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(54) Title: LAG3 BINDING PEPTIDES

IC50 in LAG3:FGL1 and LAG3:MHCII TR-FRET Assays

	LAG3:FGL1 TR-FRET Screen (BPS)			LAG3:MHC TR-FRET Screen (Cisbio)			Notes
	% Inhibition at 100 µM	% Inhibition at 33 µM	% Inhibition at 11 µM	% Inhibition at 100 µM	% Inhibition at 6.25 µM	% Inhibition at 0.39 µM	
6	100	100	99	67	24	8	high FGL low MHC
41	99	99	98	50	29	2	high FGL low MHC
68	97	89	78	70	48	24	high FGL medium MHC
69	99	85	73	57	11	-2	high FGL low MHC
115	99	99	98	70	66	65	high FGL high MHC
146	92	90	89	65	46	50	high FGL high MHC
154	99	93	79	53	26	37	high FGL medium MHC
155	99	97	93	67	53	61	high FGL high MHC
	LAG3:FGL1 TR-FRET Screen (BPS)			LAG3:MHC TR-FRET Screen (Cisbio)			Notes
	% Inhibition at 100 µM	% Inhibition at 33 µM	% Inhibition at 11 µM	% Inhibition at 100 µM	% Inhibition at 6.25 µM	% Inhibition at 0.39 µM	
11	62	49	66	68	66	49	medium FGL high MHC
16	99	94	47	68	58	56	high FGL high MHC
62	7	3	5	68	60	35	high FGL medium MHC
68	97	89	78	70	48	24	high FGL medium MHC
115	99	99	98	70	66	65	high FGL high MHC
141	80	25	7	63	44	33	medium FGL medium MHC
146	92	90	89	65	46	50	high FGL high MHC
155	99	97	93	67	53	61	high FGL high MHC

Figure 1

(57) Abstract: This disclosure provides peptides that bind to LAG3 and can be used to block its interaction with other molecules such as MHC-II, FGL1, and α-synuclein. These peptides, with their demonstrated activity and short half-life, can be used to activate cellular immunity while significantly reducing the potential for adverse events that is often associated with the antibodybased checkpoint inhibitors. The peptides can be used as stand-alone therapeutics or can be used as immune modulators in combination with other therapies.



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## LAG3 BINDING PEPTIDES

[01] This application incorporates by reference the contents of a 3.72 kb text filed created on July 20, 2020 and named “00047900277sequencelisting,” 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] Lymphocyte activation gene 3 (LAG3, also known as LAG-3, LAG 3, Lag3, CD223, FDC protein) is a member of the immunoglobulin superfamily of receptors.

[05] LAG3 is expressed on immune cells (activated T cells, Huard et al., 1994; natural killer cells, Triebel et al., 1990; B cells, Kisielow et al., 2005; plasmacytoid dendritic cells, Workman et al., 2009), where it binds to MHC class II (MHC-II) and serves as an immune checkpoint receptor. LAG3 also binds to fibrinogen-like protein (FGL1), and disrupting this binding can potentiate anti-tumor immunity (Wang et al., 2019). There is a continuing need for useful modulators of immune checkpoint pathways.

[06] LAG3 is also expressed on neurons, where it serves as a receptor for the  $\alpha$ -synuclein aggregates characteristic of synucleinopathies (Mao et al., 2016). Synucleinopathies are disorders characterized by the abnormal accumulation of aggregates of  $\alpha$ -synuclein protein in neurons, nerve fibers, or glial cells. Synucleinopathies include idiopathic and inherited forms of Parkinson's disease (PD); Diffuse Lewy Body (DLB) disease, also known as Dementia with Lewy Bodies or Lewy body dementia; incidental Lewy body disease; Lewy body variant of Alzheimer's disease (LBV); Combined Alzheimer's and Parkinson disease (CAPD); pure autonomic failure (PAF); multiple system atrophy (MSA), such as olivopontocerebellar atrophy, striatonigral degeneration, and Shy-Drager Syndrome; pantothenate kinase-associated neurodegeneration; Down's Syndrome; Gaucher disease-related synucleinopathies; and

neurodegeneration with brain iron accumulation. There is a continuing need for therapeutic agents for treating or managing symptoms of synucleinopathies.

**BRIEF DESCRIPTION OF THE DRAWING**

[07] Figure 1 shows the results of fluorescent resonance energy transfer (FRET) assays.

**DETAILED DESCRIPTION**

[08] This disclosure provides peptides that bind to LAG3 and can be used to block its interaction with other molecules such as MHC-II, FGL1, and  $\alpha$ -synuclein. The amino acid sequences of these peptides are shown in Table 1, below.

**Table 1. Peptide Sequences**

peptide	amino acid sequence	SEQ ID NO:
6	ITMWGYDTDEDDNLVIYLTLD	1
11	RERGGDDFAQDDYYVTIFYR	2
16	QGSTSGWGF GAMYELTYDVY	3
41	TVDADGDLYFDMEEGVLVFM	4
62	LAGHYHYLTAGSQDIYEYND	5
68	EDKAPWWQLDMEFEFRLDRV	6
69	AEFDHFDVVGQTLDIASWDSY	7
115	RTDRYTLSYIWT SDEWMLFD	8
141	GGVAIFLALFGGMVSSWEWD	9
146	VFRMDWDEKEVRLYADDILL	10
154	EHNREQVLEVMYSFQVNDYF	11
155	SLPYSNIYIELD SGYWNSES	12

[09] These peptides, with their demonstrated activity and short half-life, can be used to activate cellular immunity while significantly reducing the potential for adverse events that is often associated with the antibody-based checkpoint inhibitors. The peptides can be used as stand-alone therapeutics or can be used as immune modulators in combination with other

therapies. They can be genetically encoded, for example, within CAR T and oncolytic viral platforms to effectively target the tumor microenvironment.

**[10]** 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.

**[11]** 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).

**[12]** 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 any of SEQ ID NOS:1-7.

**[13]** 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.

[14] 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).

[15] 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.

[16] 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.

[17] 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.

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

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

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

[21] 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).

- [22] Examples of oncolytic herpes simplex virus include NV1020 (Geevarghese et al., 2010) and T-VEC (Andtbacka et al., 2013).
- [23] Examples of oncolytic morbillivirus virus include oncolytic measles viruses such as MV-Edm (McDonald et al., 2006) and HMWMAA (Kaufmann et al., 2013).
- [24] Examples of oncolytic retrovirus are disclosed in Lu et al., 2012.
- [25] Examples of oncolytic influenza virus are disclosed, for example, in US 2018/0057594.
- [26] Examples of oncolytic Sindbis virus are disclosed, for example, in Lundstrom, 2017.
- [27] Examples of oncolytic poxvirus are disclosed, for example, in Chan & McFadden, 2014.
- [28] Examples of oncolytic adenovirus include ONYX-015 (Khuri et al., 2000) and H101 or Oncorine (Liang, 2018).

### Therapeutic Uses

- [29] The peptides and modified versions thereof disclosed herein have a number of therapeutic applications, including treating hyperproliferative disorders (*e.g.*, cancer). “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. The peptides and modified versions thereof may also be useful for reducing one or more symptoms of or for treating synucleopathies, infectious diseases, and sepsis and for enhancing a response to vaccination.
- [30] “Administer” as used herein includes administration of a disclosed peptide or modified version thereof itself as well as administration by various vehicles described below.
- [31] 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.
- [32] In some embodiments, an engineered T cell that expresses and secretes one or more disclosed peptides can be used to deliver LAG3 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.

[33] 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.

[34] 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).

[35] 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( $\beta$ -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).

[36] 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-difluorotoluy 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.

[37] 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**

[38] 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.

[39] 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.

[40] 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.

[41] 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.

[42] 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.

[43] 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**

[44] 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.

[45] 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

[46] 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.

[47] 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).

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

[49] In some embodiments, the second therapy comprises an agent that reduces or blocks the activity of LAG3 or other inhibitory checkpoint molecules and/or molecules that suppress the immune system. These molecules include, but are not limited to:

1. V-domain Immunoglobulin Suppressor of T cell Activation (VISTA, also known as c10orf54, PD-1H, DD1 $\alpha$ , Gi24, Dies1, and SISP1; see US 2017/0334990, US 2017/0112929, Gao et al., 2017, Wang et al., 2011; Liu et al., 2015);
2. T-cell Immunoglobulin domain and Mucin domain 3 (TIM-3; see US 2017/0198041, US 2017/0029485, US 2014/0348842, Sakuishi et al., 2010);
3. killer immunoglobulin-like receptors (KIRs; see US 2015/0290316);
4. agents that inhibit indoleamine (2,3)-dioxygenase (IDO; see Mellemegaard et al., 2017);
5. B and T Lymphocyte Attenuator (BTLA; see US 2016/09222114); and
6. 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).

[50] Agents that reduce or block the activity of LAG3 include, but are not limited to, BMS-986016, IMP321, and GSK2831781 (He et al., 2016).

[51] 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).

[52] 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.

[53] 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.

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

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

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

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

[58] 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).

[59] 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.

[60] 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.

- [61] Agonists of GITR include, but are not limited to, MEDI1873. See, *e.g.*, Schaer et al., 2012; Tigue et al., 2017.
- [62] 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.
- [63] 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.
- [64] 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).
- [65] 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.
- [66] 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.
- [67] 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.

### **Treatment of Synucleinopathies**

[68] In some embodiments, one or more of the peptides, modified peptides, nucleic acid molecules, CAR-T cells, and/or oncolytic viruses described above may be useful to reduce a symptom of a synucleinopathy, either alone or in combination with other therapeutic interventions such as L-DOPA, dopamine agonists (*e.g.*, ropinirole, pramipexole), dopamine reuptake inhibitors (*e.g.*, amantadine), and cholinesterase inhibitors (*e.g.*, donepezil, rivastigmine, galantamine). Examples of synucleinopathies include idiopathic and inherited forms of Parkinson's disease (PD); Diffuse Lewy Body (DLB) disease, also known as Dementia with Lewy Bodies or Lewy body dementia; incidental Lewy body disease; Lewy body variant of Alzheimer's disease (LBV); Combined Alzheimer's and Parkinson disease (CAPD); pure autonomic failure (PAF); multiple system atrophy (MSA), such as olivopontocerebellar atrophy, striatonigral degeneration, and Shy-Drager Syndrome; pantothenate kinase-associated neurodegeneration; Down's Syndrome; Gaucher disease-related synucleinopathies; and neurodegeneration with brain iron accumulation.

### **Treatment of Sepsis**

[69] LAG3 expression is up-regulated in sepsis (Patil et al., 2017). Accordingly, one or more of the peptides, modified peptides, or nucleic acids described above may be useful to treat sepsis, either alone or in combination with other therapeutic interventions such as antibiotics, intravenous fluids, and vasopressors.

### **Treatment of Infectious Diseases**

[70] In some embodiments, one or more of the disclosed peptides, modified peptides, or nucleic acids described above can be administered to treat infectious diseases, including chronic infections, caused, *e.g.*, by viruses, fungi, bacteria, and protozoa, and helminths, either alone or in combination with other therapeutic interventions.

[71] 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.

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

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

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

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

### Use as Vaccine Adjuvants

[76] In some embodiments one or more of the disclosed peptides, modified peptides, or nucleic acids described above can be administered as a vaccine adjuvant in conjunction with a vaccine 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.

### EXAMPLE 1. Peptide Disruption of LAG3-MHC-II Interaction

[77] A Homogeneous Time-resolved Fluorescence (HTRF) LAG3/MHC-II binding assay (Cisbio US Inc.) was used to measure the interaction between MHC-II and LAG3 in the presence of peptides 11, 16, 62, 68, 115, 141, 146, and 155. In this assay, the interaction between Tag1-LAG3 and Tag2-MHC-II is detected by using anti-Tag1-Terbium (HTRF donor) and anti-Tag2-XL665 (HTRF acceptor). When the donor and acceptor antibodies are brought into close proximity due to LAG3 and MHC-II binding, excitation of the donor antibody triggers fluorescent resonance energy transfer (FRET) towards the acceptor antibody, which in turn emits specifically at 665 nm. This specific signal is directly proportional to the extent of LAG3/MHC-II interaction. Thus, an agent that blocks the interaction between LAG3 and MHC-II will cause a reduction in HTRF ratio.

[78] Tag1-LAG3 protein (10 nM), Tag2-MHCII protein (20 nM), and peptides (3-fold dilutions beginning with 100  $\mu$ M) were combined and incubated for 15 minutes. Anti-Tag1-Tb and anti-Tag2-XL665 antibodies were added and incubated for one hour, then simultaneous

measurement of both 620 nM cryptate and 665 nm acceptor emissions were read. Peptide LG11 (SAPWEPLHWPEDWWQGTGEW; SEQ ID NO:13) served as a positive peptide control. Peptide 93 (RVPAPVKEQVQKQYPNAGAI ; SEQ ID NO:15) served as a negative or low activating peptide control. An anti-human LAG3 antibody (Novoprotein #GMP-A092, Lot 0331158) served as an antibody positive control. The results are shown in Figure 1, and the determined IC<sub>50</sub> values are shown in Table 2, below.

**Table 2. Peptide Disruption of LAG3-MHC-II Interaction**

Peptide ID	IC <sub>50</sub> in LAG3:MHCII TR-FRET Assay, $\mu$ M
11	0.353
16	0.220
62	0.308
68	2.940
115	0.154
141	6.690
146	0.199
155	0.130
Anti-LAG3 mAb	0.001
LG11	18.4
Low Activity Peptide	63

**EXAMPLE 2. Peptide Disruption of LAG3-FGL1 Interaction**

[79] This example tested the ability of peptides to inhibit the interaction between human LAG3 and FGL1. The peptides were tested using a Human LAG3/FGL1 TR-FRET Binding Assay (BPS Bioscience) carried out according to the manufacturer's instructions.

[80] Peptides 6, 41, 68, 69, 115, 146, 154, and 155 were tested at 100, 33, and 11  $\mu$ M. Peptide LG56 (HIQNWSYWLNQDMMNQQVWKS; SEQ ID NO:14) served as a peptide positive control. Peptide 93 (SEQ ID NO:15) served as a negative or low activating peptide control. A

neutralizing anti-human LAG3 antibody (BPS Bioscience Cat. #71219) was used as an antibody positive control.

[81] Reaction mixes were incubated 1 hr at RT before development. After development, plate was read in a Tecan M1000 TR-FRET instrument. The results are shown in Figure 1, and the determined IC<sub>50</sub> values are shown in Table 3, below.

**Table 3. Peptide Disruption of LAG3-FGL1 Interaction**

Peptide ID	IC <sub>50</sub> in LAG3:FGL1 TR-FRET Assay, $\mu$ M
6	2.60
41	9.20
68	>21
69	3.30
115	9.10
146	7.20
154	7.20
155	7.60
Anti-LAG3 mAb	0.02
LG56	14.0
Low Activity Peptide	>100

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**CLAIMS**

1. A peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, and SEQ ID NO:9.
2. A pharmaceutical composition, comprising:
  - (a) a peptide selected from the group consisting of peptide 6 (SEQ ID NO:1), peptide 11 (SEQ ID NO:2), peptide 16 (SEQ ID NO:3), peptide 41 (SEQ ID NO:4), peptide 62 (SEQ ID NO:5), peptide 68 (SEQ ID NO:6), peptide 69 (SEQ ID NO:7), peptide 115 (SEQ ID NO:8), peptide 141 (SEQ ID NO:9), peptide 146 (SEQ ID NO:10), peptide 154 (SEQ ID NO:11), and peptide 155 (SEQ ID NO:12); and
  - (b) a pharmaceutically acceptable vehicle.
3. A method of inhibiting the progression of a hyperproliferative disorder, inhibiting the progression of a synucleinopathy, inhibiting the progression of sepsis, inhibiting the progression of an infectious disease, or enhancing a response to a vaccine, comprising administering to an individual in need thereof an effective amount of a peptide selected from the group consisting of peptide 6 (SEQ ID NO:1), peptide 11 (SEQ ID NO:2), peptide 16 (SEQ ID NO:3), peptide 41 (SEQ ID NO:4), peptide 62 (SEQ ID NO:5), peptide 68 (SEQ ID NO:6), peptide 69 (SEQ ID NO:7), peptide 115 (SEQ ID NO:8), peptide 141 (SEQ ID NO:9), peptide 146 (SEQ ID NO:10), peptide 154 (SEQ ID NO:11), and peptide 155 (SEQ ID NO:12).
4. The method of claim 3, wherein the peptide is administered to inhibit progression of the hyperproliferative disorder.
5. The method of claim 3, wherein the hyperproliferative disorder is a cancer.
6. The method of claim 4, wherein the cancer is a melanoma.
7. The method of claim 3, further comprising administering a second therapy.
8. The method of claim 6, 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 (LAG3), 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.

9. The method of any one of claims 3-8, wherein the peptide is selected from the group consisting of peptides peptide 11 (SEQ ID NO:2), peptide 16 (SEQ ID NO:3), peptide 62 (SEQ ID NO:5), peptide 68 (SEQ ID NO:6), peptide 115 (SEQ ID NO:8), peptide 141 (SEQ ID NO:9), peptide 146 (SEQ ID NO:10), and peptide 155 (SEQ ID NO:12).

10. The method of claim 3, wherein the peptide is administered to inhibit the progression of a synucleinopathy.

11. The method of claim 10, wherein the synucleinopathy is selected from the group consisting of Parkinson's disease (PD), dementia with Lewy bodies (DLB), pure autonomic failure (PAF), and multiple system atrophy (MSA).

12. The method of claim 10 or claim 11, wherein the peptide is selected from the group consisting of peptide 6 (SEQ ID NO:1), peptide 41 (SEQ ID NO:4), peptide 68 (SEQ ID NO:6), peptide 115 (SEQ ID NO:8), peptide 146 (SEQ ID NO:10), peptide 154 (SEQ ID NO:11), and peptide 155 (SEQ ID NO:12).

13. The method of claim 3, wherein the peptide is administered to inhibit the progression of sepsis.

14. The method of claim 3, wherein the peptide is administered to inhibit the progression of an infectious disease.

15. The method of claim 3, wherein the peptide is administered to enhance a response to a vaccine.

16. The method of any one of claims 3-15, wherein the peptide is selected from the group consisting of peptide 68 (SEQ ID NO:6), peptide 115 (SEQ ID NO:8), peptide 146 (SEQ ID NO:10), peptide 155 (SEQ ID NO:12).

IC50 in LAG3:FGL1 and LAG3:MHCII TR-FRET Assays

LAG3:FGL1 TR-FRET Screen (BPS)		LAG3:MHC TR-FRET Screen (Cisbio)			
% Inhibition at 100 µM	% Inhibition at 33 µM	% Inhibition at 11 µM	% Inhibition at 6.25 µM	% Inhibition at 0.39 µM	Notes
6	100	99	24	8	high FGL low MHC
41	99	98	29	2	high FGL low MHC
68	97	78	48	24	high FGL medium MHC
69	99	73	11	-2	high FGL low MHC
115	99	98	66	65	high FGL high MHC
146	92	89	46	50	high FGL high MHC
154	99	79	26	37	high FGL medium MHC
155	99	93	53	61	high FGL high MHC
LAG3:FGL1 TR-FRET Screen (BPS)		LAG3:MHC TR-FRET Screen (Cisbio)			
% Inhibition at 100 µM	% Inhibition at 33 µM	% Inhibition at 11 µM	% Inhibition at 6.25 µM	% Inhibition at 0.39 µM	Notes
11	62	66	66	49	medium FGL high MHC
16	99	47	58	56	high FGL high MHC
62	7	5	60	35	high FGL medium MHC
68	97	78	48	24	high FGL medium MHC
115	99	98	66	65	high FGL high MHC
141	80	7	44	33	medium FGL medium MHC
146	92	89	46	50	high FGL high MHC
155	99	93	53	61	high FGL high MHC

Figure 1