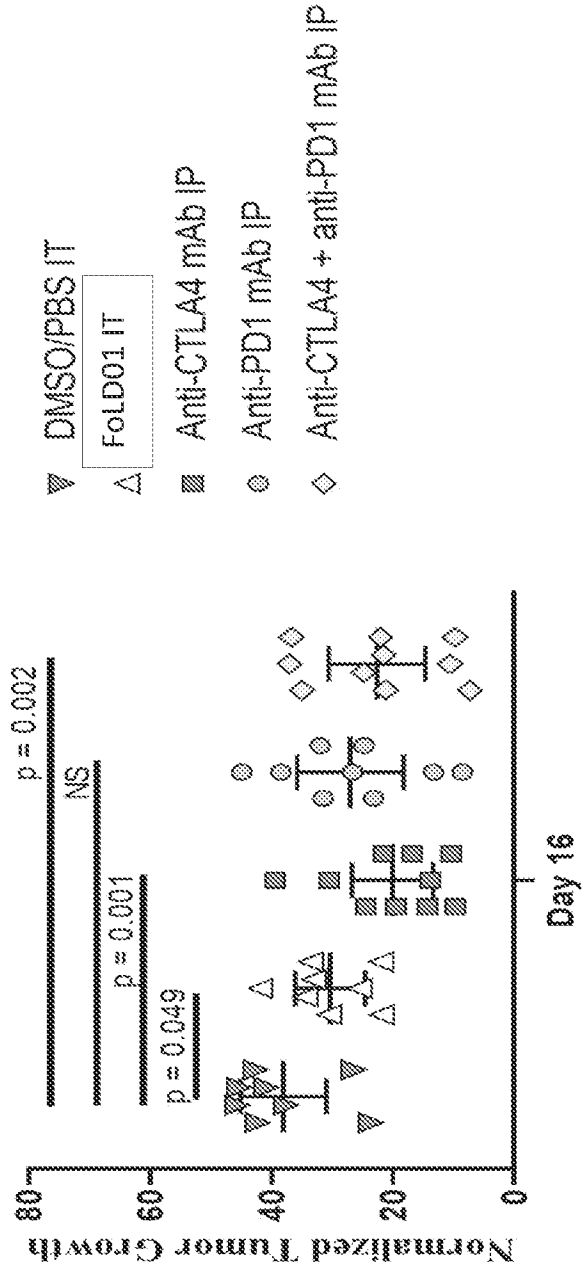

DMSO/PBS IT
FoLD01 IT
Anti-CTLA4 mAb IP
Anti-PD1 mAb IP
Anti-CTLA4 + anti-PD1 mAb IP
p = 0.002
NS
p = 0.001
p = 0.049
Normalized Tumor Growth
80
60
40
20
0
Day 16

FIG. 3C

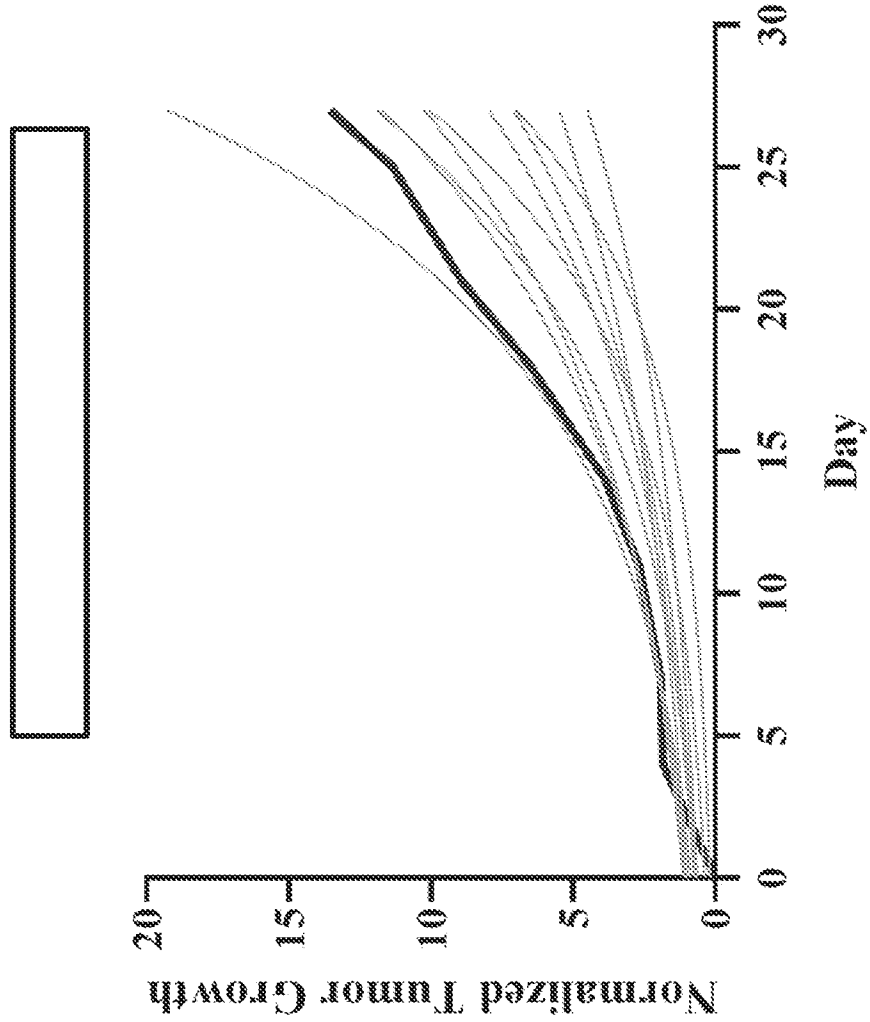


FIG. 4A

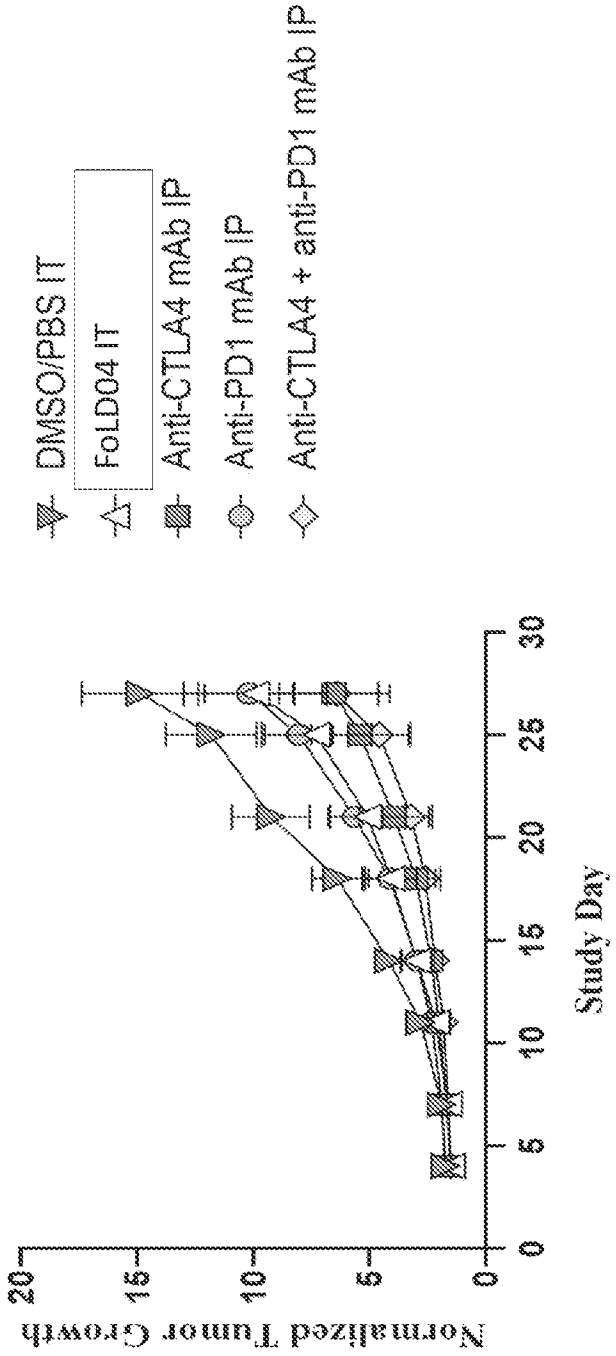

DMSO/PBS IT
FoLD04 IT
Anti-CTLA4 mAb IP
Anti-PD1 mAb IP
Anti-CTLA4 + anti-PD1 mAb IP
Normalized Tumor Growth
0
5
10
15
20
0
5
10
15
20
25
30
Study Day

FIG. 4B

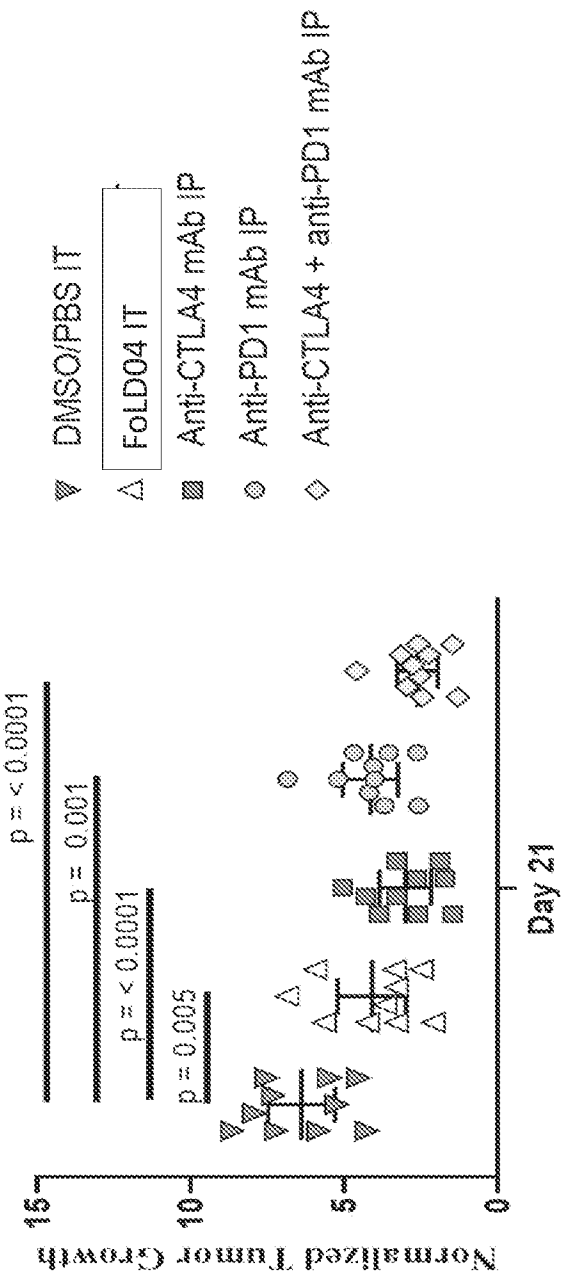

DMSO/PBS IT
FoLD04 IT
Anti-CTLA4 mAb IP
Anti-PD1 mAb IP
Anti-CTLA4 + anti-PD1 mAb IP
p = < 0.0001
p = 0.001
p = < 0.0001
p = 0.005
Normalized Tumor Growth
15
10
5
0
Day 21

FIG. 4C

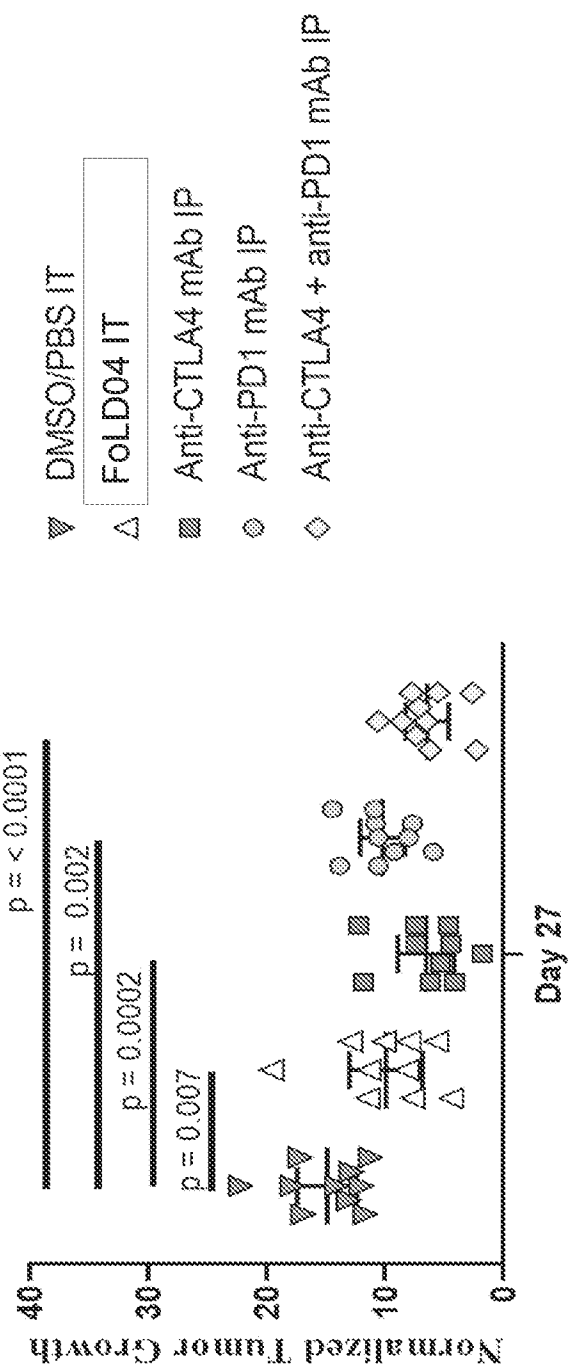

DMSO/PBS IT
FoLD04 IT
Anti-CTLA4 mAb IP
Anti-PD1 mAb IP
Anti-CTLA4 + anti-PD1 mAb IP
p = < 0.0001
p = 0.002
p = 0.0002
p = 0.007
Normalized Tumor Growth
40
30
20
10
0
Day 27

FIG. 4D

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/054127

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P35/00 A61K38/10 C07K7/08
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)
A61P 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, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/218137 A1 (LEIDOS INC [US]) 29 November 2018 (2018-11-29) claims 1-19; sequences 12, 14 -----	1-19
A	LI-JUN MA ET AL: "Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium", NATURE, vol. 464, no. 7287, 18 March 2010 (2010-03-18), pages 367-373, XP055357917, London ISSN: 0028-0836, DOI: 10.1038/nature08850 the whole document -/--	1-19

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

[x] 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

"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
31 January 2022	08/02/2022

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	Westphal-Daniel, K

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& Ma J L ET AL: "hypothetical protein FOXG_05320 [Fusarium oxysporum f. sp. lycopersici - Protein - NCBI", Nature, 26 September 2016 (2016-09-26), XP055879013, Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/protein/XP_018240489 [retrieved on 2022-01-14]	
A	WO 2018/053218 A1 (LEIDOS INC [US]) 22 March 2018 (2018-03-22) paragraph [0035] - paragraph [0074]	1-19
A	WO 2019/168524 A1 (LEIDOS INC [US]) 6 September 2019 (2019-09-06) paragraph [0035] - paragraph [0116]	1-19
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	1-19
A	PHARES T W ET AL: "Enhancement of the immune response to plasmodium yoelii circumsporozoite protein by PD-1 inhibitors", THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE, AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE, US, vol. 97, no. 5, Supplement 1, 1 November 2017 (2017-11-01), page 330, XP009508330, ISSN: 0002-9637 the whole document	1-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/054127

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

      [ ] 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 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [X] furnished subsequent to the international filing date for the purposes of international search only:

      [X] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

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

Information on patent family members

International application No
PCT/US2021/054127

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018218137 A1	29-11-2018	AU 2018273958 A1	02-01-2020
		CA 3064023 A1	29-11-2018
		EP 3630807 A1	08-04-2020
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		EP 3795167 A1	24-03-2021
		JP 6928083 B2	01-09-2021
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		US 2018185474 A1	05-07-2018
		US 2021000948 A1	07-01-2021
		WO 2018053218 A1	22-03-2018
WO 2019168524 A1	06-09-2019	AU 2018410849 A1	10-09-2020
		CA 3091414 A1	06-09-2019
		EP 3758732 A1	06-01-2021
		JP 2021514632 A	17-06-2021
		WO 2019168524 A1	06-09-2019