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(71) Applicant: THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL [US/US]; 109 Church Street, Chapel Hill, North Carolina 27516 (US).

(72) Inventors: LI, Chengwen; 1809 Spring Overlook Lane, Hillsborough, North Carolina 27278 (US). SUN, Junjiang; 117 Vintage Drive, Chapel Hill, North Carolina 27516 (US). LI, Wenjun; 202 McGregor Drive, Chapel Hill, North Carolina 27514 (US). LOESER, Richard; 12008 Wicker Drive, Chapel Hill, North Carolina 27517 (US).

(74) Agent: SCHWARTZMAN, Robert, A.; 4130 Parklake Avenue, Ste. 250, Raleigh, North Carolina 27612 (US).

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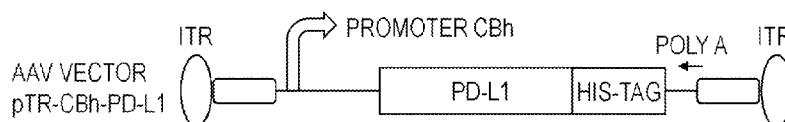


FIG. 1A

(57) Abstract: Provided herein according to some embodiments are methods of treating an autoimmune condition such as arthritis or graft rejection in a subject in need thereof, comprising administering an effective amount of an immune checkpoint protein, functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein. Methods of delaying onset of an autoimmune condition, e.g., arthritis, or graft rejection in a subject, and compositions useful for the treatment of the autoimmune condition or graft rejection are also provided.

COMPOSITIONS AND METHODS FOR TREATMENT OF AUTOIMMUNE CONDITIONS

STATEMENT OF PRIORITY

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 63/480,462, filed January 18, 2023, the entire contents of which are incorporated by reference herein.

FIELD OF THE INVENTION

[0002] This invention relates to compositions and methods for delaying the onset of or treating an autoimmune condition, *e.g.*, arthritis, or graft rejection in a subject in need thereof; including the use of compositions of checkpoint proteins or nucleic acid delivery vectors encoding checkpoint proteins, for example, PD-L1, for delaying the onset of or treating an autoimmune condition or graft rejection in a subject in need thereof.

STATEMENT REGARDING ELECTRONIC FILING OF A SEQUENCE LISTING

[0003] A Sequence Listing in XML format, entitled 5470-944WO_ST26.xml, 43,741 bytes in size, generated on January 12, 2024, and filed herewith, is hereby incorporated by reference in its entirety for its disclosures.

BACKGROUND

[0004] Autoimmune and alloimmune responses against self and non-self antigens are destructive immune responses that can result in tissue damage and graft rejection. Autoimmune conditions, despite their differences, share some common symptomatic threads including fatigue, low-grade fever, malaise, muscle aches, joint pain and/or skin rashes. The impact of autoimmune conditions can range from localized damage to certain tissues, alteration in organ growth and function, to more systemic effects when multiple tissues throughout the body are affected. Commonly known autoimmune conditions include, *e.g.*, celiac disease, Graves' disease, inflammatory bowel disease, multiple sclerosis, arthritis, systemic lupus erythematosus and Type 1 diabetes.

[0005] Rheumatoid arthritis (RA) and osteoarthritis (OA) are the most common types of arthritis. Current therapies for arthritis have aimed at reducing synovial inflammation and pain and preventing joint destruction by targeting pro-inflammatory cytokines or immune cells. The introduction of tumor necrosis factor (TNF) antagonists and other biologics has

dramatically improved arthritis treatment, especially in patients with RA. However, a majority of patients do not respond to these drugs. Additionally, these biologics are very expensive and systemic administration induces some severe complications, such as bacterial infection. Intra-articular (IA) delivery of biologics can lower the likelihood of adverse events in non-target organs and maximizes the concentration of the therapeutics in the joints at a lower cost. However, the effectiveness of IA therapy with biologics is greatly impacted due to the rapid clearance of biologics and their efficiency in the synovial space. It is difficult to achieve a sustained and therapeutic concentration of a drug in the affected joints. Moreover, a long-term therapeutic effect requires repeated administration of drugs regardless of delivery routes.

[0006] Delaying the onset or preventing progression of autoimmune conditions such as arthritis, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, psoriasis, and the like with sustained effective treatment in affected tissues without deleterious systemic effects is needed in the art.

SUMMARY OF THE INVENTION

[0007] The present invention is based, in part, on the development of therapies targeting immune checkpoints to delay the onset of, prevent progression of, or treat an autoimmune condition or graft rejection. In some embodiments, the invention provides methods for treating an autoimmune condition or graft rejection in a subject in need thereof, comprising administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or a functional fragment or derivative thereof.

[0008] In an embodiment, a method for delaying onset of an autoimmune condition or graft rejection in a subject is provided, comprising administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or functional fragment or derivative thereof.

[0009] In some embodiments, the immune checkpoint protein is selected from PD-1, PD-L1, PD-L2, CTLA4, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, A2AR, TIM-3, and VISTA. In some embodiments, the immune checkpoint protein is PD-L1 protein, or a nucleic acid molecule encoding PD-L1 protein. The PD-L1 protein can be a soluble protein or a transmembrane protein. In an aspect, the immune checkpoint protein further comprises a signal peptide, *e.g.*, a fibronectin, collagen II, or *Gaussia luciferase* signal peptide.

[0010] In some methods, administering is by intraarticular (IA), intramuscular (IM) or intravenous (IV) injection. In an aspect, delivery is via a nucleic acid delivery vector, *e.g.*, a viral vector, nanoparticle, or hydrogel. In some embodiments, the viral vector is an AAV vector.

[0011] In an aspect, the autoimmune condition is multiple sclerosis, systemic lupus erythematosus, systemic sclerosis, an idiopathic inflammatory myopathy, systemic vasculitis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, diabetes, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, autoimmune hepatitis, primary biliary cirrhosis, inflammatory bowel disease, polymyalgia rheumatica, alopecia, vasculitis, celiac disease, a bullous skin disease, psoriasis, asthma, uveitis, autoimmune retinopathy, or arthritis, *e.g.*, osteoarthritis, juvenile arthritis, reactive arthritis, gout, psoriatic arthritis, fibromyalgia, ankylosing spondylitis or rheumatoid arthritis, Felty's syndrome, diffuse idiopathic skeletal hyperostosis (DISH), Behçet's disease, inflammatory arthritis, mixed connective tissue disease, infectious arthritis, Granulomatosis with polyangiitis (GPA), Mixed Connective Tissue disease (MCTD), myositis (dermatomyositis, polymyositis), Paget's disease, Pseudogout, polymyalgia rheumatica with giant cell arteritis, Raynaud's Phenomenon, scleroderma, systemic lupus erythematosus, or Sjögren syndrome.

[0012] Nucleic acid delivery vectors comprising a nucleic acid encoding an immune checkpoint protein or a functional fragment or derivative thereof are also provided herein.

[0013] These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **FIGS. 1A-1D.** PD-L1 gene expression and function *in vitro*. **FIG. 1A.** Schematic diagram of PD-L1 cassette. **FIG. 1B.** Immunoblotting analysis of PD-L1 protein in HEK-293 cells. a, PD-L1; b, GFP; c, recombinant PD-L1 positive control. **FIG. 1C.** Representative flow cytometry data for proliferation of pan T cells with the cell trace violet staining. **FIG. 1D.** Quantification of T cell proliferation *in vitro*. Data were analyzed using one-way ANOVA. * $p < 0.05$.

[0015] **FIGS. 2A-2D.** Prevention effect of PD-L1 on arthritis in CIA mice. **FIG. 2A.** Schematic diagram of PD-L1 gene delivery in CIA mice. **FIG. 2B.** Representative histopathology of joints. **FIG. 2C.** The size change of knee joint swelling upon prophylaxis treatment. **FIG. 2D.** The scores (0-12) of knee joint histological analysis by prophylaxis treatment. Data were analyzed using one-way ANOVA.

[0016] **FIGS. 3A-3C.** Infiltration of T-cells and macrophages in the synovium in CIA mice with PD-L1 prophylaxis treatment. **FIG. 3A.** Representative images of in situ immunohistochemical staining of T cells and macrophages (magnification, $\times 400$). **FIG. 3B.** The CD3 positive staining cell percentage. **FIG. 3C.** The CD68 positive staining cell percentage. Data were analyzed using one-way ANOVA.

[0017] **FIG. 4.** Cytokine levels in synovial fluid of joints treated with AAV5-PD-L1 in CIA mice. Data were analyzed using unpaired t test or one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, ***, $p < 0.005$.

[0018] **FIGS. 5A-5C** Therapeutic effect of PD-L1 on arthritis in CIA mice. **FIG. 5A.** Schematic diagram of PD-L1 gene delivery in CIA mice. **FIG. 5B.** The size change of knee joints upon therapeutic treatment. **FIG. 5C.** Knee joint pathohistological scores (0-12) upon therapeutic treatment. Data were analyzed using one-way ANOVA, ****, $p < 0.001$.

[0019] **FIG. 6.** PD-L1 was detected in knee joint but not in sera of CIA mice. Lane 1: Sera at week 1 post-AAV5-PD-L1 intra-articular injection; Lane 2: Sera at week 7 post-AAV5-PD-L1 intra-articular injection; Lane 3: Knee at week 7 post-AAV5-luc intra-articular injection; Lane 4: Knee at week 7 post-AAV5-PD-L1 intra-articular injection.

[0020] **FIG. 7** AAV-PD-L1 vector bio-distribution in CIA mice after intra-articular injection. AAV copy number was detected by qPCR in liver, knee, heart, lung, spleen, and kidney. Data were analyzed using one-way ANOVA. ****, $p < 0.001$.

[0021] **FIG. 8** Cytokine levels in serum with intra-articular injection of AAV5-PD-L1 in CIA mice. Data were analyzed using unpaired t test or one-way ANOVA.

[0022] **FIG. 9** AAV5-PD-L1 intra-articular gene therapy prevented OA progression.

[0023] **FIG. 10** Diagrams of exemplary PD-L1 cassettes: wtPD-L1 (**SEQ ID NO:1**) encoding wtPD-L1 transmembrane protein (**SEQ ID NO:10**); h-PD-L1 (**SEQ ID NO:3**) encoding h-PD-L1 soluble protein (**SEQ ID NO:19**); sec-PD-L1 (**SEQ ID NO:4**) encoding sec-PD-L1 soluble protein (**SEQ ID NO:12**); and sh-PD-L1 (**SEQ ID NO:2**) encoding sh-PD-L1 soluble protein (**SEQ ID NO:18**).

[0024] **FIG. 11.** Expression of secreted PD-L1 in supernatant. a, Cell supernatant; b, Cell lysate.

[0025] **FIGS. 12A-12C** High PD-L1 expression from secreted PD-L1 cassette. **FIG. 12A.** PD-L1 expression in supernatant (a) and cell lysate (b). **FIG. 12B.** the whole PD-L1 expression, *i.e.*, a mixture of supernatant and cell lysates (c). **FIG. 12C.** Quantitation of relative whole PD-L1 expression.

[0026] **FIGS. 13A-13C** Soluble PD-L1 from AAV5-shPD-L1 intra-articular injection prevented arthritis development in CIA mice. **FIG. 13A.** A representative histology of the joints. **FIG. 13B.** The change of joint swelling. **FIG. 13C.** Histological analysis of joint inflammation at week 7 (scores 1-12).

[0027] **FIGS. 14A-14B.** Inhibition of immune cell infiltration after intra-articular administration of AAV5-sh-PD-L1. **FIG. 14A.** Percent positive staining in CD3 T cells. **FIG. 14B.** Percent positive staining in CD68 macrophages.

[0028] **FIG. 15.** Intra-articular injection of AAV5 vectors encoding sh-PD-L1 induced better arthritis prevention in CIA mice.

[0029] **FIG. 16.** Intravenous administration of AAV8-sh-PD-L1 prevents arthritis in CIA mice.

[0030] **FIG. 17.** Intravenous administration of AAV8-sh-PD-L1 decreases the production of inflammatory cytokines in CIA mice.

[0031] **FIG. 18.** Intramuscular injection of AAV6-sh-PD-L1 improves arthritis in CIA mice.

[0032] **FIG. 19.** Intramuscular injection of AAV6-sh-PD-L1 induces lower inflammatory cytokines in CIA mice.

DETAILED DESCRIPTION

[0033] The present invention is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure which do not depart from the instant invention. Hence, the following specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations, and variations thereof.

[0034] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically

intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0035] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. All publications, patent applications, patents, patent publications and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

[0036] Amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by either the one-letter code, or the three-letter code, both in accordance with 37 C.F.R. §1.831 and established usage.

[0037] Except as otherwise indicated, standard methods known to those skilled in the art may be used for cloning genes, amplifying, and detecting nucleic acids, and the like. Such techniques are known to those skilled in the art. *See, e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual 4th Ed. (Cold Spring Harbor, NY, 2012); Ausubel *et al.* Current Protocols in Molecular Biology (Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York).

[0038] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination.

[0039] Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted.

[0040] To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[0041] As used in the description of the invention and the appended claims, the singular forms “a,” “an,” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0042] Also as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0043] The term “about,” as used herein when referring to a measurable value such as an amount of polypeptide, dose, time, temperature, enzymatic activity or other biological

activity and the like, is meant to encompass variations of $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

[0044] As used herein, the transitional phrase “consisting essentially of” (and grammatical variants) is to be interpreted as encompassing the recited materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term “consisting essentially of” as used herein should not be interpreted as equivalent to “comprising.”

[0045] The term “consists essentially of” (and grammatical variants), as applied to a polypeptide or polynucleotide sequence of this invention, means a polypeptide or polynucleotide that consists of both the recited sequence (*e.g.*, SEQ ID NO) and a total of ten or less (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) additional amino acids on the N-terminal and/or C-terminal ends of the recited sequence or additional nucleotides on the 5' and/or 3' ends of the recited sequence such that the function of the polypeptide or polynucleotide is not materially altered. The total of ten or less additional amino acids or nucleotides includes the total number of additional amino acids or nucleotides on both ends added together. The term “materially altered,” as applied to polypeptides of the invention, refers to an increase or decrease in biological activities/properties (*e.g.*, PD-L1 protein activity) of at least about 50% or more as compared to the activity of a polypeptide consisting of the recited sequence.

[0046] The term “modified” protein as used herein refers to the addition, deletion, and/or substitution of one or more amino acids from the wild-type protein, wherein the modified protein substantially retains at least one biological activity normally associated with that polypeptide (*e.g.*, wild-type immune checkpoint protein or fragment thereof). In some embodiments, modified immune checkpoint protein is a modified PD-L1 protein, *e.g.*, a soluble PD-L1 protein.

[0047] As used herein, the term “polypeptide” encompasses both peptides and proteins, unless indicated otherwise.

[0048] As used herein, a “functional” polypeptide or “functional fragment” is one that substantially retains at least one biological activity normally associated with that polypeptide (*e.g.*, wild-type protein or fragment thereof). In particular embodiments, the “functional” polypeptide or “functional fragment” substantially retains all of the activities possessed by the unmodified polypeptide (*e.g.*, wild-type protein or fragment thereof). By “substantially retains” biological activity, it is meant that the polypeptide retains at least about 20%, 30%, 40%, 50%, 60%, 75%, 85%, 90%, 95%, 97%, 98%, 99%, or more, of the biological activity

of the native polypeptide (and can even have a higher level of activity than the native polypeptide). A “non-functional” polypeptide is one that exhibits little or essentially no detectable biological activity normally associated with the polypeptide (*e.g.*, at most, only an insignificant amount, *e.g.*, less than about 10% or even 5%). Biological activities such as binding activity and/or activation or proliferation of various cells, including T-cells and macrophages, can be measured using assays that are well known in the art and as described herein.

[0049] The term “fragment,” as applied to a peptide, will be understood to mean an amino acid sequence of reduced length relative to a reference peptide (*e.g.*, wild-type protein) or amino acid sequence and comprising, consisting essentially of, and/or consisting of an amino acid sequence of contiguous amino acids identical to the reference peptide or amino acid sequence. Such a peptide fragment according to the invention may be, where appropriate, included in a larger polypeptide of which it is a constituent. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of peptides having a length of at least about 5, 10, 15, 20, 25, 30, 35, 46, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 or more consecutive amino acids of a peptide or amino acid sequence according to the invention.

[0050] A “derivative” as applied to a peptide, will be understood to mean an amino acid sequence that is a conjugate or mutant derived from a reference peptide, with a mutant having a sequence in which one or more amino acid residues has been altered relative to the reference peptide or wherein one or more amino acids have been inserted into or deleted from the sequence of the reference peptide. Derivatives include conjugates of a peptide having an amino acid sequence derived from reference peptide that is contiguous with one or more polypeptides having amino acid sequences derived from proteins other than the protein of interest (*e.g.*, signal sequence); and other conjugates, such as those wherein the reference peptide or a fragment derived therefrom is chemically bonded to one or more non-proteinaceous moieties.

[0051] The terms “polynucleotide,” “nucleic acid,” “nucleic acid molecule,” and “oligonucleotide” are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof, or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotides). Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant

polynucleotides, branched polynucleotides, plasmids, vectors, genomic DNA, chimeras of RNA and DNA, isolated DNA of any sequence, isolated RNA of any sequence, synthetic DNA of any sequence (*e.g.*, chemically synthesized), synthetic RNA of any sequence (*e.g.*, chemically synthesized), nucleic acid probes and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs or derivatives (*e.g.*, inosine or phosphorothioate nucleotides). Such nucleotides can be used, for example, to prepare nucleic acid molecules that have altered base-pairing abilities or increased resistance to nucleases.

[0052] If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules.

[0053] The term “regulatory element” refers to a genetic element which controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element which facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements are splicing signals, polyadenylation signals, termination signals, *etc.* The region in a nucleic acid sequence or polynucleotide in which one or more regulatory elements are found may be referred to as a “regulatory region.”

[0054] As used herein, “expression” refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[0055] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) having a certain percentage (for example, 80%, 85%, 90%, or 95%) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. The alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example, those described in Current Protocols in Molecular Biology (Ausubel *et al.*, eds. 1987) Supplement 30, section 7.7.18, Table 7.7.1. Sequence identity or similarity may be determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the sequence identity alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444 (1988), by

computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux *et al.*, *Nucl. Acid Res.* 12:387 (1984), preferably using the default settings, or by inspection. An example of a useful algorithm is the BLAST algorithm, described in Altschul *et al.*, *J. Mol. Biol.* 215:403 (1990) and Karlin *et al.*, *Proc. Natl. Acad. Sci. USA* 90:5873 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul *et al.*, *Meth. Enzymol.*, 266:460 (1996); blast.wustl.edu/blast/README.html. WU-BLAST-2 uses several search parameters, which are preferably set to the default values. The parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity. An additional useful algorithm is gapped BLAST as reported by Altschul *et al.*, *Nucleic Acids Res.* 25:3389 (1997).

[0056] A signal peptide is a short peptide of around 15 to 40 amino acids in length typically found at the N-terminus on proteins that signals location for protein transfer, for example, for secretion. Signal peptides typically contain an h-region, which is a stretch of hydrophobic amino acids, *e.g.*, 5 to 16 residues in length, that often form a single alpha helix. Edman degradation can be utilized to identify the N-terminal signal peptide. In an aspect, a signal peptide may be modified to adjust the efficiency of protein secretion. A signal peptide from another protein can be used with a protein of interest to modify secretion properties, *e.g.*, operably linked to express a protein of the invention with enhanced secretion properties in a joint.

[0057] As used herein, the term “operably linked” means that the promoter and coding sequence are joined together in a manner that allows them to carry out their normal functions, *i.e.*, transcription of the coding sequence is under the control of the promoter and the transcript produced is correctly translated into the desired product.

[0058] Those skilled in the art will appreciate that a variety of promoters may be used depending on the level and specific expression desired. The promoter may be constitutive or regulatable, depending on the pattern of expression desired. The promoter may be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced.

[0059] The promoter can be native to the target cell or subject to be treated and/or can be native to the heterologous nucleotide sequence. The promoter is generally chosen so that it will function in the target cell(s) of interest. The promoter can optionally be a mammalian promoter. The promoter may further be constitutive or regulatable (*e.g.*, inducible).

[0060] Promoters for nucleic acid delivery can be tissue-preferred and/or tissue-specific promoters. In some embodiments, the promoter is joint-specific or joint-preferred.

[0061] The term “vector” is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be “exogenous,” which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. A “delivery vector” refers to a vector used as a vehicle to carry foreign nucleic acids into another cell, where it can be replicated and/or expressed. A cloning vector containing foreign nucleic acid is termed a recombinant vector. In addition to a nucleic acid of interest, a vector may also comprise one or more regulatory regions, and/or selectable markers useful in selecting, measuring, and monitoring nucleic acid transfer results (delivery to specific tissues, duration of expression, etc.). Recombinant vectors typically contain an origin of replication, a multicloning site, and a selectable marker. The nucleic acid sequence typically consists of an insert (recombinant nucleic acid or transgene) and a larger sequence that serves as the “backbone” of the vector. Expression vectors (expression constructs or expression cassettes) are for the expression of the exogenous gene in the target cell, and generally have a promoter sequence that drives expression of the exogenous gene/open reading frame (ORF). Insertion of a vector into the target cell is referred to transformation or transfection for bacterial and eukaryotic cells, although insertion of a viral vector is often called transduction. The term “vector” may also be used in general to describe vehicles that serve to carry foreign genetic material into another cell, such as, but not limited to, a transformed cell or a nanoparticle.

[0062] Vectors include plasmids, cosmids, and viruses (bacteriophage, animal viruses, and plant viruses). In some embodiments, the vector is a viral vector, optionally an adeno-associated virus (AAV) vector. Viral vectors have been used in a wide variety of gene delivery applications in cells, as well as living animal subjects. Viral vectors that can be used include, but are not limited to, retrovirus, lentivirus, adeno-associated virus, poxvirus, alphavirus, baculovirus, vaccinia virus, herpes virus, Epstein-Barr virus, and/or adenovirus vectors. Non-viral vectors include, but are not limited to, plasmids, liposomes, electrically

charged lipids (cytofectins), nucleic acid-protein complexes, and biopolymers. Vectors may be introduced into the desired cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a nucleic acid vector transporter (*see, e.g.*, Wu *et al.*, J. Biol. Chem. 267:963 (1992); Wu *et al.*, J. Biol. Chem. 263:14621 (1988); and Hartmut *et al.*, Canadian Patent Application No. 2,012,311, filed Mar. 15, 1990).

[0063] A “recombinant AAV vector genome” or “rAAV genome” is an AAV genome (*i.e.*, vDNA) that comprises at least one inverted terminal repeat (*e.g.*, one, two or three inverted terminal repeats) and one or more heterologous nucleotide sequences. rAAV vectors generally retain the 145 base terminal repeat(s) (TR(s)) in *cis* to generate virus; however, modified AAV TRs and non-AAV TRs including partially or completely synthetic sequences can also serve this purpose. All other viral sequences are dispensable and may be supplied in *trans* (Muzyczka, (1992) *Curr. Topics Microbiol. Immunol.* 158:97). The rAAV vector optionally comprises two TRs (*e.g.*, AAV TRs), which generally will be at the 5' and 3' ends of the heterologous nucleotide sequence(s), but need not be contiguous thereto. The TRs can be the same or different from each other. The vector genome can also contain a single ITR at its 3' or 5' end.

[0064] The term “terminal repeat” or “TR” includes any viral terminal repeat or synthetic sequence that forms a hairpin structure and functions as an inverted terminal repeat (ITR) (*i.e.*, mediates the desired functions such as replication, virus packaging, integration and/or provirus rescue, and the like). The TR can be an AAV ITR or a non-AAV TR. For example, a non-AAV TR sequence such as those of other parvoviruses (*e.g.*, canine parvovirus (CPV), mouse parvovirus (MVM), human parvovirus B-19) or the SV40 hairpin that serves as the origin of SV40 replication can be used as a TR, which can further be modified by truncation, substitution, deletion, insertion and/or addition. Further, the TR can be partially or completely synthetic, such as the “double-D sequence” as described in United States Patent No. 5,478,745 to Samulski *et al.*

[0065] Parvovirus genomes have palindromic sequences at both their 5' and 3' ends. The palindromic nature of the sequences leads to the formation of a hairpin structure that is stabilized by the formation of hydrogen bonds between the complementary base pairs. This hairpin structure is believed to adopt a “Y” or a “T” shape. *See, e.g.*, Fields, *et al.*, *Virology*, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0066] An “AAV inverted terminal repeat” or “AAV ITR” may be from any AAV, including but not limited to serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 or any other AAV now known or later discovered (*see, e.g., Table 1*). An AAV ITR need not have the native ITR sequence (*e.g., a native AAV ITR sequence may be altered by insertion, deletion, truncation and/or missense mutations*), as long as the ITR mediates the desired functions, *e.g., replication, virus packaging, integration, and/or provirus rescue, and the like.*

[0067] The terms “rAAV particle” and “rAAV virion” are used interchangeably here. A “rAAV particle” or “rAAV virion” comprises a rAAV vector genome packaged within an AAV capsid.

[0068] As used herein, the term “adeno-associated virus” (AAV) includes but is not limited to, AAV serotype 1 (AAV1), AAV2, AAV3 (including types 3A and 3B), AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, and any other AAV now known or later discovered. *See, e.g., Bernard N. Fields et al., Virology, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers).* Recently, a number of putative new AAV serotypes and clades have been identified (*see, e.g., Gao et al., (2004) J. Virology 78:6381-6388; Moris et al., (2004) Virology 33:375-383; and Table 1*). An AAV can be selected for tissue-specific delivery. AAV9 variants, for example AAV-PHP.B can be used for where desired to cross the blood brain barrier. AAV variants with reduced immunogenicity may also be utilized, and may comprise chimeric AAV, for example, AAV-DJ. Design strategies for AAV vectors may also be employed for the delivery of the [modified protein] according to the present invention. *See, e.g., Lee, et al. (2018) Adeno-associated virus (AAV) vectors: rational design strategies for capsid engineering. Curr. Opin. Biomed. Eng., 7, 58-63; see also Parambi et al., 2021 Oct 15, Mol Neurobiol. 2022; 59(1): 191-233, doi:10.1007/s12035-021-02555-y, incorporated herein by reference in its entirety, and specifically Table 1 for teachings of viral vectors.*

[0069] The genomic sequences of various serotypes of AAV and the autonomous parvoviruses, as well as the sequences of the native ITRs, Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. *See, e.g., GenBank Accession Numbers NC_002077, NC_001401, NC_001729, NC_001863, NC_001829, NC_001862, NC_000883, NC_001701, NC_001510, NC_006152, NC_006261, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, AY631966, AX753250, EU285562, NC_001358, NC_001540, AF513851, AF513852 and AY530579; the disclosures of which are incorporated by reference herein for teaching parvovirus and*

AAV nucleic acid and amino acid sequences. *See also, e.g., Bantel-Schaal et al., (1999) J. Virol.* 73: 939; Chiorini *et al., (1997) J. Virol.* 71:6823; Chiorini *et al., (1999) J. Virol.* 73:1309; Gao *et al., (2002) Proc. Nat. Acad. Sci. USA* 99:11854; Moris *et al., (2004) Virol.* 33-:375-383; Mori *et al., (2004) Virol.* 330:375; Muramatsu *et al., (1996) Virol.* 221:208; Ruffing *et al., (1994) J. Gen. Virol.* 75:3385; Rutledge *et al., (1998) J. Virol.* 72:309; Schmidt *et al., (2008) J. Virol.* 82:8911; Shade *et al., (1986) J. Virol.* 58:921; Srivastava *et al., (1983) J. Virol.* 45:555; Xiao *et al., (1999) J. Virol.* 73:3994; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Patent No. 6,156,303; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. *See also Table 1.* An early description of the AAV1, AAV2 and AAV3 ITR sequences is provided by Xiao, X., (1996), “Characterization of Adeno-associated virus (AAV) DNA replication and integration,” Ph.D. Dissertation, University of Pittsburgh, Pittsburgh, PA (incorporated herein in its entirety).

Table 1

AAV Serotypes/ Isolates	GenBank Accession Number	AAV Serotypes/ Isolates	GenBank Accession Number	AAV Serotypes/ Isolates	GenBank Accession Number
Clonal Isolates		Hu S17	AY695376	Cy3	AY243019
Avian AAV ATCC VR- 865	AY186198, AY629583, NC_004828	Hu T88	AY695375	Cy5	AY243017
Avian AAV strain DA-1	NC_006263, AY629583	Hu T71	AY695374	Rh13	AY243013
Bovine AAV	NC_005889, AY388617	Hu T70	AY695373		
AAV4	NC_001829	Hu T40	AY695372	Clade E	
AAV5	AY18065, AF085716	Hu T32	AY695371	Rh38	AY530558
Rh34	AY243001	Hu T17	AY695370	Hu66	AY530626
Rh33	AY243002	Hu LG15	AY695377	Hu42	AY530605
Rh32	AY243003			Hu67	AY530627
AAV10	AY631965	Clade C		Hu40	AY530603
AAV11	AY631966	AAV 3	NC_001729	Hu41	AY530604
AAV12	DQ813647	AAV 3B	NC_001863	Hu37	AY530600
AAV13	EU285562	Hu9	AY530629	Rh40	AY530559
		Hu10	AY530576	Rh2	AY243007
Clade A		Hu11	AY530577	Bb1	AY243023
AAV1	NC_002077, AF063497	Hu53	AY530615	Bb2	AY243022
AAV6	NC_001862	Hu55	AY530617	Rh10	AY243015
Hu.48	AY530611	Hu54	AY530616	Hu17	AY530582
Hu 43	AY530606	Hu7	AY530628	Hu6	AY530621
Hu 44	AY530607	Hu18	AY530583	Rh25	AY530557
Hu 46	AY530609	Hu15	AY530580	Pi2	AY530554

		Hu16	AY530581	Pi1	AY530553
Clade B		Hu25	AY530591	Pi3	AY530555
Hu19	AY530584	Hu60	AY530622	Rh57	AY530569
Hu20	AY530586	Ch5	AY243021	Rh50	AY530563
Hu23	AY530589	Hu3	AY530595	Rh49	AY530562
Hu22	AY530588	Hu1	AY530575	Hu39	AY530601
Hu24	AY530590	Hu4	AY530602	Rh58	AY530570
Hu21	AY530587	Hu2	AY530585	Rh61	AY530572
Hu27	AY530592	Hu61	AY530623	Rh52	AY530565
Hu28	AY530593			Rh53	AY530566
Hu29	AY530594	Clade D		Rh51	AY530564
Hu63	AY530624	Rh62	AY530573	Rh64	AY530574
Hu64	AY530625	Rh48	AY530561	Rh43	AY530560
Hu13	AY530578	Rh54	AY530567	AAV8	AF513852
Hu56	AY530618	Rh55	AY530568	Rh8	AY242997
Hu57	AY530619	Cy2	AY243020	Rh1	AY530556
Hu49	AY530612	AAV7	AF513851		
Hu58	AY530620	Rh35	AY243000	Clade F	
Hu34	AY530598	Rh37	AY242998	AAV9 (Hu14)	AY530579
Hu35	AY530599	Rh36	AY242999	Hu31	AY530596
AAV2	NC 001401	Cy6	AY243016	Hu32	AY530597
Hu45	AY530608	Cy4	AY243018		
Hu47	AY530610				
Hu51	AY530613				
Hu52	AY530614				
Hu T41	AY695378				

[0070] A “chimeric” AAV nucleic acid capsid coding sequence or AAV capsid protein is one that combines portions of two or more capsid sequences. A “chimeric” AAV virion or particle comprises a chimeric AAV capsid protein.

[0071] The term “tropism” as used herein refers to preferential but not necessarily exclusive entry of the vector (*e.g.*, virus vector) into certain cell or tissue type(s) and/or preferential but not necessarily exclusive interaction with the cell surface that facilitates entry into certain cell or tissue types, optionally and preferably followed by expression (*e.g.*, transcription and, optionally, translation) of sequences carried by the vector contents (*e.g.*, viral genome) in the cell, *e.g.*, for a recombinant virus, expression of the heterologous nucleotide sequence(s). Those skilled in the art will appreciate that transcription of a heterologous nucleic acid sequence from the viral genome may not be initiated in the absence of trans-acting factors, *e.g.*, for an inducible promoter or otherwise regulated nucleic acid sequence. In the case of a rAAV genome, gene expression from the viral genome may be from a stably integrated provirus and/or from a non-integrated episome, as well as any other form which the virus nucleic acid may take within the cell.

[0072] The term “tropism profile” refers to the pattern of transduction of one or more target cells, tissues and/or organs. Representative examples of chimeric AAV capsids have a tropism profile characterized by efficient transduction of cells of the central nervous system (CNS) with only low transduction of peripheral organs (*see e.g.*, US Patent No. 9,636,370 McCown *et al.*, and US patent publication 2017/0360960 Gray *et al.*). Vectors (*e.g.*, virus vectors, *e.g.*, AAV capsids) expressing specific tropism profiles may be referred to as “tropic” for their tropism profile, *e.g.*, neuro-tropic, liver-tropic, *etc.*

[0073] As used herein, “transduction” of a cell by a virus vector (*e.g.*, an AAV vector) means entry of the vector into the cell and transfer of genetic material into the cell by the incorporation of nucleic acids into the virus vector and subsequent transfer into the cell via the virus vector. Unless indicated otherwise, “efficient transduction” or “efficient tropism,” or similar terms, can be determined by reference to a suitable positive or negative control (*e.g.*, at least about 50%, 60%, 70%, 80%, 85%, 90%, 95% or more of the transduction or tropism, respectively, of a positive control or at least about 110%, 120%, 150%, 200%, 300%, 500%, 1000% or more of the transduction or tropism, respectively, of a negative control).

[0074] As used herein, parvovirus or AAV “Rep coding sequences” indicate the nucleic acid sequences that encode the parvoviral or AAV non-structural proteins that mediate viral replication and the production of new virus particles. The parvovirus and AAV replication genes and proteins have been described in, *e.g.*, Fields *et al.*, *Virology*, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0075] The “Rep coding sequences” need not encode all of the parvoviral or AAV Rep proteins. For example, with respect to AAV, the Rep coding sequences do not need to encode all four AAV Rep proteins (Rep78, Rep 68, Rep52 and Rep40), in fact, it is believed that AAV5 only expresses the spliced Rep68 and Rep40 proteins. In representative embodiments, the Rep coding sequences encode at least those replication proteins that are necessary for viral genome replication and packaging into new virions. The Rep coding sequences will generally encode at least one large Rep protein (*i.e.*, Rep78/68) and one small Rep protein (*i.e.*, Rep52/40). In particular embodiments, the Rep coding sequences encode the AAV Rep78 protein and the AAV Rep52 and/or Rep40 proteins. In other embodiments, the Rep coding sequences encode the Rep68 and the Rep52 and/or Rep40 proteins. In a still further embodiment, the Rep coding sequences encode the Rep68 and Rep52 proteins, Rep68 and Rep40 proteins, Rep78 and Rep52 proteins, or Rep78 and Rep40 proteins.

[0076] As used herein, the term “large Rep protein” refers to Rep68 and/or Rep78. Large Rep proteins of the claimed invention may be either wildtype or synthetic. A wildtype large Rep

protein may be from any parvovirus or AAV, including but not limited to serotypes 1, 2, 3a, 3b, 4, 5, 6, 7, 8, 9, 10, 11, or 13, or any other AAV now known or later discovered (*see, e.g., Table 1*). A synthetic large Rep protein may be altered by insertion, deletion, truncation and/or missense mutations.

[0077] Those skilled in the art will further appreciate that it is not necessary that the replication proteins be encoded by the same polynucleotide. For example, for MVM, the NS-1 and NS-2 proteins (which are splice variants) may be expressed independently of one another. Likewise, for AAV, the p19 promoter may be inactivated and the large Rep protein(s) expressed from one polynucleotide and the small Rep protein(s) expressed from a different polynucleotide. Typically, however, it will be more convenient to express the replication proteins from a single construct. In some systems, the viral promoters (*e.g., AAV p19 promoter*) may not be recognized by the cell, and it is therefore necessary to express the large and small Rep proteins from separate expression cassettes. In other instances, it may be desirable to express the large Rep and small Rep proteins separately, *i.e.,* under the control of separate transcriptional and/or translational control elements. For example, it may be desirable to control expression of the large Rep proteins, so as to decrease the ratio of large to small Rep proteins. In the case of insect cells, it may be advantageous to down-regulate expression of the large Rep proteins (*e.g., Rep78/68*) to avoid toxicity to the cells (*see, e.g., Urabe et al., (2002) Human Gene Therapy 13:1935*).

[0078] As used herein, the parvovirus or AAV “cap coding sequences” encode the structural proteins that form a functional parvovirus or AAV capsid (*i.e.,* can package DNA and infect target cells). Typically, the cap coding sequences will encode all of the parvovirus or AAV capsid subunits, but less than all of the capsid subunits may be encoded as long as a functional capsid is produced. Typically, but not necessarily, the cap coding sequences will be present on a single nucleic acid molecule.

[0079] The term “expression vector” refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules or ribozymes. Expression vectors can contain a variety of “control sequences,” which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described *infra*.

[0080] The virus vectors of the invention can further be “targeted” virus vectors (*e.g.*, having a directed tropism) and/or a “hybrid” parvovirus (*i.e.*, in which the viral ITRs and viral capsid are from different parvoviruses) as described in international patent publication WO 00/28004 and Chao *et al.*, (2000) *Mol. Therapy* 2:619.

[0081] As used herein, the term “host cell” refers to a cell that is engineered to express the modified polypeptide or functional fragment thereof (*e.g.*, a modified full length protein or a fragment thereof). “Host cell” refers not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[0082] Host cells may be derived from prokaryotes or eukaryotes, depending upon whether the desired result is replication of the vector or expression of part or all of the vector-encoded nucleic acid sequences. Prokaryotes include gram negative or positive cells. Numerous cell lines and cultures are available for use as a host cell, and they can be obtained through the American Type Culture Collection (ATCC), which is an organization that serves as an archive for living cultures and genetic materials (www.atcc.org). An appropriate host can be determined by one of skill in the art based on the vector backbone and the desired result. A plasmid or cosmid, for example, can be introduced into a prokaryote host cell for replication of many vectors. Bacterial cells used as host cells for vector replication and/or expression include DH5 α , JM109, and KC8, as well as a number of commercially available bacterial hosts such as SURE® Competent Cells and SOLOPACK™ Gold Cells (STRATAGENE®, La Jolla). Alternatively, bacterial cells such as *E. coli* LE392 could be used as host cells for phage viruses.

[0083] “Pharmaceutically acceptable carrier” (sometimes referred to as a “carrier”) refers to a carrier or excipient that is useful in preparing a pharmaceutical or therapeutic composition that is generally safe and non-toxic and includes a carrier that is acceptable for veterinary and/or human pharmaceutical or therapeutic use. The terms “carrier” or “pharmaceutically acceptable carrier” can include, but are not limited to, phosphate buffered saline solution, water, emulsions (such as an oil/water or water/oil emulsion) and/or various types of wetting agents. As used herein, the term “carrier” encompasses, but is not limited to, any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, lipid, stabilizer, or other material well known in the art for use in pharmaceutical formulations and as described further herein.

[0084] The term “modulate,” “modulates,” or “modulation” refers to enhancement (*e.g.*, an increase) or inhibition (*e.g.*, a decrease) in the specified level or activity.

[0085] The term “enhance” or “increase” refers to an increase in the specified parameter of at least about 1.25-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, twelve-fold, or even fifteen-fold and/or can be expressed in the enhancement and/or increase of a specified level and/or activity of at least about 1%, 5%, 10%, 15%, 25%, 35%, 40%, 50%, 60%, 75%, 80%, 90%, 95% or more.

[0086] “Inhibit” or “reduce” or grammatical variations thereof as used herein refers to a decrease or diminishment in the specified level or activity of at least about 1, 5, 10, 15%, 25%, 35%, 40%, 50%, 60%, 75%, 80%, 90%, 95% or more. In particular embodiments, the inhibition or reduction results in little or essentially no detectable activity (at most, an insignificant amount, *e.g.*, less than about 10% or even 5%).

[0087] The term “contact” or grammatical variations thereof as used with respect to a polypeptide and a cell or aggregate, refers to bringing the polypeptide and the cell or aggregate in sufficiently close proximity to each other for one to exert a biological effect on the other. In some embodiments, the term contact means binding of the polypeptide to the cell or aggregate.

[0088] The term “autoimmune condition” refers to a condition that occurs when a host's own immune system attacks the host's own cells, causing various symptoms.

[0089] The term “arthritis” refers to a joint disorder or condition that involves inflammation of one or more joints.

[0090] A “subject” may be any vertebrate organism in various embodiments. A subject may be individual to whom an agent is administered, *e.g.*, for experimental, diagnostic, and/or therapeutic purposes or from whom a sample is obtained or on whom a procedure is performed. In some embodiments a subject is a mammal, *e.g.*, a human, non-human primate, lagomorph (*e.g.*, rabbit), or rodent (*e.g.*, mouse, rat). In some embodiments a human subject is a neonate, child, adult, or geriatric subject. In some embodiments a human subject is at least 50, 60, 70, 80, or 90 years old.

[0091] “Treat,” “treating” and similar terms as used herein in the context of treating a subject refer to providing medical and/or surgical management of a subject. Treatment may include, but is not limited to, administering an agent or composition (*e.g.*, a pharmaceutical composition) to a subject. Treatment is typically undertaken in an effort to alter the course of a disease (which term is used to indicate any disease, disorder, syndrome, or undesirable condition warranting or potentially warranting therapy) in a manner beneficial to the subject.

The effect of treatment may include reversing, alleviating, reducing severity of, delaying the onset of, curing, inhibiting the progression of, and/or reducing the likelihood of occurrence or recurrence of the disease or one or more symptoms or manifestations of the disease. A therapeutic agent may be administered to a subject who has a disease or is at increased risk of developing a disease relative to a member of the general population. In some embodiments a therapeutic agent may be administered to a subject who has had a disease but no longer shows evidence of the disease. The agent may be administered *e.g.*, to reduce the likelihood of recurrence of evident disease. A therapeutic agent may be administered prophylactically, *i.e.*, before development of any symptom or manifestation of a disease. “Prophylactic treatment” refers to providing medical and/or surgical management to a subject who has not developed a disease or does not show evidence of a disease in order, *e.g.*, to reduce the likelihood that the disease will occur, delay the onset of the disease, or to reduce the severity of the disease should it occur. The subject may have been identified as being at risk of developing the disease (*e.g.*, at increased risk relative to the general population or as having a risk factor that increases the likelihood of developing the disease).

[0092] As used herein, the term “prevent,” “prevents,” or “prevention” (and grammatical equivalents thereof) means to delay or inhibit the onset of a disease. The terms are not meant to require complete abolition of disease, and encompass any type of prophylactic treatment to reduce the incidence of the condition or delays the onset of the condition.

[0093] A “treatment effective” amount as used herein is an amount that is sufficient to provide some improvement or benefit to the subject. Alternatively stated, a “treatment effective” amount is an amount that will provide some alleviation, mitigation, decrease or stabilization in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

[0094] A “prevention effective” amount as used herein is an amount that is sufficient to prevent and/or delay the onset of a disease, disorder and/or clinical symptoms in a subject and/or to reduce and/or delay the severity of the onset of a disease, disorder and/or clinical symptoms in a subject relative to what would occur in the absence of the methods of the invention. Those skilled in the art will appreciate that the level of prevention need not be complete, as long as some benefit is provided to the subject.

[0095] Grammatical variations of “administer,” “administration,” and “administering” to a subject include any route of introducing or delivering to a subject an agent. Administration can be carried out by any suitable route, including oral, topical, intravenous, subcutaneous,

transcutaneous, transdermal, intramuscular, intra-joint, parenteral, intra-arteriole, intradermal, intraventricular, intracranial, intraperitoneal, intralesional, intranasal, rectal, vaginal, by inhalation, via an implanted reservoir, parenteral (*e.g.*, subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intraperitoneal, intrahepatic, intralesional, and intracranial injections or infusion techniques), and the like.

“Concurrent administration,” “administration in combination,” “simultaneous administration,” or “administered simultaneously” as used herein, means that the compounds are administered at the same point in time, overlapping in time, or one following the other. In the latter case, the two compounds are administered at times sufficiently close that the results observed are indistinguishable from those achieved when the compounds are administered at the same point in time. “Systemic administration” refers to the introducing or delivering to a subject an agent via a route which introduces or delivers the agent to extensive areas of the subject’s body (*e.g.*, greater than 50% of the body), for example through entrance into the circulatory or lymph systems. By contrast, “local administration” refers to the introducing or delivery to a subject an agent via a route which introduces or delivers the agent to the area or area immediately adjacent to the point of administration and does not introduce the agent systemically in a therapeutically significant amount. For example, locally administered agents are easily detectable in the local vicinity of the point of administration but are undetectable or detectable at negligible amounts in distal parts of the subject's body. Administration includes self-administration and the administration by another.

[0096] In some embodiments, methods for treating an autoimmune condition (*e.g.*, arthritis) or graft rejection in a subject in need thereof are provided, comprising administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or functional fragment or derivative thereof, thereby treating the subject. Treatment can involve a reduction in one or more effects or symptoms of the autoimmune condition or graft rejection. For example, in arthritis treatment can reduce pain, stiffness, swelling, fever, joint inflammation or joint tenderness. Treatment can also involve a reduction in the underlying pathology rather than just the symptoms. The treatment can be any reduction and can be, but is not limited to, the complete ablation of the autoimmune condition or graft rejection, or signs or symptoms of the autoimmune condition or graft rejection. Treatment can include the complete amelioration of the autoimmune condition or graft rejection as detected by art-known techniques. Art recognized methods are available to detect autoimmune conditions such as arthritis and its symptoms. For example, the detection of arthritis may include, but is

not limited to, radiological examination, joint aspiration, blood tests (for example, detection of rheumatoid factors or an anti-CCP test) or MRI, to name a few. For example, a disclosed method is considered to be a treatment if there is about a 10% reduction in one or more symptoms of the autoimmune condition (*e.g.*, arthritis) or graft rejection in a subject when compared to the subject prior to treatment or control subjects. Thus, the reduction can be about a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between.

[0097] Methods for delaying onset of an autoimmune condition or graft rejection in a subject are also provided and comprise administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or functional fragment or derivative thereof, thereby delaying onset of the autoimmune condition or graft rejection in the subject. For example, the disclosed method is considered to delay the onset of an autoimmune condition or graft rejection if there is a delay in onset, incidence, severity, or recurrence of the autoimmune condition or graft rejection or one or more symptoms of the autoimmune condition or graft rejection (for example, pain, stiffness, swelling, fever, joint inflammation or joint tenderness in arthritis) in a subject susceptible to the autoimmune condition or graft rejection as compared to control subjects susceptible to the autoimmune condition or graft rejection that did not receive a treatment disclosed herein. The disclosed method is also considered to delay the onset of an autoimmune condition or graft rejection if there is a delay in onset, incidence, severity, or recurrence of the autoimmune condition or graft rejection or one or more symptoms of the autoimmune condition or graft rejection in a subject susceptible to the autoimmune condition or graft rejection after receiving a treatment disclosed herein as compared to the subject's progression prior to receiving treatment. Thus, the delay in onset, incidence, severity, or recurrence of the autoimmune condition or graft rejection can be about a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between.

[0098] In embodiments, administering the compositions comprising an immune checkpoint protein or a functional fragment or derivative thereof (*e.g.*, PD-L1) to a subject with or susceptible to arthritis reduces swelling of a joint, *e.g.*, at least about a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, or more reduction, as compared to the subject prior to treatment or control subjects. In embodiments, administering the composition decreases the infiltration of T cells and macrophages, *e.g.*, in a joint of a subject, *e.g.*, at least about a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, or more decrease, as compared to the subject prior to treatment or control subjects. In embodiments, administering the composition decreases one or more pro-inflammatory cytokines, *e.g.*, in a joint, *e.g.*, at least about a 5%, 10%, 15%,

20%, 25%, 30%, 35%, 40%, 50%, or more decrease, as compared to the subject prior to treatment or control subjects. In embodiments, administering the composition prevents or inhibits the increase of one or more pro-inflammatory cytokines, *e.g.*, in a joint. In embodiments, administering the composition prevents or minimizes the presence of (*e.g.*, maintains absence of) one or more pro-inflammatory cytokines, *e.g.*, in a joint. In embodiments, the pro-inflammatory cytokines are IL-1, IL-6, IL-17, TNF α , or a combination thereof. In embodiments pertaining to arthritis, joint histopathology may be improved, stabilized without further worsening, or a rate of worsening slowed with the methods and composition disclosed herein. By way of example, scoring of joints in subjects with osteoarthritis can be scored according to the Osteoarthritis Research Society International (OARSI) system to determine stability, worsening or improvement of joint histopathology, as is well known in the art. See, *e.g.*, *Osteoarthritis Cartilage* 19:324–331; *Osteoarthritis Cartilage* 20:476–485. In some embodiments, administering the composition decreases the OARSI score by at least about 1, 2, 3, 4, 5, 6, 7, 8, or 9 as compared to the subject prior to treatment or control subjects.

[0099] Immune checkpoint proteins are regulators of the immune system. Immune checkpoint proteins are well known in the art and include, without limitation, PD-1 (*e.g.*, GENBANK Accession No. NP_005009.2), PD-L1 (also referred to as CD274, *e.g.*, GENBANK Accession No. NP_054862.1), PD-L2 (*e.g.*, GENBANK Accession No. NP_079515.2), CTLA4 (*e.g.*, GENBANK Accession No. NP_001032720.1 or NP_005205.2), B7-H3 (*e.g.*, GENBANK Accession No. NP_001019907.1, NP_001316557.1, NP001316558.1, or NP_079516.1), B7-H4 (*e.g.*, GENBANK Accession No. NP_001240778.1, NP_001240779.1, or NP_078902.2), BTLA (*e.g.*, GENBANK Accession No. NP_001078826.1 or NP_861445.4), IDO (*e.g.*, GENBANK Accession No. NP_002155.1), KIR (*e.g.*, GENBANK Accession No. NP_001309097.1, NP_037421.2, NP_001074239.1, NP_001074241.1, NP_055034.2, NP_055033.2, or NP_056952.2), LAG3 (*e.g.*, GENBANK Accession No. NP_001401105.1, NP_001401106.1 or NP_002277.4), A2AR (*e.g.*, GENBANK Accession No. NP_00066.2, NP_001265426.1, NP_001265427.1, NP_001265428.1, NP_001265429.1), TIM-3 (*e.g.*, GENBANK Accession No. NP_116171.3), and VISTA (*e.g.*, GENBANK Accession No. NP_071436.1). In some embodiments, the immune checkpoint protein is a human immune checkpoint protein. In some embodiments, the immune checkpoint protein administered to the subject is PD-L1 protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the PD-L1 protein or functional fragment or derivative thereof. See generally, Wen et al., *Nature*

Communications 12:5106 (2021). The PD-L1 protein can be a soluble protein or a transmembrane protein. Soluble PD-L1 is typically generated following proteolytic cleavage of membrane bound PD-L1 by translation of alternatively spliced mRNA or matrix metalloproteinase and has been associated with immune response and a potential prognostic predictor in forms of cancer. See, e.g., *Front Immunol.* 13: 827921 (2022); doi:10.3389/fimmu.2022.827921; see Fig. 1B, specifically incorporated herein by reference. In some embodiments, the PD-L1 protein is human PD-L1 (**SEQ ID NOs:10-12, 19 or 20**) or sh-PD-L1 (**SEQ ID NO:18**). In some embodiments, the PD-L1 protein is encoded by the nucleotide sequence of human PD-L1 (**SEQ ID NO:1**) or sh-PD-L1 (**SEQ ID NO:2**). In an embodiment, the PD-L1 protein comprises exons 1, 2, 3, 4 and a polyadenylation signal. In an embodiment, the PD-L1 protein further comprises a signal sequence, as detailed elsewhere herein. In an embodiment, the PD-L1 comprises a protein, functional fragment, or derivative thereof, of one of the proteins of **Table 2**. In an embodiment, the functional fragment of the PD-L1 protein has an amino acid sequence of **SEQ ID NO:18**.

Table 2. Example PD-L1 proteins

Description	Ref. Seq. Protein	SEQ ID NO:	Ref. Seq. Transcript.	SEQ ID NO:
Programmed Cell Death 1 Ligand 1 Isoform a Precursor [<i>Homo sapiens</i>]	NP_054862.1	10	NM_014143.4	14
Programmed Cell Death 1 Ligand 1 Isoform b Precursor [<i>Homo sapiens</i>]	NP_001254635.1	11	NM_001267706.2	15
Programmed Cell Death 1 Ligand 1 Isoform c Precursor [<i>Homo sapiens</i>]	NP_001300958.1	12	NM_001314029.2	16
Programmed Cell Death 1 Ligand 1 Precursor [<i>Mus musculus</i>]	NP_068693.1	13	NM_021893.3	17

[0100] The immune checkpoint protein (e.g., PD-L1) may be administered to the subject by any route of administration found to be effective. The most suitable route will depend on the subject and/or condition being treated. In some embodiments, the immune checkpoint protein is administered intraarticularly, intramuscularly or intravenously. In some embodiments, the immune checkpoint protein or a functional fragment or derivative thereof is administered to the subject by a route selected from oral, rectal, transmucosal, intranasal,

inhalation (*e.g.*, via an aerosol), buccal (*e.g.*, sublingual), vaginal, intrathecal, intraocular, intravitreal, intracochlear, transdermal, intraendothelial, in utero (or in ovo), parenteral (*e.g.*, intravenous, subcutaneous, intradermal, intracranial, intramuscular (including administration to skeletal, diaphragm and/or cardiac muscle], intrapleural, intracerebral, and intraarticular), topical (*e.g.*, to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intralymphatic, and the like, as well as direct tissue or organ injection (*e.g.*, to liver, eye, skeletal muscle, cardiac muscle, diaphragm muscle or brain).

[0101] In an embodiment, the immune checkpoint protein or a functional fragment or derivative thereof or a nucleic acid encoding the protein is administered via a nucleic acid delivery vector. In embodiments, the immune checkpoint protein or functional fragment or derivative thereof or nucleic acid encoding the protein is delivered via a viral vector, nanoparticle, or hydrogel. In an embodiment, the immune checkpoint protein or a functional fragment or derivative thereof is administered via a nucleic acid delivery vector comprising a nucleic acid encoding an immune checkpoint protein. In an embodiment, the delivery vehicle may comprise a nanoparticle comprising modified dendrimers for the enclosure of the delivery of a nucleic acid, *e.g.*, a nucleic acid encoding an immune checkpoint protein. Exemplary dendrimers include polyester dendrimers, which may be modified with amine linkers, fatty acid derivatives. Exemplary molecules and methods of making nanoparticle compositions may be found at International Publication WO 2020/132196. Lipid particles, for example, lipid nanoparticles and liposomes may also be used. In an example embodiment, the lipid particles comprise one or more polynucleotides encoding an immune checkpoint polypeptide (*e.g.*, PD-L1) according to the present invention. Example lipid nanoparticles can be found in the art, for example, in U.S. Pat. Nos. 9,868,692, 10,266,485, 10,442,756, and 10,272,150. Liposomes and stable nucleic acid lipid particles (SNALPs) can also be used for delivery. Intra-articular drug delivery vehicles can include those described in *Pharmaceutics*, 2021 Dec; 13(12): 2166; doi: 10.3390/pharmaceutics1312166, incorporated herein by reference, with specific reference to Table 1 (identifying different drug delivery systems investigated for intra-articular osteoarthritis therapy).

[0102] In an embodiment, vectors comprising one or more of the nucleic acid molecules as taught herein are contemplated for use in the compositions and methods of the invention. The vector can be a viral vector or a non-viral vector. In an aspect, the viral vector is an AAV vector. The AAV vector may be a serotype listed in **Table 1**. The AAV vector can be an AAV2, AAV5, AAV6 or AAV8 serotype vector. In an aspect, the vector comprises a nucleic acid construct, which can include in 5' to 3' order, a first AAV ITR (*e.g.*, an AAV2 ITR), a

promoter operably linked to the nucleic acid encoding the immune checkpoint protein, *e.g.*, a PD-L1 protein, a transcription termination sequence, and a second AAV ITR (*e.g.*, an AAV2 ITR). In an aspect, the first and second AAV ITRs utilized can be selected based on transgene expression profiles. See, Earley et al., “Adeno-Associated Virus Serotype-Specific Inverted Terminal Repeat Sequence Role in Vector Transgene Expression,” *Hum Gene Ther.* February 2020; 31(3-4):151-162; doi:10.1089/hum.2019.274. Nucleic acids encoding the immune checkpoint protein, functional fragment or a derivative thereof, can further comprise a sequence encoding a signal peptide. In an aspect, the signal peptide is from a protein specifically expressed in joint cells. Example signal sequences include the signal sequence from fibronectin (SEQ ID NO:24), collagen II (SEQ ID NO:25), and *Gaussia luciferase* (SEQ ID NO:26). In an aspect, the nucleic acid encoding a PD-L1 protein and a signal sequence comprises a nucleotide sequence selected from SEQ ID NOs:6-8. In an embodiment, a PD-L1 protein and a signal sequence comprises a protein sequence selected from SEQ ID NOs:21-23.

[0103] In embodiments, the autoimmune condition is Acromegaly, Acquired aplastic anemia, Acquired hemophilia, Agammaglobulinemia, primary, Alopecia areata, Ankylosing spondylitis (AS), Anti-NMDA receptor encephalitis, Antiphospholipid syndrome (APS), Arteriosclerosis, Autoimmune Addison’s disease (AAD), Autoimmune autonomic ganglionopathy (AAG), Autoimmune encephalitis | acute disseminated encephalomyelitis (ADEM), Autoimmune gastritis, Autoimmune hemolytic anemia (AIHA), Autoimmune hepatitis (AIH), Autoimmune hyperlipidemia, Autoimmune hypophysitis/lymphocytic hypophysitis, Autoimmune inner ear disease (AIED), Autoimmune lymphoproliferative syndrome (ALPS), Autoimmune myelofibrosis, Autoimmune myocarditis, Autoimmune oophoritis, Autoimmune pancreatitis (AIP), Autoimmune polyglandular syndromes, Autoimmune progesterone dermatitis, Autoimmune retinopathy (AIR), Autoimmune sudden sensorineural hearing loss (SNHL), Balo disease, Behçet’s disease, Birdshot chorioretinopathy, Bullous pemphigoid, Castleman disease, Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic autoimmune urticaria, Churg-Strauss syndrome, Cogan’s syndrome, Cold agglutinin disease, CREST syndrome, Crohn’s disease (CD), Cronkhite-Canada syndrome (CSS), Cryptogenic organizing pneumonia (COP), Dermatitis herpetiformis, Dermatomyositis, Diabetes, type 1, Discoid lupus, Dressler’s syndrome, Eczema/Atopic Dermatitis, Endometriosis, Eosinophilic esophagitis/eosinophilic gastroenteritis, Eosinophilic fasciitis, Erythema nodosum, Essential mixed cryoglobulinemia, Evans syndrome, Fibrosing alveolitis/Idiopathic pulmonary fibrosis

(IPF), Giant cell arteritis, Giant Cell Myocarditis, Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with polyangiitis (GPA), Graves' disease, Guillain-Barré syndrome (GBS), Hashimoto's thyroiditis, Henoch-Schönlein purpura, Hidradenitis suppurativa, Hurst's disease, Hypogammaglobulinemia, Berger's disease, Immune-mediated necrotizing myopathy (IMNM), Immune thrombocytopenia (ITP), Inclusion body myositis, IgG4-related sclerosing disease (ISD), Interstitial cystitis, Juvenile idiopathic arthritis, Juvenile polymyositis, Kawasaki disease, Lambert-Eaton myasthenic syndrome (LEMS), Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus nephritis, chronic Lyme disease, Lymphocytic colitis/microscopic colitis, Lymphocytic hypophysitis/autoimmune hypophysitis, Ménière's disease, Microscopic polyangiitis (MPA)/ANCA-associated vasculitis, Mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Multifocal motor neuropathy, Multiple sclerosis (MS), Myalgic encephalomyelitis (ME), Myasthenia gravis (MG), Narcolepsy, Neuromyelitis Optica/Devic's disease, Ocular cicatricial pemphigoid, Opsoclonus-myoclonus syndrome (OMS), Palindromic rheumatism, Paraneoplastic cerebellar degeneration, Paraneoplastic pemphigus, Parry-Romberg syndrome (PRS)/Hemifacial atrophy (HFA)/Progressive facial hemiatrophy, Paroxysmal nocturnal hemoglobinuria (PNH), Peripheral uveitis/pars planitis, PANS/PANDAS, Parsonage-Turner syndrome, Pemphigus gestationis, Pemphigus foliaceus, Pemphigus vulgaris, Pernicious anemia, POEMS syndrome, Polyarteritis nodosa, Polymyalgia rheumatica, Polymyositis, Postural orthostatic tachycardia syndrome (POTS), Primary biliary cirrhosis (PBC), Primary sclerosing cholangitis (PSC), Psoriasis, Palmoplantar Pustulosis, Psoriatic arthritis, Pulmonary fibrosis, idiopathic (IPF), Pure red cell aplasia (PRCA), Pyoderma gangrenosum, Rasmussen's encephalitis, Raynaud's syndrome/phenomenon, Reiter's syndrome, Reflex sympathetic dystrophy syndrome (RSD), Relapsing polychondritis, Restless leg syndrome (RLS), Rheumatic fever, Rheumatoid arthritis, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma, Sclerosing Mesenteritis, Serpiginous choroidopathy, Sjögren's syndrome, Stiff person syndrome (SPS), Small fiber sensory neuropathy, Systemic lupus erythematosus (SLE), Subacute bacterial endocarditis (SBE), Subacute cutaneous lupus, Susac syndrome, Sydenham's chorea, Sympathetic ophthalmia, Takayasu's arteritis (vasculitis), Testicular autoimmunity (vasculitis, orchitis), Tolosa-Hunt syndrome, Transverse myelitis (TM), Tubulointerstitial nephritis uveitis syndrome (TINU), Ulcerative colitis (UC), Undifferentiated connective tissue disease (UCTD), Uveitis, Vasculitis, VEXAS Syndrome, Vitiligo, or Vogt-Koyanagi-Harada syndrome (VKH). In some embodiments, the

autoimmune condition is multiple sclerosis, systemic lupus erythematosus, systemic sclerosis, an idiopathic inflammatory myopathy, systemic vasculitis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, diabetes, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, autoimmune hepatitis, primary biliary cirrhosis, inflammatory bowel disease, polymyalgia rheumatica, alopecia, vasculitis, celiac disease, a bullous skin disease, psoriasis, asthma, uveitis, autoimmune retinopathy, or arthritis.

[0104] In embodiments, the arthritis is osteoarthritis, juvenile arthritis, reactive arthritis, gout, psoriatic arthritis, fibromyalgia, ankylosing spondylitis or rheumatoid arthritis, Felty's syndrome, diffuse idiopathic skeletal hyperostosis (DISH), Behçet's disease, inflammatory arthritis, mixed connective tissue disease, infectious arthritis, Granulomatosis with polyangiitis (GPA), Mixed Connective Tissue disease (MCTD), myositis (dermatomyositis, polymyositis), Paget's disease, Pseudogout, polymyalgia rheumatica with giant cell arteritis, Raynaud's Phenomenon, scleroderma, systemic lupus erythematosus, or Sjögren syndrome.

[0105] Some embodiments concern immune checkpoint protein compositions disclosed herein to reduce or prevent graft rejection. In other embodiments, immune checkpoint protein compositions disclosed herein can be used to reduce the incidence or prevent Graft versus Host disease (GVHD). In other embodiments, compositions disclosed herein can be used to reduce cellular transplant rejections or side effects thereof. Cellular transplantation can include islet cell, stem cell, corneal epithelial cells, liver cells, skin or other similar transplantation. Some embodiments concern reducing inflammatory activity and/or adverse immune responses in a subject undergoing a transplant (e.g., an organ or cellular transplant).

[0106] In an aspect, the immune checkpoint protein compositions and methods herein can be utilized with a mycoplasma-derived protein and its analogues, termed protein M, to enable successful gene delivery by preventing neutralization of heterologous agents (e.g., nucleic acid delivery vector; an immune checkpoint protein) by neutralizing antibodies (NAbs) upon administration of the heterologous agent to a subject. Protein M has been shown to block mammalian IgG, IgM, and IgA antibody classes in a species- and antigen-independent manner by universally binding to conserved regions on the antibody light and heavy chains, causing structural interference with the antigen recognizing CDR regions. Protein M binds to antibodies with nanomolar affinity, and prevents antigen-antibody union for a variety of different tested immunoglobulin/antigen pairs. Reference is made to International Patent Publication WO 2021/022187, incorporated herein by reference for its teachings. Briefly, it has been shown that protein M blocks antibody recognition of AAV and prevents antibody-mediated neutralization of AAV. It was demonstrated that protein M can be administered

alone prior to AAV administration, or formulated with AAV for NAb evasion. The effectiveness of this approach depends on the interaction of protein M with immunoglobulin before AAV is neutralized. This approach can be used to overcome NABs to multiple heterologous agents (*e.g.*, AAV vector serotypes) while maintaining the unique or beneficial properties of each agent for specific gene therapy applications. This approach can be used in methods of inhibiting neutralization of a heterologous agent (*e.g.*, a nucleic acid delivery vector and/or immune checkpoint protein of the invention) by neutralizing antibodies upon administration of the heterologous agent to a subject, comprising administering to the subject an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby inhibiting neutralization of the heterologous agent. A further aspect relates to a method of expressing a polypeptide or functional nucleic acid in a subject, *e.g.*, immune checkpoint protein, comprising administering to the subject (a) a nucleic acid delivery vector encoding the polypeptide or functional nucleic acid, and (b) an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby expressing the polypeptide or functional nucleic acid in the subject. Exemplary approaches and modified mycoplasma protein M are further detailed in International Patent Publication WO 2021/022187, in particular, at [0161]-[0176], Tables 4-7 and Examples 1-7. In an aspect, administration of mycoplasma M or a functional fragment or derivative thereof may allow for repeat administration of the compositions detailed herein while overcoming neutralizing antibodies which interfere with therapy applications.

[0107] Any administration regimen well known to those skilled in the art for regulating the timing and sequence of drug delivery can be used and repeated as necessary to effect treatment in the methods of the invention. For example, the dosage forms of the invention may be administered 1, 2, 3, or 4 times daily, by a single dose, multiple discrete doses, or continuous infusion. The administering step of any one of the methods described herein can include at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten dosages. The administering step can be performed before the subject exhibits disease symptoms (*e.g.*, prophylactically), or during or after disease symptoms occur. The administering step can be performed prior to, concurrent with, or subsequent to administration of other agents to the subject. In some embodiments, the administering step is performed prior to, concurrent with, or subsequent to the administration of one or more additional diagnostic or therapeutic agents.

[0108] In some embodiments, a subsequent administration is provided at least one day after a prior administration, or at least two days, at least three days, at least four days, at least five

days, or at least six days after a prior administration. In some embodiments, a subsequent administration is provided at least one week after a prior administration, or at least two weeks, at least three weeks, or at least four weeks after a prior administration. In some embodiments, a subsequent administration is provided at least one month, at least two months, at least three months, at least six months, or at least twelve months after a prior administration.

[0109] As a further aspect, the invention provides pharmaceutical formulations and methods of administering the same to achieve any of the therapeutic effects (*e.g.*, treatment of arthritis) discussed above. The pharmaceutical formulation may comprise any of the reagents discussed above in a pharmaceutically acceptable carrier.

[0110] By “pharmaceutically acceptable” it is meant a material that is not biologically or otherwise undesirable, *i.e.*, the material can be administered to a subject without causing any undesirable biological effects such as toxicity.

[0111] The formulations of the invention can optionally comprise medicinal agents, pharmaceutical agents, carriers, adjuvants, dispersing agents, diluents, and the like.

[0112] A further aspect of the invention is a pharmaceutical composition comprising an immune checkpoint protein or functional fragment or derivative thereof, nucleic acid molecules encoding the immune checkpoint proteins, or vectors of the invention, and a pharmaceutically acceptable carrier. Suitable carriers include, but are not limited to, salts, diluents (*e.g.*, Tris-HCl, acetate, phosphate), preservatives (*e.g.*, Thimerosal, benzyl alcohol, parabens), binders, fillers, solubilizers, disintegrants, sorbents, solvents, pH modifying agents, antioxidants, anti-infective agents, suspending agents, wetting agents, viscosity modifiers, tonicity agents, stabilizing agents, and other components and combinations thereof.

[0113] Suitable pharmaceutically acceptable carriers are preferably selected from materials which are generally recognized as safe (GRAS) and may be administered to an individual without causing undesirable biological side effects or unwanted interactions.

[0114] Suitable pharmaceutical carriers and their formulations are described in *Remington's Pharmaceutical Sciences*, 23rd ed. 2020, Academic Press. In addition, such compositions can be complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Suitable dosage forms for administration, *e.g.*, parenteral administration, include solutions, suspensions, and emulsions. Typically, the components of

the formulation are dissolved or suspended in a suitable solvent such as, for example, water, Ringer's solution, phosphate buffered saline (PBS), or isotonic sodium chloride. The formulation may also be a sterile solution, suspension, or emulsion in a nontoxic, parenterally acceptable diluent or solvent such as 1,3-butanediol. In some cases, formulations can include one or more tonicity agents to adjust the isotonic range of the formulation. Suitable tonicity agents are well known in the art and include glycerin, mannitol, sorbitol, sodium chloride, and other electrolytes. In some cases, the formulations can be buffered with an effective amount of buffer necessary to maintain a pH suitable for parenteral administration. Suitable buffers are well known by those skilled in the art and some examples of useful buffers are acetate, borate, carbonate, citrate, and phosphate buffers. In some embodiments, the formulation can be distributed or packaged in a liquid form, or alternatively, as a solid, obtained, for example by lyophilization of a suitable liquid formulation, which can be reconstituted with an appropriate carrier or diluent prior to administration. The pharmaceutical compositions comprise the immune checkpoint protein (*e.g.*, PD-L1) or functional fragment thereof, nucleic acid molecules as taught herein, or any one of the vectors as taught herein. The pharmaceutical compositions can be formulated for medical and/or veterinary use.

[0115] Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described. For example, in one aspect of the present invention, there is provided an injectable, stable, sterile composition comprising a compound of the invention, in a unit dosage form in a sealed container. The composition can be provided in the form of a lyophilizate which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection thereof into a subject. The unit dosage form typically comprises from about 10 mg to about 10 grams of the compound or salt. When the compound or salt is substantially water-insoluble, a sufficient amount of emulsifying agent which is pharmaceutically acceptable can be employed in sufficient quantity to emulsify the compound or salt in an aqueous carrier. Depots and sustained release formulation are also contemplated.

[0116] The amount of the disclosed compositions administered to a subject will vary from subject to subject, depending on the nature of the disclosed compositions and/or formulations, the species, gender, age, weight and general condition of the subject, the mode of administration, and the like. Effective dosages and schedules for administering the compositions may be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the disclosed compositions are

those large enough to produce the desired effect (*e.g.*, to treat or delay the onset of arthritis). The dosage should not be so large as to outweigh benefits by causing extensive or severe adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like, although some adverse side effects may be expected. The dosage can be adjusted by the individual clinician in the event of any counterindications. Generally, the disclosed compositions and/or formulations are administered to the subject at a dosage of active component(s) ranging from 0.1 mg/kg body weight to 100 g/kg body weight. In some embodiments, the disclosed compositions and/or formulations are administered to the subject at a dosage of active component(s) ranging from 1 mg/kg to 10 g/kg, from 10 mg/kg to 1 g/kg, from 10 mg/kg to 500 mg/kg, from 10 mg/kg to 100 mg/kg, from 10 mg/kg to 10 mg/kg, from 10 mg/kg to 1 mg/kg, from 10 mg/kg to 500 mg/kg, or from 10 mg/kg to 100 mg/kg body weight. Dosages above or below the range cited above may be administered to the individual subject if desired. The compositions can be administered in any herein disclosed pharmaceutical composition comprising a pharmaceutically acceptable carrier.

[0117] A further aspect of the invention relates to kits for use in the methods of the invention. The kit can comprise composition of the invention in a form suitable for administration to a subject or sample or in a form suitable for compounding into a formulation. The kit can further comprise other therapeutic agents, carriers, buffers, containers, devices for administration, and the like, including modulating agents as detailed elsewhere herein. The kit can comprise an immune checkpoint protein, or a fragment or derivative thereof for a therapeutic use. The detection compositions can be formulated for administration to a subject. The kit can further comprise labels and/or instructions for use, carriers, buffers, containers, devices for administration, and the like.

[0118] Having described the present invention, the same will be explained in greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the invention.

EXAMPLES

Example 1. Expression of functional PD-L1 from the AAV vector cassette.

[0119] First, a clone (pTR-CBh-PD-L1) was made in which wild-type human PD-L1 (wtPD-L1 or oriPD-L1) is driven by the CBh promoter and the expression cassette is flanked by AAV2 ITRs (**FIG. 1A**). To verify the PD-L1 protein expression, the plasmid pTR-CBh-PD-L1 was transfected in HEK-293 cell line, both supernatant and cell lysate were collected for

analysis of PD-L1 expression. PD-L1 was detected at around the size of 33 kDa in cell lysates but not in supernatant (**FIG. 1B**).

[0120] To verify the function of the PD-L1 protein produced from transfection of the plasmid pTR-CBh-PD-L1, PD-L1 purified from cell lysate was used to incubate with violet dye-conjugated activated T cells for 72 h. T cell proliferation was analyzed with flow cytometry. The proliferated T cells in both purified PD-L1 group and positive control were $17.2\pm3.8\%$ and $16.2\pm4.2\%$ respectively, while the PBS group was $28.43\pm4\%$ (**FIGS. 1C and 1D**). T cells without activation had less proliferation ($2.2\pm0.9\%$). There was a significant difference between PBS group and PD-L1-treated groups ($P<0.05$). These results indicate that the functional PD-L1 is produced from the AAV cassette pTR-CBh-PD-L1.

Example 2. Intra-articular injection of AAV5-CBh-PD-L1 vectors prevents arthritis in CIA mice.

[0121] It has been demonstrated that AAV serotype 5 vector efficiently transduces joint after intra-articular injection. To investigate the efficacy of local gene therapy for the prevention of knee joint inflammation, AAV5-CBh-PD-L1 vectors were intra-articularly injected in the left leg of DBA/1J mice and AAV5-luc vectors in the right leg when mice received the first immunization of bovine collagen to induce arthritis. At day 21, mice were treated with the booster of collagen (**FIG. 2A**). The joint swelling was monitored over 7 weeks after AAV injection. Compared to the size of joint at the day of AAV injection, 2 weeks later after the booster of collagen, the change of joint size in AAV-PD-L1-treated knees was significantly less than that with PBS control (**FIG. 2C**). At week 7 post-AAV injection, mice were euthanized and the knees were collected for histopathological score analysis (**FIGS. 2C and 2D**). In the joints treated with AAV5-PDL1, a much lower histopathological score of 3.6 ± 1 was observed when compared to the joints with AAV5-luc (8.2 ± 1.5 , $p<0.05$).

Example 3. PD-L1 decreased the infiltration of T cells and macrophages in joints of CIA mice.

[0122] Immune cells play a role in the initiation and progression of RA. To determine the effect of PD-L1 expression in joints on immune cells, the infiltration of T cells and macrophages in the synovium was investigated after intra-articular injection of AAV5-PD-L1 (**FIG. 3**). Compared to the knees in untreated mice, both T cells and macrophages were increased in synovium (**FIGS. 3B and 3C**). The CD3⁺ T cells in AAV5-PD-L1 group were greatly decreased when compared to that in AAV5-luc group ($25\pm4.3\%$ vs $47.4\pm4.3\%$,

respectively, $p < 0.05$), and higher than that in naïve mice ($5.8 \pm 2.8\%$). Similarly, CD68⁺ macrophages in the AAV5-PD-L1 group were less than that in the AAV5-luc group ($59.3 \pm 9\%$ vs $30.2 \pm 11\%$, $p < 0.05$), but naïve mice had a much lower percentage of macrophages in the joints ($7.4 \pm 2.7\%$).

Example 4. PD-L1 decreased production of inflammatory cytokines in joints.

[0123] Cytokine profiles in local knee joints were investigated. As shown in **FIG. 4**, the level of pro-inflammatory cytokines IL-1, IL-6, IL-17 and TNF α in the knee tissue homogenization with AAV5-PD-L1 treatment was significantly lower than that in the control groups treated with AAV5-luc vectors ($P < 0.05$), while IL-10 did not show a difference. The cytokine levels in naïve mice knee joint were undetectable.

Example 5. Intra-articular injection of AAV5-CBh-PD-L1 vectors slows down arthritis progression in CIA mice.

[0124] To study the therapeutic effect of PD-L1 on arthritis, AAV5-PD-L1 vectors were intra-articularly administered at day 21 when the booster immunization with collagen was applied (**FIG. 5A**), and the joint swelling and histopathology were examined (**FIGS. 5B** and **5C**). Consistent with the data when AAV vectors were injected at the day of primary collagen immunization, the treatment with AAV5-PD-L1 vectors afforded much less joint swelling when compared to control group treated with PBS (**FIG. 5B**). Also, lower histopathological scores were observed in the joints of CIA mice treated with AAV5-PD-L1 than PBS (2.2 ± 0.8 vs 8.8 ± 1.4 , respectively, $p < 0.05$, **FIG. 5C**). In naïve mice, the histopathological scores were 0.8 ± 0.4 (**FIG. 5C**).

Example 6. Articular expression of PD-L1 did not impact systemic immunity.

[0125] At sacrifice, PD-L1 was detected in knee joint lysate of the AAV5-PD-L1 group, but not in knee joint of the AAV5-luc group. However, PD-L1 was not detected in serum at weeks 1 and 7 after AAV5-PD-L1 injection (**FIG. 6**). To study the biodistribution of AAV5-PD-L1 vectors after intra-articular injection, AAV genome copy numbers in different tissues were examined based on qPCR assay. As shown in **FIG. 7**, the AAV vector genome copy number in knee joints was approximately 200-fold higher than liver. Very low genome copy number was detected in other tissues including heart, spleen, lung, kidney, and serum.

[0126] In serum, similar high levels of cytokines were detected in both AAV5-PD-L1-treated and control groups in CIA mice when compared to that in naïve mice (**FIG. 8**). The profiles

of lymphocytes in the spleen were similar in CIA mice regardless of the intra-articular treatment with AAV5-PD-L1 vectors.

Example 7. Intra-articular injection of AAV5-PD-L1 prevented the development of osteoarthritis.

[0127] Applicant also explored whether intra-articular injection of AAV5-PD-L1 was able to prevent the development of osteoarthritis in a mouse model. After intra-articular injection of AAV5-PD-L1, the joint capsule immediately medial to the patellar tendon was incised to induce osteoarthritis. At week 8 post-AAV injection, joints were collected for histopathological analysis, and a score was assigned for joint pathological change based on the OARSI grading system. As shown in **FIG. 9**, a very low score was observed in the joints treated with AAV5-PD-L1 vectors when compared to that in control OA joints without treatment.

Example 8. Effective secretion of PD-L1 with modification of transmembrane domain.

[0128] Wild-type PD-L1 (**SEQ ID NO:10**) has a transmembrane domain and is expressed on the surface of AAV transduced cells with rare secretion, which could influence the effect of PD-L1 on immune cells in the joints. Applicant hypothesized that soluble PD-L1 would distribute evenly in AAV vector transduced joints and execute a better preventive or therapeutic effect on arthritis than wtPD-L1. First, three clones with the potential to secrete PD-L1 by modifying the transmembrane domain were made (**FIG. 10**) and the plasmids transfected into 293 cells. As shown in **FIG. 11**, PD-L1 was only detected in the supernatant from cells transfected with the sh-PD-L1 construct but not with other two constructs h-PD-L1 and sec-PDL1. However, PD-L1 was detected in cell lysate from all three constructs.

[0129] Next, Applicant compared the whole PD-L1 expression from sh-PD-L1 and wtPD-L1 (oriPD-L1) from cell lysate and supernatant in the transfected cells and found much higher expression of PD-L1 in sh-PD-L1-transfected cells than that in wtPD-L1-treated cells (around 7 fold, **FIGS. 12A-12C**).

Example 9. Soluble PD-L1 from AAV5-sh-PD-L1 joint transduction prevented arthritis development in CIA mice.

[0130] AAV5 vectors encoding sh-PD-L1 were made and injected them into the joints of DBA-1 mice. After AAV injection, mice were treated with collagen to induce arthritis. At different time points, joint swelling was measured. At the end of the experiment (week 7

post-AAV administration), the joints were collected for histopathological analysis. Compared to AAV5-wtPD-L1, similar improvement of arthritis was observed in mice receiving intra-articular injection of AAV5-sh-PD-L1 including joint swelling (**FIG. 13B**) and histopathological scores (**FIGS. 13A and 13C**). Also, low numbers of immune cells including T cells and macrophages were observed in CIA mouse joints after intra-articular injection of AAV5-sh-PD-L1 vectors (**FIGS. 14A and 14B**).

Example 10. Intra-articular injection of AAV5-sh-PD-L1 induced a better prevention of arthritis development in CIA mice.

[0131] AAV5 vectors encoding either sh-PD-L1 or wtPD-L1 at different doses were intra-articularly injected into the joints of CIA mice on day 0 followed by immunization of collagen for arthritis development. At the end of experiments, the joints were collected for pathological score analysis. As showed in **FIG. 15**, similar low pathological score was achieved when the dose of AAV vectors was greater than 5×10^8 particles, regardless of the encoded transgene sh-PD-L1 or wtPD-L1. When 5×10^7 particles of AAV vectors were administered, AAV5-sh-PD-L1 vectors provided a significantly lower pathological score than AAV5-wtPD-L1 vectors.

Example 11. Intravenous injection of AAV8-sh-PD-L1 prevented arthritis development in CIA mice.

[0132] Intra-articular injection of AAV5-PD-L1 vectors prevented and treated arthritis in the AAV5-treated joints without impacting other joints or tissues. Since joint inflammation results from systemic immune dysfunction in RA, Applicants determined whether systemic expression of PD-L1 would inhibit the immune response and treat arthritis. AAV8 was selected as the vector to transduce the mouse liver for systemic administration. AAV8 vectors encoding sh-PD-L1 were administered to CIA mice and it was found that the development of arthritis was almost completely inhibited based on the pathological score in the joints (**FIG. 16**). Also, systemic administration of AAV8-sh-PD-L1 vectors suppressed the production of inflammatory cytokines in CIA mice, including IL-1, IL-6, IL-17 and TNF α (**FIG. 17**).

Example 12. Intramuscular injection of AAV6-sh-PD-L1 blocked arthritis development in CIA mice.

[0133] In clinical trials, AAV liver-targeted gene delivery induces liver injury in some patients. Thus, Applicant explored whether direct muscle delivery of AAV vectors would

provide for systemic expression of sh-PD-L1 and a therapeutic effect. AAV6 is the best serotype to transduce muscles in mice. AAV6-sh-PD-L1 vectors were directly administered into muscles and, similar to that observed with AAV8-sh-PD-L1 delivery, a similar preventive effect on arthritis development in CIA mice was observed, including reduced joint pathological scores (**FIG. 18**) and reduced inflammatory cytokines in blood (**FIG. 19**).

[0134] The foregoing examples are illustrative of the present invention and are not to be construed as limiting thereof. Although the invention has been described in detail with reference to preferred embodiments, variations and modifications exist within the scope and spirit of the invention as described and defined in the following claims.

SEQUENCES

SEQ ID NO:1. Wild type PD-L1:

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CACTAATTGTCTATTGGGAAATGGAGGATAAGAACATTATTCAATTTGTGCATGG
AGAGGAAGACCTGAAGGTTTCAGCATAGTAGCTACAGACAGAGGGCCCGGCTGTT
GAAGGACCAGCTCTCCCTGGGAAATGCTGCACTTCAGATCACAGATGTGAAATT
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CCCAAGGCCGAAGTCATCTGGACAAGCAGTGACCATCAAGTCCTGAGTGGTAAG
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SEQ ID NO:2. shPD-L1 Hind III-Not I

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CAGGATGCAGGGGTGTACCGCTGCATGATCAGCTATGGTGGTGCCGACTACAAG
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acatcctccaatgaaaggtaaagc

SEQ ID NO:3. hPD-L1-1 Hind III – Not I

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SEQ ID NO:4. SecPD-L1 Hind III – Not I

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SEQ ID NO:5. PD-L1 transgene sequence.

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SEQ ID NO:6. PD-L1 fibronectin SP Age I – Mfe I

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GGACAAGCAGTGACCATCAAGTCCTGAGTGGTAAGACCACCACCACCAATTCCA
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SEQ ID NO:7. PD-L1 collagen II SP Age I – MfeI

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SEQ ID NO:8. PD-L1 Gaussia Luc SP Age I- MfeI

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SEQ ID NO:9.

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SEQ ID NO:10. Human PD-L1 Isoform a

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WEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVY

RCMISYGGADYKRITVKVNAPYNKINQRILVVDPTSEHELTCQAEGYPKAEVIWTS
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LPLAHPNERTHLVILGAILLCLGVALTFFIFRLRKGRMMDVKKCGIQDTSKKQSDT
HLEET

SEQ ID NO:11. Human PD-L1 Isoform b

MRIFAVFIFMTYWHLLNAPYNKINQRILVVDPTSEHELTCQAEGYPKAEVIWTSSD
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LAHPNERTHLVILGAILLCLGVALTFFIFRLRKGRMMDVKKCGIQDTSKKQSDTHL
EET

SEQ ID NO:12. Human PD-L1 Isoform c, secPD-L1

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SEQ ID NO:13. Mouse PD-L1

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EET

SEQ ID NO:14. Human PD-L1 Isoform a

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SEQ ID NO:15. Human PD-L1 Isoform b

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SEQ ID NO:18. shPD-L1

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RCMISYGGADYKRITVKVNAPYNKINQRILVDPVTSEHELTCQAEGYPKAEVIWTS
SDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE
LPLAHPNER

SEQ ID NO:19. hPD-L1-1

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SDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE
LPLAHPNERTHLVILGAILLCLGVALTFFIFRLRKDTHLEET

SEQ ID NO:20. secPD-L1 transgene sequence.

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVY
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RCMISYGGADYKRITVKVNAPYNKINQRILVDPVTSEHELTCQAEGYPKAEVIWTS
SDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPE
LPLAHPNERTHLVILGAILLCLGVALTFFIFRLRKGRMMDVKKCGIQDTNSKKQSDT
HLEETHHHHHH

SEQ ID NO:21. PD-L1 fibronectin SP

MLRGPGPGLLLLAVQCLGTAVPSTGASKSKRFTVTVPKDLYVVEYGSNMTIECKFPV
EKQLDLAALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQI
TDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVDPVTSEHELTCQA
EGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDP
EENHTAELVIPELPLAHPNER

SEQ ID NO:22. PD-L1 collagen II SP

MIRLGAPQTLVLLTLLVA AVLRCQGFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDL
AALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKL
QDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVDPVTSEHELTCQAEGYPK
AEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHT
AELVIPELPLAHPNER

SEQ ID NO:23. PD-L1 Gaussia Luc SP

MGVKVLFALICIAVAEAFVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWE
MEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRC
MISYGGADYKRITVKVNAPYNKINQRILVDPVTSEHELTCQAEGYPKAEVIWTSSD
HQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPEL
LAHPNER

SEQ ID NO:24. Fibronectin signal peptide

MLRGPGPGLLLLAVQCLGTAVPSTGASKSKR

SEQ ID NO:25. collagen II signal peptide
MIRLGAPQTLVLLTLLVAAVLRCQG

SEQ ID NO:26. Gaussia Luc signal peptide
MGVKVLFALICIAVAEA

CLAIMS

What is claimed is:

1. A method for treating an autoimmune condition or graft rejection in a subject in need thereof, comprising administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or a functional fragment or derivative thereof, thereby treating the autoimmune condition or graft rejection in the subject.
2. The method of claim 1, wherein the immune checkpoint protein is selected from PD-1, PD-L1, PD-L2, CTLA4, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, A2AR, TIM-3, and VISTA.
3. The method of claim 1, wherein the immune checkpoint protein is PD-L1 protein, or a nucleic acid molecule encoding the PD-L1 protein.
4. The method of claim 3, wherein the PD-L1 protein is a soluble protein or a transmembrane protein.
5. The method of claim 4, wherein the PD-L1 protein is human PD-L1 having an amino acid sequence of any one or **SEQ ID NOs:10, 11, 12, 19 or 20**, or sh-PD-L1 having an amino acid sequence of **SEQ ID NO:18**.
6. The method of claim 4, wherein the PD-L1 protein is encoded by the nucleotide sequence human PD-L1 (**SEQ ID NO:1**) or sh-PD-L1 (**SEQ ID NO:2**).
7. The method of any one of claims 1 to 6, wherein the immune checkpoint protein comprises a fibronectin, collagen II, or *Gaussia luciferase* signal peptide.
8. The method of claim 7, wherein the immune checkpoint protein has an amino acid sequence of any one of **SEQ ID NOs:21-23**.

9. The method of claim 7, wherein the immune checkpoint protein is encoded by a nucleic acid comprising one of **SEQ ID NOs:6-8**.
10. The method of any one of claims 1 to 9, wherein the administering is by intraarticular (IA), intramuscular (IM) or intravenous (IV) injection.
11. The method of any one of claims 1 to 10, wherein the autoimmune condition is multiple sclerosis, systemic lupus erythematosus, systemic sclerosis, an idiopathic inflammatory myopathy, systemic vasculitis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, diabetes, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, autoimmune hepatitis, primary biliary cirrhosis, inflammatory bowel disease, polymyalgia rheumatica, alopecia, vasculitis, celiac disease, a bullous skin disease, psoriasis, asthma, uveitis, autoimmune retinopathy, or graft rejection.
12. The method of any one of claims 1 to 10, wherein the autoimmune condition is arthritis.
13. The method of claim 12, wherein the arthritis is osteoarthritis, juvenile arthritis, reactive arthritis, gout, psoriatic arthritis, fibromyalgia, ankylosing spondylitis or rheumatoid arthritis, Felty's syndrome, diffuse idiopathic skeletal hyperostosis (DISH), Behçet's disease, inflammatory arthritis, mixed connective tissue disease, infectious arthritis, Granulomatosis with polyangiitis (GPA), Mixed Connective Tissue disease (MCTD), myositis (dermatomyositis, polymyositis), Paget's disease, Pseudogout, polymyalgia rheumatica with giant cell arteritis, Raynaud's Phenomenon, scleroderma, systemic lupus erythematosus, or Sjögren syndrome.
14. The method of any one of claims 1 to 13, wherein administering is via a viral vector, nanoparticle, or hydrogel.
15. The method of claim 14, wherein the viral vector is an AAV vector.
16. The method of claim 15, wherein the AAV vector is an AAV2, AAV5, AAV6 or AAV8 serotype vector.

17. A method for delaying onset of an autoimmune condition or graft rejection in a subject, comprising administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or functional fragment or derivative thereof, thereby delaying onset of the autoimmune condition or graft rejection in the subject.

18. The method of claim 17, wherein the immune checkpoint protein is selected from PD-1, PD-L1, PD-L2, CTLA4, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, A2AR, TIM-3, and VISTA.

19. The method of claim 18, wherein the immune checkpoint protein is PD-L1 protein, or a nucleic acid molecule encoding the PD-L1 protein.

20. The method of claim 19, wherein the PD-L1 protein is a soluble protein or transmembrane protein.

21. The method of claim 20, wherein the PD-L1 protein is human PD-L1 having an amino acid sequence of any one or **SEQ ID NOs:10, 11, 12, 19 or 20**, or sh-PD-L1 having an amino acid sequence of **SEQ ID NO:18**.

22. The method of claim 20, wherein the PD-L1 protein is encoded by the nucleotide sequence human PD-L1 (**SEQ ID NO:1**) or sh-PD-L1 (**SEQ ID NO:2**).

23. The method of any one of claims 17 to 22, wherein the immune checkpoint protein comprises a fibronectin, collagen II, or *Gaussia luciferase* signal peptide.

24. The method of claim 23, wherein the immune checkpoint protein has an amino acid sequence of any one or **SEQ ID NOs:21-23**.

25. The method of claim 23, wherein the immune checkpoint protein is encoded by a nucleic acid comprising one of **SEQ ID NOs:6-8**.

26. The method of any one of claims 17 to 25, wherein the administering is by intraarticular (IA), intramuscular (IM) or intravenous (IV) injection.

27. The method of any one of claims 17 to 26, wherein the autoimmune condition is multiple sclerosis, systemic lupus erythematosus, systemic sclerosis, an idiopathic inflammatory myopathy, systemic vasculitis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, diabetes, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, autoimmune hepatitis, primary biliary cirrhosis, inflammatory bowel disease, polymyalgia rheumatica, alopecia, vasculitis, celiac disease, a bullous skin disease, psoriasis, asthma, uveitis, autoimmune retinopathy, or graft rejection.

28. The method of any one of claims 17 to 26, wherein the autoimmune condition is arthritis.

29. The method of claim 28, wherein the arthritis is osteoarthritis, juvenile arthritis, reactive arthritis, gout, psoriatic arthritis, fibromyalgia, ankylosing spondylitis or rheumatoid arthritis, Felty's syndrome, diffuse idiopathic skeletal hyperostosis (DISH), Behçet's disease, inflammatory arthritis, mixed connective tissue disease, infectious arthritis, Granulomatosis with polyangiitis (GPA), Mixed Connective Tissue disease (MCTD), myositis (dermatomyositis, polymyositis), Paget's disease, Pseudogout, polymyalgia rheumatica with giant cell arteritis, Raynaud's Phenomenon, scleroderma, systemic lupus erythematosus, or Sjögren syndrome.

30. The method of any one of claims 17 to 29, wherein delivery is via a viral vector, nanoparticle, or hydrogel.

31. The method of claim 30, wherein the viral vector is an AAV vector.

32. The method of claim 31, wherein the AAV vector is an AAV2, AAV5, AAV6 or AAV8 serotype vector.

33. The method of any one of claims 1 to 32, wherein the administering reduces swelling of a joint.
34. The method of any one of claims 1 to 32, wherein the administering decreases the infiltration of T cells and macrophages in a joint.
35. The method of any one of claims 1 to 32, wherein the administering decreases or prevents the increase of or the presence of one or more pro-inflammatory cytokines in a joint.
36. The method of claim 35, wherein the pro-inflammatory cytokines are IL-1, IL-6, IL-17, TNF α , or a combination thereof.
37. A nucleic acid delivery vector comprising a nucleic acid encoding an immune checkpoint protein or a functional fragment or derivative thereof.
38. The nucleic acid delivery vector of claim 37, wherein the immune checkpoint protein is selected from PD-1, PD-L1, PD-L2, CTLA4, B7-H3, B7-H4, BTLA, IDO, KIR, LAG3, A2AR, TIM-3, and VISTA.
39. The nucleic acid delivery vector of claim 37, wherein the immune checkpoint protein is PD-L1 protein.
40. The nucleic acid delivery vector of claim 39, wherein the PD-L1 protein is a soluble protein or transmembrane protein.
41. The nucleic acid delivery vector of claim 40, wherein the PD-L1 protein is human PD-L1 having an amino acid sequence of any one or **SEQ ID NOs:10, 11, 12, 19 or 20**, or sh-PD-L1 having an amino acid sequence of **SEQ ID NO:18**.

42. The nucleic acid delivery vector of claim 40, wherein the PD-L1 protein is encoded by the nucleotide sequence human PD-L1 (**SEQ ID NO:1**) or sh-PD-L1 (**SEQ ID NO:2**).

43. The nucleic acid delivery vector of any one of claims 37 to 42, wherein the immune checkpoint protein comprises a fibronectin, collagen II, or *Gaussia luciferase* signal peptide.

44. The nucleic acid delivery vector of claim 43, wherein the immune checkpoint protein has an amino acid sequence of any one or **SEQ ID NOs:21-23**.

45. The nucleic acid delivery vector of claim 43, wherein the immune checkpoint protein is encoded by a nucleic acid comprising one of **SEQ ID NOs:6-8**.

46. The nucleic acid delivery vector of any one of claims 37 to 45, wherein the nucleic acid delivery vector is a viral vector.

47. The nucleic acid delivery vector of claim 46, wherein the viral vector is an AAV vector.

48. The nucleic acid delivery vector of claim 47, wherein the AAV is AAV2, AAV5, AAV6 or AAV8.

49. The nucleic acid delivery vector of claim 47 or claim 48, comprising a nucleic acid construct that comprises, in 5' to 3' order, a first AAV2 ITR, a promoter operably linked to the nucleic acid encoding the PD-L1 protein, a transcription termination sequence, and a second AAV2 ITR.

50. The nucleic acid delivery vector of any one of claims 37 to 49, wherein the promoter is an inducible promoter.

51. The nucleic acid delivery vector of any one of claims 37 to 49, wherein the promoter is a constitutive promoter.

52. A method for treating an autoimmune condition or graft rejection in a subject in need thereof, comprising administering to the subject an effective amount of the nucleic acid delivery vector of any one of claims 37 to 51.

53. The method of claim 52, wherein the autoimmune condition is arthritis.

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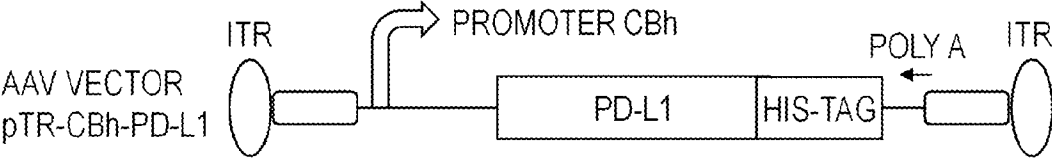


FIG. 1A

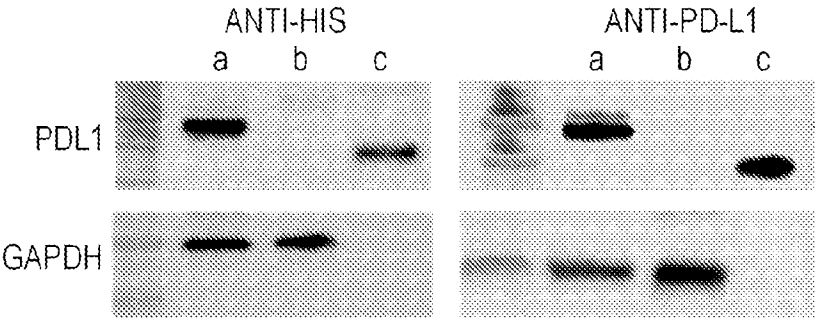


FIG. 1B

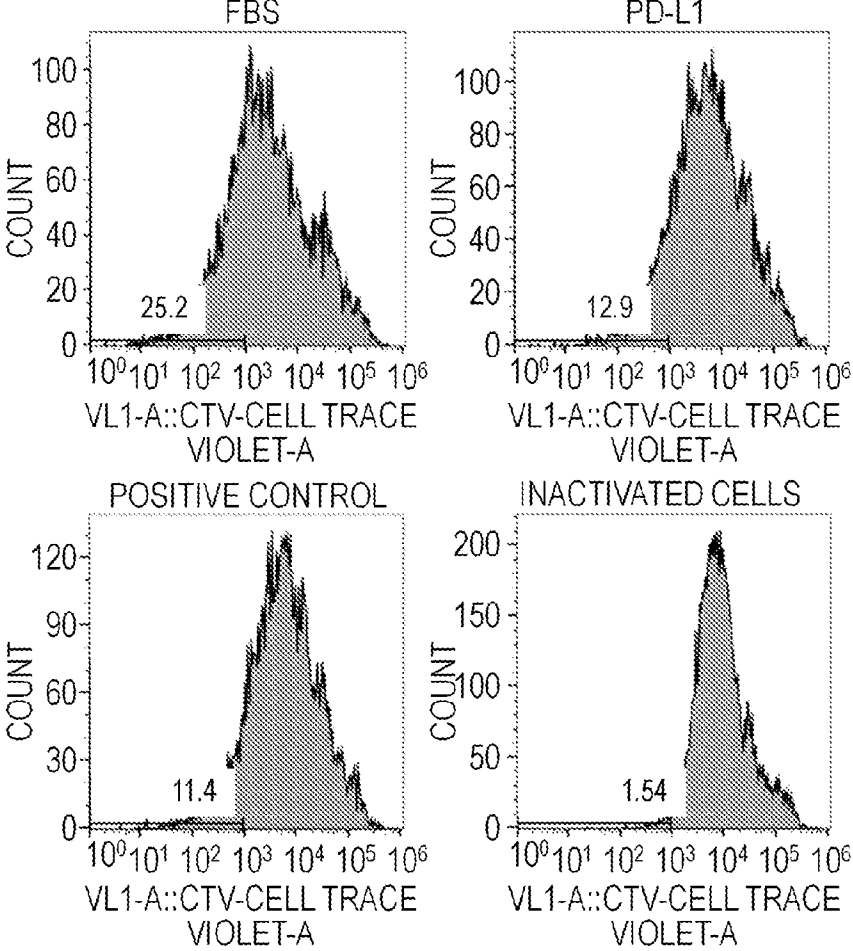


FIG. 1C

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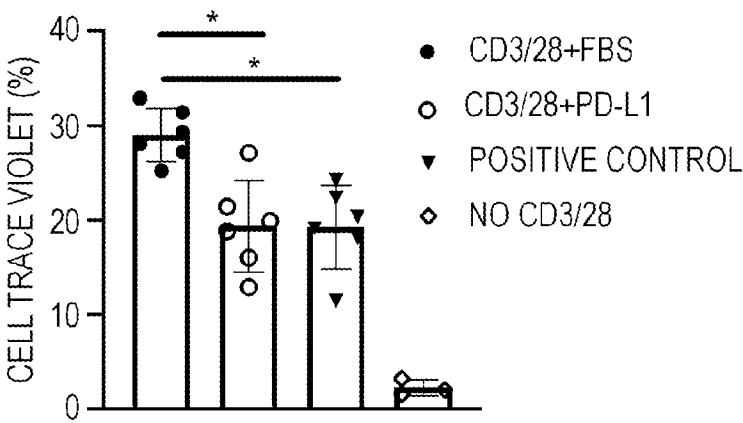


FIG. 1D

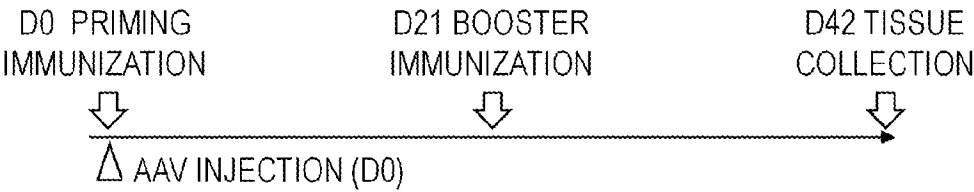


FIG. 2A

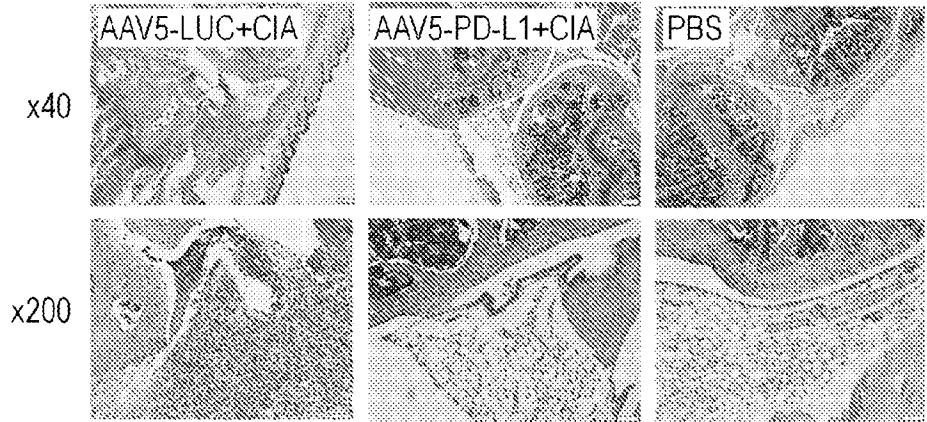


FIG. 2B

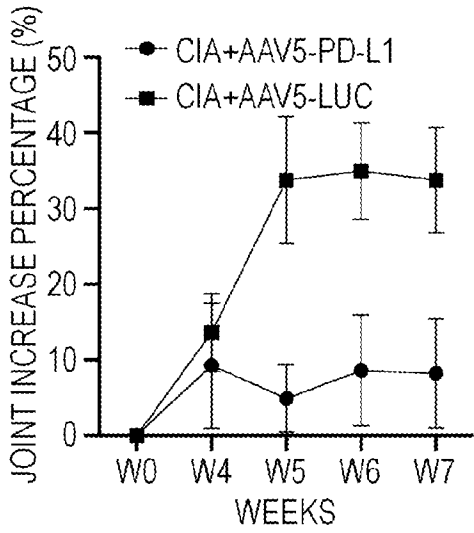


FIG. 2C

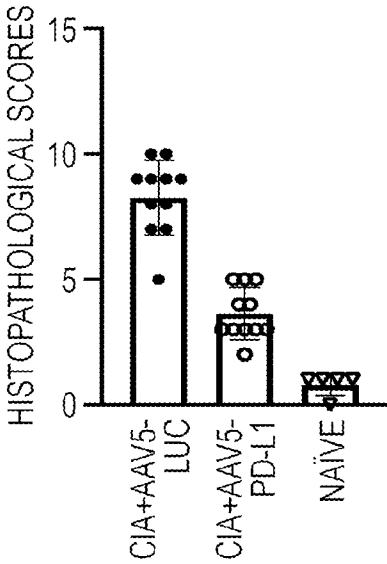


FIG. 2D

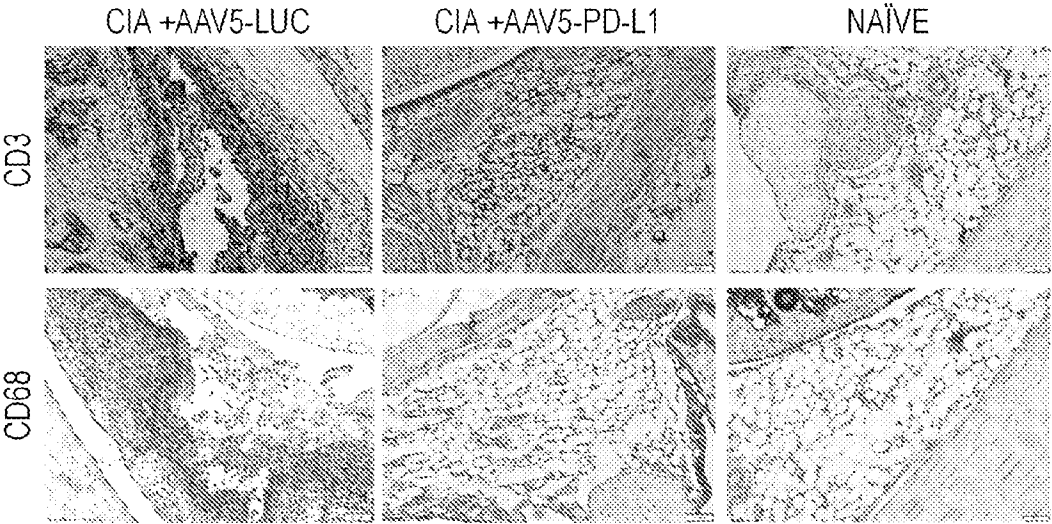


FIG. 3A

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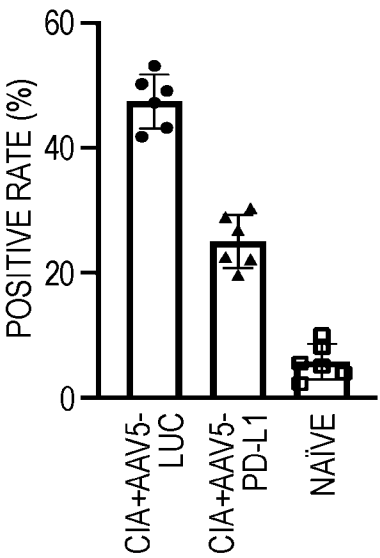


FIG. 3B

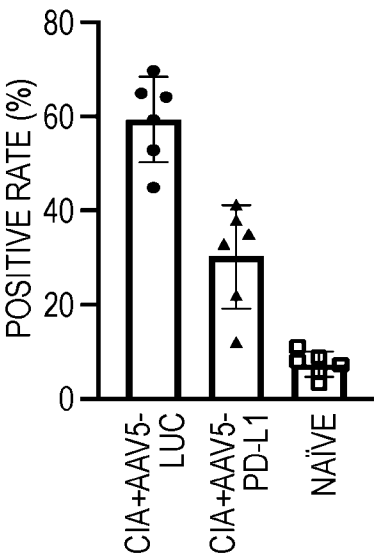


FIG. 3C

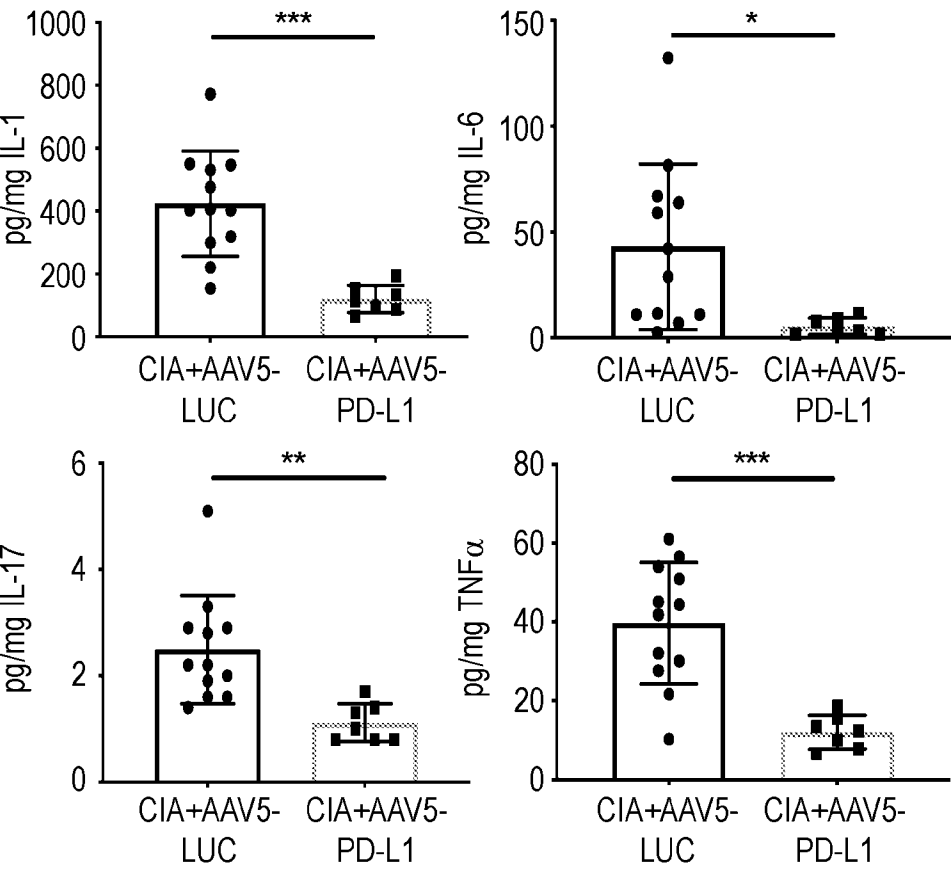


FIG. 4

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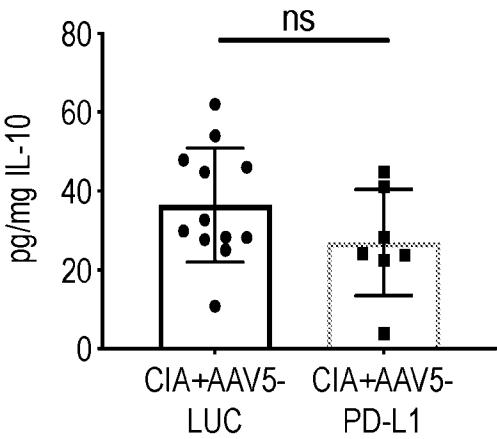


FIG. 4 (cont.)

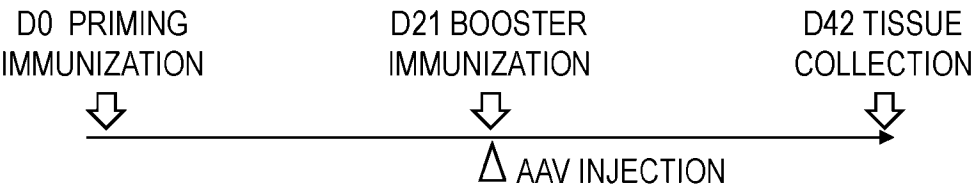


FIG. 5A

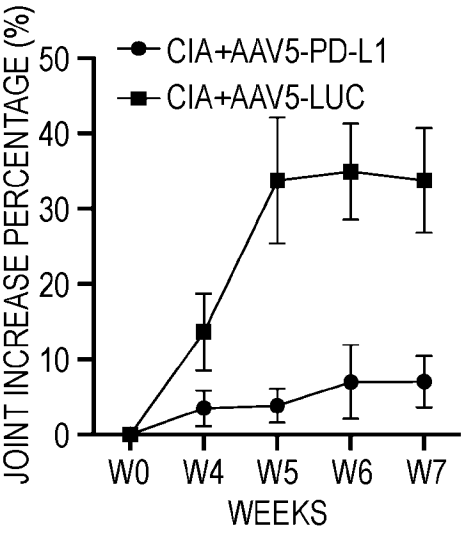


FIG. 5B

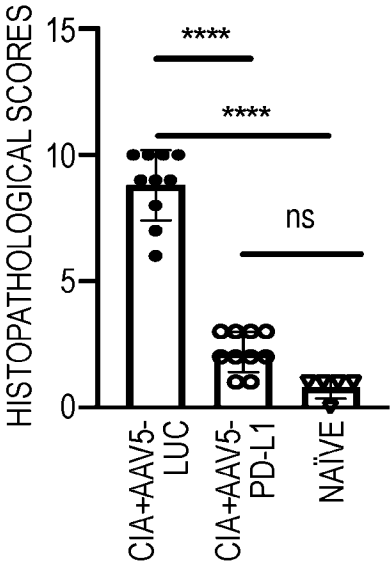


FIG. 5C

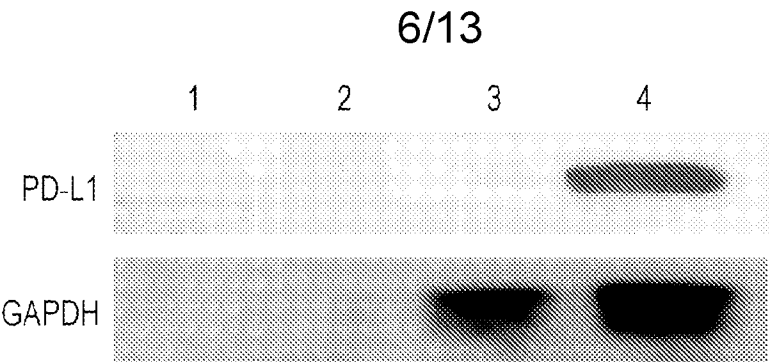


FIG. 6

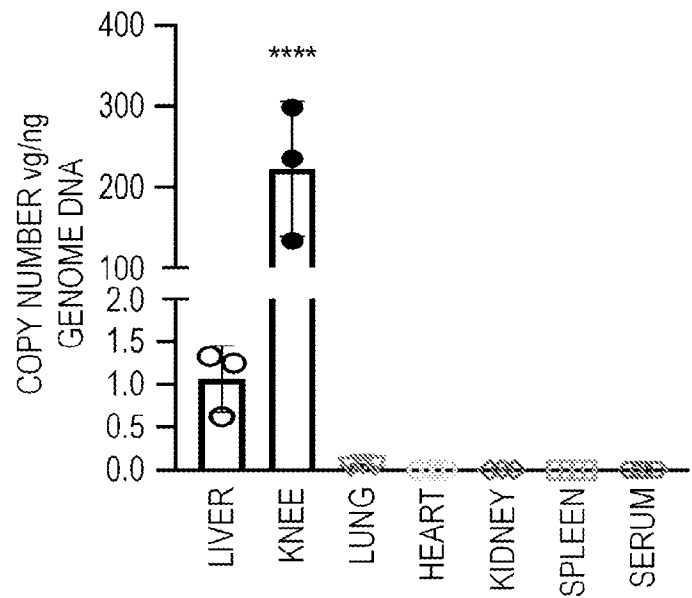


FIG. 7

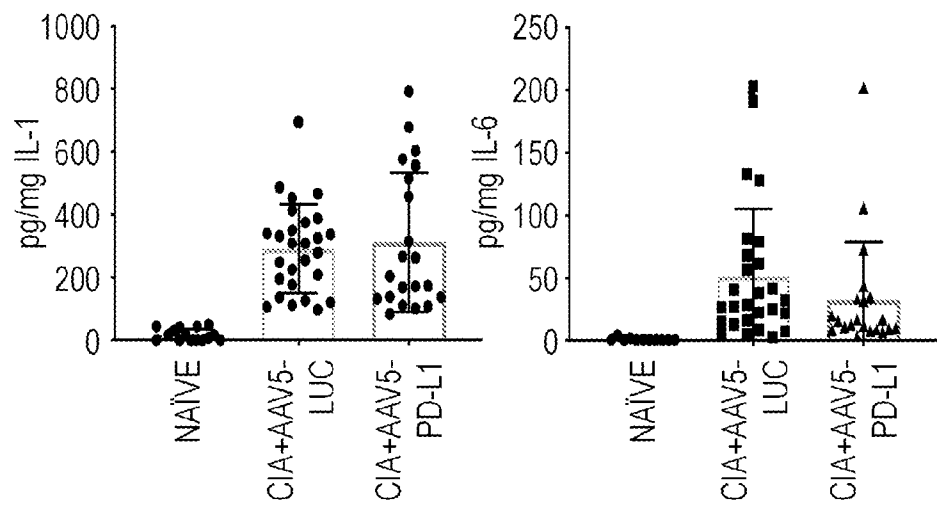


FIG. 8

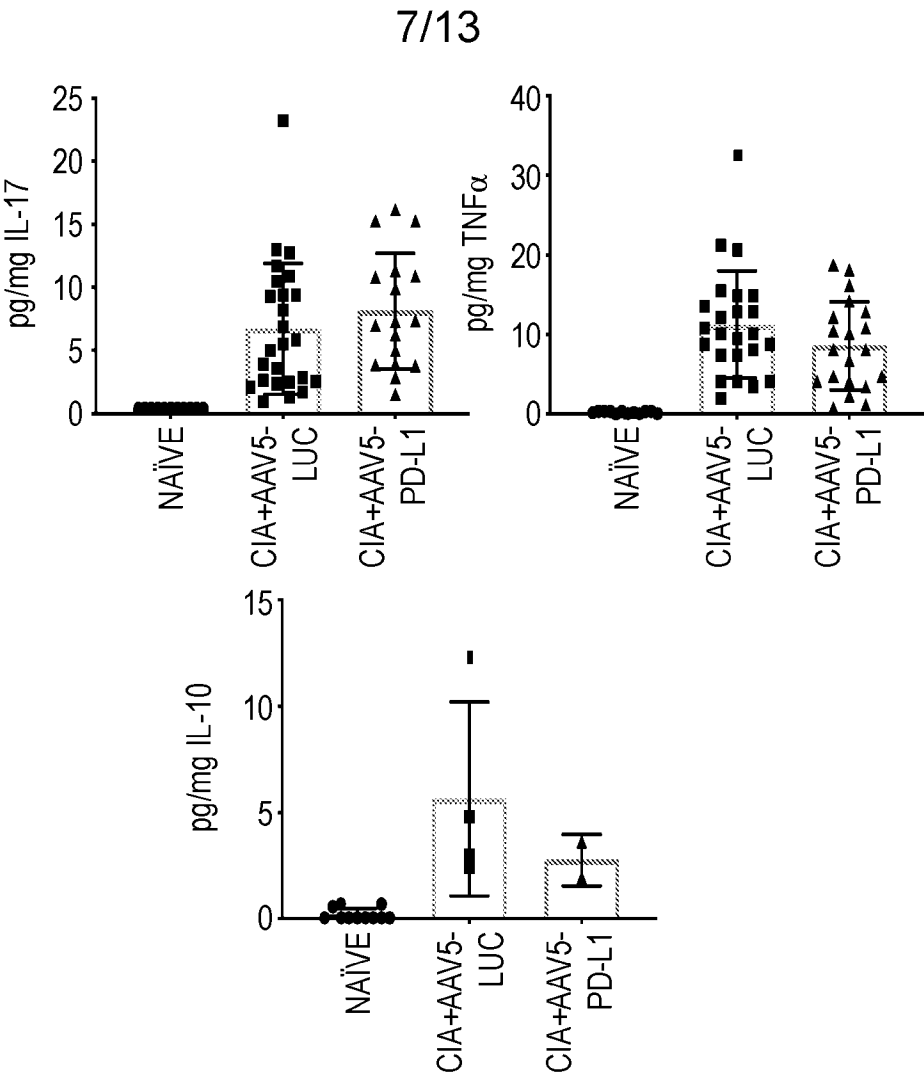


FIG. 8 (cont.)

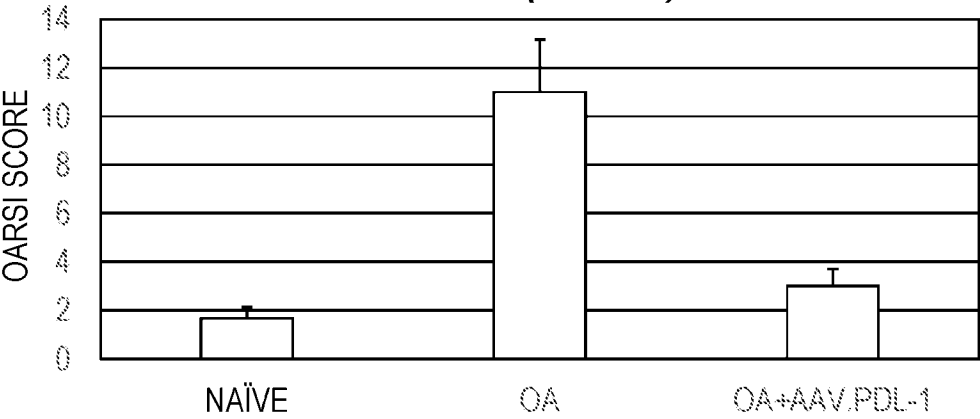


FIG. 9

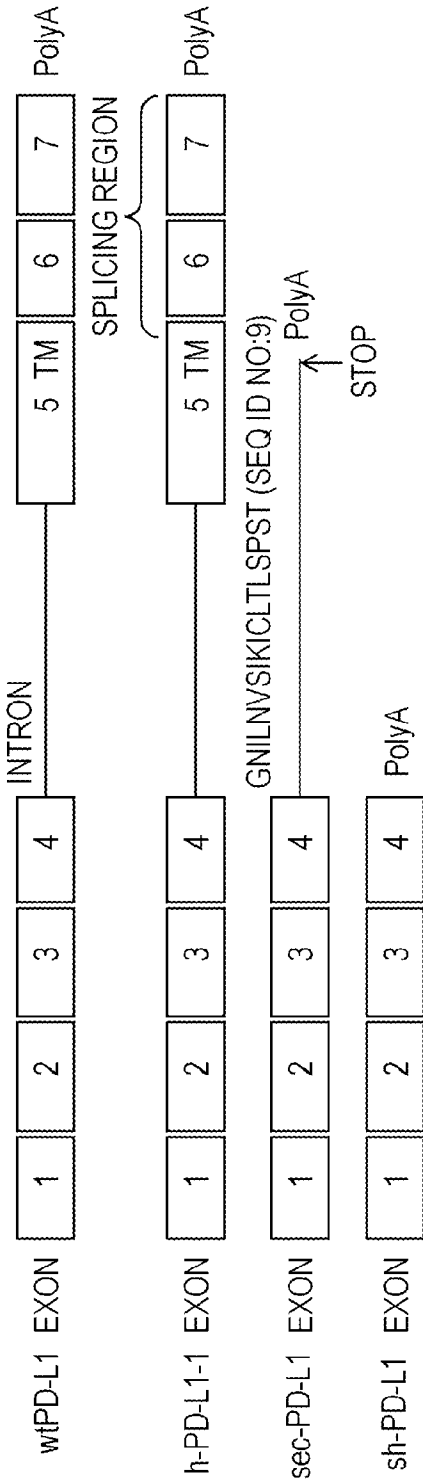


FIG. 10

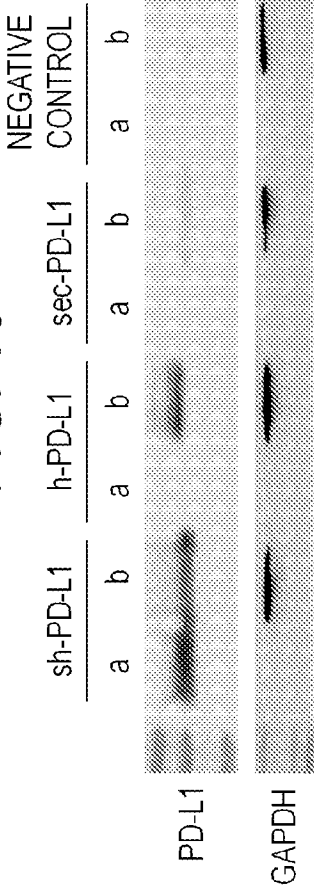


FIG. 11

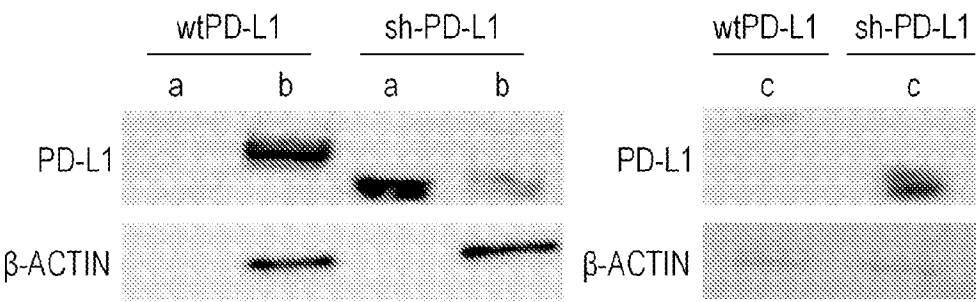


FIG. 12A

FIG. 12B

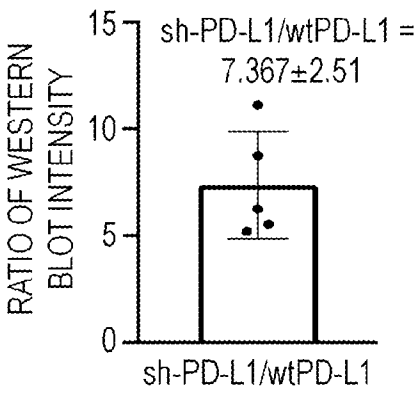


FIG. 12C

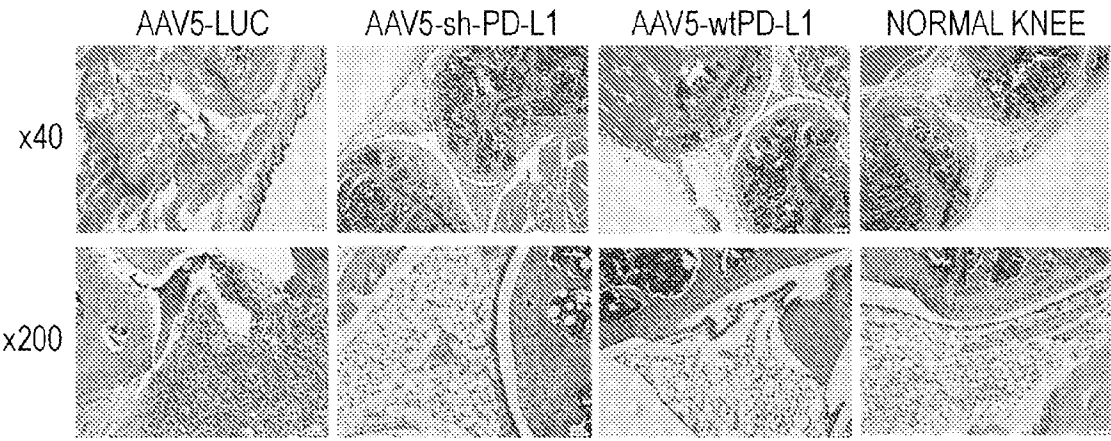


FIG. 13A

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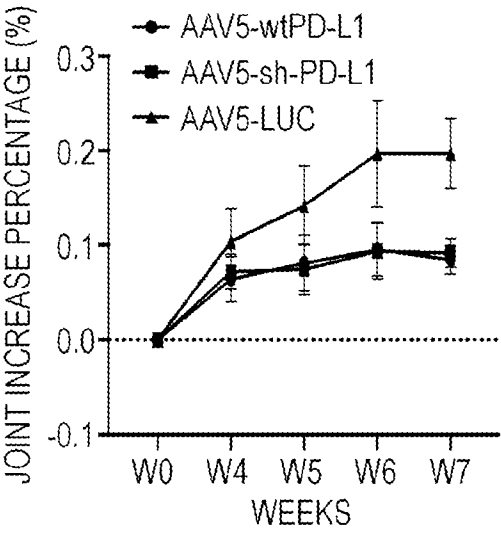


FIG. 13B

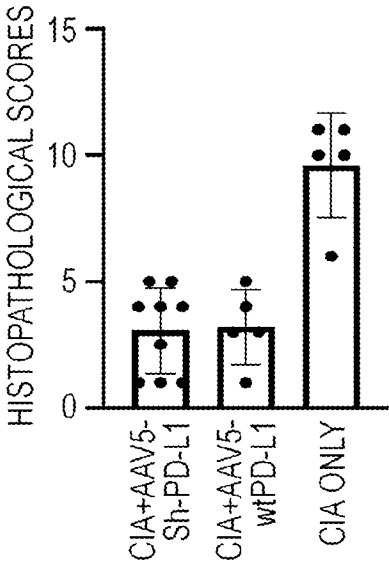


FIG. 13C

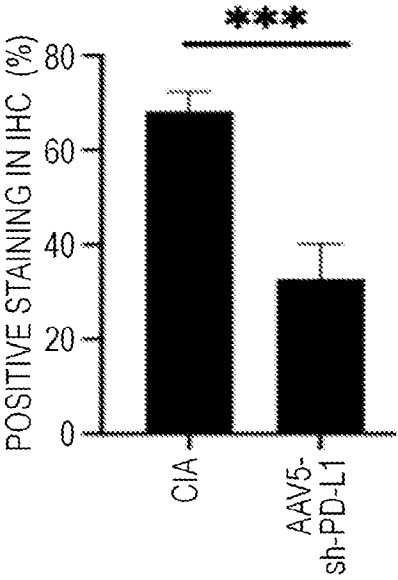


FIG. 14A

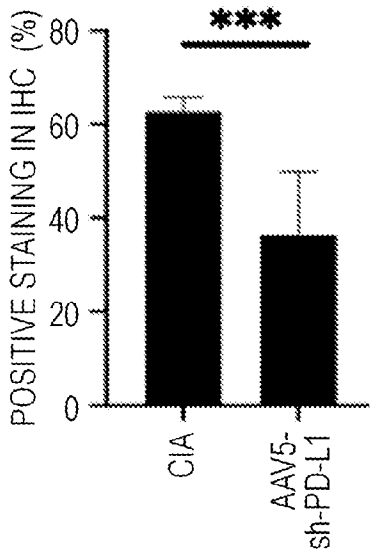


FIG. 14B

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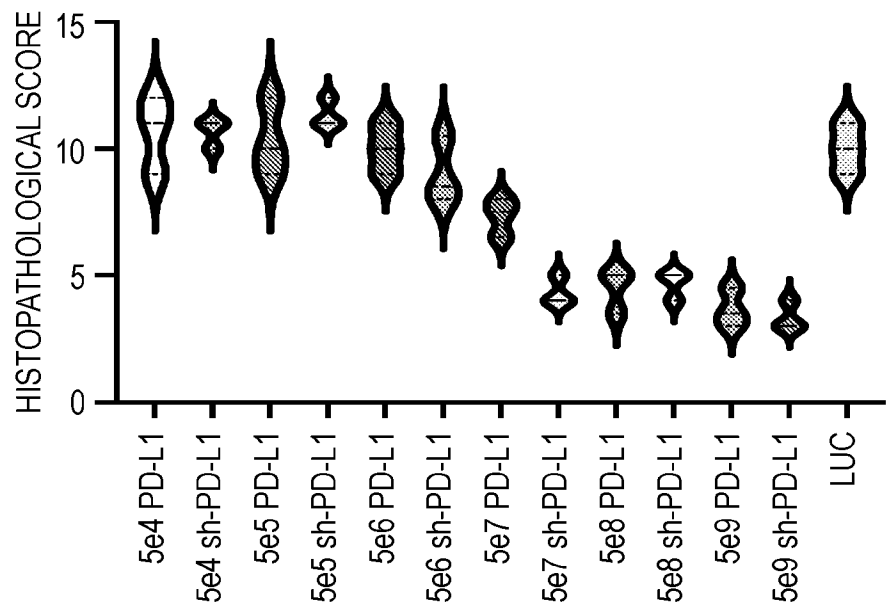


FIG. 15

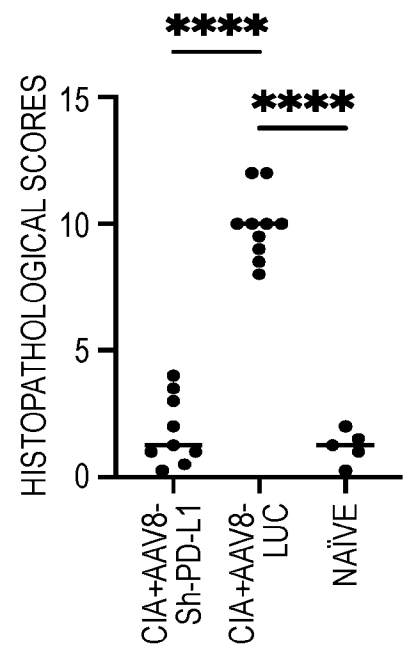


FIG. 16

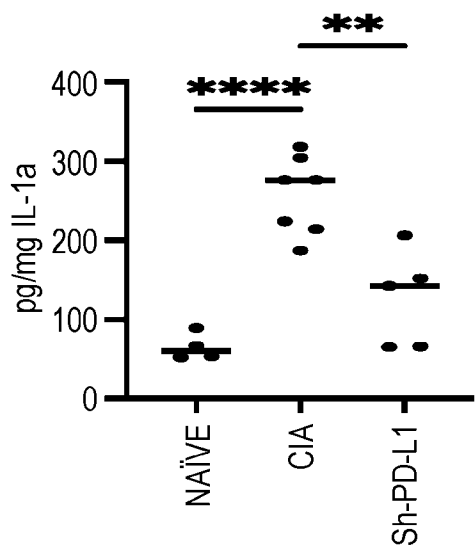


FIG. 17

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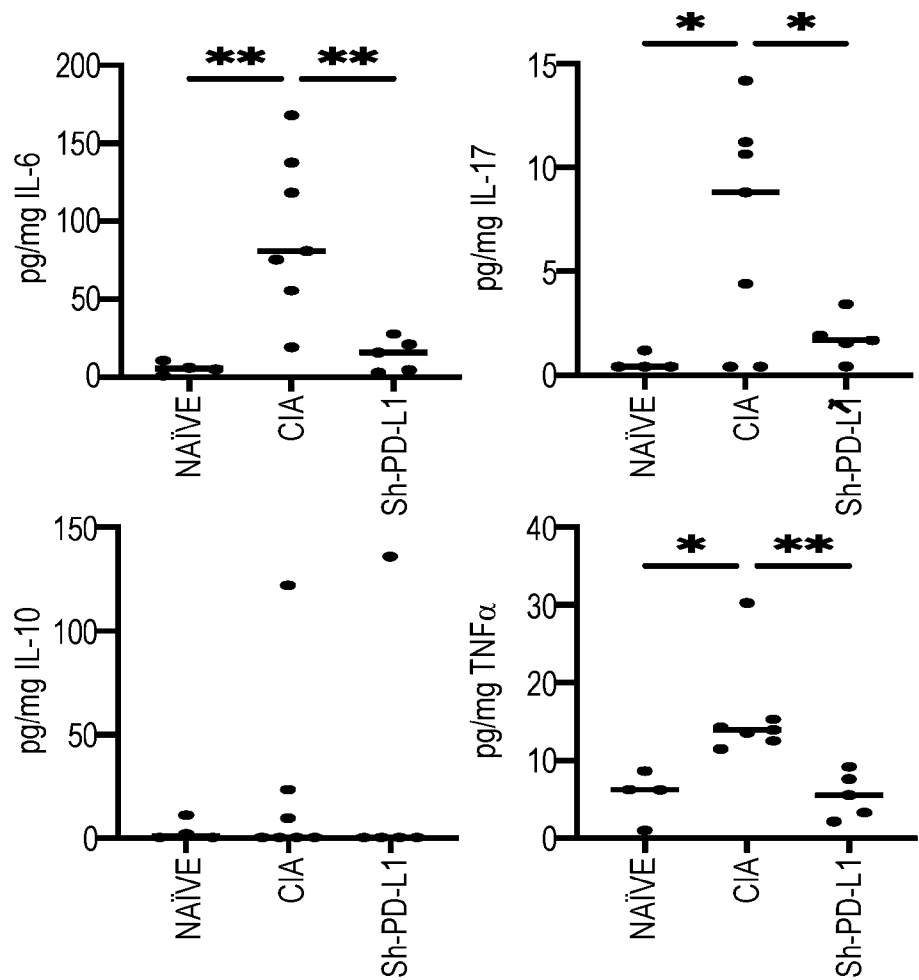


FIG. 17 (cont.)

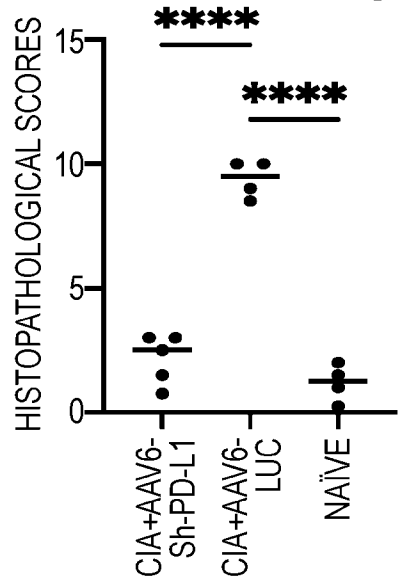


FIG. 18

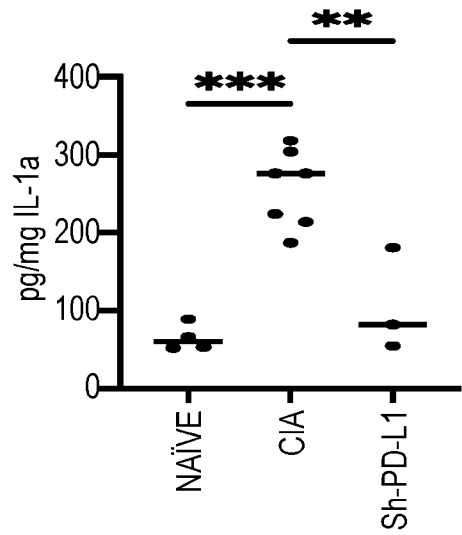


FIG. 19

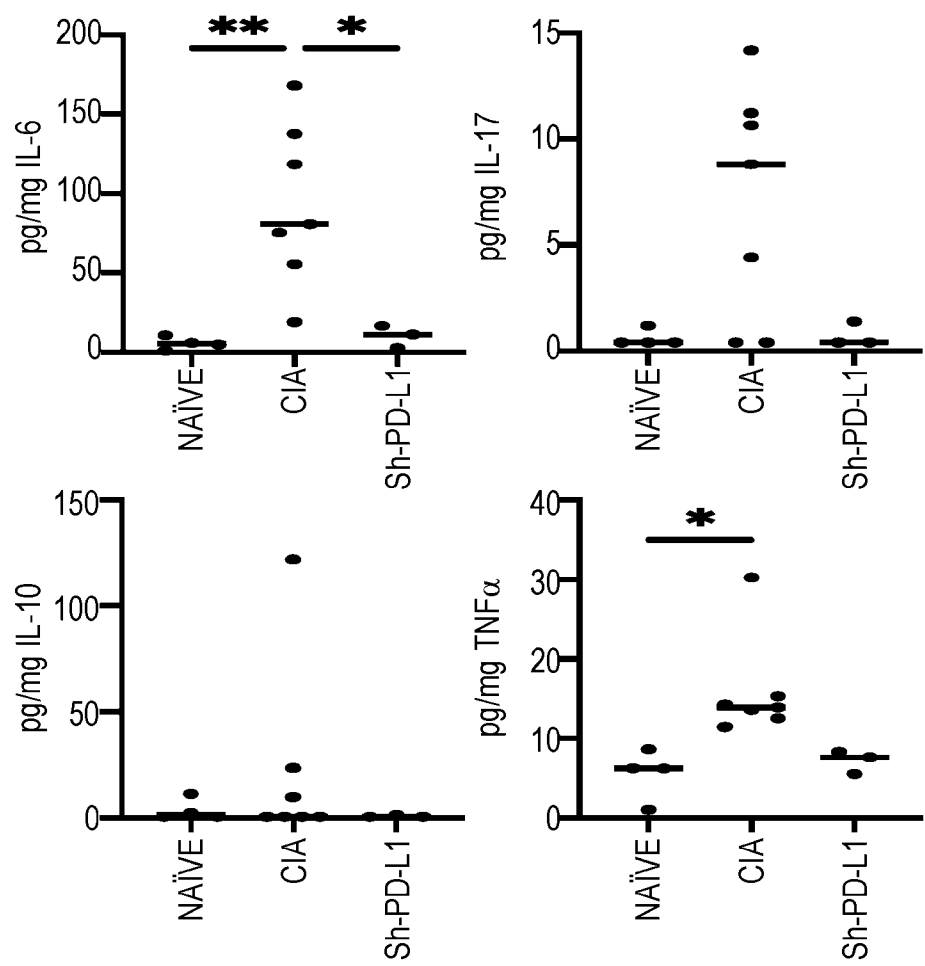


FIG. 19 (cont.)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/011743

A. CLASSIFICATION OF SUBJECT MATTERIPC: **A61K 38/17** (2024.01); **A61P 37/06** (2024.01); **C07K 14/705** (2024.01); **C12N 15/85** (2024.01)CPC: **A61P 37/06**; **A61K 38/1774**; **C07K 14/70532**; **C12N 15/85**; **A61K 39/4621**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2006/0003323 A1 (ALSOBROOK et al.) 05 January 2006 (05.01.2006) entire document	1-7, 17-23, 37-43
A	US 2002/0055139 A1 (HOLTZMAN et al.) 09 May 2002 (09.05.2002) entire document	1-7, 17-23, 37-43
A	WO 2021/197430 A1 (NANJING LEGEND BIOTECH CO. LTD.) 07 October 2021 (07.10.2021) entire document	1-7, 17-23, 37-43



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“D” document cited by the applicant in the international application

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

21 March 2024 (21.03.2024)

Date of mailing of the international search report

14 May 2024 (14.05.2024)

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

MATOS
TAINA

Telephone No. 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/011743

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.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **10-16, 26-36, 46-53**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Group I+: claims 1-9, 17-25, and 37-45 are drawn to methods for treating an autoimmune condition or graft rejection in a subject in need thereof, methods for delaying onset of an autoimmune condition or graft rejection in a subject, and nucleic acid delivery vectors.

The first invention of Group I+ is restricted to an immune checkpoint protein comprising SEQ ID NO: 10 encoded by a nucleic acid sequence comprising SEQ ID NO: 1, methods for treating an autoimmune condition or graft rejection in a subject in need thereof comprising the same, methods for delaying onset of an autoimmune condition or graft rejection in a subject comprising the same, and nucleic acid delivery vectors comprising the same. The first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the first named invention was selected based on the first listed compound species presented in the claims (see claims 5 and 6). It is believed that claims 1-7, 17-23, and 37-43 read on this first named invention and thus these claims will be searched without fee to the extent that they read on an immune checkpoint protein comprising SEQ ID NO: 10 encoded by a nucleic acid sequence comprising SEQ ID NO: 1, methods for treating an autoimmune condition or graft rejection in a subject in need thereof comprising the same, methods for delaying onset of an autoimmune condition or graft rejection in a subject comprising the same, and nucleic acid delivery vectors comprising the same.

Applicant is invited to elect additional immune checkpoint proteins and nucleic acids encoding the same to be searched in a specific combination by paying an additional fee for each election. An exemplary election would be an immune checkpoint protein comprising SEQ ID NO: 18 encoded by the nucleic acid comprising SEQ ID NO: 2, methods for treating an autoimmune condition or graft rejection in a subject in need thereof comprising the same, methods for delaying onset of an autoimmune condition or graft rejection in a subject comprising the same, and nucleic acid delivery vectors comprising the same. Additional immune checkpoint proteins will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

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

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for treating autoimmune conditions requiring the selection of alternative immune checkpoint proteins “wherein the PD-L1 protein is human PD-L1 having an amino acid sequence of any one or SEQ ID NOs:10, 11, 12, 19 or 20, or sh-PD-L1 having an amino acid sequence of SEQ ID NO:18” and “wherein the PD-L1 protein is encoded by the nucleotide sequence human PD-L1 (SEQ ID NO:1) or sh-PD-L1 (SEQ ID NO:2).”

Additionally, even if Groups I+ were considered to share the technical features of a method for treating an autoimmune condition or graft rejection in a subject in need thereof, comprising administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or a functional fragment or derivative thereof, thereby treating the autoimmune condition or graft rejection in the subject; a method for delaying onset of an autoimmune condition or graft rejection in a subject, comprising administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or functional fragment or derivative thereof, thereby delaying onset of the autoimmune condition or graft rejection in the subject; and a nucleic acid delivery vector comprising a nucleic acid encoding an immune checkpoint protein or a functional fragment or derivative thereof, these shared technical features do not represent a contribution over the prior art.

Specifically, WO 2021/197430 A1 to Nanjing Legend Biotech Co. Ltd. teaches a method for treating an autoimmune condition or graft rejection in a subject in need thereof, comprising administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or a functional fragment or derivative thereof, thereby treating the autoimmune condition or graft rejection in the subject (a method of reducing graft rejection of allogeneic therapeutic cells in an individual in need thereof, comprising administering to the individual an effective amount of the allogeneic therapeutic cells, wherein the allogeneic therapeutic cells comprise a first heterologous nucleic acid sequence encoding an immune checkpoint ligand (ICL), Para. [0016]; the ICL is selected from the group consisting of PD-L1, Para. [0018]); a method for delaying onset of an autoimmune condition or graft rejection in a subject, comprising administering to the subject an effective amount of an immune checkpoint protein or a functional fragment or derivative thereof, or a nucleic acid molecule encoding the immune checkpoint protein or functional fragment or derivative thereof, thereby delaying onset of the autoimmune condition or graft rejection in the subject (a method of reducing graft rejection of allogeneic therapeutic cells in an individual in need thereof, comprising administering to the individual an effective amount of the allogeneic therapeutic cells, wherein the allogeneic therapeutic cells comprise a first heterologous nucleic acid sequence encoding an immune checkpoint ligand (ICL), Para. [0016]; the ICL is selected from the group consisting of PD-L1, Para. [0018]; “treatment” or “treating” is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following:... delay or slowing the progression of the disease, Para. [0044]); and a nucleic acid delivery vector comprising a nucleic acid encoding an immune checkpoint protein or a functional fragment or derivative thereof (the heterologous nucleic acid sequence (s) are present in a vector. In some embodiments, the modified therapeutic cell comprises a vector comprising a first heterologous nucleic acid sequence encoding an ICL, Para. [0245]; the ICL is selected from the group consisting of PD-L1, Para. [0018]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/011743

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-7, 17-23, 37-43**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.