ADENO-ASSOCIATED VIRUS COMPOSITIONS AND METHODS OF USE THEREOF FOR HUMAN CELLS

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] The present application claims benefit of priority to U.S. Provisional Patent Application No. 63/556,179, filed February 21, 2024, which is incorporated by reference for all purposes.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Federal Grant No. R01HL089221 and R01GM127708 awarded by the National Institutes of Health. The Federal Government has certain rights to this invention.

BACKGROUND OF THE INVENTION

[0003] Adeno-associated virus (AAV) vectors have become a leading platform for gene delivery for the treatment of a variety of diseases. Although there has been clinical success using AAV gene therapies, limitations and challenges associated with use of this gene delivery platform remain. Gene therapy with vectors (viral or non-viral) is sometimes complicated because of an immune response against the vector carrying the gene. Viral vectors are the most likely to induce an immune response, especially those like adenovirus and AAV that express immunogenic epitopes within the organism. Immunity against vectors and their contents can substantially reduce the efficiency of gene therapy. A strong immune response against the constituents of the vector or the transgene leads to rejection of the cells infected by the vector and, therefore, to a reduction in the duration of expression of the therapeutic protein. Due to the many different and complex roles they play in host immune responses, immune cells have been identified as important targets for treatment of immunodeficiencies and cancer and for the development of cell-based immune-mediated therapeutics, such as chimeric antigen receptor (CAR) T cells. As such, immune cells, such as T cells and NK cells, can be important targets for AAV-mediated gene therapies. Such efforts have been hampered, however, in that AAV has generally been considered inefficient

at transducing T cells. Further, because immune cells are ubiquitously found throughout the body in blood and other lymphoid and non-lymphoid tissues, AAV-mediated gene therapies targeting immune cells would require systemic delivery at high doses, further triggering the undesired immune responses to the AAV vectors. As such, there is a need in the field for improved AAV vectors for therapeutic gene delivery, particularly for use in AAV-mediated immune cell gene therapies. Additionally, there is a need to develop AAV-based gene therapies that can selectively and specifically target tissues of interest, including tissues that have been difficult to target using known AAV serotypes, including multiple immune cell types such as T cells and NK cells.

[0004] WO2023/004407 shows that Ark313, a synthetic AAV that exhibits high transduction efficiency in murine T cells, can be used for nucleofection-free DNA delivery, CRISPR/Cas9-mediated gene knockouts, and targeted integration of large transgenes with efficiencies up to 50%.

BRIEF SUMMARY OF THE INVENTION

[0005] In some embodiments, a nucleic acid molecule is provided comprising a polynucleotide encoding an adeno- associated virus (AAV) capsid protein variant, wherein the encoded AAV capsid protein variant comprises the sequence of SEQ ID NO:1, except wherein amino acids 454-460 of the capsid protein variant are selected from the group of SEQ ID NOS: 6-94. In some embodiments, the capsid protein variant comprises a sequence selected from SEQ ID NO:2, 4, or 95-102.

[0006] In some embodiments, an AAV capsid protein variant is provided comprising a sequence having at least 90% or 95% identity to the sequence set forth in SEQ ID NO:1, wherein amino acids of the capsid protein variant corresponding to 454-460 of SEQ ID NO:1 comprise the sequence set forth in any one of SEQ ID NOS: 6-94. In some embodiments, the AAV capsid protein variant comprises a sequence selected from SEQ ID NO:2, 4, or 95-102.

[0007] In some embodiments, a recombinant AAV capsid is provided comprising about 60 copies of the AAV capsid protein variant as described above or elsewhere herein.

[0008] In some embodiments, a recombinant AAV (rAAV) vector is provided comprising: a vector genome, wherein the vector genome is encapsidated by an AAV capsid comprising the AAV capsid protein variant as described above or elsewhere herein. In some embodiments, the vector genome comprises a first inverted terminal repeat (ITR) and a second ITR. In some embodiments, the vector genome comprises a heterologous

polynucleotide located between the first ITR and the second ITR. In some embodiments, the heterologous polynucleotide comprises a transgene encoding a therapeutic RNA or a therapeutic protein. In some embodiments, the heterologous polynucleotide comprises a donor polynucleotide for genetic editing. In some embodiments, the transgene encodes a gene-editing molecule. In some embodiments, the gene-editing molecule comprises a nuclease, epigenome-editing enzyme or a base-pair editing enzyme. In some embodiments, the nuclease comprises a Cas9 nuclease.

[0009] In some embodiments, the gene-editing molecule comprises one or more single guide RNA (sgRNA). In some embodiments, the single guide RNA (sgRNA) targets a gene in a T cell or NK cell.

[0010] Also provided is a pharmaceutical composition comprising the rAAV vector as described above or elsewhere herein and at least one pharmaceutically acceptable carrier.

[0011] Also provided is a method of delivering a heterologous polynucleotide to a human target cell. In some embodiments, the method comprises contacting the rAAV vector as described above or elsewhere herein to the human cell, wherein the rAAV vector delivers the heterologous polynucleotide to the cell.

[0012] Also provided is a method of delivering a transgene to a target cell in a human subject. In some embodiments, the method comprises administering to the subject a therapeutically effective amount of the rAAV vector as described above or elsewhere herein, or the pharmaceutical composition as described above or elsewhere herein. In some embodiments, the target cell is an immune cell. In some embodiments, the immune cell comprises a T cell, a NK cell, or a combination thereof.

[0013] Also provided is a method of alleviating and/or treating a disease or a condition in a subject in need thereof, the method comprising: administering to the subject a therapeutically effective amount of the rAAV vector as described above or elsewhere herein, or the pharmaceutical composition as described above or elsewhere herein.

[0014] Also provided is a method of alleviating and/or treating a disease or a condition in a human subject in need thereof. In some embodiments, the method comprises: administering to the subject one or more cells that have been contacted ex vivo with the rAAV vector as described above or elsewhere herein, or the pharmaceutical composition as described above

or elsewhere herein. In some embodiments, the disease or condition comprises an autoimmune disease or an immune deficiency disease.

[0015] In some embodiments, following the administering of the rAAV or the pharmaceutical composition, one or more aspects of T cell and/or NK cell cellular homeostasis and/or T cell and/or NK cell cellular functionality in the subject is improved and/or restored.

[0016] In some embodiments, the method further comprises repeating one or more times the administering step.

[0017] In some embodiments, the method further comprises monitoring the subject for adverse effects. In some embodiments, in the absence of adverse effects, the method further comprises continuing to treat the subject.

DEFINITIONS

[0018] For the purposes of promoting an understanding of the principles of the present disclosure, reference will now be made to preferred embodiments and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the disclosure is thereby intended, such alteration and further modifications of the disclosure as illustrated herein, being contemplated as would normally occur to one skilled in the art to which the disclosure relates.

[0019] As used in the specification, articles "a" and "an" are used herein to refer to one or to more than one (i.e., at least one) of the grammatical object of the article. By way of example, "an element" means at least one element and can comprise more than one element.

[0020] "About" is used to provide flexibility to a numerical range endpoint by providing that a given value can be "slightly above" or "slightly below" the endpoint without affecting the desired result. The term "about" in association with a numerical value means that the numerical value can vary plus or minus by 5% or less of the numerical value.

[0021] Throughout this specification, unless the context requires otherwise, the word "comprise" and "include" and variations (e.g., "comprises," "comprising," "includes," "including") will be understood to imply the inclusion of a stated component, feature, element, or step or group of components, features, elements or steps but not the exclusion of any other integer or step or group of integers or steps.

[0022] 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 where interpreted in the alternative ("or").

[0023] Moreover, the present disclosure also contemplates that 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.

[0024] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise-indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure.

[0025] As used herein, the term "adeno-associated virus" (AAV), includes but is not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, AAV type rh32.33, AAV type rh8, AAV type rh10, 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). A number of 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).

[0026] The genomic sequences of various serotypes of AAV and the autonomous parvoviruses, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences can be found in the literature or in public databases such as GenBank, such as, for example, 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, NC_001358, NC_001540, AF513851, AF513852, AY530579; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences.

Table 1 – Identification of Various AAV Serotypes and Clades

	GenBank Accession Number		GenBank Accession Number		GenBank Accession Number
Complete Genomes		Clade C		Rh57	AY530569
Adeno-associated virus 1	NC_00207, AF063497	Hu9	AY530629	Rh50	AY530563
Adeno-associated virus 2	NC 001401	Hu10	AY530576	Rh49	AY530562
Adeno-associated virus 3	NC 001729	Hu11	AY530577	Hu39	AY530601
Adeno-associated virus 3B	NC 001863	Hu53	AY530615	Rh58	AY530570
Adeno-associated virus 4	NC 001829	Hu55	AY530617	Rh61	AY530572
Adeno-associated virus 5	Y18065, AF085716	Hu54	AY530616	Rh52	AY530565
Adeno-associated virus 6	NC 001862	Hu7	AY530628	Rh53	AY530566
Avian AAV ATCC VR-865	AY186198, AY629583, NC 004828	Hu18	AY530583	Rh51	AY530564
Avian AAV strain DA-1	NC_006263, AY629583	Hu15	AY530580	Rh64	AY530574
Bovine AAV	NC_005889, AY388617, AAR26465	Hu16	AY530581	Rh43	AY530560
AAV11	AAT46339, AY631966	Hu25	AY530591	AAV8	AF513852
AAV12	AB116639, DQ813647	Hu60	AY530622	Rh8	AY242997
Clade A		Ch5	AY243021	Rh1	AY530556
AAV1	NC_002077, AF063497	Hu3	AY530595	Clade F	

	GenBank Accession Number		GenBank Accession Number		GenBank Accession Number
AAV6	NC_001862	Hu1	AY530575	Hu14 (AAV9)	AY530579
Hu.48	AY530611	Hu4	AY530602	Hu31	AY530596
Hu 43	AY530606	Hu2	AY530585	Hu32	AY530597
Hu 44	AY530607	Hu61	AY530623	HSC1	M1332400.1
Hu 46	AY530609	Clade D		HSC2	M1332401.1
Clade B		Rh62	AY530573	HSC3	M1332402.1
Hu. 19	AY530584	Rh48	AY530561	HSC4	M1332403.1
Hu. 20	AY530586	Rh54	AY530567	HSC5	M1332405.1
Hu 23	AY530589	Rh55	AY530568	HSC6	M1332404.1
Hu22	AY530588	Cy2	AY243020	HSC7	M1332407.1
Hu24	AY530590	AAV7	AF513851	HSC8	M1332408.1
Hu21	AY530587	Rh35	AY243000	HSC9	M1332409.1
Hu27	AY530592	Rh37	AY242998	HSC11	M1332406.1
Hu28	AY530593	Rh36	AY242999	HSC12	M1332410.1
Hu 29	AY530594	Cy6	AY243016	HSC13	M1332411.1
Hu63	AY530624	Cy4	AY243018	HSC14	M1332412.1
Hu64	AY530625	Cy3	AY243019	HSC15	M1332413.1
Hu13	AY530578	Cy5	AY243017	HSC16	M1332414.1
Hu56	AY530618	Rh13	AY243013	HSC17	M1332415.1
Hu57	AY530619	Clade E		Hu68	

	GenBank Accession Number		GenBank Accession Number		GenBank Accession Number
Hu49	AY530612	Rh38	AY530558	Clonal Isolate	
Hu58	AY530620	Hu66	AY530626	AAV5	Y18065, AF085716
Hu34	AY530598	Hu42	AY530605	AAV 3	NC_001729
Hu35	AY530599	Hu67	AY530627	AAV 3B	NC_001863
AAV2	NC_001401	Hu40	AY530603	AAV4	NC_001829
Hu45	AY530608	Hu41	AY530604	Rh34	AY243001
Hu47	AY530610	Hu37	AY530600	Rh33	AY243002
Hu51	AY530613	Rh40	AY530559	Rh32	AY243003
Hu52	AY530614	Rh2	AY243007	Others	
Hu T41	AY695378	Bb1	AY243023	Rh74	
Hu S17	AY695376	Bb2	AY243022	Bearded Dragon AAV	
Hu T88	AY695375	Rh10	AY243015	Snake AAV	NC_006148.1
Hu T71	AY695374	Hu17	AY530582		
Hu T70	AY695373	Hu6	AY530621		
Hu T40	AY695372	Rh25	AY530557		
Hu T32	AY695371	Pi2	AY530554		
Hu T17	AY695370	Pi1	AY530553		
Hu LG15	AY695377	Pi3	AY530555		

[0027] The terms "heterologous nucleotide sequence" and "heterologous nucleic acid" are used interchangeably herein and refer to a sequence that is not naturally occurring in the virus. Generally, the heterologous nucleic acid comprises an open reading frame that encodes a polypeptide or nontranslated RNA of interest (e.g., for delivery to a cell or subject).

[0028] A "polynucleotide" or "nucleotide" as used herein refers to a sequence of nucleotide bases, and can be RNA, DNA or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotide), but in representative aspects are either single or double stranded DNA sequences.

[0029] As used herein, the term "peptide" refers to a short amino acid sequence. The term peptide can be used to refer to portion or region of an AAV capsid amino acid sequence. The peptide can be a peptide that naturally occurs in a native AAV capsid, or a peptide that does not naturally occur in a native AAV capsid. Naturally occurring AAV peptides in an AAV capsid can be substituted by non-naturally occurring peptides. For example, a non-naturally occurring peptide can be substituted into an AAV capsid to provide a modified capsid, such that the naturally-occurring peptide is replaced by the non-naturally occurring peptide. As used herein, the term "polypeptide" encompasses both peptides and proteins, unless indicated otherwise.

[0030] As used herein, the term "amino acid" encompasses any naturally occurring amino acid, modified forms thereof, and synthetic amino acids. Alternatively, an amino acid herein can be a modified amino acid residue and/or can be an amino acid that is modified by post-translation modification (e.g., acetylation, amidation, formylation, hydroxylation, methylation, phosphorylation or sulfatation). Naturally occurring, levorotatory (L-) amino acids are shown in Table 2.

Table 2 – Listing of Amino Acids and Corresponding Codes

Amino Acid Residue	3-Letter Code	1-Letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid (Aspartate)	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid (Glutamate)	Glu	E

Amino Acid Residue	3-Letter Code	1-Letter Code
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0031] Alternatively, the amino acid can be a modified amino acid residue (nonlimiting examples are shown in Table 3) and/or can be an amino acid that is modified by post-translation modification (e.g., acetylation, amidation, formylation, hydroxylation, methylation, phosphorylation or sulfatation).

Table 3 – Listing of Modified Amino Acid Residues

Modified Amino Acid Residue	Abbreviation
2-Aminoadipic acid	Aad
3-Aminoadipic acid	bAad
beta-Alanine, beta-Aminoproprionic acid	bAla
2-Aminobutyric acid	Abu
4-Aminobutyric acid, Piperidinic acid	4Abu
6-Aminocaproic acid	Acp
2-Aminoheptanoic acid	Ahe
2-Aminoisobutyric acid	Aib
3-Aminoisobutyric acid	bAib
2-Aminopimelic acid	Apm
t-butylalanine	t-BuA

Modified Amino Acid Residue	Abbreviation
Citrulline	Cit
Cyclohexylalanine	Cha
2,4-Diaminobutyric acid	Dbu
Desmosine	Des
2,2′-Diaminopimelic acid	Dpm
2,3-Diaminoproprionic acid	Dpr
N-Ethylglycine	EtGly
N-Ethylasparagine	EtAsn
Homoarginine	hArg
Homocysteine	hCys
Homoserine	hSer
Hydroxylysine	Hyl
Allo-Hydroxylysine	aHyl
3-Hydroxyproline	3Hyp
4-Hydroxyproline	4Hyp
Isodesmosine	Ide
allo-Isoleucine	aIle
Methionine sulfoxide	MSO
N-Methylglycine, sarcosine	MeGly
N-Methylisoleucine	MeIle
6-N-Methyllysine	MeLys
N-Methylvaline	MeVal
2-Naphthylalanine	2-Nal
Norvaline	Nva
Norleucine	Nle
Ornithine	Orn
4-Chlorophenylalanine	Phe(4-Cl)
2-Fluorophenylalanine	Phe(2-F)
3-Fluorophenylalanine	Phe(3-F)
4-Fluorophenylalanine	Phe(4-F)
Phenylglycine	Phg

Modified Amino Acid Residue	Abbreviation
Beta-2-thienylalanine	Thi

[0032] Further, the non-naturally occurring amino acid can be an "unnatural" amino acid as described by Wang et al., *Annu Rev Biophys Biomol Struct.* 35:225-49 (2006). These unnatural amino acids can advantageously be used to chemically link molecules of interest to the AAV capsid protein.

[0033] As used herein, the terms "virus vector," "vector" or "gene delivery vector" refer to a virus (e.g., AAV) particle that functions as a nucleic acid delivery vehicle, and which comprises the vector genome (e.g., viral DNA or vDNA) packaged within a virion. Alternatively, in some contexts, the term "vector" can be used to refer to the vector genome/vDNA alone.

[0034] A "rAAV vector genome" or "rAAV genome" as used herein is an AAV genome (i.e., vDNA) that comprises one or more heterologous nucleic acid sequences. rAAV vectors generally require only the terminal repeat(s) (TR(s)) in cis to generate virus. All other viral sequences are dispensable and can be supplied in trans (Muzyczka, (1992) *Curr. Topics Microbiol. Immunol.* 158:97). Typically, the rAAV vector genome will only retain the one or more TR sequence so as to maximize the size of the transgene that can be efficiently packaged by the vector. The structural and non-structural protein coding sequences can be provided in trans (e.g., from a vector, such as a plasmid, or by stably integrating the sequences into a packaging cell). In an aspect, a disclosed rAAV vector genome comprises at least one TR sequence (e.g., AAV TR sequence), optionally two TRs (e.g., two AAV TRs), which typically will be at the 5′ and 3′ ends of the vector genome and flank the heterologous nucleic acid, but need not be contiguous thereto. The TRs can be the same or different from each other.

[0035] 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 (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 TR 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 any other suitable virus sequence (e.g., 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 U.S. Pat. No. 5,478,745 to Samulski et al.

[0036] An "AAV terminal repeat" or "AAV TR" can be from any AAV, including but not limited to serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or any other AAV now known or later discovered (see, e.g., Table 1). An AAV terminal repeat need not have the native terminal repeat sequence (e.g., a native AAV TR sequence can be altered by insertion, deletion, truncation and/or missense mutations), as long as the terminal repeat mediates the desired functions, e.g., replication, virus packaging, integration, and/or provirus rescue, and the like.

[0037] An AAV vector typically comprises a protein-based capsid, and a nucleic acid encapsidated by the capsid. The nucleic acid can be, for example, a vector genome comprising a heterologous polynucleotide (e.g., a transgene or donor polynucleotide sequence, e.g., an HDRT or HITI sequence) flanked by inverted terminal repeats. The AAV "capsid" is a near-spherical protein shell that comprises individual "capsid proteins" or "subunits." AAV capsids typically comprise about 60 capsid protein subunits, associated and arranged with T=1 icosahedral symmetry. When an AAV vector is described herein as comprising an AAV capsid protein, it will be understood that the AAV vector comprises a capsid, wherein the capsid comprises one or more AAV capsid proteins (i.e., subunits). Also described herein are "viral-like particles" or "virus-like particles," which refers to a capsid that does not comprise any vector genome or nucleic acid comprising a transgene.

[0038] The virus vectors of the present disclosure can further be "targeted" virus vectors (e.g., having a directed tropism) and/or a "hybrid" parvovirus (i.e., in which the viral TRs and viral capsid are from different parvoviruses) as described in international patent publication WO 00/28004 and Chao et al. (2000) Molecular Therapy. 2:619.

[0039] The virus vectors of the present disclosure can further be duplexed parvovirus particles as described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety). Thus, in an aspect, double stranded (duplex) genomes can be packaged into a disclosed virus capsids. Further, the viral capsid or genomic elements can contain other modifications, including insertions, deletions and/or substitutions.

[0040] The term "self-complimentary AAV" or "scAAV" refers to a recombinant AAV vector which forms a dimeric inverted repeat DNA molecule that spontaneously anneals, resulting in earlier and more robust transgene expression compared with conventional single-strand (ss) AAV genomes. See, e.g., McCarty, D.M., et al., Gene Therapy 8, 1248- 1254 (2001). Unlike conventional ssAAV, scAAV can bypass second-strand synthesis, the rate-limiting step for gene expression. Moreover, double-stranded scAAV is less prone to DNA degradation after viral transduction, thereby increasing the number of copies of stable episomes. Notably, scAAV can typically only hold a genome that is about 2.4 kb, half the size of a conventional AAV vector. In an aspect, the AAV vectors described herein are self-complementary AAVs.

[0041] A "therapeutic polypeptide" or "therapeutic protein" is a polypeptide or protein that can alleviate, reduce, prevent, delay and/or stabilize symptoms that result from an absence or defect in a protein in a cell or subject and/or is a polypeptide that otherwise confers a benefit to a subject, e.g., anti-cancer effects or improvement in transplant survivability.

[0042] By the terms "treat," "treating" or "treatment of" (and grammatical variations thereof) it is meant that the severity of the subject's condition is reduced, at least partially improved or stabilized and/or that some alleviation, mitigation, decrease or stabilization in at least one clinical symptom is achieved and/or there is a delay in the progression of the disease or disorder.

[0043] The terms "prevent," "preventing" and "prevention" (and grammatical variations thereof) refer to prevention and/or delay of the onset of a disease, disorder and/or a clinical symptom(s) in a subject and/or a reduction in the severity of the onset of the disease, disorder and/or clinical symptom(s) relative to what would occur in the absence of the methods of the invention. The prevention can be complete, e.g., the total absence of the disease, disorder and/or clinical symptom(s). The prevention can also be partial, such that the occurrence of the disease, disorder and/or clinical symptom(s) in the subject and/or the severity of onset is less than what would occur in the absence of the present invention.

[0044] As used herein, the term "subject" and "patient" are used interchangeably herein and refer to both human and nonhuman animals. The term "nonhuman animals" of the disclosure includes all vertebrates, e.g., mammals and non-mammals, such as nonhuman primates, sheep, dog, cat, horse, cow, chickens, amphibians, reptiles, and the like. In an

aspect, the subject can comprise a human. In an aspect, the subject can comprise a mouse. In an aspect, the subject can comprise a human in need of one or more gene therapies.

[0045] 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. The therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

[0046] 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. The level of prevention need not be complete, as long as some benefit is provided to the subject.

[0047] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] FIG. 1A-B: A) Schematic representation of the AAV evolution in serum conditions. Figure discloses SEQ ID NO: 103. B) Bubble pot depicting evolved AAV6 human T-cell tropic mutants compared to parental library. Each bubble represents a unique amino acid variant represented within the sequencing, y-axis depicts the log of percent reads for each amino acid variant detected and the x-axis is dimensionless. Bubble size represents enrichment of unique amino acid variants from the parental library as calculated by percent reads in the evolved library over the percent reads in the unselected library for each detected variant. Strong selection of motif AAV6 VP1 numbering S455A A456P Q457R was present in all enriched capsids when evolved on human T-cells. The figure discloses SEQ ID NOS 8, 104, 9, 7, 105, 5, 106, 13, 107, 20, 108, 18, 23, 30, 29, 27, 109, 32, 31, 34, 72, 36, 110, 39, and 111, respectively, in order of appearance.

[0049] FIG. 2: Ark315 and Ark316 packaging a self-comp CBh-GFP Cassette infected on primary human CD3/CD28 stimulated T-cells three days post-infection at 1E5, 1E4, 1E3 and 1E2 vg/cell compared to wildtype AAV6 capsid. 315 and 316 shows enhanced GFP

expression of primary human T-cells as compared to wildtype AAV6 vector across all multiplicities of infection.

[0050] FIG. 3: Non-activated primary human T-cells infected with 1E5 vg/cell with AAV6 and human T-cell tropic variants Ark315 and Ark316 packaging a self-complementary CBh-GFP cassette for two days. Virus in media or membrane bound was removed and genomic DNA was extracted. Viral genomes per cell was made by comparing the viral titer to the relative abundance of LAMB1. Human T-cell tropic variants Ark315 and Ark316 have 11.4, 9.6 and 6.9 more self-complementary CBh-GFP genomes per cell than the wildtype AAV6 indicating non-activated human T-cells are highly infected with variants Ark315 and Ark316.

[0051] FIG. 4A-C: Evolution in SF Media Reveals Capsid Mutants Ark325 and Ark397. (A) Graphical representation of high-throughput sequencing data. Each bubble represents a unique amino acid variant sequenced within the evolved library, with the y-axis being the log10 of the percent reads, the x-axis being dimensionless and the bubble size being the enrichment of a given variant from the parental library. The figure discloses SEQ ID NOS 5, 50, 112, 106, 105, 93, 84, 58, 79, 52, 67, 92, 104, 113, 80, 61, 72, and 114-115, respectively, in order of appearance. (B) and (C) Transduction of activated CD3/CD28 IL-2 activated human T-cells with AAV6, Ark325 and Ark397 packaging a scCBh-GFP genome assessed by flow cytometry in (B) serum or (C) SF conditions. (n=1).

[0052] FIG. 5: Activated human T cells were cultivated in the indicated serum and transduced at the indicated MOI of AAV6, Ark312, Ark315 or Ark313 (murine T cell specific) delivering a GFP expressing cassette.

[0053] FIG. 6A-C: Ark315 improves gene targeting rate at the CLTA locus in human serum condition. A) Integration of GFP HDRT at the CLTA locus to generate a GFP-CLTA fusion using Cas9-RNP nucleofection and AAV transduction. B and C) GFP integration was analyzed by flow cytometry. Knock-in efficiency was compared for AAV6 and Ark315 across a range of MOIs.

DETAILED DESCRIPTION OF THE INVENTION

[0054] Known AAV serotypes each have a specific tissue tropism, and there are some tissues (e.g., immune cells) that cannot be easily targeted using these AAVs. Delivery of therapeutic genes using AAV vectors for treating disorders like immunodeficiencies and some cancers can be particularly difficult as AAV-mediated gene therapies targeting immune

cells would require systemic delivery at high doses, thus triggering a subject's immune response against the vector carrying the therapeutic gene. Aspects provided in the present disclosure will help a) expand the eligible cohort of human patients suitable for AAV-based gene therapy and b) allow multiple, repeat administrations of AAV-based gene therapy vectors. The present disclosure provides AAV variants that have tropism to human T-cells and in some embodiments reduced recognition by human neutralizing antibodies.

[0055] Adeno-associated virus (AAV), a member of the Parvovirus family, is a small, non-enveloped virus. Wildtype AAV is composed of an icosahedral protein capsid which encloses a single-stranded DNA genome. In wildtype AAVs, inverted terminal repeats (ITRs) flank the coding nucleotide sequences (e.g., a polynucleotides) for the non-structural proteins (encoded by Rep genes) and the structural proteins (encoded by capsid genes or Cap genes). Rep genes encode the non-structural proteins that regulate functions comprising the replication of the AAV genome. Cap genes encode the structural proteins, VP1, VP2 and/or VP3 that assemble to form the capsid.

[0056] The present disclosure provides recombinant AAV capsid proteins (VP1, VP2 and/or VP3) comprising a modification (e.g., a substitution) in the amino acid sequence relative to a wildtype capsid protein, and AAV capsids and AAV vectors comprising the modified AAV capsid protein. As detailed herein, modifications of disclosed capsid proteins can confer one or more desirable properties to virus vectors comprising the modified AAV capsid protein variants herein, including without limitation, the ability to evade neutralizing antibodies and/or the ability to specifically and selectively target a cell or tissue of interest, i.e., human T-cells. Thus, the present disclosure addresses some of the limitations associated with conventional AAV vectors.

[0057] In an aspect, AAV vectors herein can be engineered to include one or more capsid protein variants. In an aspect, AAV vectors herein can be engineered to include at least one or more amino acid substitutions, wherein the one or more substitutions can modify one or more antigenic sites on the AAV capsid protein. The modification of the one or more antigenic sites can result in inhibition of binding by an antibody to the one or more antigenic sites and/or inhibition of neutralization of infectivity of a virus particle comprising said a capsid protein variant herein.

[0058] Accordingly, in an aspect herein, the present disclosure provides an adeno-associated virus (AAV) capsid protein variant, comprising one or more amino acid

modifications (e.g., substitutions and/or deletions), wherein the one or more modifications modify one or more antigenic sites on the AAV capsid protein. In an aspect, modification of the one or more antigenic sites can result in inhibition of binding by an antibody to the one or more antigenic sites and/or inhibition of neutralization of infectivity of a virus particle comprising said AAV capsid protein. In an aspect, the modified antigenic site can prevent antibodies from binding or recognizing or neutralizing AAV capsids. In an aspect, the antibody can be an IgG (including IgG1, IgG2a, IgG2b, IgG3), IgM, IgE or IgA. In an aspect, the modified antigenic site can prevent binding, recognition, or neutralization of AAV capsids by antibodies from different animal species, wherein the animal is human, canine, porcine, bovine, non-human primate, rodent (e.g., mouse), feline or equine.

[0059] In an aspect, modification of the one or more antigenic sites can result in tropism of the AAV vectors herein to human T-cells, e.g. a CD8+ T cell or CD4+ T cell. As used herein, "tropism" refers to preferential entry of the virus into certain cells or tissues, optionally followed by expression (e.g., transcription and, optionally, translation) of a sequence(s) carried by the viral genome in the cell, e.g., for a recombinant virus, expression of a heterologous nucleic acid(s) of interest, for example preferential entry compared to a native AAV vector (e.g., such as AAV6).. In an aspect, modification of the one or more antigenic sites can result in AAV vectors herein that can exhibit tropism to one or more human hematopoietic progenitor cells.

[0060] In an aspect, the one or more amino acid modifications (e.g., substitutions and/or deletions) within capsid protein variants herein, can be in one or more antigenic footprints identified by peptide epitope mapping and/or cryo-electron microscopy studies of AAV-antibody complexes containing AAV capsid proteins. In an aspect, the one or more antigenic sites herein that can be subject to one or more amino acid modifications can be antigenic motifs (CAMs) as described in WO 2017/058892, which is incorporated herein by reference in its entirety.

[0061] In an aspect, the one or more antigenic sites herein that can be subject to one or more amino acid modifications can be in a variable region (VR) of an AAV capsid protein. An AAV capsid contains 60 copies (in total) of three VPs (VP1, VP2, VP3) that are encoded by the *cap* gene and have overlapping sequences. Each VP can contain an eight-stranded β-barrel motif (βB to βI) and/or an α-helix (αA) conserved in autonomous parvovirus capsids. Structurally variable regions (VRs) can occur in the surface loops that connect the β-strands,

which cluster to produce local variations in the capsid surface. In an aspect, the one or more amino acid modifications herein that modify one or more antigenic sites in AAV capsid protein variants herein can be in VR-I, VR-II, VR-III, VR-IV, VR-V, VR-VI, VR-VII, VR-VI II, VR-IX, or any combination thereof. In an aspect, one or more antigenic sites can be in the HI loop of the AAV capsid protein variants herein.

[0062] In an aspect, AAV vectors herein can comprise (i) a AAV capsid protein variant disclosed herein, and (ii) a cargo nucleic acid encapsidated by the capsid protein. In an aspect, an AAV vector comprising an AAV capsid protein variant described herein can have a phenotype of: selective tropism to human T-cells, and further also optionally evading neutralizing antibodies and/or enhanced or maintained transduction efficiency.

[0063] In an aspect, the AAV vectors disclosed herein can exhibit at least about 2-fold (for example, about 4-fold, about 5-fold, about 7-fold, about 10-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 20-fold, about 25-fold, or about 30-fold, including all values and subranges that lie there between) higher transduction in a human immune cell (e.g., a T cell, a NK cell) compared to parental AAV6.

[0064] In an aspect, AAV capsid protein variants disclosed herein can include at least one or more amino acid substitutions wherein about 1 amino acid residue to about 50 amino acid residues (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50) can be substituted from the amino acid residues comprising an amino acid sequence of a naturally occurring capsid protein.

[0065] In an aspect, AAV capsid protein variants disclosed herein can have an amino acid sequence with about 85% (e.g., about 85%, 90%, 95%, 99%, 100%) similarity to a naturally occurring capsid protein. As used herein, "naturally occurring" or "wild-type" means existing in nature without modification by man. In an aspect, a naturally occurring capsid protein herein can be derived from a single species. Non-limiting examples of species that can be the origin of a naturally occurring capsid protein herein include those from a general organism such as a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate (e.g., monkey, chimpanzee, baboon, gorilla) bird, reptile, worm, fish, and the like. In an aspect, AAV capsid protein variants having at least one amino acid substitution as disclosed herein can have an amino acid sequence with about 85% (e.g., about 85%, 90%, 95%, 99%, 100%) similarity to a naturally occurring capsid protein having an amino acid

sequence referenced by 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, NC_001358, NC_001540, AF513851, AF513852, AY530579, and any combination thereof.

[0066] Methods of determining sequence similarity or identity between two or more amino acid sequences are known in the art. Sequence similarity or identity can be determined using standard techniquesfor example the BLAST algorithm, described in Altschul et al., *J Mol. Biol.* 215, 403-410, (1990) and Karlin et al., *Proc. Natl. Acad. Sci. USA* 90, 5873- 5787 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul et al., Methods in Enzymology, 266, 460-480 (1996). WU-BLAST-2 uses several search parameters, which are optionally 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 can be adjusted to increase sensitivity. Further, an additional useful algorithm is gapped BLAST as reported by Altschul et al, (1997) Nucleic Acids Res. 25, 3389-3402. For purposes of the instant disclosure, unless otherwise indicated, percent identity is calculated using the Basic Local Alignment Search Tool (BLAST) available online at blast.ncbi.nlm.nih.gov/Blast.cgi. The skilled artisan will understand that other algorithms can be substituted as appropriate.

[0067] In an aspect, AAV capsid protein variants herein or fragments thereof can have an amino acid sequence with about 85% (e.g., about 85%, 90%, 95%, 99%, 100%) identity to a naturally occurring VP1 capsid protein or fragment thereof. In an aspect, capsid protein variants herein can comprise an amino acid substitution at one or more (e.g., 2, 3, 4, 5, 6, or 7, e.g., as shown in in Table 4 or 5) of amino acid residues 454-460 of AAV6 (VP1 numbering), in any combination, or the equivalent amino acid residues in AAV2, AAV3, AAV4, AAV5, AAV1, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, bovine AAV or avian AAV.

[0068] In an aspect, capsid protein variants herein can have at least 90% (e.g., about 90%, 95%, 99%, 100%) sequence identity to the native sequence of the AAV6 capsid (SEQ ID NO:1) and have enhanced tropism for human T-cells compared to native AAV6. In an aspect, capsid protein variants herein can have at least 90% (e.g., about 90%, 95%, 99%,

100%) sequence identity to a protein encoded by native nucleic acid sequence of the AAV6 (SEQ ID NO:2), but include one set of amino acid substitutions listed in Table 4 or 5.

[0069] In an aspect, AAV vectors herein can comprise (i) a AAV6 capsid protein variant and (ii) a cargo nucleic acid encapsidated by the capsid protein. In an aspect, AAV vectors herein can comprise (i) a AAV6 capsid protein variant and (ii) a cargo nucleic acid encapsidated by the capsid protein wherein the capsid protein can comprise a peptide having any one of the sequences in Table 4 or 5 at amino acids 454-460 (VP1 numbering) of a native AAV6 capsid protein, (SEQ ID NO:1), wherein the peptide does not occur in the native AAV6 capsid protein sequence.

[0070] In an aspect, capsid protein variants herein can comprise a peptide wherein the amino acids corresponding to amino acid position 454-460 (VP1 numbering) of a native AAV6 capsid protein, (SEQ ID NO:1) can be substituted with amino acids corresponding to any one of SEQ ID NO:6 – SEQ ID NO:54. Table 4 and 5 below provides amino acids corresponding to any one of SEQ ID NO:6 – SEQ ID NO:54 (as well as AAV6 wild-type sequence shown as SEQ ID NO:5).

Table 4 – Listing of AAV6 Capsid Variants selected with serum and Sequence Identifiers

Designation	Sequence (positions 454-460 relative to SEQ ID NO:1)	SEQ ID NO:
AAV6 (WT)	**GSAQNKD (G454 S455 A456 Q457 N458 K459 D460S)**	**5**
Ark315	VAPRDSS	6
Ark316	EAPRAMS	7
Ark317	LAPRDRE	8
Ark318	EAPRSVS	9
Ark321	EAPRESA	10
Ark323	DAPRLGG	11
Ark324	PAPRESS	12
Ark326	LAPREAL	13
Ark328	LAPREWE	14
Ark329	VAPRDAV	15
Ark330	DAPRQTM	16

Ark331	EAPRQTS	17
Ark332	EAPRLDA	18
Ark333	MAPRVVD	19
Ark334	TAPRETN	20
Ark335	MAPRDAG	21
Ark336	EAPRAVQ	22
Ark337	VAPREVM	23
Ark338	EAPRQPT	24
Ark339	NAPREVN	25
Ark340	PAPRDGM	26
Ark341	EAPRDVI	27
Ark342	EAPRFGG	28
Ark343	EAPRSQW	29
Ark344	IAPRGSD	30
Ark345	PAPRMIE	31
Ark346	IAPRSVG	32
Ark347	FPAGSME	33
Ark348	EAPRSRD	34
Ark349	EAPRRLE	35
Ark350	VAPRVAG	36
Ark351	GLGLGNG	37
Ark352	YVHSAEE	38
Ark353	DAPRIVK	39
Ark354	EAPRVWS	40
Ark355	QGPGLLG	41
Ark356	SAPRDHG	42
Ark357	YAPRELG	43
Ark358	LAPREPG	44
Ark359	VAPRAGA	45
Ark360	DAPRVQL	46
Ark361	AAPRSVA	47

Ark362	SAPREME	48
Ark363	QTDAQPR	49

Table 5 – Listing of AAV6 Capsid Variants selected without serum and Sequence Identifiers

Designation	Sequence (positions 454-460 relative to SEQ ID NO:1)	SEQ ID NO:
AAV6 WT	GSAQNKD (G454 S455 A456 Q457 N458 K459 D460S)	5
Ak325	-KEGA- - (dashes are not present, meaning positions 454, 459 and 460 are absent)	50
Ark396	NAPREVN	51
Ark397	PVGDVGK	52
Ark600	VQKGSYE	53
Ark601	RPDGNSL	54
Ark602	KEGQAIQ	55
Ark603	KGSEGIS	56
Ark604	AKEVERH	57
Ark605	SEKGASS	58
Ark606	SSSGDNM	59
Ark607	KSTNDLS	60
Ark608	LDSKRLE	61
Ark609	EAPRESA	62
Ark610	SETEARK	63
Ark611	GVADGVK	64
Ark612	GPDDTRK	65
Ark613	SEKMGFN	66
Ark614	RSTESIE	67
Ark615	LQKDGAA	68
Ark616	SSTVERV	69
Ark617	LAPRSPD	70
Ark618	GSERPNV	71

Ark619	AAPRECG	72
Ark620	STDRQRD	73
Ark621	ASTGEGR	74
Ark622	VASGSPS	75
Ark623	VSKDVPN	76
Ark624	SNEVKIG	77
Ark625	TTGTDRG	78
Ark626	VGSDGVR	79
Ark627	LEGKERG	80
Ark628	TTTADKA	81
Ark629	SGVREGV	82
Ark630	ADRDASA	83
Ark631	TNTSDKL	84
Ark632	GDGKGQW	85
Ark633	AVDKERL	86
Ark634	PTAKDMA	87
Ark635	HQEGERM	88
Ark636	REDDGRC	89
Ark637	KEEASRH	90
Ark638	SVSVDRH	91
Ark639	TDKDGMS	92
Ark640	TESGALS	93
Ark641	HDIDSPR	94

[0071] In an aspect, capsid protein variants herein can comprise a peptide wherein the amino acids corresponding to amino acid position 454-460 (VP1 numbering) of a native AAV6 capsid protein, (SEQ ID NO:1) can be substituted with amino acids corresponding to VAPRDSS (SEQ ID NO:6, e.g., Ark315) or with any other of SEQ ID Nos: 7-94.

[0072] In an aspect, capsid protein variants herein can share at least about 85% (e.g., about 85%, 90%, 95%, 99%, or 100%) amino acid sequence similarity with any one of the

sequences set forth in SEQ ID NO:1 or SEQ ID NO:2, 4, or 95-102. In an aspect, capsid protein variants herein can comprise SEQ ID NO: 2 or a species equivalent thereof. In an aspect, capsid protein variants herein can be encoded from a polynucleotide sharing at least about 85% (e.g., about 85%, 90%, 95%, 99%, or 100%) nucleic acid sequence similarity with the sequence set forth in SEQ ID NO:3. Amino acid sequences of native AAV6 capsid protein (SEQ ID NO:1) and SEQ ID NO:2 (Ark315), SEQ ID NO:4 (Ark316), SEQ ID NO:95 (Ark317), SEQ ID NO:96 (Ark318), SEQ ID NO:97 (Ark321), SEQ ID NO:98 (Ark325), SEQ ID NO:99 (Ark396), SEQ ID NO:100 (Ark397), SEQ ID NO:101 (Ark398), and SEQ ID NO:102 (Ark399), are provided below. The nucleic acid sequence of native AAV6 capsid protein (SEQ ID NO:3) is provided below, and can be modified to encode any of the above sequences.

[0073] In an aspect, a disclosed wild-type AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**GSAQNKD**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT
GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMI
TDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQ
GPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRY
LTRPL (SEQ ID NO:1).

[0074] In an aspect, a disclosed Ark315 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**VAPRDSS**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT
GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMI

TDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQ
GPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRY
LTRPL (SEQ ID NO:2).

[0075] In an aspect, a disclosed Ark316 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**EAPRAMS**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT
GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMI
TDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQ
GPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRY
LTRPL (SEQ ID NO:4).

[0076] In an aspect, a disclosed Ark317 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**LAPRDRE**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT
GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMI
TDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQ
GPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRY
LTRPL (SEQ ID NO:95).

[0077] In an aspect, a disclosed Ark318 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP

AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**EAPRSVS**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTG
ASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMIT
DEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQG
PIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYST
GQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYL
TRPL (SEQ ID NO:96).

[0078] In an aspect, a disclosed Ark321 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**EAPRESA**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT
GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMI
TDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQ
GPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRY
LTRPL (SEQ ID NO:97).

[0079] In an aspect, a disclosed Ark325 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**KEGA**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGAS
KYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMITDEE
EIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQGPIW
AKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQ
VSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRP
L (SEQ ID NO:98).

[0080] In an aspect, a disclosed Ark396 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**NAPREVN**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT
GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMI
TDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQ
GPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRY
LTRPL (SEQ ID NO:99).

[0081] In an aspect, a disclosed Ark397 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**PVGDVGK**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT
GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMI
TDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQ
GPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRY
LTRPL (SEQ ID NO:100).

[0082] In an aspect, a disclosed Ark398 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT

QNQS**VQKGSYE**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT
GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMI
TDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQ
GPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRY
LTRPL (SEQ ID NO:101).

[0083] In an aspect, a disclosed Ark399 AAV6 capsid protein can comprise the sequence set forth below:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPFGLVEEGAKTAPGKKRPVEQSPQEPDSSSGIGKTGQQP
AKKRLNFGQTGDSESVPDPQPLGEPPATPAAVGPTTMASGGGAPMADNNEGADGV
GNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYS
TPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIA
NNLTSTVQVFSDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRS
SFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRT
QNQS**RPDGNSL**LLFSRGSPAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWT
GASKYNLNGRESIINPGTAMASHKDDKDKFFPMSGVMIFGKESAGASNTALDNVMI
TDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMGALPGMVWQDRDVYLQ
GPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENSKRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRY
LTRPL (SEQ ID NO:102).

[0084] In an aspect, a disclosed wild-type AAV6 capsid protein can encoded by the sequence set forth below, optionally altered to encode the variations described herein, and optionally codon-optimized for a human or other cell:

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGG
TCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCA
GAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGGAGGGGTGGAGTCGTGA
CGTGAATTACGTCATAGGGTTAGGGAGGTCCTGTATTAGAGGTCACGTGAGTGTT
TTGCGACATTTTGCGACACCATGTGGTCACGCTGGGTATTTAAGCCCGAGTGAGC
ACGCAGGGTCTCCATTTTGAAGCGGGAGGTTTGAACGCGCAGCGCCATGCCGGG
GTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGACGAGCATCTGCCCGGC
ATTTCTGACAGCTTTGTGAACTGGGTGGCCGAGAAGGAATGGGAGTTGCCGCCA
GATTCTGACATGGATCTGAATCTGATTGAGCAGGCACCCCTGACCGTGGCCGAG
AAGCTGCAGCGCGACTTCCTGGTCCAGTGGCGCCGCGTGAGTAAGGCCCCGGAG
GCCCTCTTCTTTGTTCAGTTCGAGAAGGGCGAGTCCTACTTCCACCTCCATATTCT
GGTGGAGACCACGGGGGTCAAATCCATGGTGCTGGGCCGCTTCCTGAGTCAGAT
TAGGGACAAGCTGGTGCAGACCATCTACCGCGGGATCGAGCCGACCCTGCCCAA
CTGGTTCGCGGTGACCAAGACGCGTAATGGCGCCGGAGGGGGGAACAAGGTGGT
GGACGAGTGCTACATCCCCAACTACCTCCTGCCCAAGACTCAGCCCGAGCTGCA
GTGGGCGTGGACTAACATGGAGGAGTATATAAGCGCGTGTTTAAACCTGGCCGA
GCGCAAACGGCTCGTGGCGCACGACCTGACCCACGTCAGCCAGACCCAGGAGCA
GAACAAGGAGAATCTGAACCCCAATTCTGACGCGCCTGTCATCCGGTCAAAAAC

CTCCGCACGCTACATGGAGCTGGTCGGGTGGCTGGTGGACCGGGGCATCACCTC
CGAGAAGCAGTGGATCCAGGAGGACCAGGCCTCGTACATCTCCTTCAACGCCGC
CTCCAACTCGCGGTCCCAGATCAAGGCCGCTCTGGACAATGCCGGCAAGATCAT
GGCGCTGACCAAATCCGCGCCCGACTACCTGGTAGGCCCCGCTCCGCCCGCCGA
CATTAAAACCAACCGCATTTACCGCATCCTGGAGCTGAACGGCTACGACCCTGCC
TACGCCGGCTCCGTCTTTCTCGGCTGGGCCCAGAAAAGGTTCGGAAAACGCAAC
ACCATCTGGCTGTTTGGGCCGGCCACCACGGGCAAGACCAACATCGCGGAAGCC
ATCGCCCACGCCGTGCCCTTCTACGGCTGCGTCAACTGGACCAATGAGAACTTTC
CCTTCAACGATTGCGTCGACAAGATGGTGATCTGGTGGGAGGAGGGCAAGATGA
CGGCCAAGGTCGTGGAGTCCGCCAAGGCCATTCTCGGCGGCAGCAAGGTGCGCG
TGGACCAAAAGTGCAAGTCGTCCGCCCAGATCGATCCCACCCCCGTGATCGTCAC
CTCCAACACCAACATGTGCGCCGTGATTGACGGGAACAGCACCACCTTCGAGCA
CCAGCAGCCGTTGCAGGACCGGATGTTCAAATTTGAACTCACCCGCCGTCTGGAG
CATGACTTTGGCAAGGTGACAAAGCAGGAAGTCAAAGAGTTCTTCCGCTGGGCG
CAGGATCACGTGACCGAGGTGGCGCATGAGTTCTACGTCAGAAAGGGTGGAGCC
AACAAGAGACCCGCCCCCGATGACGCGGATAAAAGCGAGCCCAAGCGGGCCTG
CCCCTCAGTCGCGGATCCATCGACGTCAGACGCGGAAGGAGCTCCGGTGGACTT
TGCCGACAGGTACCAAAACAAATGTTCTCGTCACGCGGGCATGCTTCAGATGCTG
TTTCCCTGCAAAACATGCGAGAGAATGAATCAGAATTTCAACATTTGCTTCACGC
ACGGGACCAGAGACTGTTCAGAATGTTTCCCCGGCGTGTCAGAATCTCAACCGGT
CGTCAGAAAGAGGACGTATCGGAAACTCTGTGCCATTCATCATCTGCTGGGGCG
GGCTCCCGAGATTGCTTGCTCGGCCTGCGATCTGGTCAACGTGGATCTGGATGAC
TGTGTTTCTGAGCAATAAATGACTTAAACCAGGTATGGCTGCCGATGGTTATCTT
CCAGATTGGCTCGAGGACAACCTCTCTGAGGGCATTCGCGAGTGGTGGGACTTG
AAACCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCAGGACGACGGCCG
GGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGACCCTTCAACGGACTCGACAAG
GGGGAGCCCGTCAACGCGGCGGATGCAGCGGCCCTCGAGCACGACAAGGCCTAC
GACCAGCAGCTCAAAGCGGGTGACAATCCGTACCTGCGGTATAACCACGCCGAC
GCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGTCTTTTGGGGGCAACCTCGGG
CGAGCAGTCTTCCAGGCCAAGAAGAGGGTTCTCGAACCTTTTGGTCTGGTTGAGG
AAGGTGCTAAGACGGCTCCTGGAAAGAAACGTCCGGTAGAGCAGTCGCCACAAG
AGCCAGACTCCTCCTCGGGCATTGGCAAGACAGGCCAGCAGCCCGCTAAAAAGA
GACTCAATTTTGGTCAGACTGGCGACTCAGAGTCAGTCCCCGACCCACAACCTCT
CGGAGAACCTCCAGCAACCCCCGCTGCTGTGGGACCTACTACAATGGCTTCAGG
CGGTGGCGCACCAATGGCAGACAATAACGAAGGCGCCGACGGAGTGGGTAATG
CCTCAGGAAATTGGCATTGCGATTCCACATGGCTGGGCGACAGAGTCATCACCA
CCAGCACCCGAACATGGGCCTTGCCCACCTATAACAACCACCTCTACAAGCAAA
TCTCCAGTGCTTCAACGGGGGCCAGCAACGACAACCACTACTTCGGCTACAGCA
CCCCCTGGGGGTATTTTGATTTCAACAGATTCCACTGCCATTTCTCACCACGTGAC
TGGCAGCGACTCATCAACAACAATTGGGGATTCCGGCCCAAGAGACTCAACTTC
AAGCTCTTCAACATCCAAGTCAAGGAGGTCACGACGAATGATGGCGTCACGACC
ATCGCTAATAACCTTACCAGCACGGTTCAAGTCTTCTCGGACTCGGAGTACCAGT
TGCCGTACGTCCTCGGCTCTGCGCACCAGGGCTGCCTCCCTCCGTTCCCGGCGGA
CGTGTTCATGATTCCGCAGTACGGCTACCTAACGCTCAACAATGGCAGCCAGGCA
GTGGGACGGTCATCCTTTTACTGCCTGGAATATTTCCCATCGCAGATGCTGAGAA
CGGGCAATAACTTTACCTTCAGCTACACCTTCGAGGACGTGCCTTTCCACAGCAG
CTACGCGCACAGCCAGAGCCTGGACCGGCTGATGAATCCTCTCATCGACCAGTA
CCTGTATTACCTGAACAGAACTCAGAATCAGTCCGGAAGTGCCCAAAACAAGGA
CTTGCTGTTTAGCCGGGGGTCTCCAGCTGGCATGTCTGTTCAGCCCAAAAACTGG
CTACCTGGACCCTGTTACCGGCAGCAGCGCGTTTCTAAAACAAAAACAGACAAC

AACAACAGCAACTTTACCTGGACTGGTGCTTCAAAATATAACCTTAATGGGCGTG
AATCTATAATCAACCCTGGCACTGCTATGGCCTCACACAAAGACGACAAAGACA
AGTTCTTTCCCATGAGCGGTGTCATGATTTTTGGAAAGGAGAGCGCCGGAGCTTC
AAACACTGCATTGGACAATGTCATGATCACAGACGAAGAGGAAATCAAAGCCAC
TAACCCCGTGGCCACCGAAAGATTTGGGACTGTGGCAGTCAATCTCCAGAGCAG
CAGCACAGACCCTGCGACCGGAGATGTGCATGTTATGGGAGCCTTACCTGGAAT
GGTGTGGCAAGACAGAGACGTATACCTGCAGGGTCCTATTTGGGCCAAAATTCC
TCACACGGATGGACACTTTCACCCGTCTCCTCTCATGGGCGGCTTTGGACTTAAG
CACCCGCCTCCTCAGATCCTCATCAAAAACACGCCTGTTCCTGCGAATCCTCCGG
CAGAGTTTTCGGCTACAAAGTTTGCTTCATTCATCACCCAGTATTCCACAGGACA
AGTGAGCGTGGAGATTGAATGGGAGCTGCAGAAAGAAAACAGCAAACGCTGGA
ATCCCGAAGTGCAGTATACATCTAACTATGCAAAATCTGCCAACGTTGATTTCAC
TGTGGACAACAATGGACTTTATACTGAGCCTCGCCCCATTGGCACCCGTTACCTC
ACCCGTCCCCTGTAATTGTGTGTTAATCAATAAACCGGTTAATTCGTGTCAGTTG
AACTTTGGTCTCATGTCGTTATTATCTTATCTGGTCACCATAGCAACCGGTTACAC
ATTAACTGCTTAGTTGCGCTTCGCGAATACCCCTAGTGATGGAGTTGCCCACTCC
CTCTATGCGCGCTCGCTCGCTCGGTGGGGCCGGCAGAGCAGAGCTCTGCCGTCTG
CGGACCTTTGGTCCGCAGGCCCCACCGAGCGAGCGAGCGCGCATAGAGGGAGTG
GGCAA (SEQ ID NO:3).

[0085] In an aspect, capsid protein variants of the present disclosure can be produced by modifying the capsid protein of any AAV capsid protein now known or later discovered using the methodology described herein. Further, the AAV capsid protein that is to be modified according to the present disclosure can be a naturally occurring AAV capsid protein (e.g., an AAV2, AAV3a or 3b, AAV4, AAV5, AAV8, AAV9, AAV10 or AAV11 capsid protein or any of the AAV shown in **Table 1**) but is not so limited. A variety of manipulations to the AAV capsid proteins are known in the art and the invention is not limited to modifications of naturally occurring AAV capsid proteins. For example, the capsid protein to be modified can already have one or more alterations as compared with naturally occurring AAV (e.g., is derived from a naturally occurring AAV capsid protein, e.g., AAV2, AAV3a, AAV3b, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 or any other AAV now known or later discovered). Such AAV capsid proteins are also within the scope of the present disclosure.

[0086] In an aspect, disclosed herein are virus capsids which can have one or more of any of the capsid protein variants disclosed herein. In an aspect, a virus capsid herein can be a parvovirus capsid, which can further be an autonomous parvovirus capsid or a dependovirus capsid. Optionally, a virus capsid herein can be an AAV capsid. In an aspect, AAV capsids of the present disclosure can be an AAV1, AAV2, AAV3a, AAV3b, AAV4, AAV5, AAV6,

AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAVrh32.33, bovine AAV capsid, avian AAV capsid and/or any other AAV now known or later identified.

[0087] In an aspect, modified virus capsids herein can be used as capsid vehicles. In an aspect, molecules can be packaged by the modified virus capsids herein and transferred into a cell wherein the molecules can include heterologous DNA, RNA, polypeptides, small organic molecules, metals, or combinations of the same. Heterologous molecules are defined herein as those that are not naturally found in an AAV infection, e.g., those not encoded by a wild-type AAV genome. Further, therapeutically useful molecules for use herein can be associated with the outside of the chimeric virus capsid for transfer of the molecules into one or more host target cells. Such associated molecules can include DNA, RNA, small organic molecules, metals, carbohydrates, lipids and/or polypeptides. In an aspect, a therapeutically useful molecule herein can be covalently linked (i.e., conjugated or chemically coupled) to a capsid proteins.

[0088] In an aspect, modified virus capsids herein can be used in raising antibodies against the capsid protein variants disclosed herein. As a further alternative, an exogenous amino acid sequence can be inserted into the modified virus capsid for antigen presentation to a cell, e.g., for administration to a subject to produce an immune response to the exogenous amino acid sequence.

[0089] In an aspect, modified virus capsids herein can be a targeted virus capsid, comprising a targeting sequence (e.g., substituted or inserted in the viral capsid) that can direct the virus capsid to interact with cell-surface molecules present on desired target tissue(s) (see, e.g., international patent publication WO 00/28004 and Hauck et al. (2003) J. Virology, 77:2768-2774); Shi et al. (2006) Human Gene Ther. 17:353-361 describing insertion of the integrin receptor binding motif RGD at positions 520 and/or 584 of the AAV capsid subunit; and U.S. Pat. No. 7,314,912 describing insertion of the P1 peptide containing an RGD motif following amino acid positions 447, 534, 573 and 587 of the AAV2 capsid subunit). Other positions within the AAV capsid subunit that tolerate insertions are known in the art (e.g., positions 449 and 588 described by Grifman et al. (2001) Molecular Therapy 3:964-975).

[0090] A virus capsid of the present disclosure has tropism toward certain target cells of interest (e.g., immune cells, such as T cells). A targeting sequence can advantageously be incorporated into these vectors to thereby further confer to the virus capsid a higher tropism

and, optionally, selective tropism for particular tissue(s). AAV capsid proteins, capsids and vectors comprising targeting sequences are described, for example in international patent publication WO 00/28004. As another example, one or more non-naturally occurring amino acids as described by Wang et al., *Annu Rev Biophys Biomol Struct.* 35:225-49 (2006) can be incorporated into an AAV capsid subunit of this disclosure at an orthogonal site as a means of redirecting a vector to desired target tissue(s). These unnatural amino acids can advantageously be used to chemically link molecules of interest to the AAV capsid protein including without limitation: glycans (mannose-dendritic cell targeting); RGD, bombesin or a neuropeptide for targeted delivery to specific cancer cell types; RNA aptamers or peptides selected from phage display targeted to specific cell surface receptors such as growth factor receptors, integrins, and the like. Methods of chemically modifying amino acids are known in the art (see, e.g., Greg T. Hermanson, *Bioconjugate Techniques,* 1st edition, Academic Press, 1996).

[0091] In an aspect, the targeting sequence can be a virus capsid sequence (e.g., an autonomous parvovirus capsid sequence, AAV capsid sequence, or any other viral capsid sequence) that directs infection to a particular cell type(s).

[0092] In an aspect, an exogenous targeting sequence for use herein can be any amino acid sequence encoding a peptide that alters the tropism of a virus capsid or virus vector comprising the modified AAV capsid protein. In an aspect, the targeting peptide or protein can be naturally occurring or, alternately, completely or partially synthetic. In an aspect, targeting sequences can include ligands and other peptides that bind to cell surface receptors and glycoproteins, such as RGD peptide sequences, bradykinin, hormones, peptide growth factors (e.g., epidermal growth factor, nerve growth factor, fibroblast growth factor, platelet-derived growth factor, insulin-like growth factors I and II, etc.), cytokines, melanocyte stimulating hormone (e.g., α, β or γ), neuropeptides and endorphins, and the like, and fragments thereof that retain the ability to target cells to their cognate receptors. Other illustrative peptides and proteins include, but are not limited to substance P, keratinocyte growth factor, neuropeptide Y, gastrin releasing peptide, interleukin 2, hen egg white lysozyme, erythropoietin, gonadoliberin, corticostatin, β-endorphin, leu-enkephalin, rimorphin, α-neo-enkephalin, angiotensin, pneumadin, vasoactive intestinal peptide, neurotensin, motilin, and fragments thereof as described above. As yet a further alternative, the binding domain from a toxin (e.g., tetanus toxin or snake toxins, such as α-bungarotoxin, and the like) can be substituted into the capsid protein as a targeting sequence. In an aspect, a

AAV capsid protein herein can be modified by substitution of a "nonclassical" import/export signal peptide (e.g., fibroblast growth factor-1 and -2, interleukin 1, HIV-1 Tat protein, herpes virus VP22 protein, and the like) as described by Cleves (*Current Biology* 7:R318 (1997)) into the AAV capsid protein. In an aspect, a targeting sequence for use herein can be a peptide that can be used for chemical coupling (e.g., can comprise arginine and/or lysine residues that can be chemically coupled through their R groups) to another molecule that targets entry into a cell.

[0093] In an aspect, capsid protein variants, virus capsids and/or AAV vectors disclosed herein can have equivalent or enhanced transduction efficiency relative to the transduction efficiency of the AAV serotype from which the capsid protein variant, virus capsid and/or vector originated. In an aspect, capsid protein variants, virus capsids and/or vectors disclosed herein can have reduced transduction efficiency relative to the transduction efficiency of the AAV serotype from which the capsid protein variant, virus capsid and/or vector originated. In an aspect, capsid protein variants, virus capsids and/or vectors disclosed herein can have equivalent or enhanced tropism relative to the tropism of the AAV serotype from which capsid protein variant, virus capsid and/or vector originated. In an aspect, capsid protein variants, virus capsids and/or vectors disclosed herein can have an altered or different tropism relative to the tropism of the AAV serotype from which the capsid protein variant, virus capsid and/or vector originated. In an aspect, capsid protein variants, virus capsids and/or vectors disclosed herein can have or be engineered to have tropism for immune cells (e.g., T cells, NK cells). In an aspect, capsid protein variants, virus capsids and/or vectors disclosed herein can have or be engineered to have enhanced tropism for immune cells (e.g., T cells, NK cells). In an aspect, capsid protein variants, virus capsids and/or AAV vectors disclosed herein can produce an attenuated immunological response relative to the immunological response of the AAV serotype from which the capsid protein variant, virus capsid and/or vector originated. In an aspect, capsid protein variants, virus capsids and/or AAV vectors disclosed herein can be administered to a subject in multiple dosages (e.g., about two doses, about three doses, about four doses, about 5 doses, about 10 doses, about 15 doses, about 20 doses, about 40 doses, as many doses as needed to observe one or more desired responses) relative to the number of doses that can be administered using the AAV serotype from which the capsid protein variant, virus capsid and/or vector originated.

[0094] In an aspect, the present disclosure provides AAV vectors comprising one or more of the capsid protein variants disclosed herein. As used herein, a "vector" refers to any

molecule or moiety which transports, transduces or otherwise acts as a carrier of a heterologous molecule. A "viral vector" is a vector which comprises one or more polynucleotide regions encoding or comprising a payload molecule of interest, e.g., a heterologous polynucleotide, a donor polynucleotide, a transgene, a polynucleotide encoding a polypeptide or multi-polypeptide or a modulatory nucleic acid. Viral vectors of the present invention can be produced recombinantly using methods known in the art. Such techniques are explained fully in the literature, such as in Molecular Cloning: A Laboratory Manual, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed. 1984); Methods in Molecular Biology, Humana Press; and Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1989) Academic Press.

[0095] In an aspect, AAV viral particles disclosed herein can have a vector genome for expressing one or more of the capsid protein variants disclosed herein. The vector genome of the AAV vector can, in an aspect, be derived from the wild type genome of a virus, such as AAV, by using molecular methods to remove the wild type genome from the virus (e.g., AAV6), and replacing with a non-native nucleic acid, such as a heterologous polynucleotide sequence (e.g., a coding sequence for a transgene of interest or a donor polynucleotide, e.g., such as an HDR template or HITI donor sequence). Typically, for AAV vectors, one or both inverted terminal repeat (ITR) sequences of the wild type AAV genome are retained in the AAV vector whereas other parts of the wild type viral genome are replaced with a non-native sequence such as a heterologous polynucleotide sequence between the retained ITRs. The vector genomes disclosed herein can encompass AAV genome-derived backbone elements, a coding sequence for a capsid protein variant disclosed herein, and a suitable promoter in operable linkage to the coding sequence. In an aspect, vector genomes disclosed herein can further comprise regulatory sequences regulating expression and/or secretion of the encoded protein. Examples include, but are not limited to, enhancers, polyadenylation signal sites, internal ribosome entry sites (IRES), sequences encoding protein transduction domains (PTD), microRNA-target sites, or a combination thereof.

[0096] In an aspect, vector genomes described herein can be single stranded. In other examples, vector genomes disclosed herein can be double stranded. For example, a vector genome described herein can be a self-complementary AAV vector genome capable of comprising double stranded portions therein.

[0097] In an aspect, vector genomes disclosed herein can have one or more AAV-genome derived backbone elements, which refer to the minimum AAV genome elements required for the bioactivity of the AAV vectors. For example, the AAV-genome derived backbone elements may include the packaging site for the vector to be assembled into an AAV viral particle, one or more of the capsid protein variants disclosed herein, elements needed for vector replication, and/or expression of a transgene-encoding sequence comprised therein in host cells.

[0098] In an aspect, vector genome backbones disclosed herein may include at least one inverted terminal repeat (ITR) sequence. In an aspect, vector genome backbones herein may include two ITR sequences. In an aspect, one ITR sequence can be 5' of a heterologous polynucleotide sequence, e.g., coding for a transgene. In an aspect, one ITR sequence can be 3' of a heterologous polynucleotide sequence, e.g., coding for a transgene. In an aspect, a heterologous polynucleotide sequence, e.g., coding for a transgene, herein can be flanked on either side by an ITR sequence. Accordingly, in an aspect, a vector genome can comprise heterolohous polynucleotide located between the first ITR and the second ITR.

[0099] In an aspect, vector genomes herein may include sequences or components originating from at least one distinct AAV serotype. In an aspect, AAV vector genome backbones disclosed herein can include at least ITR sequence from one distinct AAV serotype. In an aspect, AAV vector genome backbones disclosed herein may include at least ITR sequence from one distinct human AAV serotype. Such a human AAV can be derived from any known serotype, e.g., from any one of serotypes 1-11. In an aspect, AAV serotypes used herein have a tropism for human immune cells, such as but not limited to a hematopoietic progenitor cell, a T-cell (CD4 T cell and/or CD8 T cell), a B-cell or a natural killer (NK) cell. In an aspect, AAV vector genome backbones disclosed herein may have an ITR sequence of serotype AAV6.

[0100] In an aspect, AAV vectors herein can be a pseudotyped AAV vector, (i.e., comprises sequences or components originating from at least two distinct AAV serotypes). In an aspect, a pseudotyped AAV vector herein may include an AAV genome backbone derived from one AAV serotype, and a capsid protein derived at least in part from a distinct AAV serotype. In an aspect, pseudotyped AAV vectors herein can have an AAV2 vector genome backbone and a capsid protein derived from an AAV serotype having a tropism toward immune cells (e.g., T cells, NK cells).

[0101] To analyze the success of viral vector-mediated gene transfer, it can be useful to be able to monitor both the distribution of the vector and the effectiveness of vector-mediated gene expression. This can be achieved by subcloning a reporter gene into the vector genome backbone. In an aspect, AAV vector genome backbones disclosed herein may contain a reporter gene. Several reporter genes are commonly used for this purpose and include, but are not limited to, fluorescent proteins of various colors (including green fluorescent protein (GFP), red fluorescent protein (RFP)), *E. coli* β-galactosidase (*LacZ*), and various forms of luciferase *(Luc)*. In an aspect, AAV vector backbones disclosed herein may contain GFP.

[0102] The vector constructs disclosed herein can be prepared using known techniques. (*See e.g., Current Protocols in Molecular Biology*, Ausubel., F. et al., eds, Wiley and Sons, New York 1995). Fragment length can be chosen so that the recombinant genome does not exceed the packaging capacity of the AAV particle. If necessary, a "stuffer" DNA sequence can be added to the construct to maintain standard AAV genome size for comparative purposes. Such a fragment can be derived from such non-viral sources, e.g., lacZ, or other genes which are known and available to those skilled in the art.

[0103] In an aspect, AAV vectors disclosed herein can be self-complementary AAV (scAAV) vectors. Self-complementary AAV (scAAV) vectors contain complementary sequences that are capable of spontaneously annealing (folding back on itself to form a double-stranded genome) when entering into infected cells, thus circumventing the need for converting a single-stranded DNA vector using the cell's DNA replication machinery. An AAV herein having a self-complementing genome can quickly form a double stranded DNA molecule by virtue of its partially complementing sequences (e.g., complementing coding and non-coding strands of a transgene-encoding sequence).

[0104] In an aspect, a scAAV viral vector disclosed herein can comprise a first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence, which can form intrastrand base pairs. In an aspect, the first heterologous polynucleotide sequence and the second heterologous polynucleotide sequence are linked by a sequence that facilitates intrastrand base pairing, e.g., to form a hairpin DNA structure. In an aspect, the dimeric structure of a scAAV vector upon entering a cell can be stabilized by a mutation or a deletion of one of the two terminal resolution sites (trs). As trs are Rep-binding sites contained within each ITR, a mutation or a deletion of such trs can prevent cleavage of a dimeric structure of a scAAV vector by AAV Rep proteins to form monomers. In an aspect,

a scAAV viral vector disclosed herein can include a truncated 5' inverted terminal repeats (ITR), a truncated 3' ITR, or both. In an aspect, a scAAV vector disclosed herein can comprise a truncated 3' ITR, in which the D region or a portion thereof (e.g., the terminal resolution sequence therein) can be deleted. Such a truncated 3' ITR can be located between the first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence noted above.

[0105] In an aspect, AAV vectors disclosed herein can comprise further elements necessary for expression, such as at least one suitable promoter which controls the expression of the transgene-encoding sequence. Such a promoters can be ubiquitous, tissue-specific, strong, weak, regulated, chimeric, etc., to allow efficient and suitable production of the protein in the infected tissue. The promoter can be homologous to the encoded protein, or heterologous, including cellular, viral, fungal, plant or synthetic promoters. Most preferred promoters for use herein can be functional in human cells. Non-limiting examples of ubiquitous promoters include viral promoters, particularly the CMV promoter, the RSV promoter, the SV40 promoter, etc. and cellular promoters such as the PGK (phosphoglycerate kinase) promoter. In an aspect, viral promoters herein can be a CMV promoter, a SV40 promoter, or any combination thereof.

[0106] In an aspect, AAV vectors disclosed herein can comprise further elements necessary for expression, such as at least one suitable promoter which controls the expression of the transgene-encoding sequence after infection of the appropriate cells. Suitable promoters for use herein include, in addition to the AAV promoters, e.g. the cytomegalovirus (CMV) promoter or the chicken beta actin/cytomegalovirus hybrid promoter (CAG), an endothelial cell-specific promoter such as the VE-cadherin promoter, as well as steroid promoters and metallothionein promoters. In an aspect, the promoter used in the vectors disclosed herein can be a CAG promoter.

[0107] In an aspect, a disclosed transgene-encoding sequence can comprise a tissue specific promoter which is functionally linked to the transgene-encoding sequence to be expressed. Accordingly, the specificity of the vectors according to the disclosure for the tissue (e.g., immune cells such as T cells, B cells, HSCs and NK cells) can be further increased. In an aspect, a vector disclosed herein can have a tissue-specific promoter whose activity in the specific tissue is at least about 2-fold, 5-fold, 10-fold, 20-fold, 50-fold or 100-fold higher than in a tissue which is not the specific tissue. In an aspect, a tissue specific promoter herein is a

human a tissue specific promoter. In an aspect, the expression cassette can also include an enhancer element for increasing the expression levels of exogenous protein to be expressed. Furthermore, the expression cassette can further comprise polyadenylation sequences, such as the SV40 polyadenylation sequences or polyadenylation sequences of bovine growth hormone.

[0108] In an aspect, AAV vectors disclosed herein can include one or more control elements which are operably linked to the transgene-encoding sequence in a manner which permits its transcription, translation and/or expression in a cell transfected with the plasmid vector or infected with the virus produced by the invention. As used herein, "operably linked" sequences can include both expression control sequences that are contiguous with the transgene-encoding sequence and expression control sequences that act in trans or at a distance to control the transgene-encoding sequence. Expression control sequences can further comprise appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (e.g., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A great number of expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art and can be utilized herein.

[0109] In an aspect, an AAV vector disclosed herein can include a modified capsid, including proteins or peptides of non-viral origin or structurally modified, to alter the tropism of the vector. For example, the capsid can include a ligand of a particular receptor, or a receptor of a particular ligand, to target the vector towards cell type(s) expressing said receptor or ligand, respectively.

[0110] In an aspect, AAV vectors disclosed herein can be prepared or derived from various serotypes of AAVs. The term "serotype" is a distinction with respect to an AAV having a capsid which is serologically distinct from other AAV serotypes. Serologic distinctiveness is determined on the basis of the lack of cross-reactivity between antibodies to the AAV as compared to other AAV. Cross-reactivity can be measured using methods known in the art. For example, cross-reactivity herein can be measured using a neutralizing antibody assay. For this assay polyclonal serum is generated against a specific AAV in a rabbit or other suitable animal model using the adeno-associated viruses. In this assay, the serum generated against a

specific AAV is then tested in its ability to neutralize either the same (homologous) or a heterologous AAV. The dilution that achieves 50% neutralization is considered the neutralizing antibody titer. If for two AAVs the quotient of the heterologous titer divided by the homologous titer is lower than 16 in a reciprocal manner, those two vectors are considered as the same serotype. Conversely, if the ratio of the heterologous titer over the homologous titer is 16 or more in a reciprocal manner the two AAVs are considered distinct serotypes.

[0111] In an aspect, AAV vectors herein can be mixed of at least two serotypes of AAVs or with other types of viruses to produce chimeric (e.g., pseudotyped) AAV viruses. In an aspect, AAV vectors herein can be a human serotype AAV vector. Such a human AAV can be derived from any known serotype, e.g., from any one of serotypes 1-11.

[0112] In an aspect, AAV vector genomes described herein can be packaged into virus particles which can be used to deliver to the genome for transgene-encoding sequence expression or donor polynucleotide insertion in target cells. In an aspect, AAV vector genomes disclosed herein can be packaged into particles by transient transfection, use of producer cell lines, combining viral features into Ad-AAV hybrids, use of herpesvirus systems, or production in insect cells using baculoviruses.

[0113] A method of generating a packaging cell for use herein can involve creating a cell line that stably expresses all the necessary components for AAV particle production. For example, a plasmid (or multiple plasmids) comprising a rAAV genome lacking AAV rep and cap genes, AAV rep and cap genes separate from the rAAV genome, and a selectable marker, such as a neomycin resistance gene, are integrated into the genome of a cell. AAV genomes have been introduced into bacterial plasmids by procedures such as GC tailing, addition of synthetic linkers containing restriction endonuclease cleavage sites, or by direct, blunt-end ligation. The packaging cell line is then infected with a helper virus, such as adenovirus. The advantages of this method are that the cells are selectable and are suitable for large-scale production of rAAV. Examples of suitable methods herein employ adenovirus or baculovirus, rather than plasmids, to introduce rAAV genomes and/or rep and cap genes into packaging cells.

[0114] In an aspect, AAV vectors and/or AAV particles herein can have one or more improvements compared to naturally isolated AAV vectors. As used herein, a "naturally isolated AAV vector" refers to a vector that does not comprise one or more of the capsid

protein variants disclosed herein. In an aspect, AAV vectors and/or AAV particles herein can have increased gene transfer efficiency in a cell compared to naturally isolated AAV vectors. In an aspect, AAV vectors and/or AAV particles herein can have at least about 2-fold to about 50-fold (e.g., about 2-, 4-, 6-, 8-, 10-, 20-, 30-, 40-, 50-fold) increased gene transfer efficiency in a cell compared to naturally isolated AAV vectors.

[0115] In an aspect, AAV vectors and/or AAV particles herein can have increased gene transfer efficiency in the cell and/or tissue of one or more mammalian species. In an aspect, AAV vectors and/or AAV particles herein can have increased gene transfer efficiency in the cell and/or tissue of a human, e.g., a human T-cell.

[0116] In an aspect, AAV vectors and/or AAV particles herein can have a higher vector titer compared to naturally isolated AAV vectors. In an aspect, AAV vectors and/or AAV particles herein can have at least about 2-fold to about 50-fold (e.g., about 2-, 4-, 6-, 8-, 10-, 20-, 30-, 40-, 50-fold) higher vector titer compared to naturally isolated AAV vectors.

[0117] In an aspect, AAV vectors and/or AAV particles herein can be less susceptible to antibody-mediated neutralization compared to naturally isolated AAV vectors. In an aspect, AAV vectors and/or AAV particles herein can be less susceptible to antibody-mediated neutralization by about 2-fold to about 50-fold (e.g., about 2-, 4-, 6-, 8-, 10-, 20-, 30-, 40-, 50-fold) compared to naturally isolated AAV vectors. In an aspect, AAV vectors and/or AAV particles herein can be less susceptible to antibody-mediated neutralization for at least about 1 hour to about 24 hours (e.g., about 1, 2, 4, 8, 12, 16, 20, 24 hours) after administration to a subject compared to naturally isolated AAV vectors.

[0118] In an aspect, AAV vectors and/or AAV particles herein can produce lower levels of anti-AAV antibodies after at least one administration to a subject herein compared to naturally isolated AAV vectors. In an aspect, AAV and/or AAV particles herein can produce about 2-fold to about 50-fold (e.g., about 2-, 4-, 6-, 8-, 10-, 20-, 30-, 40-, 50-fold) less anti-AAV antibodies after at least one administration to a subject herein compared to naturally isolated AAV vectors. In an aspect, gene therapies comprising AAV vectors and/or AAV particles herein can be administered about 2 times to about 10 times (e.g., about 2, 3, 4, 5, 6, ,7, 8, 9, 10) to a subject herein without becoming susceptible to antibody-mediated neutralization.

[0119] In an aspect, AAV vectors and/or AAV particles herein can have expression in any cell or tissue type of more than one mammal. In an aspect, AAV vectors and/or AAV

particles herein can have expression in any cell or tissue type of more than one mammal comprising a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate (e.g., monkey, chimpanzee, baboon, gorilla). In an aspect, AAV vectors and/or AAV particles herein can have expression in any cell or tissue type of a human, a mouse, a dog, and a non-human primate.

[0120] Disclosed herein is a polynucleotide (e.g., DNA or RNA) encoding an AAV capsid protein variant as described herein. In some embodiments, the polynucleotide encodes an AAV capsid protein variant having at least 90%, 95% or 98% identity to the sequence of SEQ ID NO:1, wherein the amino acids corresponding to amino acids 454-460 of SEQ ID NO:1 are substituted with a peptide having a sequence of any one of SEQ ID NO:6 – SEQ ID NO:94.

[0121] Disclosed herein is a recombinant AAV vector comprising a disclosed AAV capsid protein. Disclosed herein is a recombinant AAV vector comprising a disclosed AAV capsid variant protein. In an aspect, a disclosed recombinant AAV vector can comprise a vector genome. A vector genome can be encapsidated by a disclosed AAV capsid comprising a disclosed AAV capsid protein or a disclosed AAV capsid protein variant. In an aspect, a disclosed vector genome can comprise a first inverted terminal repeat (ITR) and a second ITR. In an aspect, a disclosed vector genome can comprise a heterologous polynucleotide (e.g., a transgene or donor polynucleotide) located between the first ITR and the second ITR. In an aspect, a heterologous polynucleotide (e.g., a transgene or donor polynucleotide) can comprise a therapeutic RNA, a therapeutic protein, or a gene-editing molecule, e.g., a gene-editing nuclease, a prime editing enzyme (i.e., a dCas enzyme linked to a reverse transcriptase) or other enzyme that makes epigenetic or base (e.g., base editing) changes or a donor polynucleotide, e.g., for use as an HDRT or HITI template for insertion into a nicked genome. Exemplary prime editing systems are described in, e.g., Anzalone, *et al.*, *Nature* Vol 576, 5 December 2019). Exemplary base editing enzymes are described in, e.g., Komor, et al., Nature Vol. 533, May 19, 2016. Exemplary Nakamura, et al., *Nature Cell Biology* vol.23, January 2021, pp. 11–22. In an aspect, a gene-editing molecule can comprise a nuclease. In an aspect, a nuclease can comprise Cas9. In an aspect, a gene-editing molecule can be a single guide RNA (sgRNA). Disclosed herein is a AAV capsid protein variant comprising a peptide having the sequence of any one of SEQ ID NO:6 – SEQ ID NO:94. Disclosed in an AAV capsid protein variant comprising an amino acid sequence having at least 90%, 95% or 98% identity to the sequence of SEQ ID NO:1, wherein the amino acids

corresponding to amino acids 454-460 of SEQ ID NO:1 are substituted with a peptide having a sequence of any one of SEQ ID NO:6 – SEQ ID NO:94. Disclosed herein is an AAV capsid protein variant comprising an amino acid sequence of SEQ ID NO:2 or a sequence with at least 90% or at least 95% identity thereto. In an aspect, a disclosed AAV capsid can comprise a disclosed AAV capsid protein variant.

[0122] In an aspect, any of the disclosed AAV vectors, virus capsids, and/or AAV viral particles disclosed herein can be formulated to form a pharmaceutical composition. In an aspect, pharmaceutical compositions herein can further include a pharmaceutically acceptable carrier, diluent or excipient. Any of the pharmaceutical compositions to be used in the present methods can comprise pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formations or aqueous solutions.

[0123] The carrier in the pharmaceutical composition must be "acceptable" in the sense that it is compatible with the active ingredient of the composition, and preferably, capable of stabilizing the active ingredient and not deleterious to the subject to be treated. For example, "pharmaceutically acceptable" can refer to molecular entities and other ingredients of compositions comprising such that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (*e.g.*, a human, a mouse). In an aspect, the "pharmaceutically acceptable" carrier used in the pharmaceutical compositions disclosed herein can be those approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

[0124] Pharmaceutically acceptable carriers, including buffers, are well known in the art, and can comprise phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; amino acids; hydrophobic polymers; monosaccharides; disaccharides; and other carbohydrates; metal complexes; and/or non-ionic surfactants. See, *e.g.* Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover.

[0125] In an aspect, the pharmaceutical compositions or formulations herein are for parenteral administration, such as intravenous, intracerebroventricular injection, intra-cisterna magna injection, intra-parenchymal injection, or a combination thereof. Such pharmaceutically acceptable carriers can be sterile liquids, such as water and oil, including

those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and the like. Saline solutions and aqueous dextrose, polyethylene glycol (PEG) and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Pharmaceutical compositions disclosed herein can further comprise additional ingredients, for example preservatives, buffers, tonicity agents, antioxidants and stabilizers, nonionic wetting or clarifying agents, viscosity-increasing agents, and the like. The pharmaceutical compositions described herein can be packaged in single unit dosages or in multidosage forms.

[0126] Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which can contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which can include suspending agents and thickening agents. Aqueous solutions can be suitably buffered (preferably to a pH of from 3 to 9). The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

[0127] The pharmaceutical compositions to be used for in vivo administration should be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. Sterile injectable solutions are generally prepared by incorporating the active (e.g., AAV vectors virus capsids, and/or AAV viral particles) in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique that yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

[0128] The pharmaceutical compositions disclosed herein can also comprise other ingredients such as diluents and adjuvants. Acceptable carriers, diluents and adjuvants are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such

as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, pluronics or polyethylene glycols.

[0129] Disclosed herein is a method of alleviating and/or treating a disease or a condition comprising administering to a subject in need thereof a therapeutically effectively amount of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0130] Disclosed herein is a method of alleviating and/or treating a disease or a condition comprising administering to a subject in need thereof a therapeutically effectively amount of a pharmaceutical composition comprising a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0131] Disclosed herein is a method of alleviating and/or treating a disease or a condition comprising administering to a subject in need thereof a therapeutically effectively amount of cells that have been generated using a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof. For example, in an aspect, a disclosed method can comprise administering CAR T cells made by using compositions (e.g., one or more of a disclosed AAV vector, disclosed AAV particle, disclosed AAV genome, disclosed viral capsid, a disclosed viral capsid protein, or any combination thereof) disclosed herein. For example, in an aspect, CAR T cells can be made using a disclosed AAV capsid protein comprising the sequence set forth in SEQ ID NO:1, wherein amino acids 454-460 of the capsid protein are substituted with the sequence set forth in any one of SEQ ID NO:6 – SEQ ID NO:94. In an aspect, CAR T cells can be made using a disclosed AAV capsid protein comprising the sequence set forth in SEQ ID NO:2.

[0132] Any of the compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) described herein can be used for alleviating and/or treating a disease or a condition. In an aspect, any of the compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) described herein can be used for

alleviating and/or treating a disease or a condition by systemic administration. In an aspect, any of the compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) described herein can be used for alleviating and/or treating a disease or a condition by genetically modifying a subject's immune cells ex vivo. In an aspect, any of the compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) described herein can be used for alleviating and/or treating a disease or a condition by modifying an immune cell to have one or more genetic modifications to enable expression of chimeric antigen receptors (CARs).

[0133] Thus, in an aspect, the present disclosure provides methods for alleviating one or more symptoms and/or for treating a disease or a condition in a subject in need of treatment by compositions disclosed herein, as well as a pharmaceutical composition comprising such. In an aspect, a subject of the methods herein can be a human subject. In an aspect, the subject can be a subject that has not been previously exposed to wild-type AAV or a recombinant (rAAV) vector. In an aspect, the subject can be a subject that has not been previously administered a rAAV vector. In an aspect, the subject is a subject that has been previously administered a rAAV vector, e.g., a rAAV vector described herein. A subject that has been exposed or administered an AAV or rAAV can be identified using methods known in the art, e.g., by PCR detection of viral DNA or by measuring antibody titer to AAV or rAAV, either the capsid or the transgene. In an aspect, the subject can be a subject that has not been administered an enzyme replacement therapy (e.g., by administration of the enzyme protein). A subject that has been administered an enzyme replacement therapy can be identified using methods known in the art, e.g., by measuring antibody titer to the enzyme. However, in an aspect the subject has previously been treated with an enzyme replacement therapy. In an aspect, the subject is a subject that has undergone one or more approaches to clear neutralizing antibodies (NAbs) (e.g., plasmapheresis, immunosuppression, enzymatic degradation). In an aspect, a subject suitable of methods of use herein cannot need to clear neutralizing antibodies (NAbs) before administration of any of the compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) described herein.

[0134] In an aspect, the subject has or is suspected of having a disease that can be treated with gene therapy. Illustrative diseases or a conditions that can be treated using the methods disclosed herein can include, but are not limited to: cystic fibrosis (cystic fibrosis transmembrane regulator protein) and other diseases of the lung, hemophilia A (Factor VIII), hemophilia B (Factor IX), thalassemia (β-globin), anemia (erythropoietin) and other blood disorders, Alzheimer's disease (GDF; neprilysin), multiple sclerosis (β-interferon), Parkinson's disease (glial-cell line derived neurotrophic factor GDNF), Huntington's disease (RNAi to remove repeats), amyotrophic lateral sclerosis, epilepsy (galanin, neurotrophic factors), and other neurological disorders, cancer (endostatin, angiostatin, TRAIL, FAS-ligand, cytokines including interferons; RNAi including RNAi against VEGF or the multiple drug resistance gene product, mir-26a, e.g., for hepatocellular carcinoma), diabetes mellitus (insulin), muscular dystrophies including Duchenne (dystrophin, mini-dystrophin, insulin-like growth factor I, a sarcoglycan e.g., a, β, γ, RNAi against myostatin, myostatin propeptide, follistatin, activin type II soluble receptor, anti-inflammatory polypeptides such as the I-kappa B dominant mutant, sarcospan, utrophin, mini-utrophin, antisense or RNAi against splice junctions in the dystrophin gene to induce exon skipping (see, e.g., WO/2003/095647), antisense against U7 snRNAs to induce exon skipping (see, e.g., WO/2006/021724), and antibodies or antibody fragments against myostatin or myostatin propeptide) and Becker, Gaucher disease (glucocerebrosidase), Hurler's disease (α-L-iduronidase), adenosine deaminase deficiency (adenosine deaminase), glycogen storage diseases (e.g., Fabry disease α-galactosidase and Pompe disease lysosomal acid α-glucosidase) and other metabolic disorders, congenital emphysema (αl-antitrypsin), Lesch-Nyhan Syndrome (hypoxanthine guanine phosphoribosyl transferase), Niemann-Pick disease (sphingomyelinase), Tay Sachs disease (lysosomal hexosaminidase A), Maple Syrup Urine Disease (branched-chain keto acid dehydrogenase), retinal degenerative diseases (and other diseases of the eye and retina; e.g., PDGF for macular degeneration and/or vasohibin or other inhibitors of VEGF or other angiogenesis inhibitors to treat/prevent retinal disorders, e.g., in Type I diabetes), diseases of solid organs such as brain (including Parkinson`s Disease GDNF, astrocytomas endostatin, angiostatin and/or RNAi against VEGF, glioblastomas endostatin, angiostatin and/or RNAi against VEGF), liver, kidney, heart including congestive heart failure or peripheral artery disease (PAD) (e.g., by delivering protein phosphatase inhibitor I (I-l) and fragments thereof (e.g., IIC), serca2a, zinc finger proteins that regulate the phospholamban gene, Barkct, P2-adrenergic receptor, p2-adrenergic receptor kinase (BARK), phosphoinositide-3 kinase (PI3 kinase), S100A1, parvalbumin, adenylyl cyclase type 6, a molecule that effects G-protein

coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct; calsarcin, RNAi against phospholamban; phospholamban inhibitory or dominant-negative molecules such as phospholamban S16E, etc.), arthritis (insulin-like growth factors), joint disorders (insulin-like growth factor 1 and/or 2), intimal hyperplasia (e.g., by delivering enos, inos), improve survival of heart transplants (superoxide dismutase), AIDS (soluble CD4), muscle wasting (insulin-like growth factor I), kidney deficiency (erythropoietin), anemia (erythropoietin), arthritis (anti-inflammatory factors such as IRAP and TNFa soluble receptor), hepatitis (a-interferon), LDL receptor deficiency (LDL receptor), hyperammonemia (ornithine transcarbamylase), Krabbe's disease (galactocerebrosidase), Batten's disease, spinal cerebral ataxias including SCA1, SCA2 and SCA3, phenylketonuria (phenylalanine hydroxylase), autoimmune diseases, and the like.

[0135] To perform the methods disclosed herein, an effective amount of the compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) or a pharmaceutical composition comprising a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof, or cells generated by using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof can be administered to a subject who needs treatment *via* a suitable route (*e.g.*, oral, intramuscular, intravenous, intracerebroventricular injection, intra-cisterna magna injection, intravitreal, subretinal, subconjuctival, retrobulbar, intracameral, suprachoroidal, intracoronary injection, intraarterial injection, and/or intra-parenchymal injection) at a suitable amount as disclosed herein.

[0136] In an aspect, the present disclosure also provides for methods of introducing one or more AAV vectors to a cell, comprising contacting the cell with a composition disclosed herein. In an aspect, methods herein can include delivering one or more AAV vectors herein to a cell, comprising contacting the cell or layer with a viral vector wherein the viral vector can comprise an AAV capsid protein variant disclosed herein. In an aspect of this method, AAV vectors herein can deliver one or more heterologous molecules to a cell. In an aspect, AAV vectors herein can deliver one or more therapeutic heterologous molecules to a cell. In an aspect, one or more therapeutic heterologous molecules delivered to a cell using the methods herein can be a therapeutic protein, a therapeutic DNA, and/or therapeutic RNA. In

an aspect, the therapeutic protein can be a monoclonal antibody or a fusion protein. In an aspect, the therapeutic DNA and/or RNA can be an antisense oligonucleotide, siRNA, shRNA, mRNA, a DNA oligonucleotide, and the like.

[0137] In an aspect, the present disclosure also provides for methods of introducing an AAV vector to a hematopoietic progenitor cell, a T-cell (CD4 T cell and/or CD8 T cell), a B-cell, natural killer (NK) cell or any combination thereof, comprising contacting the cell with a virus vector and/or composition disclosed herein. In an aspect, AAV vectors herein can be delivered to a specific tissue by administering AAV particles having one or more AAV capsid protein variants disclosed herein with enhanced tropism to a hematopoietic progenitor cell, a T-cell (CD4 T cell and/or CD8 T cell), a B-cell, a natural killer (NK) cell, or any combination thereof.

[0138] In an aspect, methods of administering at least one of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof having one or more nucleic acid molecules herein to a tissue substantially modulates expression of the at least one protein and/or gene as compared to baseline. As used herein, "baseline" refers to the expression of the at least one transgene (and the encoded product of the transgene) before the AAV vectors herein were administered. As used herein, "substantially modulates expression" refers to at least a 1-fold change in expression (e.g., increased expression, decreased expression) as compared to baseline. In an aspect, methods of administering at least one of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof disclosed herein to a tissue modulates expression of the at least one protein and/or gene as compared to baseline by at least about 2-fold to about 50-fold (e.g., about 2-, 4-, 6-, 8-, 10-, 20-, 30-, 40-, 50-fold). In an aspect, methods of administering at least one AAV particle or AAV vector having one or more AAV capsid protein variants disclosed herein to a tissue modulates expression of the at least one protein and/or gene as compared to baseline by at least about 2-fold to about 50-fold (e.g., about 2-, 4-, 6-, 8-, 10-, 20-, 30-, 40-, 50-fold) when the at least one AAV particle or AAV vector is delivered to a hematopoietic progenitor cell, a T-cell (CD4 T cell and/or CD8 T cell), a B-cell, a natural killer (NK) cell, a dendritic cell, a macrophage or any combination thereof.

[0139] In any of the methods disclosed herein, an effective amount of the compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) described herein can be given to a subject in need thereof to alleviate one or more symptoms associated with a disease and or condition. "An effective amount" as used herein refers to a dose of a disclosed composition which is sufficient to confer a therapeutic effect on a subject having a disease and or condition. In an aspect, an effective amount can be an amount that reduces at least one symptom of disease or condition in the subject.

[0140] In an aspect, methods of administering at least one AAV as disclosed herein can have increased gene transfer efficiency in a cell compared to naturally isolated AAV vectors. In an aspect, methods of administering at least one AAV vector as disclosed herein can have at least about 2-fold to about 50-fold (e.g., about 2-, 4-, 6-, 8-, 10-, 20-, 30-, 40-, 50-fold) increased gene transfer efficiency in a cell compared to naturally isolated AAV vectors. In an aspect, methods of administering at least one AAV vector as disclosed herein can have increased gene transfer efficiency in a tissue compared to naturally isolated AAV vectors. In an aspect, methods of administering at least one AAV vector as disclosed herein can have at least about 2-fold to about 50-fold (e.g., about 2-, 4-, 6-, 8-, 10-, 20-, 30-, 40-, 50-fold) increased gene transfer efficiency in a tissue compared to naturally isolated AAV vectors. In an aspect, methods of administering at least one AAV vector as disclosed herein can have increased gene transfer efficiency in a subject compared to naturally isolated AAV vectors. In an aspect, methods of administering at least one AAV vector as disclosed herein can have at least about 2-fold to about 50-fold (e.g., about 2-, 4-, 6-, 8-, 10-, 20-, 30-, 40-, 50-fold) increased gene transfer efficiency in a subject compared to naturally isolated AAV vectors.

[0141] In an aspect, methods herein can include administering at least one AAV vector to a subject at least once. In an aspect, methods herein can include administering at least one AAV particle and/or at least one AAV vector to a subject more than once. In an aspect, methods herein can include administering at least one AAV vector herein to a subject between at least once to at least 10 times (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 times). In an aspect, methods herein can include administering at least one AAV vector herein to a subject at least twice, at least 3 times, at least 4 times, or at least 5 times. In an aspect, methods herein can include administering at least one AAV vector herein to a subject once a day, once every other day, once a week, once every two weeks, once every three weeks, once a month, once every other month, once every three months, once every four months, once a year, or

twice a year. In an aspect, methods herein can include administering at least one AAV vector herein to a subject at many times as needed to see the desired response. In an aspect, the desired response can be attenuation of at least one symptom of a disease and/or condition in a subject after administration of a dose of an AAV vector herein compared to before administration of the AAV vector. One of skill in the art will appreciate that dosing regimens can be optimized according to disease/condition, disease/condition severity, characteristics of the subject (e.g., age, gender, weight), and the like.

[0142] In an aspect, an AAV vector herein can be used for the delivery of cre-recombinase. In an aspect, an AAV vector herein can be used for the delivery of cre-recombinase to result in a conditional activation, a conditional inactivation, an activation, an inactivation, or any combination thereof of one or more genes in a cell, tissue, and/or subject. In an aspect, an AAV vector herein can deliver cre-recombinase to one or more specific cell and/or tissue types (e.g., immune cells such as T cells and NK cells).

[0143] In an aspect, an AAV vector herein can be used for the delivery of a CRISPR-Cas system. The "CRISPR/Cas9" system or "CRISPR/Cas9-mediated gene editing" refers to a type II CRISPR/Cas system that has been modified for genome editing/engineering. It is typically comprised of a "guide" RNA (gRNA) and a non-specific CRISPR-associated endonuclease (Cas9). "Guide RNA (gRNA)" is used interchangeably herein with "short guide RNA (sgRNA)" or "single guide RNA (sgRNA). The sgRNA is a short synthetic RNA composed of a "scaffold" sequence necessary for Cas9-binding and a user-defined ~20 nucleotide "spacer" or "targeting" sequence which defines the genomic target to be modified. The genomic target of Cas9 can be changed by changing the targeting sequence present in the sgRNA.

[0144] In an aspect, an AAV vector can comprise a vector genome, wherein the vector genome encodes a gene-editing molecule. In an aspect, the gene-editing molecule is a nuclease. In an aspect, the nuclease is a Cas9 nuclease or a variant thereof, e.g., dCas9. In an aspect, the nuclease is a Cpf1, C2c2, or Cas12a nuclease. In an aspect, the gene editing molecule is a sgRNA. In another aspect, the nuclease is a Zinc Finger Nuclease (ZFN), a Transcription Activator-Like Effector Nuclease (TALEN) or a Meganuclease.

[0145] In an aspect, methods provided herein can include generating a cell to express any of the polynucleotides and/or vectors described herein. In an aspect, cells for use herein can be one or more immune cells. As used herein an "immune cell" can refer to a cell of the

immune system. Immune cells can be categorized as lymphocytes, neutrophils, granulocytes, mast cells, monocytes/macrophages, and dendritic cells. In an aspect, cells for use herein can be one or more lymphocytes. In an aspect, lymphocytes can be T-cells (CD4 T cells and/or CD8 T cells), B-cells, and/or natural killer (NK) cells. In an aspect, cells for use herein can be one or more cytotoxic lymphocytes. As used herein, a "cytotoxic lymphocyte" refers to a lymphocyte capable cytolysis. For example, but not limited to, a cytotoxic lymphocyte can be capable of killing cancer cells, cells that are infected (particularly with viruses), and cells that are damaged in one or more other ways.

[0146] In an aspect, cells for use herein can be isolated from a subject. In an aspect, cells for use herein can be isolated from peripheral blood, umbilical cord blood, and/or bone marrow. In an aspect, cells for use herein can be isolated from peripheral blood mononuclear cells (PBMCs). In an aspect, cells for use herein can be isolated from a leukapheresis sample. In an aspect, cells for use herein can be isolated from tumor-infiltrated lymphocytes, tissue-infiltrated lymphocytes, lymph nodes, thymus, and/or secondary lymphoid organs. In an aspect, cells for use herein can be isolated from autologous peripheral blood, umbilical cord blood, bone marrow, PBMCs, leukapheresis sample, tumor-infiltrated lymphocytes, tissue-infiltrated lymphocytes, lymph nodes, thymus, and/or secondary lymphoid organs. As used herein, the term "autologous" refers to peripheral blood, umbilical cord blood, bone marrow, PBMCs, leukapheresis sample, tumor-infiltrated lymphocytes, tissue-infiltrated lymphocytes, lymph nodes, thymus, and/or secondary lymphoid organs obtained from the same subject to be treated with the compositions disclosed herein. In an aspect, cells for use herein can be isolated from allogeneic peripheral blood, umbilical cord blood, bone marrow, PBMCs, leukapheresis sample, tumor-infiltrated lymphocytes, tissue-infiltrated lymphocytes, lymph nodes, thymus, and/or secondary lymphoid organs. As used herein, the term "allogeneic" refers to peripheral blood, umbilical cord blood, bone marrow, PBMCs, leukapheresis sample, tumor-infiltrated lymphocytes, tissue-infiltrated lymphocytes, lymph nodes, thymus, and/or secondary lymphoid organs obtained from a different subject of the same species as the subject to be treated with the compositions disclosed herein. In an aspect, cells for use herein can be isolated from haploidentical allogeneic peripheral blood, umbilical cord blood, bone marrow, PBMCs, leukapheresis sample, tumor-infiltrated lymphocytes, tissue-infiltrated lymphocytes, lymph nodes, thymus, and/or secondary lymphoid organs.

[0147] In an aspect, gene expression of an immune cell as disclosed herein can be modulated by any of the compositions (e.g., a disclosed AAV vector, a disclosed AAV

particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) herein to alter expression of at least one gene native to an immune cell. In an aspect, modulating gene expression of an immune cell as disclosed herein can alter expression of at least one gene native to an immune cell by about 1% to about 100%, about 5% to about 95%, about 10% to about 90%, about 15% to about 85%, or about 20% to about 80%. In an aspect, modulating gene expression of an immune cell as disclosed herein can prevent expression of at least one gene native to the immune cell. In an aspect, modulating gene expression of an immune cell as disclosed herein can lower expression of at least one gene native to the immune cell. In an aspect, modulating gene expression of an immune cell as disclosed herein can increase expression of at least one gene native to the immune cell.

[0148] In an aspect, a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof comprises a polynucleotide encoding a chimeric antigen receptor (CAR), or HLA-independent T cell receptor (HIT), optionally flanked by regions to enable homologous recombination into a cleavage site, i.e., as an HDR template. In an aspect, gene expression of an immune cell as disclosed herein can be modulated by any of the compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) herein to have one or more genetic modifications to enable expression of chimeric antigen receptors (CARs). In an aspect, immune cells with modulated gene expression can express at least one CAR with one or more genetic modifications to an extracellular antigen recognition domain of the single-chain Fragment variant (scFv) of the CAR, a transmembrane domain of the CAR, an intracellular activation domain of the CAR, or a combination thereof.

[0149] The extracellular target-binding domain comprises a polypeptide that binds to target polypeptide. In some embodiments, the extracellular antigen-binding domain of a comprises an scFv. The scFv can be derived from fusing the variable heavy and light regions (V_H and V_L, respectively) of an antibody, or derived from an Fab or $F(ab)_2$. Other antibody-based recognition domains (cAb VHH (camelid antibody variable domains) and humanized versions, IgNAR VH (shark antibody variable domains) and humanized versions, sdAb VH (single domain antibody variable domains) and "camelized" antibody variable domains are suitable. In some embodiments, the antigen-binding domain is a nanobody.

[0150] In some embodiments, the extracellular target-binding domain binds to a target polypeptide with a dissociation constant (K_d) of about 2×10^{-7} M or less. In some embodiments, the K_d is about 2×10^{-7} M or less, about 1×10^{-7} M or less, about 9×10^{-8} M or less, about 1×10^{-8} M or less, about 9×10^{-9} M or less, about 5×10^{-9} M or less, about 4×10^{-9} M or less, about 3×10^{-9} or less, about 2×10^{-9} M or less, or about 1×10^{-9} M or less. In certain non-limiting embodiments, the K_d is about 3×10^{-9} M or less. In certain non-limiting embodiments, the K_d is from about 1×10^{-9} M to about 3×10^{-7} M. In certain non-limiting embodiments, the K_d is from about 1.5×10^{-9} M to about 3×10^{-7} M. In certain non-limiting embodiments, the K_d is from about 1.5×10^{-9} M to about 2.7×10^{-7} M. In certain non-limiting embodiments, the K_d is from about 1×10^{-4} M to about 1×10^{-6} M. In certain non-limiting embodiments, the K_d is from about 1×10^{-13} M to about 1×10^{-15} M.

[0151] In some embodiments, the extracellular target-binding domain binds to a target polypeptide associated with cancer, such as a cancer antigen or a tumor antigen, e.g., CD3, CD5, CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, CD152, CD19, CD200, CD221, CD23 (igE receptor), CD28, CD4, CD40, CD51, CD52, CD80, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, ERBB2, MAGEA3, p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-A, VEGFR-1, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, PRAME, CCR4, CD5, CD3, TRBC1, TRBC2, TIM-3, Integrin B7, ICAM-1, CD70, Tim3, CLEC12A, ERBB, Her2/neu, CA125, MUC-1, prostate-specific membrane antigen (PSMA), high molecular weight-melanoma associated antigen (HMW-MAA), 4-1BB, adenocarcinoma antigen, α-fetoprotein (AFP), BAFF, B-lymphoma cell, C242 antigen, carbonic anhydrase 9 (CA-IX), C-MET, CEA, FAP, ibronectin extra domain-B, folate receptor 1, GD2, GD3 ganglioside, glycoprotein 75, GPNMB, HER2/neu, HGF, human scatter factor receptorkinase, IGF-1 receptor,IGF-I, IgG1, L1-CAM, IL-13, IL-6, insulin-like growth factorI receptor,integrin α5β1, integrin αvβ3, MORAb-009, MS4A1, MUC1, mucin CanAg, N-glycolylneuraminic acid, NPC-1C, PDGF-R α, PDL192, phosphatidylserine, prostatic carcinoma cells, RANKL, RON, ROR1, SCH 900105, SDC1, SLAMF7, TAG-72, tenascin

C, TGF beta 2, TGF-β, TRAIL-R1, TRAIL-R2, tumor antigen CTAA16.88, and vimentin. In some embodiments, the extracellular target-binding domain binds to a CD3 polypeptide.

[0152] In some embodiments, the extracellular target-binding domain comprises V_H and V_L amino acid sequences of cancer-associated antigen-binding antibodies; many examples are known in the art, as are the light chain and heavy chain CDRs of such antibodies. See, e.g., Ling et al. (2018) Frontiers Immunol. 9:469; International Patent Application Publication No. WO 2005/012493 and U.S. Patent Application Publication Nos. US 2019/0119375 and US 2013/0066055. The following are non-limiting examples of antibodies that bind cancer-associated antigens.

[0153] In some embodiments, the extracellular target-binding domain comprises an anti-CD19 antibody (e.g., an anti-CD19 scFv or an anti-CD19 nanobody). Anti-CD19 antibodies are known in the art; and the V_H and V_L, or the V_H and V_L CDRs, of any anti-CD19 antibody can be included in a CAR. See e.g., International Patent Application Publication No. WO 2005/012493.

[0154] The transmembrane domain links the extracellular target-binding domain and the intracellular signaling domain, and anchors the heterologous polypeptide to the plasma membrane of the host cell that is modified to express the heterologous polypeptide (e.g., the plasma membrane of a human T cell). Any transmembrane domain suitable for use in a cell receptor construct may be employed. A transmembrane domain incorporated into a cell receptor construct may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. Such transmembrane domains, include, but are not limited to, all or part of the transmembrane domain of the α, beta or ζ chain of the T-cell receptor, CD28, CD27, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, a transmembrane domain may include at least the transmembrane region(s) of, e.g., KIRDS2, OX40, CD2, CD27, LFA-1 (CD 11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGAl, VLAl, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGB 1, CD29, ITGB2, CD 18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100, (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150,

IPO-3), BLAME, (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, or NKG2C. In some embodiments, the transmembrane domain is derived from the CD28 polypeptide.

[0155] The hinge domain links the extracellular target-binding domain and the transmembrane domain for positioning the extracellular target-binding domain. In some embodiments, the cell receptor may contain one or more hinge domains that link the extracellular target-binding domain and the transmembrane domain for positioning the extracellular target-binding domain. Such a hinge domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. The hinge domain can include the amino acid sequence of a naturally occurring immunoglobulin hinge region, e.g., a naturally occurring human immunoglobulin hinge region, or an altered immunoglobulin hinge region. Illustrative hinge domains suitable for use in a cell receptors include the hinge region derived from the extracellular regions of type 1 membrane proteins such as CD8 α, CD4, CD28, PD1, CD152, and CD7, which may be wildtype hinge regions from these molecules or may be altered. In some embodiments, the hinge domain is based on the hinge region of a human immunoglobulin IgG1 or IgG4. In some embodiments, the hinge region includes the IgG1 or IgG4's CH2 region, which may comprise one or more mutations. In some embodiments, the mutation is L235E, N297Q, or both L235E and N297Q (which is known as the EQ mutation in the IgG4 hinge region). In some embodiments, the hinge domain is derived from the CD28 polypeptide.

[0156] A cell receptor can include one or more intracellular signaling domains, also referred to herein as co-stimulatory domains, activation domains, or cytoplasmic domains that activate or otherwise modulate an immune cell. The intracellular signaling domain is generally responsible for activation of at least one of the normal effector functions of the immune cell in which the cell receptor has been introduced. For example, a "first-generation chimeric antigen receptor" ("CAR") generally has a CD3 zeta (CD3ζ) signaling domain. Additional costimulatory intracellular domains may also be introduced (e.g., second and third generation CARS) and further domains including homing and suicide domains may be included in CAR constructs.

[0157] In some embodiments, an intracellular signaling domain is used that increases immune cell (e.g., T cell) cytokine production. In some embodiments, an intracellular signaling domain is used that facilitates immune cell (e.g., T cell) replication. In some embodiments, an intracellular signaling domain is used that prevents immune cell (e.g., T

cell) exhaustion. In some embodiments, an intracellular signaling domain is used that increases immune cell (e.g., T cell) antitumor activity. In some embodiments, an intracellular signaling domain is used that enhances survival of immune cells (e.g., T cells) (e.g., post-infusion into patients). Examples of intracellular signaling domains for use in a cell receptor include the cytoplasmic sequences of a cell receptor (e.g., T cell receptor (TCR)) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

[0158] A primary intracellular signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM containing primary intracellular signaling domains include those of CD3ζ, common FcR gamma, Fc gamma Rlla, FcR beta (Fc Epsilon Rib), CD3 gamma, CD3 delta, CD3 epsilon, CD79a, CD79b, DAP10, and DAP12.

[0159] An intracellular signaling domain of a cell receptor can comprise a primary intracellular signaling domain only, or may comprise additional desired intracellular signaling domain(s). For example, the intracellular signaling domain of a cell receptor can comprise a CD3ζ chain portion and a costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the cell receptor comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that binds to CD83, and the like. For example, CD27 costimulation has been demonstrated to enhance expansion, effector function, and survival of human CART cells in vitro and augments human T cell persistence and antitumor activity in vivo (Song et al. Blood. 2012; 119(3):696-706). Further examples of such costimulatory molecules include CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD 160, CD 19, CD4, CD8α, CD8β, IL2Rbeta, IL2R gamma, IL7R α, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-1, ITGAM, CDl lb, ITGAX, CDl lc, ITGB 1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL, DNAM1

(CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), NKG2D, CEACAMl, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM, (SLAMFl, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, and CD19a.

[0160] In some embodiments, the intracellular signaling domain of the cell receptor comprises a modified CD3ζ polypeptide comprising or consisting essentially of or consisting of an ITAM1 variant comprising one or more loss-of-function mutations, an ITAM2 variant comprising one or more loss-of-function mutations, an ITAM3 variant comprising one or more loss-of-function mutations, or a combination thereof. In some embodiments, the loss-of-function mutation comprises a Tyr to Phe mutation.

[0161] In some embodiments, the intracellular signaling domain of the cell receptor comprises a modified CD3ζ polypeptide comprising an ITAM2 variant comprising one or more (e.g., two) loss-of-function mutations and an ITAM3 variant comprising one or more (e.g., two) loss-of-function mutations. In some embodiments, the intracellular signaling domain of the cell receptor comprises a modified CD3ζ polypeptide comprising a native ITAM1, an ITAM2 variant comprising two loss-of-function mutations and an ITAM3 variant comprising two loss-of-function mutations. In some embodiments the CAR can comprise one or more immunoreceptor tyrosine activation motif (ITAM). In some embodiments, the ITAM is modified, e.g., as described in Feucht et al (*Nat Med.* 2019 25: 82-88, e.g., the single iTAM-containing 1928ζ mutants termed 1XX, X2X, and XX3 or double X23 mutants described therein.

[0162] In an aspect, gene expression of an immune cell as disclosed herein can be modulated by any of the compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) herein to have one or more genetic modifications to T-cell receptors (TCRs). In an aspect, immune cells with modulated gene expression and/or native immune cells can have one or more genetic modifications to an alpha-chain of a TCR, a beta-chain of a TCR, or a combination thereof, or can comprise a recombinant TCR. In an aspect, immune cells with modulated gene expression and/or native immune cells according to methods disclosed herein can have one or more genetic modifications to increase secretion of one or more antibodies, one or more cytokines, one or more proteins, or a combination thereof.

[0163] Disclosed herein is a method of generating an immune cell therapy comprising administering to a subject in need thereof a therapeutically effectively amount of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0164] Disclosed herein is a method of generating an immune cell therapy comprising administering to a subject in need thereof a therapeutically effectively amount of a pharmaceutical composition comprising a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0165] Disclosed herein is a method of generating an immune cell therapy comprising administering to a subject in need thereof a therapeutically effectively amount of cells that have been generated using a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof. For example, in an aspect, a disclosed method can comprise administering CAR T cells made by using compositions (e.g., one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) disclosed herein. For example, in an aspect, CAR T cells can be made using a disclosed AAV capsid protein comprising the sequence set forth in SEQ ID NO:1, wherein amino acids 454-460 of the capsid protein are substituted with the sequence set forth in any one of SEQ ID NO:6 – SEQ ID NO:94.

[0166] In an aspect, compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) herein can be used in methods of generating an immune cell therapy composition. In an aspect, an immune cell therapy composition disclosed herein can include at least one immune cell with modulated gene expression. As used herein, the term "immune cell therapy" or "immunotherapy" refers to a therapeutic approach of activating or suppressing the immune system for the treatment of disease. In an aspect, an immune cell therapy composition disclosed herein encompasses adoptive cell therapy. As used herein, the term "adoptive cell therapy" refers to the transfer of ex vivo grown immune cells into a subject for treatment of a disease. In an aspect, immune cell therapy compositions disclosed herein include at least one lymphocyte with modulated gene expression. In an

aspect, a lymphocyte with modulated gene expression for use in an immune cell therapy composition can be a cytotoxic lymphocyte. In an aspect, a cytotoxic lymphocyte for use in an immune cell therapy composition can be a NK cell, a CD4 T cell, and/or a CD8 T cell.

[0167] In an aspect, immune cell therapy compositions disclosed herein can be administered to a subject in need thereof. A suitable subject includes a mammal, a human, a livestock animal, a companion animal, a lab animal, or a zoological animal. In an aspect, the subject can be a rodent, e.g., a mouse, a rat, a guinea pig, etc. In an aspect, the subject can be a livestock animal. Non-limiting examples of suitable livestock animals can include pigs, cows, horses, goats, sheep, llamas and alpacas. In an aspect, the subject can be a companion animal. Non-limiting examples of companion animals can include pets such as dogs, cats, rabbits, and birds. In an aspect, the subject can be a zoological animal. As used herein, a "zoological animal" refers to an animal that can be found in a zoo. Such animals can include non-human primates, large cats, wolves, and bears. In an aspect, the animal is a laboratory animal. Non-limiting examples of a laboratory animal can include rodents, canines, felines, and non-human primates. In an aspect, the animal is a rodent. Non-limiting examples of rodents can include mice, rats, guinea pigs, etc. In an aspect, the subject is a human.

[0168] In an aspect, a subject in need thereof can have been diagnosed with a cancer. By example, but not limited to, a subject can have been diagnosed with nasopharyngeal cancer, synovial cancer, hepatocellular cancer, renal cancer, cancer of connective tissues, melanoma, lung cancer, bowel cancer, colon cancer, rectal cancer, colorectal cancer, brain cancer, throat cancer, oral cancer, liver cancer, bone cancer, pancreatic cancer, choriocarcinoma, gastrinoma, pheochromocytoma, prolactinoma, T-cell leukemia/lymphoma, neuroma, von Hippel-Lindau disease, Zollinger-Ellison syndrome, adrenal cancer, anal cancer, bile duct cancer, bladder cancer, ureter cancer, brain cancer, oligodendroglioma, neuroblastoma, meningioma, spinal cord tumor, bone cancer, osteochondroma, chondrosarcoma, Ewing`s sarcoma, cancer of unknown primary site, carcinoid, carcinoid of gastrointestinal tract, fibrosarcoma, breast cancer, Paget's disease, cervical cancer, colorectal cancer, rectal cancer, esophagus cancer, gall bladder cancer, head cancer, eye cancer, neck cancer, kidney cancer, Wilms' tumor, liver cancer, Kaposi's sarcoma, prostate cancer, lung cancer, testicular cancer, Hodgkin's disease, non-Hodgkin's lymphoma, oral cancer, skin cancer, mesothelioma, multiple myeloma, ovarian cancer, endocrine pancreatic cancer, glucagonoma, pancreatic cancer, parathyroid cancer, penis cancer, pituitary cancer, soft tissue sarcoma, retinoblastoma, small intestine cancer, stomach cancer, thymus cancer, thyroid cancer, trophoblastic cancer,

hydatidiform mole, uterine cancer, endometrial cancer, vagina cancer, vulva cancer, acoustic neuroma, mycosis fungoides, insulinoma, carcinoid syndrome, somatostatinoma, gum cancer, heart cancer, lip cancer, meninges cancer, mouth cancer, nerve cancer, palate cancer, parotid gland cancer, peritoneum cancer, pharynx cancer, pleural cancer, salivary gland cancer, tongue cancer, tonsil cancer, or a combination thereof.

[0169] In an aspect, a subject in need thereof can have been diagnosed with an infectious disease. By example, but not limited to, a subject can have been diagnosed with chickenpox, common cold, diphtheria, E. coli, giardiasis, HIV/AIDS, infectious mononucleosis, influenza, Lyme disease, malaria, measles, meningitis, mumps, poliomyelitis (polio), pneumonia, Rocky mountain spotted fever, rubella (German measles), Salmonella infections, severe acute respiratory syndrome (SARS), sexually transmitted diseases, shingles (herpes zoster), tetanus, toxic shock syndrome, tuberculosis, viral hepatitis, West Nile virus, whooping cough (pertussis), or a combination thereof.

[0170] In an aspect, a subject in need thereof can have been diagnosed with an autoimmune disease. By example, but not limited to, a subject can have been diagnosed with diabetes (Type 1), lupus, multiple sclerosis, rheumatoid arthritis, celiac disease, or a combination thereof.

[0171] In an aspect, a subject in need thereof can have been diagnosed with an immune deficiency disease. By example, but not limited to, a subject can have been diagnosed with autoimmune lymphoproliferative syndrome (ALPS), autoimmune polyglandular syndrome type 1 (APS-1), BENTA disease, caspase eight deficiency state (CEDS), CARD9 deficiency and other syndromes of susceptibility to *Candidiasis*, chronic granulomatous disease (CGD), common variable immunodeficiency (CVID), congenital neutropenia syndromes, CTLA4 deficiency, DOCK8 deficiency, *GATA2* deficiency, hyper-immunoglobulin E syndrome (HIES), hyper-immunoglobulin M (IgM) syndrome, leukocyte adhesion deficiency (LAD), *LRBA* deficiency, PI3 kinase disease, PLAID and/or PLAID-like disease, severe combined immunodeficiency (SCID), *STAT3* gain-of-function disease, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis (WHIM) Syndrome, Wiskott-Aldrich syndrome (WAS), X-linked agammaglobulinemia (XLA), XMEN disease, lupus or Type I or Type II diabetes or a combination thereof.

[0172] In an aspect, compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid

protein, or any combination thereof) herein can be used in methods of generating an immune cell therapy composition that can increase cytolytic activity in immune cells with modulated gene expression as disclosed herein compared to cytolytic activity of native immune cells. In an aspect, an immune cell therapy composition disclosed herein can increase cytolytic activity immune cells with modulated gene expression as disclosed herein by about 1% to about 100%, about 10% to about 90%, or about 20% to about 80% compared to native immune cells. In an aspect, an immune cell therapy composition disclosed herein can increase cytolytic activity in immune cells with modulated gene expression as disclosed herein by about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 100% compared to native immune cells. In an aspect, an immune cell therapy composition disclosed herein can increase cytolytic activity of immune cells with modulated gene expression as disclosed herein against leukemia cells, lymphoma cells, tumor cells, metastasizing cells of solid tumors compared to cytolytic activity of native immune cells. In an aspect, an immune cell therapy composition disclosed herein can increase cytolytic activity of immune cells with modulated gene expression as disclosed herein from subjects with viral, mycotic or bacterial infectious diseases compared to cytolytic activity of native immune cells.

[0173] In an aspect, compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) herein can be used in methods of generating CAR T cells, e.g., as descried herein.

[0174] Disclosed herein is a method of making CAR T cells using a therapeutically effectively amount of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0175] The present disclosure also provides methods of making CAR T cells using compositions (e.g., one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) disclosed herein. For example, in an aspect, a disclosed AAV capsid protein can comprise the sequence set forth in SEQ ID NO:1, wherein amino acids 454-460 of the capsid protein are substituted with the sequence set forth in any one of SEQ ID NO:6 – SEQ ID NO:94.

[0176] In an aspect, CRISPR-Cas9 gene-editing or other nuclease-targeted components can be used to introduce a site-specific disruption at a gene sequence that is associated with diseases and/or conditions of interest, such as the TCR and/or MHC. In an aspect, the gene-sequence is selected from a component of the TCR. In an aspect, the TCR component is a TRAC. In an aspect, the site-specific disruption is a permanent deletion of at least a portion of the gene. In an aspect, the site-specific disruption is a small deletion in the gene. In an aspect, the site-specific disruption is a small insertion in the gene. In an aspect, the site-specific disruption is an insertion of a nucleic acid encoding a CAR or HIT in the gene. In an aspect, a site-specific disruption of the TRAC gene provides a T cell without a functional TCR. In an aspect, a DNA double-stranded break at the TRAC locus can be repaired by homology directed repair with any of the AAV vectors (e.g., AAV6, Ark315 or others) disclosed herein. In an aspect, a DNA double-stranded break at the TRAC locus can be repaired by homology directed repair with any of the AAV vectors (e.g., AAV6, Ark315 or others) herein wherein the AAV vector can comprise a nucleotide sequence containing right and left homology arms to the TRAC locus flanking a chimeric antigen receptor (CAR) cassette.

[0177] In an aspect, compositions (e.g., a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) herein can be used in methods of generating T-cells expressing an HLA-independent T cell receptor (HIT). In some embodiments, the HIT is capable of inducing an immune response when binding to an antigen on the surface of a target cell, e.g., a cancer or tumor cell. Non-limiting examples of HITs are discussed in International Patent Application Publication No. WO 2019/157454A1 and Mansilla-Soto, Jorge et al., *Nature Medicine* vol. 28,2 (2022): 345-352.

[0178] In some embodiments, the heterologous cell receptor is a HIT comprising an extracellular antigen-binding domain derived from an antibody fragment such as an scFv or an Fab. In some embodiments, the extracellular antigen-binding domain also comprises a constant domain. In some embodiments, the extracellular antigen-binding domain is capable of dimerizing with another extracellular antigen-binding domain (e.g., forming a fragment variable (Fv)), wherein the dimerized antigen-binding domains (e.g., an Fv) specifically bind to an antigen, e.g., a tumor antigen or a pathogen antigen. In some embodiments, the HIT extracellular antigen-binding domain comprises a heavy chain variable region (VH) and/or a light chain variable region (VL) of an antibody, wherein the VH or the VL is capable of

dimerizing with another extracellular antigen binding domain comprising a VL or a VH (e.g., forming a fragment variable (Fv)). In some embodiments, a TCR's HLA-restricted Vα-Vβ pair is replaced by heterologous VL-VH binding domains for heterologous antigen-binding specificity. In some embodiments, the VL-VH binding domains confer specificity for target polypeptide. The engineered VH-Cβ and VL-Cα chains can associate to form the HIT heterodimer with the VH and VL portions forming the fragment variable Fv.

[0179] In an aspect, the present disclosure relates to an administration of a population of engineered T cells (e.g., CAR T cells or HIT T cells) with a disrupted TCR and MHC as generated by any of the AAV vectors (e.g., AAV6, Ark315 or others) disclosed herein. In an aspect, the present disclosure relates to administration of a population of cells comprising engineered T cells (e.g., engineered human CAR T cells) with a reduced risk of inducing an AAV-mediated immune response in the recipient patient. In an aspect, CRISPR-Cas9 gene-editing components are used to introduce a site-specific disruption at a TRAC locus. In an aspect, a site-specific disruption in the TRAC locus is an insertion of a nucleic acid encoding a CAR in the gene. In an aspect, a site-specific disruption in the TRAC locus provides a population of engineered T cells (e.g., engineered human CAR T cells) wherein at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% of donor T cells lack expression of a functional TCR. In an aspect, a site-specific disruption in the TRAC locus provides engineered T cells wherein at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 100% of engineered T cells lack expression of a functional TCR. In an aspect, a site-specific disruption in the TRAC locus and a purification step provides a cell population of engineered T cells (e.g., engineered human CAR T cells) wherein at least 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% of engineered T cells lack expression of a functional TCR. In an aspect, a site-specific disruption in the TRAC locus and a purification step provides engineered T cells wherein at least 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% of engineered T cells lack expression of a functional TCR. In an aspect, administration of a population of engineered T cells, wherein at least 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% of engineered T cells (e.g., engineered human CAR T cells) lack expression of a functional TCR, reduces the risk of an AAV-mediated immune response following administration to a recipient patient. In an aspect, administration of engineered T cells, wherein at least 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, or 100% of engineered T cells lack expression of a functional TCR, reduces the risk of an AAV-mediated immune response following administration to a recipient patient.

[0180] Disclosed herein is a method of treating a genetic disease or disorder comprising administering to a subject in need thereof a therapeutically effective amount of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0181] Disclosed herein is a method of treating a genetic disease or disorder comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0182] Disclosed herein is a method of treating a genetic disease or disorder comprising administering to a subject in need thereof a therapeutically effective amount of one or more cells, wherein the one or more cells have been contacted with a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0183] Disclosed herein is a method of treating a genetic disease or disorder comprising administering to a subject in need thereof a therapeutically effective amount of a pharmaceutical composition comprising one or more cells, wherein the one or more cells have been contacted with a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0184] In an aspect, the one or more cells have been contacted ex vivo. For example, in an aspect, a disclosed method can comprise administering CAR T cells made by using compositions (e.g., one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof) disclosed herein. For example, in an aspect, CAR T cells can be made using a disclosed AAV capsid protein comprising the sequence set forth in SEQ ID NO:1, wherein amino acids 454-460 of the capsid protein are substituted with the sequence set forth in any one of SEQ ID NO:6 – SEQ ID NO:94.

[0185] In an aspect, a subject can have or be suspected of having a disease or disorder that can be treated with gene therapy. In an aspect, a subject can have a genetic disease or disorder that affects the immune system.

[0186] In an aspect, a subject in need thereof can be diagnosed with an autoimmune disease. By example, but not limited to, a subject can be diagnosed with diabetes (Type 1), lupus, multiple sclerosis, rheumatoid arthritis, celiac disease, or a combination thereof.

[0187] In an aspect, a subject in need thereof can be diagnosed with an immune deficiency disease. By example, but not limited to, a subject can be diagnosed with autoimmune lymphoproliferative syndrome (ALPS), autoimmune polyglandular syndrome type 1 (APS-1), BENTA disease, caspase eight deficiency state (CEDS), CARD9 deficiency and other syndromes of susceptibility to *Candidiasis*, chronic granulomatous disease (CGD), common variable immunodeficiency (CVID), congenital neutropenia syndromes, CTLA4 deficiency, DOCK8 deficiency, *GATA2* deficiency, hyper-immunoglobulin E syndrome (HIES), hyper-immunoglobulin M (IgM) syndrome, leukocyte adhesion deficiency (LAD), *LRBA* deficiency, PI3 kinase disease, PLAID and/or PLAID-like disease, severe combined immunodeficiency (SCID), *STAT3* gain-of-function disease, Warts, Hypogammaglobulinemia, Infections, and Myelokathexis (WHIM) Syndrome, Wiskott-Aldrich syndrome (WAS), X-linked agammaglobulinemia (XLA), XMEN disease, or a combination thereof.

[0188] Other genetic diseases and disorders include, but are not limited to, diseases and disorders due to a defect in the following genes: ABCA1, ABCA12, ABCA13, ABCA2, ABCA3, ABCA4, ABCA5, ABCC1, ABCC2, ABCC6, ABCC8, ABCC9, ACAN, ADAMTS13, ADCY10, ADGRV1, AGL, AGRN, AHDC1, ALK, ALMS1, ALPK3, ALS2, ANAPC1, ANK1, ANK2, ANK3, ANKRD11, ANKRD26, APC, APC2, APOB, ARFGEF2, ARHGAP31, ARHGEF10, ARHGEF18, ARID1A, ARID1B, ARID2, ASH1L, ASPM, ASXL1, ASXL2, ASXL3, ATM, ATP7A, ATP7B, ATR, ATRX, BAZ1A, BAZ2B, BCOR, BCORL1, BDP1, BLM, BPTF, BRCA1, BRCA2, BRD4, BRWD3, C2CD3, C3, C5, CACNA1A, CACNA1B, CACNA1C, CACNA1D, CACNA1E, CACNA1F, CACNA1G, CACNA1H, CACNA1S, CAD, CAMTA1, CARMIL2, CC2D2A, CCDC88A, CCDC88C, CCNB3, CDH23, CDK13, CDK5RAP2, CELSR1, CEMIP2, CENPE, CENPF, CENPJ, CEP152, CEP164, CEP250, CEP290, CFAP43, CFAP44, CFAP65, CFTR/ABCC7, CHD1, CHD2, CHD3, CHD4, CHD7, CHD8, CIC, CIT, CLIP1, CLTC, CNOT1, CNTNAP1, COL11A1, COL11A2, COL12A1, COL17A1, COL18A1, COL1A1, COL1A2, COL27A1, COL2A1, COL3A1, COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, COL4A6, COL5A1, COL5A2, COL6A3, COL7A1, CPAMD8, CPLANE1, CPS1, CPSF1, CRB1, CREBBP, CUBN, CUL7, CUX1, DCC, DCHS1, DEPDC5, DICER1, DIP2B, DLC1, DMD,

DMXL2, DNAH1, DNAH11, DNAH17, DNAH2, DNAH5, DNAH7, DNAH8, DNAH9, DNMBP, DNMT1, DOCK2, DOCK3, DOCK6, DOCK7, DOCK8, DSCAM, DSP, DST, DUOX2, DYNC1H1, DYNC2H1, DYSF, EIF2AK4, EP300, EPG5, ERCC6, ERCC6L2, EXPH5, EYS, F5, F8, FANCA, FANCD2, FANCM, FAT1, FAT4, FBN1, FBN2, FLG, FLG2, FLNA, FLNB, FLNC, FLT4, FMN2, FN1, FRAS1, FREM1, FREM2, FSIP2, FYCO1, GLI2, GLI3, GPR179, GREB1L, GRIN2A, GRIN2B, GRIN2D, HCFC1, HECW2, HERC1, HERC2, HFM1, HIVEP1, HIVEP2, HMCN1, HSPG2, HTT, HUWE1, HYDIN, IFT140, IFT172, IGF1R, IGF2R, IGSF1, INSR, INTS1, IQSEC2, ITGB4, ITPR1, ITPR2, JMJD1C, KALRN, KANK1, KAT6A, KAT6B, KDM3B, KDM5B, KDM5C, KDM6A, KDM6B, KDR, KIAA0586, KIAA1109, KIAA1549, KIDINS220, KIF14, KIF1A, KIF1B, KIF21A, KIF26B, KIF7, KMT2A, KMT2B, KMT2C, KMT2D, KMT2E, KNL1, LAMA1, LAMA2, LAMA3, LAMA4, LAMA5, LAMB1, LAMB2, LAMC3, LCT, LOXHD1, LPA, LRBA, LRP1, LRP2, LRP4, LRP5, LRP6, LRPPRC, LRRK1, LRRK2, LTBP2, LTBP4, LYST, MACF1, MADD, MAGI2, MAP1B, MAP3K1, MAPK8IP3, MAPKBP1, MAST1, MBD5, MCM3AP, MED12, MED12L, MED13, MED13L, MED23, MEGF8, MET, MLH3, MPDZ, MSH6, MTOR, MYH10, MYH11, MYH14, MYH2, MYH3, MYH6, MYH7, MYH7B, MYH8, MYH9, MYLK, MYO15A, MYO18B, MYO3A, MYO5A, MYO5B, MYO7A, MYO9A, NALCN, NBAS, NBEA, NBEAL2, NCAPD2, NCAPD3, NEB, NEXMIF, NEXMIF, NF1, NFASC, NHS, NIN, NIPBL, NLRP1, NOTCH1, NOTCH2, NOTCH3, NPHP4, NRXN1, NRXN3, NSD1, NSD2, NUP155, NUP188, NUP205, OBSCN, OBSL1, OTOF, OTOG, OTOGL, PARD3, PBRM1, PCDH15, PCLO, PCNT, PHIP, PI4KA, PIEZO1, PIEZO2, PIK3C2A, PIKFYVE, PKD1, PKD1L1, PKHD1, PLCE1, PLEC, PLEKHG2, PNPLA6, POGZ, POLA1, POLE, POLR1A, POLR2A, POLR3A, PRG4, PRKDC, PRPF8, PRR12, PRX, PTCH1, PTPN23, PTPRF, PTPRJ, PTPRQ, PXDN, QRICH2, RAB3GAP2, RAI1, RALGAPA1, RANBP2, RB1CC1, RELN, RERE, REV3L, RIC1, RIMS1, RIMS2, RNF213, ROBO1, ROBO2, ROBO3, ROS1, RP1, RP1L1, RTTN, RUSC2, RYR1, RYR2, SACS, SAMD9, SAMD9L, SBF2, SCAPER, SCN10A, SCN11A, SCN1A, SCN2A, SCN3A, SCN4A, SCN5A, SCN8A, SCN9A, SETBP1, SETD1A, SETD1B, SETD2, SETD5, SETX, SHANK2, SHANK3, SHROOM4, SI, SIPA1L3, SLIT2, SLX4, SMARCA2, SMARCA4, SMCHD1, SNRNP200, SON, SPEF2, SPEG, SPG11, SPTA1, SPTAN1, SPTB, SPTBN2, SPTBN4, SRCAP, STRC, SVIL, SYNE1, SYNGAP1, SYNJ1, SZT2, TAF1, TANC2, TCF20, TCOF1, TDRD9, TECPR2, TECTA, TENM3, TENM4, TET3, TEX14, TEX15, TG, THOC2, TMEM94, TNC, TNIK, TNR, TNRC6B, TNXB, TOGARAM1, TONSL, TRIO, TRIOBP, TRIP11, TRIP12, TRPM1, TRPM6,

TRPM7, TRRAP, TSC2, TTC37, TTN, TUBGCP6, UBR1, UNC80, USH2A, USP9X, VCAN, VPS13A, VPS13B, VPS13C, VPS13D, VWF, WDFY3, WDR19, WDR62, WDR81, WNK1, WRN, ZFHX2, ZFYVE26, ZNF142, ZNF292, ZNF335, ZNF407, ZNF462, ZNF469, or a portion thereof.

[0189] In an aspect, a disclosed method of treating a genetic disease or disorder can restore one or more aspects of cellular homeostasis and/or cellular functionality and/or metabolic dysregulation in the subject (such as, for example, homeostasis and/or cellular function and/or metabolic dysregulation relating to the immune system). In an aspect, a disclosed method of treating a genetic disease or disorder can restore the functionality and/or structural integrity of a missing, deficient, and/or mutant protein or enzyme (such as, for example, a protein or enzyme in the immune system). In an aspect, restoring one or more aspects of cellular homeostasis and/or cellular functionality can comprise one or more of the following: (i) correcting cell starvation in one or more cell types; (ii) normalizing aspects of the autophagy pathway (such as, for example, correcting, preventing, reducing, and/or ameliorating autophagy); (iii) improving, enhancing, restoring, and/or preserving mitochondrial functionality and/or structural integrity; (iv) improving, enhancing, restoring, and/or preserving organelle functionality and/or structural integrity; (v) correcting enzyme dysregulation; (vi) reversing, inhibiting, preventing, stabilizing, and/or slowing the rate of progression of the multi-systemic manifestations of a genetic disease or disorder; (vii) reversing, inhibiting, preventing, stabilizing, and/or slowing the rate of progression of a genetic disease or disorder, or (viii) any combination thereof. In an aspect, restoring one or more aspects of cellular homeostasis can comprise improving, enhancing, restoring, and/or preserving one or more aspects of cellular structural and/or functional integrity in the subject.

[0190] In an aspect, restoring the activity and/or functionality of a missing, deficient, and/or mutant protein or enzyme (such as those, for example, contributing to immune system function) can comprise a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any amount of restoration when compared to a pre-existing level such as, for example, a pre-treatment level. In an aspect, the amount of restoration can be 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, or 90-100% more than a pre-existing level such as, for example, a pre-treatment level. In an aspect, restoration can be measured against a control level or a reference level (e.g., determined, for example, using one or more subjects not having a missing, deficient, and/or mutant protein or enzyme (such as those contributing to immune system function). In an aspect, restoration can be a partial or incomplete

restoration. In an aspect, restoration can be complete or near complete restoration such that the level of expression, activity, and/or functionality is similar to that of a wild-type or control level.

[0191] In an aspect, a therapeutically effective amount of disclosed AAV vector can comprise a range of about 1 x 10^{10} vg/kg to about 2 x 10^{14} vg/kg. In an aspect, for example, a disclosed AAV vector can be administered at a dose of about 1 x 10^{11} to about 8 x 10^{13} vg/kg or about 1 x 10^{12} to about 8 x 10^{13} vg/kg. In an aspect, a disclosed AAV vector can be administered at a dose of about 1 x 10^{13} to about 6 x 10^{13} vg/kg. In an aspect, a disclosed AAV vector can be administered at a dose of at least about 1 x 10^{10}, at least about 5 x 10^{10}, at least about 1 x 10^{11}, at least about 5 x 10^{11}, at least about 1 x 10^{12}, at least about 5 x 10^{12}, at least about 1 x 10^{13}, at least about 5 x 10^{13}, or at least about 1 x 10^{14} vg/kg. In an aspect, a disclosed AAV vector can be administered at a dose of no more than about 1 x 10^{10}, no more than about 5 x 10^{10}, no more than about 1 x 10^{11}, no more than about 5 x 10^{11}, no more than about 1 x 10^{12}, no more than about 5 x 10^{12}, no more than about 1 x 10^{13}, no more than about 5 x 10^{13}, or no more than about 1 x 10^{14} vg/kg. In an aspect, a disclosed AAV vector can be administered at a dose of about 1 x 10^{12} vg/kg. In an aspect, a disclosed AAV vector can be administered at a dose of about 1 x 10^{11} vg/kg. In an aspect, a disclosed AAV vector can be administered in a single dose, or in multiple doses (such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 doses) as needed for the desired therapeutic results. In an aspect, a therapeutically effective amount of disclosed AAV vector can comprise a range determined by a skilled person.

[0192] In an aspect of a disclosed method, techniques to monitor, measure, and/or assess the restoring one or more aspects of cellular homeostasis and/or cellular functionality can comprise qualitative (or subjective) means as well as quantitative (or objective) means. These means are known to the skilled person.

[0193] In an aspect, administering can comprise oral, intravenous, intraarterial, intramuscular, intraperitoneal, subcutaneous, intra-CSF, intrathecal, intraventricular, intrahepatic, hepatic intra-arterial, hepatic portal vein (HPV), or in utero administration. In an aspect, a disclosed composition, a disclosed pharmaceutical formulation, and/or a disclosed vector can be concurrently and/or serially administered to a subject via multiple routes of administration. For example, in an aspect, administering a disclosed vector and/or a disclosed pharmaceutical formulation can comprise intravenous administration and intra-cistern magna (ICM) or intrathecal (ITH) administration. In an aspect, a disclosed method

can employ multiple routes of administration to the subject. In an aspect, a disclosed method can employ a first route of administration that can be the same or different as a second and/or subsequent routes of administration.

[0194] In an aspect, a disclosed method of treating and/or preventing a genetic disease or disorder can further comprise administering to the subject a therapeutically effective amount of a therapeutic agent. A therapeutic agent can be any disclosed agent that effects a desired clinical outcome.

[0195] In an aspect, a disclosed method of treating and/or preventing a genetic disease or disorder can further comprise monitoring the subject for adverse effects. In an aspect, in the absence of adverse effects, the method can further comprise continuing to treat the subject. In an aspect, in the presence of adverse effects, the method can further comprise modifying the treating step. Methods of monitoring a subject's well-being can include both subjective and objective criteria (and are discussed supra). Such methods are known to the skilled person.

[0196] In an aspect, a disclosed method can further comprise administering to the subject a therapeutically effective amount of an agent that can correct one or more aspects of a dysregulated metabolic or enzymatic pathway. In an aspect, such an agent can comprise an enzyme for enzyme replacement therapy. In an aspect, a disclosed enzyme can replace any enzyme in a dysregulated or dysfunctional metabolic or enzymatic pathway. In an aspect, a disclosed method can comprise replacing one or more enzymes in a dysregulated or dysfunctional metabolic pathway.

[0197] In an aspect, a disclosed method of treating and/or preventing a genetic disease or disorder can further comprise administering one or more immune modulators. In an aspect, a disclosed immune modulator can be methotrexate, rituximab, intravenous gamma globulin, or bortezomib, or a combination thereof. In an aspect, a disclosed immune modulator can be bortezomib or SVP-Rapamycin. In an aspect, a disclosed immune modulator can be Tacrolimus. In an aspect, a disclosed method of treating and/or preventing a genetic disease or disorder can further comprise administering one or more proteasome inhibitors (e.g., bortezomib, carfilzomib, marizomib, ixazomib, and oprozomib). In an aspect, a disclosed method of treating and/or preventing a genetic disease or disorder can further comprise administering one or more immunosuppressive agents. In an aspect, an immunosuppressive agent can be, but is not limited to, azathioprine, methotrexate, sirolimus, anti-thymocyte

globulin (ATG), cyclosporine (CSP), mycophenolate mofetil (MMF), steroids, or a combination thereof.

[0198] In an aspect, a disclosed method of treating a genetic disease or disorder can comprise repeating a disclosed administering step one or more times such as, for example, repeating the administering of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0199] In an aspect, a disclosed method of treating a genetic disease or disorder can comprise repeating a disclosed administering step one or more times such as, for example, repeating the administering of a disclosed therapeutic agent, a disclosed immune modulator, a disclosed proteasome inhibitor, a disclosed immunosuppressive agent, a disclosed compound that exerts a therapeutic effect against B cells and/or a disclosed compound that targets or alters antigen presentation or humoral or cell mediated immune response.

[0200] In an aspect, a disclosed method of treating a genetic disease or disorder can comprise modifying one or more of the disclosed steps. For example, modifying one or more of steps of a disclosed method can comprise modifying or changing one or more features or aspects of one or more steps of a disclosed method. For example, in an aspect, a method can be altered by changing the amount of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof administered to a subject, or by changing the frequency of administration of one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof to a subject, or by changing the duration of time one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof are administered to a subject.

[0201] In an aspect, a method can be altered by changing the amount of a disclosed pharmaceutical composition comprising a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof administered to a subject, or by changing the frequency of administration of a disclosed pharmaceutical composition comprising one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV

viral capsid, a disclosed AAV viral capsid protein, or any combination thereof to a subject, or by changing the duration of time one or more of a disclosed pharmaceutical composition comprising a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof are administered to a subject.

[0202] In an aspect, a method can be altered by changing the amount of one or more disclosed therapeutic agents, disclosed immune modulators, disclosed proteasome inhibitors, disclosed immunosuppressive agents, disclosed compounds that exert therapeutic effect against B cells and/or disclosed compounds that targets or alters antigen presentation or humoral or cell mediated immune response administered to a subject, or by changing the frequency of administration of one or more of the disclosed therapeutic agents, disclosed immune modulators, disclosed proteasome inhibitors, disclosed immunosuppressive agents, disclosed compounds that exert therapeutic effect against B cells and/or disclosed compounds that targets or alters antigen presentation or humoral or cell mediated immune response administered to a subject.

[0203] In as aspect, a disclosed method can comprise concurrent administration of one or more of the following: one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof, a disclosed pharmaceutical composition comprising a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof, cells generated by using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof, one or more disclosed therapeutic agents, one or more disclosed immune modulators, one or more disclosed proteasome inhibitors, one or more disclosed immunosuppressive agents, one or more disclosed compounds that exert therapeutic effect against B cells, one or more disclosed compounds that targets or alters antigen presentation or humoral or cell mediated immune response, or any combination thereof.

[0204] In an aspect, a disclosed immune modulator can be administered prior to or after the administration of a disclosed therapeutic agent.

[0205] In an aspect, a disclosed method of treating and/or preventing a genetic disease or disorder can further comprise generating a disclosed AAV vector, a disclosed AAV particle,

a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof. In an aspect, a disclosed method can further comprise generating a disclosed viral vector. In an aspect, generating a disclosed viral vector can comprise generating an AAV vector or a recombinant AAV (such as those disclosed herein). In an aspect, a disclosed method can further comprise gene editing one or more relevant genes (such as, for example, a missing, deficient, and/or mutant protein or enzyme), wherein editing includes but is not limited to single gene knockout, loss of function screening of multiple genes at one, gene knockin, or a combination thereof.

[0206] In an aspect, a disclosed method can further reprogram NK cell antitumor activity. In an aspect, a disclosed method can further reducing T cell exhaustion, extending T cell effector function or improving T cell memory formation.

[0207] In an aspect, a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof can be used for the delivery of a CRISPR-Cas system.

[0208] Disclosed herein are methods of manipulating immune cells using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0209] Disclosed herein are methods of delivering CRISPR to immune cells to generate CAR sequences using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0210] Disclosed herein are methods of genetically reprogramming immune cells to reduce T cell exhaustion using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0211] Disclosed herein are methods of enhancing antitumor activity of immune cells using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0212] Disclosed herein is a preclinical model of engineering cell therapies in immunocompetent hosts using one or more of a disclosed AAV vector, a disclosed AAV

particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0213] Disclosed herein is a preclinical model of T cell function in an autoimmune disease using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0214] Disclosed herein is a method of performing homology directed repair in cells using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0215] Disclosed herein is a method of enhancing transduction efficiency in human T cells using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0216] Disclosed herein is a method of precise genome engineering in human T cells using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0217] Disclosed herein is a method of performing nucleofection-free DNA delivery using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof. In some embodiments, AAV delivery of one or more nucleic acids (e.g., one or more gRNAs and/or HDR template nucleic acids) can be combined with polypeptide delivery of Cas9, to result in an electroporation-free knockout (or knock-in if with an HDR template).

[0218] In an aspect, disclosed immune cells can comprise memory/effector T cells, naïve T cells, NK cells, or any combination thereof. In an aspect, a disclosed method can comprise contacting the disclosed immune cells *vitro*, *ex vivo*, or *in vivo*. In an aspect, immune cells can be contacted with a disclosed viral vector comprising an AAV capsid protein comprising the sequence set forth in SEQ ID NO:1, wherein amino acids 454-460 of the capsid protein are substituted with the sequence set forth in any one of SEQ ID NO:6 – SEQ ID NO:94. In an aspect, immune cells can be contacted with one or more a disclosed AAV vector, a

disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof. In an aspect, a disclosed method can reduce tumor size in a subject and/or improve survival of a subject. In an aspect, a disclosed method can be used to screen one or more libraries of genes in human T cells.

[0219] Disclosed herein is a nucleotide sequence encoding an adeno-associated virus (AAV) capsid protein variant, wherein the encoded AAV capsid protein variant has at least 90% identity to the sequence of SEQ ID NO:1, wherein the amino acids corresponding to amino acids 454-460 of SEQ ID NO:1 are substituted with a peptide having a sequence of any one of SEQ ID NO:6 – SEQ ID NO:94. Disclosed herein is a recombinant AAV capsid protein variant, wherein the capsid protein variant comprises a peptide having the sequence of any one SEQ ID NO:6 – SEQ ID NO:94. Disclosed herein is an AAV capsid protein variant, wherein the AAV capsid protein variant comprises the sequence of SEQ ID NO:2 or a sequence with at least 90% or at least 95% identity thereto. Disclosed herein is a recombinant AAV vector comprising a disclosed AAV capsid protein variant, wherein the AAV vector comprises a vector genome. In an aspect, a disclosed vector genome is encapsidated by an AAV capsid comprising a disclosed AAV capsid protein variant. In an aspect, a disclosed vector genome comprises a first inverted terminal repeat (ITR) and a second ITR. In an aspect, a disclosed vector genome comprises a heterologous polynucleotide (e.g., a transgene or donor polynucleotide) located between the first ITR and the second ITR. In an aspect, a disclosed transgene encodes a therapeutic RNA. In an aspect, a disclosed transgene encodes a therapeutic protein. In an aspect, a disclosed transgene encodes a gene-editing molecule. In an aspect, a disclosed gene-editing molecule is a nuclease or a donor polynucleotide. In an aspect, a disclosed nuclease is a Cas9 nuclease. In an aspect, a disclosed gene-editing molecule is a single guide RNA (sgRNA). Disclosed herein is an AAV capsid protein variant comprising a peptide having the sequence of any one of SEQ ID NO:6 – SEQ ID NO:94. Disclosed herein is an AAV capsid protein variant comprising an amino acid sequence having at least 90% identity to the sequence of SEQ ID NO:1, wherein the amino acids corresponding to amino acids 454-460 of SEQ ID NO:1 are substituted with a peptide having a sequence of any one of SEQ ID NO:6 – SEQ ID NO:94. Disclosed herein is an AAV capsid comprising a disclosed AAV capsid protein variant. In an aspect, a disclosed AAV capsid comprises about 60 copies of the AAV capsid protein variant, or fragments thereof. In an aspect, a disclosed AAV capsid comprises one or more copies of the AAV capsid protein variant and wherein the AAV capsid protein variants are arranged

with T=1 icosahedral symmetry. Disclosed herein is a recombinant AAV vector comprising a disclosed AAV capsid protein variant or a disclosed AAV capsid. Disclosed herein is a pharmaceutical composition comprising a disclosed recombinant AAV vector or a disclosed pharmaceutical composition. Disclosed herein is a method of introducing a recombinant AAV vector into a target cell, the method comprising contacting the target cell with a disclosed recombinant AAV vector or a disclosed pharmaceutical composition. A method of delivering a heterologous polynucleotide (e.g., a transgene or donor polynucleotide) to a target cell in a subject, the method comprising administering to the subject a disclosed recombinant AAV vector or a disclosed pharmaceutical composition. In an aspect, the target cell is an immune cell. In an aspect, a disclosed immune cell comprises a T cell, a NK cell, or a combination thereof. In an aspect, contacting of the cell is performed *in vitro*, *ex vivo*, or *in vivo*. Disclosed herein is a method of treating a subject in need thereof, comprising administering to the subject an effective amount of a disclosed recombinant AAV vector or a disclosed pharmaceutical composition. Disclosed herein is a method of treating a subject in need thereof, comprising administering to the subject a cell that has been contacted *ex vivo* with a disclosed recombinant AAV vector or a disclosed pharmaceutical composition. In an aspect, the subject comprises a mammal. In an aspect, the subject is a human or a mouse.

[0220] Disclosed herein is a kit comprising one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof. Disclosed herein is a kit comprising cells generated by using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof. Disclosed herein is a kit comprising CAR T cells generated by using one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0221] In an aspect, a disclosed kit can be used to prepare one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0222] Disclosed herein is a kit comprising a pharmaceutical formulation comprising one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a

disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0223] In an aspect, a disclosed kit can comprise at least two components constituting the kit. Together, the components constitute a functional unit for a given purpose (such as, for example, treating a subject in need thereof). Individual member components may be physically packaged together or separately. For example, a kit comprising an instruction for using the kit may or may not physically include the instruction with other individual member components. Instead, the instruction can be supplied as a separate member component, either in a paper form or an electronic form which may be supplied on computer readable memory device or downloaded from an internet website, or as recorded presentation. In an aspect, a kit for use in a disclosed method can comprise (i) one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof, and (ii) a label or package insert with instructions for use. In an aspect, suitable containers include, for example, bottles, vials, syringes, blister pack, etc. The containers can be formed from a variety of materials such as glass or plastic. The container can hold comprising one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof a disclosed pharmaceutical formulation, or any combination thereof, and can have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert can indicate one or more a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof can be used for delivering gene therapy, for delivering CAR gene therapy, for delivering CRISPR to engineer long CAR sequences, for genetically reprogramming T cells to, for example, reduce exhaustion and/or enhance NK cell antitumor activity. A disclosed kit can comprise additional components necessary for administration such as, for example, other buffers, diluents, filters, needles, and syringes.

[0224] As stated above, a disclosed kit can comprise instructions relating to the use, dosage, dosing schedule, and/or route of administration of one or more of a disclosed AAV vector, a disclosed AAV particle, a disclosed AAV genome, a disclosed AAV viral capsid, a disclosed AAV viral capsid protein, or any combination thereof.

[0225] In an aspect, a disclosed kit can provide additional components such as buffers and other interpretive information. In an aspect, the disclosure can provide articles of manufacture comprising contents of the kits described above.

EXAMPLE

[0226] Genetic manipulation of human T lymphocytes has proven useful in a wide range of applications including the generation of CAR-T cells for cancer immunotherapy, regulatory T cell therapies and anti-HIV therapies amongst others. In this regard, the use of viral vectors such as adeno-associated virus (AAV) has proven useful in enabling gene editing through homology directed repair within the human T cell genome. Currently, this approach relies on a natural AAV serotype 6, which can deliver transgenes to human T cells at moderate efficiency. Furthermore, AAV6 is known to infect hematopoietic stem cells, NK cells and B cells. Also, most humans carry AAV6 neutralizing antibodies. The lack of specificity toward human T cell and sensitivity neutralizing antibodies limit the use of AAV6 for in vivo gene delivery. We have applied a previously developed structure-guided evolution approach (see, e.g., PCT WO2023/004407) to engineer new T cell tropic AAV6 variants which may be used for modulating T cells ex vivo and possibly in vivo. This approach combines cryo-reconstruction data with capsid engineering to modify capsid receptor binding domains, and we evolve new, antigenically distinct capsids with sequences that do not as yet exist in nature or any AAV variants. The new AAV variants help address the challenges of AAV6 transduction efficiency, tropism, specificity for T-cells and neutralizing antibodies. AAV capsid libraries were generated by saturation mutagenesis of different residues within the antigenic AAV6 capsid variable region 4 (VR4) to generate the AAV640 library. This library was then used to infect human primary T-cells cultivated in with or without human serum, using two different human donors. The library was then amplified following infection using PCR and cloned back into the wtAAV6 backbone to generate the second-round library. We then cycled this back on human primary T-cells for 3 more cycles using two new donors each time to avoid any donor specific evolution. After the last round the viral library was analyzed through high throughput sequencing and revealed novel AAV6 variant capsids with high transduction efficiency in human primary T-cells.

RESULTS

AAV6 evolution on human primary T cells to identify AAV variant with improved T cells targeting

[0227] To identify an AAV variant enabling efficient DNA delivery to human T cells, we generated an AAV capsid library based on AAV6. This serotype was chosen as a template for mutagenesis and evolution due to its established ability to transduce and facilitate HDRT knockin in human T lymphocytes, NK cells, and hematopoietic stem cells. We performed saturation mutagenesis on a pseudotyped AAV2/6 wild-type genome composed of the AAV2 Rep gene and AAV6 Cap gene flanked by AAV2 inverted terminal repeats (ITRs). Saturation mutagenesis was performed on variable region IV (VR-IV) (amino acids 454–460) of the VP3 capsid protein subunit. This surface epitope has been implicated in host cell entry and antibody-mediated neutralization of different AAV serotypes. We have previously shown that targeting this region in other AAV serotypes for structure-guided evolution can yield improved variants.

[0228] Primary T cells isolated from PBMCs of healthy human donor were activated with CD3/CD28 beads, recombinant IL-7/IL-15 and human serum and then co-cultured with the capsid library at a low multiplicity of infection (MOI) of 1×10^4 (**Figure 1A**). To enrich for mutants that had undergone cellular uptake, T cells were washed post-infection to remove residual surface-bound virus. The viral DNA that remained was purified, PCR amplified, and re-cloned into the wild-type AAV plasmid backbone to generate a capsid library for a subsequent round of evolution (**Figure 1A**). After three infection cycles, the parental and evolved libraries were analyzed by next-generation sequencing (NGS) (**Figure 1B**). We identified a number of variants with significant enrichment in the evolved library compared to the parental library, indicating multiple new AAV variants with potentially improved T cells targeting and serum resistance (Figure 1B). Interestingly, many of the top enriched variants contained a stretch of three amino acids, VPR (**Figure 1B**).

Evolved AAV variants improves T cells transduction in serum conditions

[0229] Next, we sought to determine the transduction profile on primary human T cells of the highly evolved variants from the screen. A self-complementary AAV construct was used to transiently express a GFP transcript under a CBh promoter. This construct was packaged in to the parental AAV6 and three highly enriched variants names Ark315 and Ark316. Activated primary human T cells were then transduced with each AAV serotype at an MOI of 1×10^2, 1×10^3, 1×10^4, 1×10^5 or treated with PBS. The cells were cultures in fetal bovine serum and analyzed by fluorescent microscopy three days post infection. All three evolved serotypes significantly improved T cell transduction and GFP expression at all MOI, with Ark315 showing the highest transduction efficiencies (**Figure 2**). We then transduced naïve

human T cells with the same panel of GFP-expressing AAVs at a MOI of 1×10^5 followed by two days of co-cultured of T cells. Genomic DNA was extracted from the cells and analyzed by qPCR to quantify the vector copy number in per cell (**Figure 3**). We observed a 9.6-fold increase in viral uptake by the naïve T cells of Ark315 compared to AAV6 (**Figure 3**). Increases were also observed with Ark316 (**Figure 3**). While these AAV variants showed significantly improved transduction profiles of primary human T cells, we hypothesized that some of the improved was potentially due to resistance to serum. We performed the initial evolution in human serum and hypothesized that the enrichment was due to selective pressure of neutralizing antibodies in serum. AAV6 variants shown in Table 4 preferentially infected human T-cells.

AAV6 evolution on human primary T cells in serum free conditions

[0230] We repeated the AAV6 evolution on activated human T cells (Figure 1A) in serum-free conditions at a MOI of 1×10^4. After three infection cycles, the parental and evolved libraries were analyzed by NGS (**Figure 4A**). We identified a new list of evolved AAV variants, the top list included many AAV variants identified in the previous evolution, but also contained many novel variants such as Ark397 and Ark325 (**Figure 4A**).

[0231] We then transduced activated primary human T cells with scCBh-GFP packaged in to AAV6, Ark325 and Ark397 in both media with or without serum at a range of MOI (**Figure 4B, C**). All serotypes perform equally well in serum-free conditions (**Figure 4 C**). Interestingly, Ark325 and Ark397 out-perform AAV6 in serum conditions (**Figure 4B**). These results display the differences in cell culture condition during AAV evolutions and potential differences in transduction mechanisms and profiles of the evolved AAV serotypes compared to AAV6. AAV6 variants shown in Table 5 preferentially infected human T-cells.

AAV transduction profile of human T cells in different types of serum products

[0232] To further understand the transduction profile of Ark315 and AAV6 in serum conditions, we transduced activated human T cell with scCBh-GFP packaged in to AAV6 or Ark315 at a range of MOI in either serum-free media or in media supplemented with human serum or fetal bovine serum (**Figure 5**). Interestingly, AAV6 outperformed Ark315 in serum-free conditions while Ark315 performed markedly better than AAV6 in all tested serum conditions (**Figure 5**), the serum-effects were most pronounced with fetal bovine serum (**Figure 5**). Next, we compared AAV6 to Ark315 in their ability to deliver a DNA template for knockin by homologous directed repair. We packaged an AAV construct carrying a GFP

gene flanked by homology arms to fuse it to the endogenous CLTA (Figure 6A). Activated human T cells were electroporated with Cas9 and a sgRNA targeting *CLTA*, followed by AAV transduction at a range of MOI in media supplemented with human serum. GFP expression and knockin was determined by flow cytometry 72-hours post transduction (**Figure 6B**). Ark315 yielded the highest knockin efficiencies in serum-supplemented conditions while AAV6.

[0233] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a polynucleotide encoding an adeno-associated virus (AAV) capsid protein variant, wherein the encoded AAV capsid protein variant comprises the sequence of SEQ ID NO:1, except wherein amino acids 454-460 of the capsid protein variant are selected from the group of SEQ ID NOS: 6-94.

2. The nucleic acid of claim 1, wherein the capsid protein variant comprises a sequence selected from SEQ ID NO:2, 4, or 95-102.

3. An AAV capsid protein variant comprising a sequence having at least 90% or 95% identity to the sequence set forth in SEQ ID NO:1, wherein amino acids of the capsid protein variant corresponding to 454-460 of SEQ ID NO:1 comprise the sequence set forth in any one of SEQ ID NOS: 6-94.

4. The AAV capsid protein variant of claim 3, comprising a sequence selected from SEQ ID NO:2, 4, or 95-102.

5. A recombinant AAV capsid comprising about 60 copies of the AAV capsid protein variant of any one of claims 4-5.

6. A recombinant AAV (rAAV) vector comprising: a vector genome, wherein the vector genome is encapsidated by an AAV capsid comprising the AAV capsid protein variant of any one of claims 4-5.

7. The rAAV vector of claim 6, wherein the vector genome comprises a first inverted terminal repeat (ITR) and a second ITR.

8. The rAAV vector of claim 7, wherein the vector genome comprises a heterologous polynucleotide located between the first ITR and the second ITR.

9. The rAAV vector of claim 8, wherein the heterologous polynucleotide comprises a transgene encoding a therapeutic RNA or a therapeutic protein.

10. The rAAV vector of claim 8, wherein the heterologous polynucleotide comprises a donor polynucleotide for genetic editing.

11. The rAAV vector of claim 8, wherein the transgene encodes a gene-editing molecule.

12. The rAAV vector of claim 11, wherein the gene-editing molecule comprises a nuclease, epigenome-editing enzyme or a base-pair editing enzyme.

13. The rAAV vector of claim 12, wherein the nuclease comprises a Cas9 nuclease.

14. The rAAV vector of claim 17, wherein the gene-editing molecule comprises one or more single guide RNA (sgRNA).

15. The rAAV vector of claim 14, wherein the single guide RNA (sgRNA) targets a gene in a T cell or NK cell.

16. A pharmaceutical composition comprising the rAAV vector of any one of claims 6-15 and at least one pharmaceutically acceptable carrier.

17. A method of delivering a heterologous polynucleotide to a human target cell , the method comprising contacting the rAAV vector of any one of claims 10-15 to the human cell, wherein the rAAV vector delivers the heterologous polynucleotide to the cell.

18. A method of delivering a transgene to a target cell in a human subject, the method comprising:

administering to the subject a therapeutically effective amount of the rAAV vector of any one of claims 6-15, or the pharmaceutical composition of claim 16.

19. The method of claim 18, wherein the target cell is an immune cell.

20. The method of claim 19, wherein the immune cell comprises a T cell, a NK cell, or a combination thereof.

21. A method of alleviating and/or treating a disease or a condition in a subject in need thereof, the method comprising: administering to the subject a therapeutically effective amount of the rAAV vector of any one of claims 6-15, or the pharmaceutical composition of claim 16.

22. A method of alleviating and/or treating a disease or a condition in a human subject in need thereof, the method comprising: administering to the subject one or more cells that have been contacted ex vivo with the rAAV vector of any one of claims 6-15, or the pharmaceutical composition of claim 16.

23. The method of claim 21 or 22, wherein the disease or condition comprises an autoimmune disease or an immune deficiency disease.

24. The method of any one of claims 21-23, wherein following the administering of the rAAV or the pharmaceutical composition, one or more aspects of T cell and/or NK cell cellular homeostasis and/or T cell and/or NK cell cellular functionality in the subject is improved and/or restored.

25. The method of any one of claims 21-24, further comprising repeating one or more times the administering step.

26. The method of any one of claims 21-25, further comprising monitoring the subject for adverse effects.

27. The method of claim 26, wherein in the absence of adverse effects, the method further comprises continuing to treat the subject.