TREATMENT OF DANON DISEASE

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to United States Provisional Patent Application No. 63/122,249, filed December 7, 2020 and titled "Treatment of Danon Disease," which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled "ROPA_023_01WO_SeqList_ST25.txt" created on December 7, 2021 and having a size of ~62 kilobytes. The sequence listing contained in this .txt file is part of the specification and is incorporated herein by reference in its entirety.

FIELD OF INVENTION

The invention relates generally to clinical use of an adeno-associated virus (AAV) gene therapy in Danon Disease.

BACKGROUND

Lysosome-associated membrane protein 2 (LAMP-2, also known as CD107b) is a gene that encodes a lysosome-associated membrane glycoprotein. Alternative splicing of the gene produces three isoforms: LAMP-2A, LAMP-2B, and LAMP-2C. Loss-of-function mutations in LAMP-2 are associated with human diseases, including Danon disease, a familial cardiomyopathy associated with impaired autophagy.

International Patent Application Publication No. WO2017127565A1 discloses that overexpression of LAMP-2 in human induced pluripotent stem cells (hiPSCs) derived from patients with LAMP-2 mutations, as described in Hashem, et al., *Stem Cells*. 2015 Jul;33(7):2343-50, results in reduced oxidative stress levels and apoptotic cell death, confirming the importance of LAMP-2B in disease pathophysiology.

There remains a need in the art for methods and compositions related to treatment of Danon disease in human subjects. The present disclosure provides such methods of compositions.

SUMMARY OF THE INVENTION

In an embodiment, the disclosure provides a method for treating Danon disease in a subject identified as suffering from or at risk for Danon disease and/or having an inactivating mutation in one or more isoforms of the *LAMP-2* gene, comprising administering to the subject a therapeutically effective amount of a recombinant adeno-associated virus (rAAV) virion comprising a capsid and a vector genome where the vector genome comprises a polynucleotide sequence encoding a LAMP-2 protein, preferably a LAMP-2B protein.

Disclosed herein is the use of a recombinant adeno-associated virus (rAAV) virion comprising a capsid and a vector genome in the manufacture of a medicament for treating Danon disease in a subject identified as suffering from or at risk for Danon disease and/or having an inactivating mutation in one or more isoforms of the LAMP-2 gene, wherein the rAAV virion is to be administered at a therapeutically effective amount of less than about 5 × 1014 vector genomes (vg) per kilogram (kg) of the subject's body weight, wherein the vector genome comprises a polynucleotide sequence encoding a LAMP-2 protein, preferably a LAMP-2B protein.

In some embodiments, the therapeutically effective amount is less than about 2×10^{14} vector genomes (vg) per kilogram (kg) of the subject's body weight.

In some embodiments, the therapeutically effective amount is less than about 1.5×10^{14} vg/kg of the subject's body weight.

In some embodiments, the therapeutically effective amount is less than about 1×10^{14} vg/kg of the subject's body weight.

In some embodiments, the therapeutically effective amount is at least about 1×10^{12} vg/kg of the subject's body weight.

In some embodiments, the therapeutically effective amount is at least about 1×10^{13} vg/kg of the subject's body weight.

In some embodiments, the therapeutically effective amount is about 6.7×10^{13} vg/kg of the subject's body weight.

In some embodiments, the therapeutically effective amount is about 1.1×10^{14} vg/kg of the subject's body weight.

In some embodiments, the therapeutically effective amount is about 2.0×10^{14} vg/kg of the subject's body weight.

In some embodiments, the method further comprises administering to the subject an effective amount of tacrolimus.

In some embodiments, the method further comprises administering to the subject an effective amount of rituximab.

In some embodiments, the method comprises administering to the subject an effective amount of tacrolimus and administering to the subject an effective amount of rituximab.

In some embodiments, the method comprises administering to the subject an effective amount of eculizumab.

In some embodiments, the method further comprises administering to the subject an effective amount of rituximab; administering to the subject an effective amount of tacrolimus; and/or administering to the subject an effective amount of eculizumab.

In some embodiments, the subject is at risk for sequelae of complement activation, such as atypical hemolytic-uremic syndrome (aHUS), optionally aHUS resulting in reversible thrombocytopenia and/or acute kidney injury (AKI).

In some embodiments, the method further comprises administering to the subject an effective amount of corticosteroids.

In some embodiments, the method further comprises administering to the subject an effective amount of corticosteroids prior to administering the effective amount of tacrolimus.

In some embodiments, the subject is a juvenile subject, optionally having an age of 8-14 years old and/or 15-17 years old.

In some embodiments, the subject is a pediatric subject, optionally having an age of 0-8 years old.

In some embodiments, the subject is an adult subject, optionally having an age of 18 years old or older.

In some embodiments, the therapeutically effective amount of the AAV is administered intravenously.

In some embodiments, the therapeutically effective amount of the AAV is administered by direct cardiac injection, optionally by intrajugular or Swan-Ganz catheter

In some embodiments, the therapeutically effective amount of the AAV is administered by intraperitoneal injection.

In some embodiments, the method results in one or more of: a) transduction of cardiomyocyte and/or skeletal muscle by the AAV; b) expression of exogenous ribonucleic acid polynucleotide encoding LAMP-2B and/or expression of exogenous LAMP-2B protein, optionally in cardiomyocyte and/or skeletal muscle; c) correction or improvement of one or more Danon disease-associated histologic abnormalities, optionally autophagic vacuoles or myofibrillar disarray, optionally determined by histology of sampled endomyocardial biopsy; d) correction or improvement of cardiomyocyte molecular marker expression; and/or e) correction or improvement of cardiomyocyte histology.

In some embodiments, the AAV comprises an expression cassette comprising the polynucleotide sequence encoding the LAMP-2B protein operatively linked to a promoter, and wherein the polynucleotide sequence shares at least 95% identity to SEQ ID NO: 2 and/or the LAMP-2B protein shares at least 95% identity to SEQ ID NO: 1.

In some embodiments, the polynucleotide sequence comprises or consists of SEQ ID NO: 2 and/or the LAMP-2B protein comprises or consists of SEQ ID NO: 1.

In some embodiments, the promoter is a CAG promoter.

In some embodiments, the promoter comprises an enhancer/promoter region that shares at least 95% identity to SEQ ID NO: 22.

In some embodiments, the enhancer/promoter region comprises or consists of SEQ ID NO: 22.

In some embodiments, the expression cassette comprises, in 5′ to 3′ order:

(a) an enhancer/promoter region that comprises SEQ ID NO: 22;

(b) the polynucleotide sequence that encodes the LAMP-2B protein, wherein the polynucleotide sequence comprises SEQ ID NO: 3;

(c) a 3′ UTR sequence comprising SEQ ID NO: 27; and/or

(d) a poly-adenylation sequence comprising SEQ ID NO: 7.

In some embodiments, the expression cassette is flanked by: (i) a 5′ ITR that comprises SEQ ID NO: 11; and (ii) a 3′ ITR that comprises SEQ ID NO: 12.

In some embodiments, the expression cassette comprises SEQ ID NO: 8.

In some embodiments, the capsid is an AAV9 capsid.

In some embodiments, the AAV9 capsid comprises one or more capsid proteins that comprise amino acids 1 to 736 of SEQ ID NO: 28, amino acids 138 to 736 of SEQ ID NO: 28, or amino acids 203 to 736 of SEQ ID NO: 28.

In other embodiments, the disclosure provides a unit dose, pharmaceutical composition, or composition for use in treating Danon disease. The unit dose, pharmaceutical composition, or composition for use comprises a therapeutically effective amount of a recombinant adeno-associated virus (rAAV) virion comprising a capsid and a vector genome where the vector genome comprises a polynucleotide sequence encoding a LAMP-2 protein, preferably a LAMP-2B protein.

Disclosed herein is a unit dose, pharmaceutical composition, or composition for use in treating Danon disease, comprising a therapeutically effective amount of an adeno-associated virus (AAV) comprising a polynucleotide sequence encoding a LAMP-2 protein, preferably a LAMP-2B protein, wherein the therapeutically effective amount is less than about 5 × 1014 vector genomes (vg) per kilogram (kg) of the subject's body weight.

In some embodiments, the therapeutically effective amount is less than about 2×10^{14} vector genomes (vg) per kilogram (kg) of the subject's body weight.

In some embodiments, the therapeutically effective amount is less than about 1.5×10^{14} vg/kg.

In another embodiment, the disclosure provides a kit comprising the unit dose, pharmaceutical composition, or composition for use of the disclosure; and instructions for use in treating Danon disease.

Disclosed herein is a kit comprising the unit dose, pharmaceutical composition, or composition for use as defined herein; and instructions for use in treating Danon disease, optionally wherein the kit further comprises one or more unit dose, pharmaceutical composition, or composition comprising one or more of: rituximab; tacrolimus; eculizumab; and a corticosteroid.

The kit may further comprising one or more unit dose, pharmaceutical composition, or composition comprising one or more of: rituximab; tacrolimus; eculizumab; and a corticosteroid.

Further aspects and embodiments of the invention are disclosed in the Detailed Description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a diagram of the pAAV-LAMP2B transfer plasmid used to generate the adeno-associated virus (AAV) particle in the present disclosure. The AAV particle contains an expression cassette flanked by inverted terminal repeat (ITR) elements derived from AAV2, a CAG promoter comprising of cytomegalovirus immediate early (CMV IE) enhancer, chicken beta-actin (CBA) promoter, chicken beta-actin and rabbit globin introns (CBA/RbG Intron), a LAMP2B transgene, a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), and a rabbit globin poly-adenylation signal (RGpA).

FIGs. 2A-2B depict graphs of cardiac tissue analyses in WT or LAMP2 KO mice injected with PBS or varying doses of AAV9.LAMP2B (1×10^{13}, 5×10^{13}, and 1×10^{14} vg/kg). FIG. 2A shows vector copy number per nucleus (VCN/Nuclei) determined using qPCR. FIG. 2B shows quantitative RT-PCR of LAMP2B mRNA levels (fold change over PBS,

normalized by GAPDH). Primers detect both human and mouse LAMP2B mRNA. Values are Mean ±SEM. ## p<0.01, ### p<0.0001 vs KO mice injected with PBS.

FIGs. 3A-3C depict graphs of human LAMP2 and LC3-II Protein Expression in heart tissue. FIG. 3A shows protein lysates from hearts of Lamp2 KO mice injected with PBS or increasing doses of AAV9.LAMP2B (1×10^{13}, 5×10^{13}, and 1×10^{14} vg/kg), and naïve control WT mice evaluated by western blot for mouse (mLAMP2) and human LAMP2 (hLAMP2), LC3-II (marker for autophagy), GAPDH used as a loading control. FIG. 3B shows the quantification of hLAMP2 protein (densitometry using ImageJ) normalized to GAPDH. FIG. 3C shows the quantification of LC3-II (densitometry using ImageJ) normalized to GAPDH. Values are Mean ±SEM. **p<0.01vs WT; #p<0.05, ##p<0.01 vs KO mice injected with PBS.

FIG. 4 depicts transmission electron microscopy images of cardiac tissues showing vacuoles (autophagy structures as denoted by yellow arrows). Representative images from WT type or Lamp2 KO mice injected with PBS, or AAV9.LAMP2B at 5×10^{13} or 1×10^{14} vg/kg. Scale bars are 2 µm in upper panels and 0.54 µm in lower panels.

FIG. 5 depicts a graph of Vector DNA copies per diploid genome in patients treated with 6.7×10^{13} GC/kg AAV9.LAMP2B. Patients 1001, 1002 and 1005 were analyzed at Baseline, Week 8, Month 6, and Month 12 following treatment.

FIG. 6 depicts immunohistochemistry images of cardiac tissues from patient 1002 showing cardiac LAMP2B expression. Representative images from Pre-dose, Positive Control, or patient treated with 6.7×10^{13} GC/kg AAV9.LAMP2B at Week 8, Month 6, and Month 12 following treatment. Scale bars are 100 µm.

FIGs. 7A-7C depict graphs of the fold change of Brain natriuretic peptide (BNP) over baseline for patient 1001 (FIG. 7A), patient 1002 (FIG. 7B), and patient 1005 (FIG. 7C). Patients were treated with 6.7×10^{13} GC/kg AAV9.LAMP2B at BNP fold change was analyzed at Week 8, Month 6, and Month 12 following treatment.

FIG. 8 depicts a diagram of the enrollment sequence in study cohorts, in a scenario in which no DLT is identified.

FIG. 9 depicts a diagram of the enrollment sequence in any cohort in the setting of a single DLT within a given cohort. Enrollment within a cohort will be halted if a second

patient experiences DLT. The triangle/exclamation point indicates the DLT occurrence for patient 2.

FIGs. 10A-10D are charts depicting the AAV9.LAMP2B Study Assessment and Therapy Table of Events. Abbreviations: AAV9: adeno-associated virus serotype 9; ADA: anti-drug antibody; aPTT: activated partial thromboplastin time; BNP: brain natriuretic peptide; C3: complement 3; C4: complement 4; CBC: complete blood count; CK-MB: creatinine kinase-MB; D: day; d/c: discontinue; ECG: electrocardiogram; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; IgG: immunoglobulin G; IgM: immunoglobulin M; IP: Investigational Product; IV: intravenous infusion; LFT: liver function tests; M: Month; MRI: magnetic resonance imaging; PMBC: peripheral blood mononuclear cell; PRO: patient-reported outcome; PT: prothrombin time; QOL: quality-of-life; sC5b-9: serum membrane attack complex; TAT: thrombin-antithrombin complex; VO2: maximal oxygen consumption; W: week.

FIGs. 11A-11B depict graphs of cardiac contractility (FIG. 11A) and cardiac relaxation (FIG. 11B) analyzed by invasive left intraventricular pressure (dP/dt max and dP/dt min, respectively) in Lamp2 KO mice injected with PBS or increasing doses of AAV9.LAMP2B (1×10^{13}, 5×10^{13}, and 1×10^{14} vg/kg) or naïve control WT mice.

FIG. 12 depicts transmission electron microscopy images of cardiac tissues showing vacuoles (autophagy structures as denoted by yellow arrows). Representative images from WT type or Lamp2 KO mice injected with PBS, or AAV9.LAMP2B at 5×10^{13}, 1×10^{14} vg/kg or 2×10^{14} vg/kg. Scale bars are 2 μm in upper panels and 0.6 μm in lower panels.

FIGs. 13A-13B depict graphs of cardiac contractility (FIG. 13A) and cardiac relaxation (FIG. 13B) analyzed by invasive left hemodynamics (dP/dt max and dP/dt min, respectively) in Lamp2 KO mice injected with PBS or increasing doses of AAV9.LAMP2B (1×10^{13}, 5×10^{13}, 1×10^{14}, and 2×10^{14} vg/kg) or naïve control WT mice.

FIGs. 14A-14B depict LAMP2 protein expression by immunohistochemistry (FIG. 14A) and cell morphology by electron microscopy (FIG. 14B) after treatment with RP-A501 at low dose. Representative images from patient 1005 from biopsy of intra-ventricular septum are shown.

FIG. 15A depicts remodeling of ventricular hypertrophy on echocardiography with reduction or stabilization of wall thickness in both low and high dose patients. All echocardiographic parameters are from local laboratory assessment, conducted by a single reader.

FIG. 15B depicts stabilization or improvement of left ventricular (LV) ejection fraction (EF) and wall thickness in both low and high dose patients. All echocardiographic parameters are from local laboratory assessment, conducted by a single reader.

FIG. 15C depicts invasive hemodynamics demonstrated long term stabilization or improvement of diastolic dysfunction (LV filling pressure) as measured by pulmonary capillary wedge pressure in the low and high dose patients.

FIG. 15D shows hemodynamic stabilization or improvement of systolic function in both high and low dose patients.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides methods and compositions that relate to treating Danon disease in human subjects. The present inventors have demonstrated successful treatment of Danon disease in human subjects with an adeno-associated virus (AAV) designed to expression the LAMP-2B isoform of LAMP-2. The AAV may be administered in conjunction with treatment with corticosteroids, tacrolimus, rituximab, and/or eculizumab; and the AAV may be administered at various doses. As disclosed herein, doses in a range of approximately 6.7×10^{13} vg/kg, or lower, to approximately 2.0×10^{14} vg/kg, or higher, may be safe and effective in Danon disease subjects.

Vector Sequences

The wild-type polypeptide sequence of human LAMP-2B (SEQ ID NO: 1) and the wild-type polynucleotide sequence encoding human LAMP-2B (SEQ ID NO: 2) are, respectively:

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  1 MVCFRLFPVP GSGLVLVCLV LGAVRSYALE LNLTDSENAT CLYAKWQMNF TVRYETTNKT
 61 YKTVTISDHG TVTYNGSICG DDQNGPKIAV QFGPGFSWIA NFTKAASTYS IDSVSFSYNT
121 GDNTTFPDAE DKGILTVDEL LAIRIPLNDL FRCNSLSTLE KNDVVQHYWD VLVQAFVQNG
181 TVSTNEFLCD KDKTSTVAPT IHTTVPSPTT TPTPKEKPEA GTYSVNNGND TCLLATMGLQ
241 LNITQDKVAS VININPNTTH STGSCRSHTA LLRLNSSTIK YLDFVFAVKN ENRFYLKEVN
301 ISMYLVNGSV FSIANNNLSY WDAPLGSSYM CNKEQTVSVS GAFQINTFDL RVQPFNVTQG
361 KYSTAQECSL DDDTILIPII VGAGLSGLII VIVIAYVIGR RKSYAGYQT
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(SEQ ID NO: 1); and

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   1 ATGGTGTGCT TCCGCCTCTT CCCGGTTCCG GGCTCAGGGC TCGTTCTGGT CTGCCTAGTC
  61 CTGGGAGCTG TGCGGTCTTA TGCATTGGAA CTTAATTTGA CAGATTCAGA AAATGCCACT
 121 TGCCTTTATG CAAAATGGCA GATGAATTTC ACAGTTCGCT ATGAAACTAC AAATAAAACT
 181 TATAAAACTG TAACCATTTC AGACCATGGC ACTGTGACAT ATAATGGAAG CATTTGTGGG
 241 GATGATCAGA ATGGTCCCAA AATAGCAGTG CAGTTCGGAC CTGGCTTTTC CTGGATTGCG
 301 AATTTTACCA AGGCAGCATC TACTTATTCA ATTGACAGCG TCTCATTTTC CTACAACACT
 361 GGTGATAACA CAACATTTCC TGATGCTGAA GATAAAGGAA TTCTTACTGT TGATGAACTT
 421 TTGGCCATCA GAATTCCATT GAATGACCTT TTTAGATGCA ATAGTTTATC AACTTTGGAA
 481 AAGAATGATG TTGTCCAACA CTACTGGGAT GTTCTTGTAC AAGCTTTTGT CCAAAATGGC
 541 ACAGTGAGCA CAAATGAGTT CCTGTGTGAT AAAGACAAAA CTTCAACAGT GGCACCCACC
 601 ATACACACCA CTGTGCCATC TCCTACTACA ACACCTACTC CAAAGGAAAA ACCAGAAGCT
 661 GGAACCTATT CAGTTAATAA TGGCAATGAT ACTTGTCTGC TGGCTACCAT GGGGCTGCAG
 721 CTGAACATCA CTCAGGATAA GGTTGCTTCA GTTATTAACA TCAACCCCAA TACAACTCAC
 781 TCCACAGGCA GCTGCCGTTC TCACACTGCT CTACTTAGAC TCAATAGCAG CACCATTAAG
 841 TATCTAGACT TTGTCTTTGC TGTGAAAAAT GAAAACCGAT TTTATCTGAA GGAAGTGAAC
 901 ATCAGCATGT ATTTGGTTAA TGGCTCCGTT TTCAGCATTG CAAATAACAA TCTCAGCTAC
 961 TGGGATGCCC CCCTGGGAAG TTCTTATATG TGCAACAAAG AGCAGACTGT TTCAGTGTCT
1021 GGAGCATTTC AGATAAATAC CTTTGATCTA AGGGTTCAGC CTTTCAATGT GACACAAGGA
1081 AAGTATTCTA CAGCCCAAGA GTGTTCGCTG GATGATGACA CCATTCTAAT CCCAATTATA
1141 GTTGGTGCTG GTCTTTCAGG CTTGATTATC GTTATAGTGA TTGCTTACGT AATTGGCAGA
1201 AGAAAAAGTT ATGCTGGATA TCAGACTCTG TAA
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(SEQ ID NO: 2).

Disclosed herein are modified polynucleotide sequences encoding an isoform of lysosome-associated membrane protein 2 (LAMP-2) or a functional variant thereof. In certain embodiments, the modified polynucleotide sequences comprise one or more of the following modifications as compared to the wild-type polynucleotide encoding the isoform of LAMP-2: codon-optimization, CpG depletion, removal of cryptic splice sites, or a reduced number of alternative open-reading frames (ORFs). In some embodiments, the modified polynucleotide encodes LAMP-2A, LAMP-2B, LAMP-2C or a functional variant of any of these isoforms. In embodiments, the disclosure provides a polynucleotide sequence or transgene encoding LAMP-2B or a functional variant thereof comprising one or more nucleotide substitutions as compared to SEQ ID NO:2. In embodiments, the transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to a sequence selected from SEQ ID NOs: 3-5. The disclosure provides at least three illustrative variant transgene sequences encoding LAMP-2B (SEQ ID NOs: 3-5):

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   1 ATGGTCTGCT TCAGACTGTT CCCTGTCCCT GGATCTGGTC TGGTGCTTGT GTGCTTGGTG
  61 CTGGGTGCTG TGAGATCCTA TGCCCTTGAG CTGAACCTGA CTGACTCAGA AAATGCCACT
 121 TGCCTGTATG CCAAGTGGCA GATGAACTTC ACTGTGAGAT ATGAGACTAC CAACAAGACC
 181 TACAAGACTG TGACCATCTC AGACCATGGC ACTGTCACCT ACAATGGATC AATCTGTGGT
 241 GATGATCAGA ATGGCCCAAA GATAGCAGTG CAGTTTGGGC CCGGTTTTTC CTGGATTGCT
 301 AACTTCACCA AGGCAGCCTC CACCTACAGC ATTGACTCAG TCAGCTTCAG CTACAACACT
 361 GGGGATAACA CCACCTTCCC TGACGCAGAG GACAAGGGAA TCCTTACTGT GGACGAACTC
 421 CTGGCAATCA GAATCCCCCT TAACGACCTG TTCAGATGCA ACTCCCTTTC AACCCTTGAA
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 481 AAGAATGATG TGGTGCAACA CTATTGGGAC GTCCTGGTGC AAGCCTTTGT GCAGAATGGG
 541 ACAGTGAGTA CCAACGAGTT CCTCTGTGAC AAGGACAAGA CCAGCACTGT GGCCCCCACT
 601 ATCCACACCA CTGTGCCCAG CCCTACCACT ACCCCCACCC CTAAAGAGAA GCCAGAAGCT
 661 GGAACCTACT CAGTCAACAA TGGAAATGAC ACATGCCTCC TTGCCACCAT GGGACTGCAG
 721 CTGAACATCA CTCAGGACAA GGTGGCCTCA GTGATTAACA TCAACCCTAA CACCACTCAT
 781 AGCACTGGGA GCTGCAGATC ACATACAGCT CTGCTGAGGC TCAACTCCTC CACCATCAAG
 841 TACCTGGACT TTGTGTTTGC TGTGAAGAAT GAGAACAGGT TCTACCTCAA GGAAGTGAAC
 901 ATTTCCATGT ACCTGGTCAA TGGTTCAGTG TTCTCTATTG CCAACAACAA TCTGAGCTAC
 961 TGGGATGCAC CCCTGGGATC CTCCTACATG TGCAACAAGG AGCAGACTGT GAGTGTGTCA
1021 GGTGCTTTTC AGATCAACAC TTTTGACCTG AGGGTGCAGC CCTTCAATGT GACTCAGGGA
1081 AAGTACTCCA CTGCACAAGA GTGTTCCTTG GATGATGACA CTATCCTCAT CCCCATTATT
1141 GTGGGAGCTG GACTGTCAGG ATTGATTATA GTGATTGTGA TTGCTTATGT GATTGGAAGG
1201 AGAAAGAGCT ATGCTGGCTA CCAGACCCTG TAA
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(SEQ ID NO: 3);

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   1 ATGGTGTGCT TTAGACTGTT TCCTGTGCCT GGTTCAGGGC TGGTCCTGGT CTGTCTGGTG
  61 CTGGGGGCTG TCAGAAGCTA TGCCTTGGAG CTGAACCTCA CTGATAGTGA AAATGCCACT
 121 TGTCTGTATG CTAAGTGGCA GATGAACTTC ACTGTGAGAT ATGAAACCAC CAACAAGACT
 181 TACAAAACAG TGACCATCTC AGATCATGGA ACTGTGACCT ACAACGGCAG CATTTGTGGA
 241 GACGACCAGA ACGGACCAAA AATCGCTGTC CAATTTGGGC CTGGATTCTC CTGGATTGCC
 301 AATTTCACTA AAGCTGCCTC CACATATTCA ATTGACTCAG TGTCCTTCTC CTACAACACT
 361 GGGGACAACA CTACTTTCCC TGATGCTGAA GATAAGGGAA TCTTGACAGT GGATGAGCTG
 421 CTGGCTATCA GGATCCCTTT GAATGACCTG TTTAGGTGTA ATTCACTGAG CACTCTGGAG
 481 AAGAACGACG TGGTGCAGCA CTACTGGGAC GTGCTGGTGC AGGCCTTTGT GCAGAACGGC
 541 ACTGTGTCCA CCAACGAATT CCTGTGTGAT AAGGACAAAA CTTCCACTGT GGCACCTACA
 601 ATTCACACTA CTGTGCCTTC ACCTACCACC ACTCCAACTC CAAAGGAAAA GCCTGAAGCA
 661 GGAACCTACT CTGTGAACAA TGGCAATGAT ACCTGTCTGT TGGCCACCAT GGGCCTCCAA
 721 CTGAACATTA CTCAGGACAA GGTGGCCTCA GTGATTAACA TTAACCCCAA CACTACCCAC
 781 TCCACTGGCA GCTGTAGATC ACACACAGCC TTGCTCAGAC TGAATAGCAG CACCATCAAG
 841 TATTTGGATT TTGTGTTTGC AGTGAAGAAT GAAAACAGGT TCTACCTGAA GGAAGTCAAC
 901 ATCTCAATGT ACCTGGTGAA CGGCTCAGTG TTCAGCATTG CCAACAACAA CCTCTCCTAT
 961 TGGGACGCTC CACTGGGGAG CAGCTACATG TGTAACAAGG AACAGACTGT GTCAGTGTCA
1021 GGAGCCTTCC AGATTAACAC CTTTGATCTG AGGGTCCAAC CCTTTAATGT CACTCAAGGA
1081 AAGTATAGCA CTGCCCAGGA GTGCTCCCTG GATGATGACA CCATTCTGAT TCCAATCATT
1141 GTGGGTGCAG GACTTTCTGG GCTTATTATT GTGATTGTGA TTGCCTATGT GATTGGCAGA
1201 AGGAAATCCT ATGCAGGGTA CCAAACTCTG TAA
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(SEQ ID NO: 4); and

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   1 ATGGTCTGTT TTAGGCTGTT CCCTGTCCCT GGTTCAGGAC TGGTCTTAGT GTGTCTGGTG
  61 CTTGGAGCTG TCAGAAGCTA TGCCCTGGAG CTGAACCTGA CTGACTCAGA AAATGCCACT
 121 TGCCTGTATG CCAAGTGGCA GATGAACTTC ACTGTCAGAT ATGAAACCAC CAACAAGACC
 181 TATAAGACTG TGACCATCTC AGACCATGGC ACTGTGACTT ACAATGGGTC AATTTGTGGA
 241 GATGACCAGA ATGGCCCTAA GATAGCTGTC CAGTTTGGTC CAGGATTCAG CTGGATTGCC
 301 AACTTCACCA AGGCAGCCAG CACCTACAGC ATTGACTCTG TGTCCTTCTC CTACAACACA
 361 GGAGACAACA CCACTTTCCC TGATGCAGAG GACAAAGGTA TCCTGACTGT GGATGAGTTG
 421 CTGGCAATCA GGATCCCACT GAACGATCTG TTCAGGTGCA ACTCACTGTC CACTCTGGAA
 481 AAGAATGATG TGGTGCAGCA CTATTGGGAT GTGCTAGTCC AGGCCTTTGT CCAGAATGGG
 541 ACTGTGTCAA CTAATGAGTT CCTGTGTGAC AAGGACAAGA CAAGCACTGT AGCCCCCACT
 601 ATCCATACCA CAGTACCTAG CCCCACCACT ACTCCAACCC CCAAGGAGAA GCCTGAGGCT
 661 GGCACCTACT CAGTGAACAA TGGGAATGAC ACCTGTTTGC TGGCCACTAT GGGACTCCAA
 721 CTGAACATCA CCCAGGACAA AGTGGCCTCT GTGATCAATA TCAATCCCAA CACCACCCAC
 781 AGCACTGGGT CCTGCAGAAG CCACACTGCC CTCCTGAGGC TCAACTCATC AACTATCAAG
 841 TACTTGGATT TTGTGTTTGC AGTGAAGAAT GAGAACAGAT TCTACCTCAA AGAGGTCAAC
 901 ATTTCAATGT ACCTGGTGAA TGGGAGTGTG TTCTCCATTG CTAACAACAA CCTGAGCTAC
 961 TGGGATGCCC CTCTGGGCTC CTCATACATG TGCAACAAGG AACAGACTGT GAGTGTGTCA
1021 GGGGCCTTCC AGATCAACAC TTTTGACCTG AGAGTGCAGC CCTTTAATGT GACACAGGGA
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1081 AAGTACAGCA CTGCTCAGGA GTGCAGCCTG GATGATGACA CTATCCTGAT CCCTATCATT
1141 GTGGGGGCAG GCCTGTCTGG ACTCATTATT GTGATTGTGA TTGCCTATGT GATAGGGAGA
1201 AGGAAGTCTT ATGCTGGATA CCAGACCCTG TAA
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(SEQ ID NO: 5).

In an embodiment, the transgene shares at least 95% identity to a sequence selected from SEQ ID NOs: 2-5. In an embodiment, the transgene shares at least 99% identity to a sequence selected from SEQ ID NOs: 2-5. In an embodiment, the transgene comprises a sequence selected from SEQ ID NOs: 2-5. In an embodiment, the transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to SEQ ID NO: 3. In an embodiment, the transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to SEQ ID NO: 4. In an embodiment, the transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to SEQ ID NO: 5.

In some embodiments, the transgene is similar to or identical to a subsequence of any one of SEQ ID NOs: 2-5. In some embodiments, the transgene comprises a subsequence of any one of SEQ ID NOs: 2-5. In various embodiments, the subsequence may comprise any set of consecutive nucleotides (nt) in the full sequence having a length of at least about 50 nt, at least about 100 nt, at least about 150 nt, at least about 250 nt, at least about 200 nt, at least about 350 nt, at least about 450 nt, at least about 400 nt, at least about 450 nt, at least about 550 nt, at least about 600 nt, at least about 650 nt, at least about 600 nt, at least about 650 nt, at least about 700 nt, at least about 750 nt, at least about 800 nt, at least about 850 nt, at least about 900 nt, at least about 950 nt, or at least abou 1000 nt.

In some embodiments, the transgene shares at least 95% identity to a subsequence that comprises nucleotides 1-500, 250-750, 500-1000, or 750-1240 of any one of SEQ ID NO: 3-5. In an embodiment, the transgene shares at least 99% identity to a subsequence that comprises nucleotides 1-500, 250-750, 500-1000, or 750-1240 of any one of SEQ ID NO: 3-5. In an embodiment, the transgene comprises a sequence that comprises nucleotides 1-500, 250-750, 500-1000, or 750-1240 of any one of SEQ ID NOs: 2-5. In embodiment, the transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to a subsequence that comprises nucleotides 1-500, 250-750, 500-1000, or 750-1240 of any one of SEQ ID NOs: 2-5. In embodiments, the

transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to a subsequence that comprises nucleotides 1-500, 250-750, 500-1000, or 750-1240 of SEQ ID NO: 3. In embodiment, the transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to a subsequence that comprises nucleotides 1-500, 250-750, 500-1000, or 750-1240 of SEQ ID NO: 3.

In certain embodiments, the transgene encodes any of the various isoforms of LAMP-2, including any of LAMP-2A, LAMP-2B, or LAMP-2C, or a functional fragment or variant of any of these isoforms. Thus, in particular embodiments, the expression cassette is an optimized polynucleotide sequence encoding any of LAMP-2A, LAMP-2B, or LAMP-2C, or a functional fragment or variant thereof, which comprises one or more modifications as compared to the corresponding wild-type polynucleotide sequence, including one or more modification selected from: codon-optimization of the transgene sequence encoding LAMP-2A, LAMP-2B, or LAMP-2C; the expression cassette or transgene sequence contains fewer CpG sites than its corresponding wild-type sequence; the expression cassette or transgene sequence contains fewer CpG sites than its corresponding wild-type sequence; the expression cassette or transgene sequence contains fewer cryptic splice sites than its corresponding wild-type sequence; and/or the expression cassette or transgene sequence contains fewer open reading frames than its corresponding wild-type sequence. In particular embodiments, the optimized sequence is optimized for increased expression in human cells. The wild-type human polynucleotide sequences encoding the LAMP-2A and LAMP-2C isoforms are set forth in SEQ ID NOs: 29 and 30, respectively. The wild-type sequences of human LAMP-2A and LAMP-2C proteins are set forth in SEQ ID NOs: 34 and 35, respectively. The sequences of the wild-type LAMP-2 isoforms and coding sequences are also publicly available. While the specification describes specific embodiments with respect to LAMP-2B, it is understood that LAMP-2A or LAMP-2C could alternatively be used in each embodiment.

The coding sequences of wild-type LAMP-2A (SEQ ID NO: 29) and wild-type LAMP-2C (SEQ ID NO: 30) are 100% identical to the coding sequence of wild-type LAMP-2B (SEQ ID NO: 2) across at least nucleotides 1 – 1080. Accordingly, it will be readily recognized by those in the art that that transgenes, expression cassettes, and vectors disclosed herein can be adapted for expression of these isoforms of LAMP-2 by substituting the 3′ end

(nucleotides 1081 – end) of either of LAMP-2A (SEQ ID NO: 29) or wild-type LAMP-2C (SEQ ID NO: 30) in place of nucleotides 1081 – 1233 of LAMP-2B (e.g., an optimized LAMP-2B of any of SEQ ID NO: 3-5). For example, embodiments of the invention utilize nucleotides 1-1080 of the optimized LAMP-2B gene sequences, SEQ ID NOs: 3-5, which are, respectively, SEQ ID NOs: 31-33.

In an embodiment, the transgene shares at least 95% identity to a sequence selected from SEQ ID NOs: 31-33. In an embodiment, the transgene shares at least 99% identity to a sequence selected from SEQ ID NOs: 31-33. In an embodiment, the transgene comprises a sequence selected from SEQ ID NOs: 31-33. In an embodiment, the transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to SEQ ID NO: 31. In an embodiment, the transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to SEQ ID NO: 32. In an embodiment, the transgene shares at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or complete identity to SEQ ID NO: 33. In some cases, the transgene has a polynucleotide sequence that is different from the polynucleotide sequence of a reference sequence, *e.g.*, a "native" or "wild-type" LAMP-2B sequence. In some embodiments, the transgene shares at most 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, or 95% identity with a reference sequence. In some embodiments, the reference sequence is SEQ ID NO: 2. For example, SEQ ID NO: 3 shares 78.5% identity to SEQ ID NO: 2.

In some cases, the transgene has a polynucleotide sequence that is different from the polynucleotide sequence of a reference sequence, *e.g.*, a "native" or "wild-type" LAMP-2A sequence. In some embodiments, the transgene shares at most 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, or 95% identity with a reference sequence. In some embodiments, the reference sequence is the wild-type human LAMP-2A sequence set forth in SEQ ID NO: 29.

In some cases, the transgene has a polynucleotide sequence that is different from the polynucleotide sequence of a reference sequence, *e.g.*, a "native" or "wild-type" LAMP-2C sequence. In some embodiments, the transgene shares at most 70%,

71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, or 95% identity with a reference sequence. In some embodiments, the reference sequence is the wild-type human LAMP-2C sequence set forth in SEQ ID NO: 30.

In an embodiment, the transgene is codon-optimized for expression in a human host cell. In an embodiment, the transgene coding sequence is modified, or "codon optimized" to enhance expression by replacing infrequently represented codons with more frequently represented codons. The coding sequence is the portion of the mRNA sequence that encodes the amino acids for translation. During translation, each of 61 trinucleotide codons are translated to one of 20 amino acids, leading to a degeneracy, or redundancy, in the genetic code. However, different cell types, and different animal species, utilize tRNAs (each bearing an anticodon) coding for the same amino acids at different frequencies. When a gene sequence contains codons that are infrequently represented by the corresponding tRNA, the ribosome translation machinery may slow, impeding efficient translation. Expression can be improved via "codon optimization" for a particular species, where the coding sequence is altered to encode the same protein sequence, but utilizing codons that are highly represented, and/or utilized by highly expressed human proteins (Cid-Arregui et al., 2003; J. Virol. 77: 4928).

In some embodiments, the coding sequence of the transgene is modified to replace codons infrequently expressed in mammal or in primates with codons frequently expressed in primates. For example, in some embodiments, the transgene encodes a polypeptide having at least 85% sequence identity to a reference polypeptide (*e.g.* wild-type LAMP-2B; SEQ ID NO: 3)—for example, at least 90% sequence identity, at least 95% sequence identity, at least 98% identity, or at least 99% identity to the reference polypeptide—wherein at least one codon of the coding sequence has a higher tRNA frequency in humans than the corresponding codon in the sequence disclosed above or herein.

In an embodiment, the transgene comprises fewer alternative open reading frames than SEQ ID: 2. In an embodiment, the transgene is modified to enhance expression by termination or removal of open reading frames (ORFs) that do not encode the desired transgene. An open reading frame (ORF) is the nucleic acid sequence that follows a start

codon and does not contain a stop codon. ORFs may be in the forward or reverse orientation, and may be "in frame" or "out of frame" compared with the gene of interest. Such open reading frames have the potential to be expressed in an expression cassette alongside the gene of interest, and could lead to undesired adverse effects. In some embodiments the transgene has been modified to remove open reading frames by further altering codon usage. This may be done by eliminating one or more start codons (ATG) and/or introducing one or more stop codons (TAG, TAA, or TGA) in reverse orientation or out-of-frame to the desired ORF, while preserving the encoded amino acid sequence and, optionally, maintaining highly utilized codons in the gene of interest (*i.e.*, avoiding codons with frequency < 20%).

In some embodiments, the expression cassette comprises at most one, at most two, at most three, at most four, or at most five start codons 5′ to the start codon of the transgene. In some embodiments, the expression cassette comprises no start codon 5′ to the start codon of the transgene. In some embodiments, one or more ATG codons in the 5′ UTR, the promoter, the enhance, the promoter/enhancer element, or other sequences 5′ to the start codon of the transgene remain after one or more cryptic start sites are removed. In some embodiments, the expression cassette comprises no cryptic starts sites upstream of transgene to generate erroneous mRNAs.

In variations of the present disclosure, the transgene coding sequence may be optimized by either codon optimization or removal of non-transgene ORFs or using both techniques. In some cases, one removes or minimizes non-transgene ORFs after codon optimization in order to remove ORFs introduced during codon optimization.

In an embodiment, the transgene contains fewer CpG sites than SEQ ID: 2. Without being bound by theory, it is believed that the presence of CpG sites in a polynucleotide sequence is associated with the undesirable immunological responses of the host against a viral vector comprising the polynucleotide sequence. In some embodiments, the transgene is designed to reduce the number of CpG sites. Exemplary methods are provides in U.S. Patent Application Publication No. US20020065236A1.

In an embodiment, the transgene contains fewer cryptic splice sites than SEQ ID: 2. For the optimization, GeneArt® software may be used, *e.g.*, to increase the GC content and/or remove cryptic splice sites in order to avoid transcriptional silencing and, therefore, increase transgene expression. Alternatively, any optimization method known in the art may

be used. Removal of cryptic splice sites is described, for example, in International Patent Application Publication No. WO2004015106A1.

Also disclosed herein are expression cassettes and gene therapy vectors encoding LAMP-2B. In certain embodiments, the expression cassettes and gene therapy vectors comprise a codon-optimized or variant LAMP-2B polynucleotide sequence or transgene sequence disclosed herein.

In particular embodiments, an expression cassette or gene therapy vector encoding LAMP-2B comprises: a consensus optimal Kozak sequence, a full-length polyadenylation (polyA) sequence (or substitution of full-length polyA by a truncated polyA), and minimal or no upstream (*i.e.* 5′) or cryptic start codons (*i.e.* ATG sites). In some embodiments, the expression cassette comprises no start site 5′ to the transgene capable of generating alternative mRNAs. In certain embodiments, the expression cassette or gene therapy vector comprises a sequence encoding LMAP-2B, *e.g.*, a codon-optimized or variant LAMP-2B polynucleotide sequence or transgene sequence disclosed herein.

In some cases, the expression cassette contains two or more of a first inverted terminal repeat, an enhancer/promoter region, a consensus optimal Kozak sequence, a transgene (*e.g.*, a transgene encoding a LAMP-2B disclosed herein), a 3′ untranslated region including a full-length polyA sequence, and a second inverted terminal repeat. In some embodiments, one or both of the inverted terminal repeats (ITRs) are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, or AAV9 ITRs, or any one ITR known in the art. In some embodiments, the expression cassette comprises exactly two ITRs. In some embodiments, both ITRs are AAV2, AAV5, or AAV9 ITRs. In some embodiments, both ITRs are AAV2 ITRs.

In an embodiment, the expression cassette comprises a Kozak sequence operatively linked to the transgene. In an embodiment, the Kozak sequence is a consensus optimal Kozak sequence comprising or consisting of SEQ ID NO: 6:

GCCGCCACCATGG (SEQ ID NO: 6).

In various embodiments, the expression cassette comprises an alternative Kozak sequence operatively linked to the transgene. In an embodiment, the Kozak

sequence is an alternative Kozak sequence comprising or consisting of any one of SEQ ID NOs. 14-18:

(gcc)gccRccAUGG (SEQ ID NO: 14);

AGNNAUGN (SEQ ID NO: 15);

ANNAUGG (SEQ ID NO: 16);

ACCAUGG (SEQ ID NO: 17);

GACACCAUGG (SEQ ID NO: 18).

In some embodiments, the expression cassette comprises no Kozak sequence.

In SEQ ID NO: 14, a lower-case letter denotes the most common base at a position where the base can nevertheless vary; an upper-case letter indicates a highly conserved base; R indicates adenine or guanine. In SEQ ID NO: 14, the sequence in parentheses (GCC) is optional. In SEQ ID NOs: 15-17, 'N' denotes any base.

A variety of sequences can be used in place of this consensus optimal Kozak sequence as the translation-initiation site and it is within the skill of those in the art to identify and test other sequences. *See* Kozak M. An analysis of vertebrate mRNA sequences: intimations of translational control. *J. Cell Biol.* 115 (4): 887–903 (1991).

In an embodiment, the expression cassette comprises a full-length polyA sequence operatively linked to the transgene. In an embodiment, the full-length polyA sequence comprises SEQ ID NO: 7:

```
  1 TGGCTAATAA AGGAAATTTA TTTTCATTGC AATAGTGTGT TGGAATTTTT TGTGTCTCTC
 61 ACTCGGAAGG ACATATGGGA GGGCAAATCA TTTAAAACAT CAGAATGAGT ATTTGGTTTA
121 GAGTTTGGCA ACATATGCCC ATATGCTGGC TGCCATGAAC AAAGGTTGGC TATAAAGAGG
181 TCATCAGTAT ATGAAACAGC CCCCTGCTGT CCATTCCTTA TTCCATAGAA AAGCCTTGAC
241 TTGAGGTTAG ATTTTTTTTA TATTTTGTTT TGTGTTATTT TTTTCTTTAA CATCCCTAAA
301 ATTTTCCTTA CATGTTTTAC TAGCCAGATT TTTCCTCCTC TCCTGACTAC TCCCAGTCAT
361 AGCTGTCCCT CTTCTCTTAT GGAGATC
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(SEQ ID NO: 7).

Various alternative polyA sequences may be used in expression cassettes of the present disclosure, including without limitation, bovine growth hormone polyadenylation signal (bGHpA) (SEQ ID NO: 19), the SV40 early/late polyadenylation signal (SEQ ID NO: 20), and human growth hormone (HGH) polyadenylation signal (SEQ ID NO: 21):

```
  1 TCGACTGTGC CTTCTAGTTG CCAGCCATCT GTTGTTTGCC CCTCCCCCGT GCCTTCCTTG
 61 ACCCTGGAAG GTGCCACTCC CACTGTCCTT TCCTAATAAA ATGAGGAAAT TGCATCGCAT
121 TGTCTGAGTA GGTGTCATTC TATTCTGGGG GGTGGGGTGG GGCAGGACAG CAAGGGGGAG
181 GATTGGGAGG ACAATAGCAG GCATGCTGGG GATGCGGTGG GCTCTATGGC TTCTG
```

(SEQ ID NO: 19);

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  1 CAGACATGAT AAGATACATT GATGAGTTTG GACAAACCAC AACTAGAATG CAGTGAAAAA
 61 AATGCTTTAT TTGTGAAATT TGTGATGCTA TTGCTTTATT TGTAACCATT ATAAGCTGCA
121 ATAAACAAGT TAACAACAAC AATTGCATTC ATTTTATGTT TCAGGTTCAG GGGGAGATGT
181 GGGAGGTTTT TTAAAGCAAG TAAAACCTCT ACAAATGTGG TA
```

(SEQ ID NO: 20);

```
  1 CTGCCCGGGT GGCATCCCTG TGACCCCTCC CCAGTGCCTC TCCTGGCCCT GGAAGTTGCC
 61 ACTCCAGTGC CCACCAGCCT TGTCCTAATA AAATTAAGTT GCATCATTTT GTCTGACTAG
121 GTGTCCTTCT ATAATATTAT GGGGTGGAGG GGGGTGGTAT GGAGCAAGGG GCCCAAGTTG
181 GGAAGAAACC TGTAGGGCCT GC
```

(SEQ ID NO: 21).

In some embodiments, the expression cassette comprises an active fragment of a polyA sequence. In particular embodiments, the active fragment of the polyA sequence comprises or consists of less than 20 base pair (bp), less than 50 bp, less than 100 bp, or less than 150 bp, *e.g.*, of any of the polyA sequences disclosed herein.

In some cases, expression of the transgene is increased by ensuring that the expression cassette does not contain competing ORFs. In an embodiment, the expression cassette comprises no start codon within 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or 500 base pairs 5′ of the start codon of the transgene. In some embodiment, the expression cassette comprises no start codon 5′ of the start codon of the transgene. In some embodiments, the expression cassette comprises no start site 5′ to the transgene capable of generating alternative mRNAs.

In an embodiment, the expression cassette comprises operatively linked, in the 5′ to 3′ direction, a first inverted terminal repeat, an enhancer/promoter region, introns, a consensus optimal Kozak sequence, the transgene, a 3′ untranslated region including a full-length polyA sequence, and a second inverted terminal repeat, wherein the expression cassette comprises no start site 5′ to the transgene capable of generating alternative mRNAs.

In some embodiments, the enhancer/promoter region comprises, in the 5′ to 3′ direction: a CMV IE enhancer and a chicken beta-actin promoter. In an embodiment, the enhancer/promoter region comprises a CAG promoter (SEQ ID NO: 22). As used herein

"CAG promoter" refers to a polynucleotide sequence comprising a CMV early enhancer element, a chicken beta-actin promoter, the first exon and first intron of the chicken beta-actin gene, and a splice acceptor from the rabbit beta-globin gene.

```
   1 CTAGTCGACA TTGATTATTG ACTAGTTATT AATAGTAATC AATTACGGGG TCATTAGTTC
  61 ATAGCCCATA TATGGAGTTC CGCGTTACAT AACTTACGGT AAATGGCCCG CCTGGCTGAC
 121 CGCCCAACGA CCCCCGCCCA TTGACGTCAA TAATGACGTA TGTTCCCATA GTAACGCCAA
 181 TAGGGACTTT CCATTGACGT CAATGGGTGG AGTATTTACG GTAAACTGCC CACTTGGCAG
 241 TACATCAAGT GTATCATATG CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAAATGGC
 301 CCGCCTGGCA TTATGCCCAG TACATGACCT TATGGGACTT TCCTACTTGG CAGTACATCT
 361 ACGTATTAGT CATCGCTATT ACCATGGTCG AGGTGAGCCC CACGTTCTGC TTCACTCTCC
 421 CCATCTCCCC CCCCTCCCCA CCCCCAATTT TGTATTTATT TATTTTTTAA TTATTTTGTG
 481 CAGCGATGGG GGCGGGGGGG GGGGGGGGGC GCGCGCCAGG CGGGGCGGGG CGGGGCGAGG
 541 GGCGGGGCGG GGCGAGGCGG AGAGGTGCGG CGGCAGCCAA TCAGAGCGGC GCGCTCCGAA
 601 AGTTTCCTTT TATGGCGAGG CGGCGGCGGC GGCGGCCCTA TAAAAAGCGA AGCGCGCGGC
 661 GGGCGGGAGT CGCTGCGCGC TGCCTTCGCC CCGTGCCCCG CTCCGCCGCC GCCTCGCGCC
 721 GCCCGCCCCG GCTCTGACTG ACCGCGTTAC TCCCACAGGT GAGCGGGCGG GACGGCCCTT
 781 CTCCTCCGGG CTGTAATTAG CGCTTGGTTT AATGACGGCT TGTTTCTTTT CTGTGGCTGC
 841 GTGAAAGCCT TGAGGGGCTC CGGGAGGGCC CTTTGTGCGG GGGGAGCGGC TCGGGGGGTG
 901 CGTGCGTGTG TGTGTGCGTG GGGAGCGCCG CGTGCGGCTC CGCGCTGCCC GGCGGCTGTG
 961 AGCGCTGCGG GCGCGGCGCG GGGCTTTGTG CGCTCCGCAG TGTGCGCGAG GGGAGCGCGG
1021 CCGGGGGCGG TGCCCCGCGG TGCGGGGGGG GCTGCGAGGG GAACAAAGGC TGCGTGCGGG
1081 GTGTGTGCGT GGGGGGGTGA GCAGGGGGTG TGGGCGCGTC GGTCGGGCTG CAACCCCCCC
1141 TGCACCCCCC TCCCCGAGTT GCTGAGCACG GCCCGGCTTC GGGTGCGGGG CTCCGTACGG
1201 GGCGTGGCGC GGGGCTCGCC GTGCCGGGCG GGGGGTGGCG GCAGGTGGGG GTGCCGGGCG
1261 GGGCGGGGCC GCCTCGGGCC GGGGAGGGCT CGGGGGAGGG GCGCGGCGGC CCCCGGAGCG
1321 CCGGCGGCTG TCGAGGCGCG GCGAGCCGCA GCCATTGCCT TTTATGGTAA TCGTGCGAGA
1381 GGGCGCAGGG ACTTCCTTTG TCCCAAATCT GTGCGGAGCC GAAATCTGGG AGGCGCCGCC
1441 GCACCCCCTC TAGCGGGCGC GGGGCGAAGC GGTGCGGCGC CGGCAGGAAG GAAATGGGCG
1501 GGGAGGGCCT TCGTGCGTCG CCGCGCCGCC GTCCCCTTCT CCCTCTCCAG CCTCGGGGCT
1561 GTCCGCGGGG GGACGGCTGC CTTCGGGGGG GACGGGGCAG GGCGGGGTTC GGCTTCTGGC
1621 GTGTGACCGG CGGCTCTAGA GCCTCTGCTA ACCATGTTCA TGCCTTCTTC TTTTTCCTAC
1681 AGCTCCTGGG CAACGTGCTG GTTATTGTGC TGTCTCATCA TTTTGGCAAA
```

(SEQ ID NO: 22).

In some embodiments, the enhancer/promoter region comprises a ubiquitous promoter. In some embodiments, the enhancer/promoter region comprises a CMV promoter (SEQ ID NO: 23), an SV40 promoter (SEQ ID NO: 24), a PGK promoter (SEQ ID NO: 25), and/or a human beta-actin promoter (SEQ ID NO: 26). In some embodiments, the enhancer/promoter region comprises a polynucleotide that shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with any one of SEQ ID NOs: 23-26:

```
   1 GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT
  61 CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC
 121 TTTCCAAAAT GTCGTAACAA CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG
 181 TGGGAGGTCT ATATAAGCAG AGCT
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(SEQ ID NO: 23);

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   1 GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT
  61 AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA
 121 TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA
 181 CTCCGCCCAG TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG
 241 AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC TTTTTTGGAG
 301 GCCTAGGCTT TTGCAAA
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(SEQ ID NO: 24);

```
   1 GGGTAGGGGA GGCGCTTTTC CCAAGGCAGT CTGGAGCATG CGCTTTAGCA GCCCCGCTGG
  61 GCACTTGGCG CTACACAAGT GGCCTCTGGC CTCGCACACA TTCCACATCC ACCGGTAGGC
 121 GCCAACCGGC TCCGTTCTTT GGTGGCCCCT TCGCGCCACC TTCTACTCCT CCCCTAGTCA
 181 GGAAGTTCCC CCCCGCCCCG CAGCTCGCGT CGTGCAGGAC GTGACAAATG GAAGTAGCAC
 241 GTCTCACTAG TCTCGTGCAG ATGGACAGCA CCGCTGAGCA ATGGAAGCGG GTAGGCCTTT
 301 GGGGCAGCGG CCAATAGCAG CTTTGCTCCT TCGCTTTCTG GGCTCAGAGG CTGGGAAGGG
 361 GTGGGTCCGG GGGCGGGCTC AGGGGCGGGC TCAGGGGCGG GGCGGGCGCC CGAAGGTCCT
 421 CCGGAGGCCC GGCATTCTGC ACGCTTCAAA AGCGCACGTC TGCCGCGCTG TTCTCCTCTT
 481 CCTCATCTCC GGGCCTTTCG
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(SEQ ID NO: 25);

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   1 CCTGCAGGGC CCACTAGTTC CATGTCCTTA TATGGACTCA TCTTTGCCTA TTGCGACACA
  61 CACTCAATGA ACACCTACTA CGCGCTGCAA AGAGCCCCGC AGGCCTGAGG TGCCCCCACC
 121 TCACCACTCT TCCTATTTTT GTGTAAAAAT CCAGCTTCTT GTCACCACCT CCAAGGAGGG
 181 GGAGGAGGAG GAAGGCAGGT TCCTCTAGGC TGAGCCGAAT GCCCCTCTGT GGTCCCACGC
 241 CACTGATCGC TGCATGCCCA CCACCTGGGT ACACACAGTC TGTGATTCCC GGAGCAGAAC
 301 GGACCCTGCC CACCCGGTCT TGTGTGCTAC TCAGTGGACA GACCCAAGGC AAGAAAGGGT
 361 GACAAGGACA GGGTCTTCCC AGGCTGGCTT TGAGTTCCTA GCACCGCCCC GCCCCCAATC
 421 CTCTGTGGCA CATGGAGTCT TGGTCCCCAG AGTCCCCCAG CGGCCTCCAG ATGGTCTGGG
 481 AGGGCAGTTC AGCTGTGGCT GCGCATAGCA GACATACAAC GGACGGTGGG CCCAGACCCA
 541 GGCTGTGTAG ACCCAGCCCC CCCGCCCCGC AGTGCCTAGG TCACCCACTA ACGCCCCAGG
 601 CCTGGTCTTG GCTGGGCGTG ACTGTTACCC TCAAAAGCAG GCAGCTCCAG GGTAAAAGGT
 661 GCCCTGCCCT GTAGAGCCCA CCTTCCTTCC CAGGGCTGCG GCTGGGTAGG TTTGTAGCCT
 721 TCATCACGGG CCACCTCCAG CCACTGGACC GCTGGCCCCT GCCCTGTCCT GGGGAGTGTG
 781 GTCCTGCGAC TTCTAAGTGG CCGCAAGCCA CCTGACTCCC CCAACACCAC ACTCTACCTC
 841 TCAAGCCCAG GTCTCTCCCT AGTGACCCAC CCAGCACATT TAGCTAGCTG AGCCCCACAG
 901 CCAGAGGTCC TCAGGCCCTG CTTTCAGGGC AGTTGCTCTG AAGTCGGCAA GGGGGAGTGA
 961 CTGCCTGGCC ACTCCATGCC CTCCAAGAGC TCCTTCTGCA GGAGCGTACA GAACCCAGGG
1021 CCCTGGCACC CGTGCAGACC CTGGCCCACC CCACCTGGGC GCTCAGTGCC CAAGAGATGT
1081 CCACACCTAG GATGTCCCGC GGTGGGTGGG GGGCCCGAGA GACGGGCAGG CCGGGGGCAG
1141 GCCTGGCCAT GCGGGGCCGA ACCGGGCACT GCCCAGCGTG GGGCGCGGGG GCCACGGCGC
1201 GCGCCCCCAG CCCCCGGGCC CAGCACCCCA AGGCGGCCAA CGCCAAAACT CTCCCTCCTC
1261 CTCTTCCTCA ATCTCGCTCT CGCTCTTTTT TTTTTCGCA AAAGGAGGGG AGAGGGGGTA
1321 AAAAAATGCT GCACTGTGCG GCGAAGCCGG TGAGTGAGCG GCGCGGGGCC AATCAGCGTG
1381 CGCCGTTCCG AAAGTTGCCT TTTATGGCTC GAGCGGCCGC GGCGGCGCCC TATAAAACCC
1441 AGCGGCGCGA CGCGCCACCA CCGCCGAGAC CGCGTCCGCC CCGCGAGCAC AGAGCCTCGC
1501 CTTTGCCGAT CCGCCGCCCG TCCACACCCG CCGCCAGGTA AGCCCGGCCA GCCGACCGGG
1561 GCAGGCGGCT CACGGCCCGG CCGCAGGCGG CCGCGGCCCC TTCGCCCGTG CAGAGCCGCC
1621 GTCTGGGCCG CAGCGGGGGG CGCATGGGGG GGGAACCGGA CCGCCGTGGG GGGCGCGGGA
1681 GAAGCCCCTG GGCCTCCGGA GATGGGGGAC ACCCCACGCC AGTTCGGAGG CGCGAGGCCG
1741 CGCTCGGGAG GCGCGCTCCG GGGGTGCCGC TCTCGGGGCG GGGGCAACCG GCGGGGTCTT
1801 TGTCTGAGCC GGGCTCTTGC CAATGGGGAT CGCAGGGTGG GCGCGGCGGA GCCCCCGCCA
1861 GGCCCGGTGG GGGCTGGGGC GCCATTGCGC GTGCGCGCTG GTCCTTTGGG CGCTAACTGC
1921 GTGCGCGCTG GGAATTGGCG CTAATTGCGC GTGCGCGCTG GGACTCAAGG CGCTAACTGC
1981 GCGTGCGTTC TGGGGCCCGG GGTGCCGCGG CCTGGGCTGG GGCGAAGGCG GGCTCGGCCG
2041 GAAGGGGTGG GGTCGCCGCG GCTCCCGGGC GCTTGCGCGC ACTTCCTGCC CGAGCCGCTG
2101 GCCGCCCGAG GGTGTGGCCG CTGCGTGCGC GCGCGCCGAC CCGGCGCTGT TTGAACCGGG
2161 CGGAGGCGGG GCTGGCGCCC GGTTGGGAGG GGGTTGGGGC CTGGCTTCCT GCCGCGCGCC
2221 GCGGGGACGC CTCCGACCAG TGTTTGCCTT TTATGGTAAT AACGCGGCCG GCCCGGCTTC
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2281 CTTTGTCCCC AATCTGGGCG CGCGCCGGCG CCCCCTGGCG GCCTAAGGAC TCGGCGCGCC
2341 GGAAGTGGCC AGGGCGGGGG CGACCTCGGC TCACAGCGCG CCCGGCTATT CTCGCAGCTC
2401 ACC
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(SEQ ID NO: 26).

Further exemplary promoters include, but are not limited to, human Elongation Factor 1 alpha promoter (EFS), SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late promoter (Ad MLP); a herpes simplex virus (HSV) promoter, an endogenous cellular promoter that is heterologous to the gene of interest, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a Rous sarcoma virus (RSV) promoter, synthetic promoters, hybrid promoters, and the like.

In some embodiments, the 3′ UTR comprises a polynucleotide (WPRE element) that shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with SEQ ID NO: 27:

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   1 ATTCGAGCAT CTTACCGCCA TTTATTCCCA TATTTGTTCT GTTTTTCTTG ATTTGGGTAT
  61 ACATTTAAAT GTTAATAAAA CAAAATGGTG GGGCAATCAT TTACATTTTT AGGGATATGT
 121 AATTACTAGT TCAGGTGTAT TGCCACAAGA CAAACATGTT AAGAAACTTT CCCGTTATTT
 181 ACGCTCTGTT CCTGTTAATC AACCTCTGGA TTACAAAATT TGTGAAAGAT TGACTGATAT
 241 TCTTAACTAT GTTGCTCCTT TTACGCTGTG TGGATATGCT GCTTTAATGC CTCTGTATCA
 301 TGCTATTGCT TCCCGTACGG CTTTCGTTTT CTCCTCCTTG TATAAATCCT GGTTGCTGTC
 361 TCTTTATGAG GAGTTGTGGC CCGTTGTCCG TCAACGTGGC GTGGTGTGCT CTGTGTTTGC
 421 TGACGCAACC CCCACTGGCT GGGGCATTGC CACCACCTGT CAACTCCTTT CTGGGACTTT
 481 CGCTTTCCCC CTCCCGATCG CCACGGCAGA ACTCATCGCC GCCTGCCTTG CCCGCTGCTG
 541 GACAGGGGCT AGGTTGCTGG GCACTGATAA TTCCGTGGTG TTGTCGGGGA AGGGCC
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(SEQ ID NO: 27).

In some embodiment, the expression cassette shares at least 95% identity to a sequence selected from SEQ ID NOs: 8-10. In an embodiment, the expression cassette shares complete identity to a sequence selected from SEQ ID NOs: 8-10, or shares at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identity to a sequence selected from SEQ ID NOs: 8-10:

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   1 CTGCGCGCTC GCTCGCTCAC TGAGGCCGCC CGGGCAAAGC CCGGGCGTCG GGCGACCTTT
  61 GGTCGCCCGG CCTCAGTGAG CGAGCGAGCG CGCAGAGAGG GAGTGGCCAA CTCCATCACT
 121 AGGGGTTCCT TGTAGTTAAT GATTAACCCG CCATGCTACT TATCTACCAG GGTAATGGGG
 181 ATCCTCTAGA ACTATAGCTA GTCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT
 241 TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC TTACGGTAAA
 301 TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT
 361 TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT ATTTACGGTA
 421 AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT
 481 CAATGACGGT AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC
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 541 TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTCGAGG TGAGCCCCAC
 601 GTTCTGCTTC ACTCTCCCCA TCTCCCCCCC CTCCCCACCC CCAATTTTGT ATTTATTTAT
 661 TTTTTAATTA TTTTGTGCAG CGATGGGGGC GGGGGGGGGG GGGGGGCGCG CGCCAGGCGG
 721 GGCGGGGCGG GGCGAGGGGC GGGGCGGGGC GAGGCGGAGA GGTGCGGCGG CAGCCAATCA
 781 GAGCGGCGCG CTCCGAAAGT TTCCTTTTAT GGCGAGGCGG CGGCGGCGGC GGCCCTATAA
 841 AAAGCGAAGC GCGCGGCGGG CGGGAGTCGC TGCGCGCTGC CTTCGCCCCG TGCCCCGCTC
 901 CGCCGCCGCC TCGCGCCGCC CGCCCCGGCT CTGACTGACC GCGTTACTCC CACAGGTGAG
 961 CGGGCGGGAC GGCCCTTCTC CTCCGGGCTG TAATTAGCGC TTGGTTTAAT GACGGCTTGT
1021 TTCTTTTCTG TGGCTGCGTG AAAGCCTTGA GGGGCTCCGG GAGGGCCCTT TGTGCGGGGG
1081 GAGCGGCTCG GGGGGTGCGT GCGTGTGTGT GTGCGTGGGG AGCGCCGCGT GCGGCTCCGC
1141 GCTGCCCGGC GGCTGTGAGC GCTGCGGGCG CGGCGCGGGG CTTTGTGCGC TCCGCAGTGT
1201 GCGCGAGGGG AGCGCGGCCG GGGGCGGTGC CCCGCGGTGC GGGGGGGGCT GCGAGGGGAA
1261 CAAAGGCTGC GTGCGGGGTG TGTGCGTGGG GGGGTGAGCA GGGGGTGTGG GCGCGTCGGT
1321 CGGGCTGCAA CCCCCCCTGC ACCCCCCTCC CCGAGTTGCT GAGCACGGCC CGGCTTCGGG
1381 TGCGGGGCTC CGTACGGGGC GTGGCGCGGG GCTCGCCGTG CCGGGCGGGG GGTGGCGGCA
1441 GGTGGGGGTG CCGGGCGGGG CGGGGCCGCC TCGGGCCGGG GAGGGCTCGG GGGAGGGGCG
1501 CGGCGGCCCC CGGAGCGCCG GCGGCTGTCG AGGCGCGGCG AGCCGCAGCC ATTGCCTTTT
1561 ATGGTAATCG TGCGAGAGGG CGCAGGGACT TCCTTTGTCC CAAATCTGTG CGGAGCCGAA
1621 ATCTGGGAGG CGCCGCCGCA CCCCCTCTAG CGGGCGCGGG GCGAAGCGGT GCGGCGCCGG
1681 CAGGAAGGAA ATGGGCGGGG AGGGCCTTCG TGCGTCGCCG CGCCGCCGTC CCCTTCTCCC
1741 TCTCCAGCCT CGGGGCTGTC CGCGGGGGGA CGGCTGCCTT CGGGGGGGAC GGGGCAGGGC
1801 GGGGTTCGGC TTCTGGCGTG TGACCGGCGG CTCTAGAGCC TCTGCTAACC ATGTTCATGC
1861 CTTCTTCTTT TTCCTACAGC TCCTGGGCAA CGTGCTGGTT ATTGTGCTGT CTCATCATTT
1921 TGGCAAAGAA TTCGAGCGGC CGCCAGCCGC CACCATGGTC TGCTTCAGAC TGTTCCCTGT
1981 CCCTGGATCT GGTCTGGTGC TTGTGTGCTT GGTGCTGGGT GCTGTGAGAT CCTATGCCCT
2041 TGAGCTGAAC CTGACTGACT CAGAAAATGC CACTTGCCTG TATGCCAAGT GGCAGATGAA
2101 CTTCACTGTG AGATATGAGA CTACCAACAA GACCTACAAG ACTGTGACCA TCTCAGACCA
2161 TGGCACTGTC ACCTACAATG GATCAATCTG TGGTGATGAT CAGAATGGCC CAAAGATAGC
2221 AGTGCAGTTT GGGCCCGGTT TTTCCTGGAT TGCTAACTTC ACCAAGGCAG CCTCCACCTA
2281 CAGCATTGAC TCAGTCAGCT TCAGCTACAA CACTGGGGAT AACACCACCT TCCCTGACGC
2341 AGAGGACAAG GGAATCCTTA CTGTGGACGA ACTCCTGGCA ATCAGAATCC CCCTTAACGA
2401 CCTGTTCAGA TGCAACTCCC TTTCAACCCT TGAAAAGAAT GATGTGGTGC AACACTATTG
2461 GGACGTCCTG GTGCAAGCCT TTGTGCAGAA TGGGACAGTG AGTACCAACG AGTTCCTCTG
2521 TGACAAGGAC AAGACCAGCA CTGTGGCCCC CACTATCCAC ACCACTGTGC CCAGCCCTAC
2581 CACTACCCCC ACCCCTAAAG AGAAGCCAGA AGCTGGAACC TACTCAGTCA ACAATGGAAA
2641 TGACACATGC CTCCTTGCCA CCATGGGACT GCAGCTGAAC ATCACTCAGG ACAAGGTGGC
2701 CTCAGTGATT AACATCAACC CTAACACCAC TCATAGCACT GGGAGCTGCA GATCACATAC
2761 AGCTCTGCTG AGGCTCAACT CCTCCACCAT CAAGTACCTG GACTTTGTGT TTGCTGTGAA
2821 GAATGAGAAC AGGTTCTACC TCAAGGAAGT GAACATTTCC ATGTACCTGG TCAATGGTTC
2881 AGTGTTCTCT ATTGCCAACA ACAATCTGAG CTACTGGGAT GCACCCCTGG GATCCTCCTA
2941 CATGTGCAAC AAGGAGCAGA CTGTGAGTGT GTCAGGTGCT TTTCAGATCA ACACTTTTGA
3001 CCTGAGGGTG CAGCCCTTCA ATGTGACTCA GGGAAAGTAC TCCACTGCAC AAGAGTGTTC
3061 CTTGGATGAT GACACTATCC TCATCCCCAT TATTGTGGGA GCTGGACTGT CAGGATTGAT
3121 TATAGTGATT GTGATTGCTT ATGTGATTGG AAGGAGAAAG AGCTATGCTG GCTACCAGAC
3181 CCTGTAAAAG GGCGAATTCC AGCACACGCG TCCTAGGAGC TCGAGTACTA CTGGCGGCCG
3241 TTACTAGTGG ATCCGCGGTA CAAGTAAGCA TGCAAGCTTC GAGGACGGGG TGAACTACGC
3301 CTGAATCAAG CTTATCGATA AATTCGAGCA TCTTACCGCC ATTTATTCCC ATATTTGTTC
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3421 TTTACATTTT TAGGGATATG TAATTACTAG TTCAGGTGTA TTGCCACAAG ACAAACATGT
3481 TAAGAAACTT TCCCGTTATT TACGCTCTGT TCCTGTTAAT CAACCTCTGG ATTACAAAAT
3541 TTGTGAAAGA TTGACTGATA TTCTTAACTA TGTTGCTCCT TTTACGCTGT GTGGATATGC
3601 TGCTTTAATG CCTCTGTATC ATGCTATTGC TTCCCGTACG GCTTTCGTTT TCTCCTCCTT
3661 GTATAAATCC TGGTTGCTGT CTCTTTATGA GGAGTTGTGG CCCGTTGTCC GTCAACGTGG
3721 CGTGGTGTGC TCTGTGTTTG CTGACGCAAC CCCCACTGGC TGGGGCATTG CCACCACCTG
3781 TCAACTCCTT TCTGGGACTT TCGCTTTCCC CCTCCCGATC GCCACGGCAG AACTCATCGC
3841 CGCCTGCCTT GCCCGCTGCT GGACAGGGGC TAGGTTGCTG GGCACTGATA ATTCCGTGGT
3901 GTTGTCGGGG AAGGGCCTCG ATACCGTCGA TATCGATCCT GGCTAATAAA GGAAATTTAT
3961 TTTCATTGCA ATAGTGTGTT GGAATTTTTT GTGTCTCTCA CTCGGAAGGA CATATGGGAG
4021 GGCAAATCAT TTAAAACATC AGAATGAGTA TTTGGTTTAG AGTTTGGCAA CATATGCCCA
4081 TATGCTGGCT GCCATGAACA AAGGTTGGCT ATAAAGAGGT CATCAGTATA TGAAACAGCC
4141 CCCTGCTGTC CATTCCTTAT TCCATAGAAA AGCCTTGACT TGAGGTTAGA TTTTTTTTAT
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4261 AGCCAGATTT TTCCTCCTCT CCTGACTACT CCCAGTCATA GCTGTCCCTC TTCTCTTATG
4321 GAGATCGAAG CAATTCGTTG ATCTGAATTT CGACCACCCA TAATAGATCT CCCATTACCC
4381 TGGTAGATAA GTAGCATGGC GGGTTAATCA TTAACTACAA GGAACCCCTA GTGATGGAGT
4441 TGGCCACTCC CTCTCTGCGC GCTCGCTCGC TCACTGAGGC CGGGCGACCA AAGGTCGCCC
4501 GACGCCCGGG CTTTGCCCGG GCGGCCTCAG TGAGCGAGCG AGCGCGCAG
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(SEQ ID NO: 8);

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   1 CTGCGCGCTC GCTCGCTCAC TGAGGCCGCC CGGGCAAAGC CCGGGCGTCG GGCGACCTTT
  61 GGTCGCCCGG CCTCAGTGAG CGAGCGAGCG CGCAGAGAGG GAGTGGCCAA CTCCATCACT
 121 AGGGGTTCCT TGTAGTTAAT GATTAACCCG CCATGCTACT TATCTACCAG GGTAATGGGG
 181 ATCCTCTAGA ACTATAGCTA GTCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT
 241 TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC TTACGGTAAA
 301 TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT
 361 TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT ATTTACGGTA
 421 AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT
 481 CAATGACGGT AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC
 541 TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTCGAGG TGAGCCCCAC
 601 GTTCTGCTTC ACTCTCCCCA TCTCCCCCCC CTCCCCACCC CCAATTTTGT ATTTATTTAT
 661 TTTTTAATTA TTTTGTGCAG CGATGGGGGC GGGGGGGGGG GGGGGGCGCG CGCCAGGCGG
 721 GGCGGGGCGG GGCGAGGGGC GGGGCGGGGC GAGGCGGAGA GGTGCGGCGG CAGCCAATCA
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 841 AAAGCGAAGC GCGCGGCGGG CGGGAGTCGC TGCGCGCTGC CTTCGCCCCG TGCCCCGCTC
 901 CGCCGCCGCC TCGCGCCGCC CGCCCCGGCT CTGACTGACC GCGTTACTCC CACAGGTGAG
 961 CGGGCGGGAC GGCCCTTCTC CTCCGGGCTG TAATTAGCGC TTGGTTTAAT GACGGCTTGT
1021 TTCTTTTCTG TGGCTGCGTG AAAGCCTTGA GGGGCTCCGG GAGGGCCCTT TGTGCGGGGG
1081 GAGCGGCTCG GGGGGTGCGT GCGTGTGTGT GTGCGTGGGG AGCGCCGCGT GCGGCTCCGC
1141 GCTGCCCGGC GGCTGTGAGC GCTGCGGGCG CGGCGCGGGG CTTTGTGCGC TCCGCAGTGT
1201 GCGCGAGGGG AGCGCGGCCG GGGGCGGTGC CCCGCGGTGC GGGGGGGGCT GCGAGGGGAA
1261 CAAAGGCTGC GTGCGGGGTG TGTGCGTGGG GGGGTGAGCA GGGGGTGTGG GCGCGTCGGT
1321 CGGGCTGCAA CCCCCCCTGC ACCCCCCTCC CCGAGTTGCT GAGCACGGCC CGGCTTCGGG
1381 TGCGGGGCTC CGTACGGGGC GTGGCGCGGG GCTCGCCGTG CCGGGCGGGG GGTGGCGGCA
1441 GGTGGGGGTG CCGGGCGGGG CGGGGCCGCC TCGGGCCGGG GAGGGCTCGG GGGAGGGGCG
1501 CGGCGGCCCC CGGAGCGCCG GCGGCTGTCG AGGCGCGGCG AGCCGCAGCC ATTGCCTTTT
1561 ATGGTAATCG TGCGAGAGGG CGCAGGGACT TCCTTTGTCC CAAATCTGTG CGGAGCCGAA
1621 ATCTGGGAGG CGCCGCCGCA CCCCCTCTAG CGGGCGCGGG GCGAAGCGGT GCGGCGCCGG
1681 CAGGAAGGAA ATGGGCGGGG AGGGCCTTCG TGCGTCGCCG CGCCGCCGTC CCCTTCTCCC
1741 TCTCCAGCCT CGGGGCTGTC CGCGGGGGGA CGGCTGCCTT CGGGGGGGAC GGGGCAGGGC
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1861 CTTCTTCTTT TTCCTACAGC TCCTGGGCAA CGTGCTGGTT ATTGTGCTGT CTCATCATTT
1921 TGGCAAAGAA TTCGAGCGGC CGCCAGCCGC CACCATGGTG TGCTTTAGAC TGTTTCCTGT
1981 GCCTGGTTCA GGGCTGGTCC TGGTCTGTCT GGTGCTGGGG GCTGTCAGAA GCTATGCCTT
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2101 CTTCACTGTG AGATATGAAA CCACCAACAA GACTTACAAA ACAGTGACCA TCTCAGATCA
2161 TGGAACTGTG ACCTACAACG GCAGCATTTG TGGAGACGAC CAGAACGGAC CAAAAATCGC
2221 TGTCCAATTT GGGCCTGGAT TCTCCTGGAT TGCCAATTTC ACTAAAGCTG CCTCCACATA
2281 TTCAATTGAC TCAGTGTCCT TCTCCTACAA CACTGGGGAC AACACTACTT TCCCTGATGC
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2581 CACCACTCCA ACTCCAAAGG AAAAGCCTGA AGCAGGAACC TACTCTGTGA ACAATGGCAA
2641 TGATACCTGT CTGTTGGCCA CCATGGGCCT CCAACTGAAC ATTACTCAGG ACAAGGTGGC
2701 CTCAGTGATT AACATTAACC CCAACACTAC CCACTCCACT GGCAGCTGTA GATCACACAC
2761 AGCCTTGCTC AGACTGAATA GCAGCACCAT CAAGTATTTG GATTTTGTGT TTGCAGTGAA
2821 GAATGAAAAC AGGTTCTACC TGAAGGAAGT CAACATCTCA ATGTACCTGG TGAACGGCTC
2881 AGTGTTCAGC ATTGCCAACA ACAACCTCTC CTATTGGGAC GCTCCACTGG GGAGCAGCTA
2941 CATGTGTAAC AAGGAACAGA CTGTGTCAGT GTCAGGAGCC TTCCAGATTA ACACCTTTGA
3001 TCTGAGGGTC CAACCCTTTA ATGTCACTCA AGGAAAGTAT AGCACTGCCC AGGAGTGCTC
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3061 CCTGGATGAT GACACCATTC TGATTCCAAT CATTGTGGGT GCAGGACTTT CTGGGCTTAT
3121 TATTGTGATT GTGATTGCCT ATGTGATTGG CAGAAGGAAA TCCTATGCAG GGTACCAAAC
3181 TCTGTAAAAG GGCGAATTCC AGCACACGCG TCCTAGGAGC TCGAGTACTA CTGGCGGCCG
3241 TTACTAGTGG ATCCGCGGTA CAAGTAAGCA TGCAAGCTTC GAGGACGGGG TGAACTACGC
3301 CTGAATCAAG CTTATCGATA AATTCGAGCA TCTTACCGCC ATTTATTCCC ATATTTGTTC
3361 TGTTTTTCTT GATTTGGGTA TACATTTAAA TGTTAATAAA ACAAAATGGT GGGGCAATCA
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3601 TGCTTTAATG CCTCTGTATC ATGCTATTGC TTCCCGTACG GCTTTCGTTT TCTCCTCCTT
3661 GTATAAATCC TGGTTGCTGT CTCTTTATGA GGAGTTGTGG CCCGTTGTCC GTCAACGTGG
3721 CGTGGTGTGC TCTGTGTTTG CTGACGCAAC CCCCACTGGC TGGGGCATTG CCACCACCTG
3781 TCAACTCCTT TCTGGGACTT TCGCTTTCCC CCTCCCGATC GCCACGGCAG AACTCATCGC
3841 CGCCTGCCTT GCCCGCTGCT GGACAGGGGC TAGGTTGCTG GGCACTGATA ATTCCGTGGT
3901 GTTGTCGGGG AAGGGCCTCG ATACCGTCGA TATCGATCCT GGCTAATAAA GGAAATTTAT
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4021 GGCAAATCAT TTAAAACATC AGAATGAGTA TTTGGTTTAG AGTTTGGCAA CATATGCCCA
4081 TATGCTGGCT GCCATGAACA AAGGTTGGCT ATAAAGAGGT CATCAGTATA TGAAACAGCC
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4201 ATTTTGTTTT GTGTTATTTT TTTCTTTAAC ATCCCTAAAA TTTTCCTTAC ATGTTTTACT
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4441 TGGCCACTCC CTCTCTGCGC GCTCGCTCGC TCACTGAGGC CGGGCGACCA AAGGTCGCCC
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(SEQ ID NO: 9); and

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   1 CTGCGCGCTC GCTCGCTCAC TGAGGCCGCC CGGGCAAAGC CCGGGCGTCG GGCGACCTTT
  61 GGTCGCCCGG CCTCAGTGAG CGAGCGAGCG CGCAGAGAGG GAGTGGCCAA CTCCATCACT
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 301 TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT
 361 TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT ATTTACGGTA
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 601 GTTCTGCTTC ACTCTCCCCA TCTCCCCCCC CTCCCCACCC CCAATTTTGT ATTTATTTAT
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1861 CTTCTTCTTT TTCCTACAGC TCCTGGGCAA CGTGCTGGTT ATTGTGCTGT CTCATCATTT
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1981 CCCTGGTTCA GGACTGGTCT TAGTGTGTCT GGTGCTTGGA GCTGTCAGAA GCTATGCCCT
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2761 TGCCCTCCTG AGGCTCAACT CATCAACTAT CAAGTACTTG GATTTTGTGT TTGCAGTGAA
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3061 CCTGGATGAT GACACTATCC TGATCCCTAT CATTGTGGGG GCAGGCCTGT CTGGACTCAT
3121 TATTGTGATT GTGATTGCCT ATGTGATAGG GAGAAGGAAG TCTTATGCTG GATACCAGAC
3181 CCTGTAAAAG GGCGAATTCC AGCACACGCG TCCTAGGAGC TCGAGTACTA CTGGCGGCCG
3241 TTACTAGTGG ATCCGCGGTA CAAGTAAGCA TGCAAGCTTC GAGGACGGGG TGAACTACGC
3301 CTGAATCAAG CTTATCGATA AATTCGAGCA TCTTACCGCC ATTTATTCCC ATATTTGTTC
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3721 CGTGGTGTGC TCTGTGTTTG CTGACGCAAC CCCCACTGGC TGGGGCATTG CCACCACCTG
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3841 CGCCTGCCTT GCCCGCTGCT GGACAGGGGC TAGGTTGCTG GGCACTGATA ATTCCGTGGT
3901 GTTGTCGGGG AAGGGCCTCG ATACCGTCGA TATCGATCCT GGCTAATAAA GGAAATTTAT
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4021 GGCAAATCAT TTAAAACATC AGAATGAGTA TTTGGTTTAG AGTTTGGCAA CATATGCCCA
4081 TATGCTGGCT GCCATGAACA AAGGTTGGCT ATAAAGAGGT CATCAGTATA TGAAACAGCC
4141 CCCTGCTGTC CATTCCTTAT TCCATAGAAA AGCCTTGACT TGAGGTTAGA TTTTTTTTAT
4201 ATTTTGTTTT GTGTTATTTT TTTCTTTAAC ATCCCTAAAA TTTTCCTTAC ATGTTTTACT
4261 AGCCAGATTT TTCCTCCTCT CCTGACTACT CCCAGTCATA GCTGTCCCTC TTCTCTTATG
4321 GAGATCGAAG CAATTCGTTG ATCTGAATTT CGACCACCCA TAATAGATCT CCCATTACCC
4381 TGGTAGATAA GTAGCATGGC GGGTTAATCA TTAACTACAA GGAACCCCTA GTGATGGAGT
4441 TGGCCACTCC CTCTCTGCGC GCTCGCTCGC TCACTGAGGC CGGGCGACCA AAGGTCGCCC
4501 GACGCCCGGG CTTTGCCCGG GCGGCCTCAG TGAGCGAGCG AGCGCGCAG
```

(SEQ ID NO: 10).

In certain embodiments, the expression cassette comprises one or more modifications as compared to a sequence selected from SEQ ID NOs: 8-10, including but not limited to any of the modifications disclosed herein. In particular embodiments, the one or more modifications comprise one or more of: removal of one or more (*e.g.*, all) upstream ATG sequences, replacement of the Kozak sequence with an optimized consensus Kozak sequence or another Kozak sequence, including but not limited to any of those disclosed herein, and/or replacement of the polyadenylation sequence with a full-length polyadenylation sequence or

another polyadenylation sequence, including but not limited to any of those disclosed herein. An illustrative configuration of genetic elements within these exemplary expression cassettes is depicted in **FIG. 1**.

In an embodiment, the vector is an adeno-associated virus (AAV) vector. In an embodiment, the expression cassette comprises inverted terminal repeat (ITR) sequences selected from SEQ ID NOs: 11 and 12:

```
  1 CTGCGCGCTC GCTCGCTCAC TGAGGCCGCC CGGGCAAAGC CCGGGCGTCG GGCGACCTTT
 61 GGTCGCCCGG CCTCAGTGAG CGAGCGAGCG CGCAGAGAGG GAGTGGCCAA CTCCATCACT
121 AGGGGTTCCT
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(SEQ ID NO: 11);

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  1 AGGAACCCCT AGTGATGGAG TTGGCCACTC CCTCTCTGCG CGCTCGCTCG CTCACTGAGG
 61 CCGGGCGACC AAAGGTCGCC CGACGCCCGG GCTTTGCCCG GGCGGCCTCA GTGAGCGAGC
121 GAGCGCGCAG
```

(SEQ ID NO: 12).

In related embodiments, the disclosure provides gene therapy vectors comprising an expression cassette disclosed herein. Generally, the gene therapy vectors described herein comprise an expression cassette comprising a polynucleotide encoding one or more isoforms of lysosome-associated membrane protein 2 (LAMP-2), and allows for the expression of LAMP-2 to partially or wholly rectify deficient LAMP-2 protein expression levels and/or autophagic flux in a subject in need thereof (*e.g.*, a subject having Danon disease or another disorder characterized by deficient autophagic flux at least in part due to deficient LAMP-2 expression). In particular embodiments, the expression cassette comprises a polynucleotide sequence encoding LAMP-2 disclosed herein, *e.g.*, SEQ ID NOs: 2-5, or a functional variant thereof. In some embodiments, the variant sequence has at least 90%, at least 95%, at least 98%, or at least 99% identity to any of SEQ ID NOs: 2-5. In some embodiments, the variant is a fragment of any of SEQ ID NOs: 2-5, *e.g.*, a fragment having at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of the sequence of any of SEQ ID NOs: 2-5.

Gene Therapy Vectors

Gene therapy vectors can be viral or non-viral vectors. Illustrative non-viral vectors include, *e.g.*, naked DNA, cationic liposome complexes, cationic polymer complexes,

cationic liposome-polymer complexes, and exosomes. Examples of viral vectors include, but are not limited to, adenoviral, retroviral, lentiviral, herpesvirus and adeno-associated virus (AAV) vectors.

In composition and methods according to the present disclosure, the viral vector generally is an AAV vector. AAV is a 4.7 kb, single stranded DNA virus. Recombinant vectors based on AAV are associated with excellent clinical safety, since wild-type AAV is nonpathogenic and has no etiologic association with any known diseases. In addition, AAV offers the capability for highly efficient gene delivery and sustained transgene expression in numerous tissues. By an "AAV vector" is meant a vector derived from an adeno-associated virus serotype, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAVrh.10, AAVrh.74, *etc*. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, *e.g.*, the rep and/or cap genes, but retain functional flanking inverted terminal repeat (ITR) sequences. Functional ITR sequences are necessary for the rescue, replication and packaging of the AAV virion. Thus, an AAV vector is defined herein to include at least those sequences required in cis for replication and packaging (*e.g.*, functional ITRs) of the virus. The ITRs need not be the wild-type nucleotide sequences, and may be altered, *e.g.* by the insertion, deletion or substitution of nucleotides, as long as the sequences provide for functional rescue, replication and packaging. AAV vectors may comprise other modifications, including but not limited to one or more modified capsid protein (*e.g.*, VP1, VP2 and/or VP3). For example, a capsid protein may be modified to alter tropism and/or reduce immunogenicity.

Immunogenicity against AAV is anticipated. Following administration of AAV therapy, the development of antibodies to the viral capsid and/or transgene have been reported in the literature (Mendell et al. New Engl. J. Med. 377:1713-1722 (2017); Rangarajan et al. New Engl. J. Med. 377:2519-2530 (2017). Consistent with published literature, the studies conducted in the present disclosure similarly showed an increase in serum neutralizing antibodies to AAV9 at Days 30 and 91 post-dosing. The vector delivery resulted in characteristic binding antibody response to AAV9 capsid, but no significant anti-drug antibody (ADA) response was noted to LAMP2B, with no detectable consequences regarding transduction-mediated LAMP2B function in target tissues.

Recombinant vectors based on AAV are associated with excellent clinical safety, since wild-type AAV is nonpathogenic and has no etiologic association with any known

diseases. In addition, AAV offers the capability for highly efficient gene delivery and sustained transgene expression in numerous tissues. Various serotypes of AAV are known, including, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAVrh.10, AAVrh.74, *etc*.. AAV vectors can have one or more of the AAV wild-type genes deleted in whole or part, *e.g.*, the *rep* and/or *cap* genes, but retain functional flanking inverted terminal repeat (ITR) sequences. The serotype of a recombinant AAV vector is determined by its capsid. International Patent Publication No. WO2003042397A2 discloses various capsid sequences including those of AAV1, AAV2, AAV3, AAV8, AAV9, and rh10. International Patent Publication No. WO2013078316A1 discloses the polypeptide sequence of the VP1 from AAVrh74. Numerous diverse naturally occurring or genetically modified AAV capsid sequences are known in the art.

An illustrative, non-limiting capsid is an AAV9 capsid, having the sequence of SEQ ID NO: 28 (or the VP1, VP2, or VP3 fragments thereof). In some embodiments, the AAV vectors of the disclosure comprise capsid proteins that share at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% identity of the entire sequence of SEQ ID NO: 28, or over amino acids 138 to 736 of SEQ ID NO: 28, or over amino acids 203 to 736 of SEQ ID NO: 28.

```
  1 MAADGYLPDW LEDNLSEGIR EWWALKPGAP QPKANQQHQD NARGLVLPGY KYLGPGNGLD
 61 KGEPVNAADA AALEHDKAYD QQLKAGDNPY LKYNHADAEF QERLKEDTSF GGNLGRAVFQ
121 AKKRLLEPLG LVEEAAKTAP GKKRPVEQSP QEPDSSAGIG KSGAQPAKKR LNFGQTGDTE
181 SVPDPQPIGE PPAAPSGVGS LTMASGGGAP VADNNEGADG VGSSSGNWHC DSQWLGDRVI
241 TTSTRTWALP TYNNHLYKQI SNSTSGGSSN DNAYFGYSTP WGYFDFNRFH CHFSPRDWQR
301 LINNNWGFRP KRLNFKLFNI QVKEVTDNNG VKTIANNLTS TVQVFTDSDY QLPYVLGSAH
361 EGCLPPFPAD VFMIPQYGYL TLNDGSQAVG RSSFYCLEYF PSQMLRTGNN FQFSYEFENV
421 PFHSSYAHSQ SLDRLMNPLI DQYLYYLSKT INGSGQNQQT LKFSVAGPSN MAVQGRNYIP
481 GPSYRQQRVS TTVTQNNNSE FAWPGASSWA LNGRNSLMNP GPAMASHKEG EDRFFPLSGS
541 LIFGKQGTGR DNVDADKVMI TNEEEIKTTN PVATESYGQV ATNHQSAQAQ AQTGWVQNQG
601 ILPGMVWQDR DVYLQGPIWA KIPHTDGNFH PSPLMGGFGM KHPPPQILIK NTPVPADPPT
661 AFNKDKLNSF ITQYSTGQVS VEIEWELQKE NSKRWNPEIQ YTSNYYKSNN VEFAVNTEGV
721 YSEPRPIGTR YLTRNL
```

(SEQ ID NO: 28).

AAV expression vectors are constructed using known techniques to at least provide as operatively linked components in the direction of transcription, control elements including a transcriptional initiation region, the DNA of interest (*i.e.* the LAMP-2 gene) and a transcriptional termination region.

In some embodiments, the viral vector is an AAV9 vector. In some embodiments, the expression cassette of the viral vector is flanked by AAV2 inverted terminal repeats (ITRs). ITRs used in alternative embodiments of the disclosed vectors include, but are not limited to, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9. In some embodiments, the viral vector is an AAV2/9 vector. The notation AAV2/9 refers to an AAV vector have the ITRs of AAV2 and the capsid of AAV9. Other embodiments of the disclosure include without limitation AAV2/9, AAV5/9, AAVrh74, AAV2/rh74, AAV5/9, and AAV5/rh74 vectors. Other ITRs known in the art may be used. Exemplary ITRs (and other AAV components) useful in the vectors of the present disclosure include, without limitation, those described in US6936466B2, US9169494B2, US20050220766A1, US20190022249A1, and US7282199B2, which are each incorporated by reference herein in their entireties.

Gene delivery viral vectors useful in the practice of the present invention can be constructed utilizing methodologies well known in the art of molecular biology. Typically, viral vectors carrying transgenes are assembled from polynucleotides encoding the transgene, suitable regulatory elements and elements necessary for production of viral proteins, which mediate cell transduction. Such recombinant viruses may be produced by techniques known in the art, *e.g.*, by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include but are not limited to HeLa cells, SF9 cells (optionally with a baculovirus helper vector), 293 cells, etc. A Herpesvirus-based system can be used to produce AAV vectors, as described in US20170218395A1. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in W095/14785, W096/22378, U.S. Pat. No. 5,882,877, U.S. Pat. No. 6,013,516, U.S. Pat. No. 4,861,719, U.S. Pat. No. 5,278,056 and W094/19478, the complete contents of each of which is hereby incorporated by reference.

The present disclosure also provides pharmaceutical compositions comprising an expression cassette or vector (*e.g.*, gene therapy vector) disclosed herein and one or more pharmaceutically acceptable carriers, diluents or excipients. In particular embodiments, the pharmaceutical composition comprises an AAV vector comprising an expression cassette disclosed herein, *e.g.*, wherein the expression cassette comprises a codon-optimized transgene encoding LAMP-2B, *e.g.*, any of SEQ ID NOs: 3-5 and variants thereof. Provided are pharmaceutical compositions, *e.g.*, for use in preventing or treating a disorder characterized by deficient autophagic flux (*e.g.*, Danon disease) which comprises a therapeutically effective

amount of an expression cassette or vector disclosed herein that comprises a nucleic acid sequence of a polynucleotide that encodes one or more isoforms of LAMP-2.

AAV vectors useful in the practice of the present invention can be packaged into AAV virions (viral particles) using various systems including adenovirus-based and helper-free systems. Standard methods in AAV biology include those described in Kwon and Schaffer. *Pharm Res.* (2008) 25(3):489-99; Wu et al. *Mol. Ther.* (2006) 14(3):316-27. Burger et al. *Mol. Ther.* (2004) 10(2):302-17; Grimm et al. *Curr Gene Ther.* (2003) 3(4):281-304; Deyle DR, Russell DW. *Curr Opin Mol Ther.* (2009) 11(4):442-447; McCarty et al. *Gene Ther.* (2001) 8(16):1248-54; and Duan et al. *Mol Ther.* (2001) 4(4):383-91. Helper-free systems included those described in US 6,004,797; US 7,588,772; and US 7,094,604;

The pharmaceutical compositions that contain the expression cassette or vector may be in any form that is suitable for the selected mode of administration, for example, for intraventricular, intramyocardial, intracoronary, intravenous, intra-arterial, intra-renal, intraurethral, epidural or intramuscular administration. The gene therapy vector comprising a polynucleotide encoding one or more LAMP-2 isoforms can be administered, as sole active agent, or in combination with other active agents, in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. In some embodiments, the pharmaceutical composition comprises cells transduced *ex vivo* with any of the gene therapy vectors of the disclosure.

Treatment of Danon Disease

Exemplary methods of treating lysosomal disorders and/or Danon disease are provided in WO 2018/170239 A1, which is incorporated herein in its entirety.

In an aspect, the disclosure provides methods of preventing, mitigating, ameliorating, reducing, inhibiting, eliminating and/or reversing one or more symptoms of Danon disease or another autophagy disorder in a subject in need thereof, wherein the method comprises administering to the subject a gene therapy vector of the disclosure. The term “Danon disease” refers to an X-linked dominant skeletal and cardiac muscle disorder with multisystem clinical manifestations. Danon disease mutations lead to an absence of lysosome-associated membrane protein 2 (LAMP-2) protein expression. Major clinical features include skeletal and cardiac myopathy, cardiac conduction abnormalities, cognitive

difficulties, and retinal disease. Men are typically affected earlier and more severely than women.

Cardiac injection may be performed by central vein access, *e.g.*, intrajugular vein. A Swan-Ganz pulmonary artery catheter (PAC) or other PAC may be used to deliver the AAV to the heart.

In an embodiment, the vector is administered via a route selected from the group consisting of parenteral, intravenous, intra-arterial, intracardiac, intracoronary, intramyocardial, intrarenal, intraurethral, epidural, and intramuscular. In an embodiment, the vector is administered multiple times. In an embodiment, the vector is administered by intramuscular injection of the vector. In an embodiment, the vector is administered by injection of the vector into skeletal muscle. In an embodiment, the expression cassette comprises a muscle-specific promoter, optionally a muscle creatine kinase (MCK) promoter or a MCK/SV40 hybrid promoter as described in Takeshita et al. Muscle creatine kinase/SV40 hybrid promoter for muscle-targeted long-term transgene expression. *Int J Mol Med.* 2007 Feb;19(2):309-15. In an embodiment, the vector is administered by intracardiac injection.

In an embodiment, the vector, e.g., AAV vector, is administered systemically, and more particularly, intravenously. Advantageously, the vector is administered at a dose (in vg per mL, vg/kg body mass, or vg/min/kg) less than the dose required to observe the same response when an original or wild-type LAMP-2B sequence is used. In particular embodiments, the vector is an AAV2/9 vector comprising an expression cassette comprising a polynucleotide encoding LAMP-2B disclosed herein.

In some embodiments, the disclosure provides a method of expressing LAMP-2B in a subject, comprising systemically administering an adeno-associated viral (AAV) vector to the subject, wherein the AAV vector comprises an expression cassette comprising a transgene sharing at least 95% identity with SEQ ID NO: 2 or is identical to SEQ ID NO: 2, the transgene operatively linked to an enhancer/promoter region, wherein systemic administration of the AAV vector to the subject results in increased expression of LAMP-2B compared to expression of LAMP-2B prior to administration of the AAV vector or expression of LAMP-2B in an untreated control subject. In some embodiments, the AAV virion is an AAV2/9 vector, which is a vector having an AAV9 capsid and AAV2 ITRs in the

vector genome. In particular embodiments, the expression cassette comprises any of the elements disclosed herein. In some embodiment, systemic administration comprises intravenous administration. In some embodiments, the subject is exhibiting symptoms of Danon disease. In some embodiments, the subject suffers from, or is at risk for, Danon disease.

Systemic (or more particularly intravenous) administration in some embodiments results in expression of LAMP-2B polynucleotide as mRNA, in the form of an mRNA expressed from the transgene, in one or more tissues (*e.g.* heart, muscle, and/or liver) of the subject. In some embodiments, expression of the LAMP-2B polynucleotide as mRNA is increased at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2.0-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 3-fold, or at least about 4-fold in the heart compared to expression in an untreated subject or a subject treated with a control vector. In some embodiments, expression of LAMP-2B polynucleotide as mRNA is increased at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, at least 2.0-fold, at least 2.2-fold, at least 2.3-fold, at least 2.4-fold, at least 2.5-fold, at least 3-fold, or at least 4-fold in the heart compared to expression in an untreated subject or a subject treated with a control vector. In some embodiments, expression of LAMP-2B polynucleotide as mRNA is increased 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 3-fold, or 4-fold in the heart compared to expression in an untreated subject or a subject treated with a control vector.

In some embodiments, expression of LAMP-2B polynucleotide as mRNA is increased at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2.0-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 3-fold, or at least about 4-fold in the muscle compared to expression in an untreated subject or a subject treated with a control vector. In some embodiments, expression of LAMP-2B polynucleotide as mRNA is increased at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, at least 2.0-fold, at least 2.2-fold, at least 2.3-fold, at least 2.4-

fold, at least 2.5-fold, at least 3-fold, or at least 4-fold in the muscle compared to expression in an untreated subject or a subject treated with a control vector. In some embodiments, expression of LAMP-2B polynucleotide as mRNA is increased 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 3-fold, or 4-fold in the muscle compared to expression in an untreated subject or a subject treated with a control vector.

In some embodiments, the LAMP-2B transgene is expressed in the heart and not expressed in the liver of the subject. In some embodiments, expression of LAMP-2B polynucleotide as mRNA is observed to be at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2.0-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 3-fold, or at least about 4-fold in the heart compared to the liver. In some embodiments, expression of LAMP-2B polynucleotide as mRNA is observed to be at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, at least 2.0-fold, at least 2.2-fold, at least 2.3-fold, at least 2.4-fold, at least 2.5-fold, at least 3-fold, or at least 4-fold in the heart compared to the liver. In some embodiments, expression of LAMP-2B polynucleotide as mRNA is observed to be 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 3-fold, or 4-fold in the heart compared to the liver.

In some embodiments, expression of wild-type or functional LAMP-2B protein is increased at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2.0-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 3-fold, or at least about 4-fold in the heart compared to expression in an untreated subject or a subject treated with a control vector. In some embodiments, expression of wild-type or functional LAMP-2B protein is increased at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, at least 2.0-fold, at least 2.2-fold, at least 2.3-fold, at least 2.4-fold, at least 2.5-fold, at least 3-fold, or at least 4-fold in the heart compared to expression in an untreated subject or a subject treated with a control vector. In some embodiments, expression of wild-type or functional LAMP-2B protein is increased 1.2-fold,

1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 3-fold, or 4-fold in the heart compared to expression in an untreated subject or a subject treated with a control vector.

In some embodiments, expression of wild-type or functional LAMP-2B protein is observed to be at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2.0-fold, at least about 2.2-fold, at least about 2.3-fold, or at least 5-fold, in the heart compared to the liver. In some embodiments, expression of wild-type or functional LAMP-2B protein is observed to be at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, at least 2.0-fold, at least 2.2-fold, at least 2.3-fold, at least 2.4-fold, at least 2.5-fold, at least 3-fold, or at least 4-fold in the heart compared to the liver. In some embodiments, expression of wild-type or functional LAMP-2B protein is observed to be 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 3-fold, or 4-fold in the heart compared to the liver.

In some embodiments, administration of the gene therapy vector results in expression of wild-type or functional LAMP-2B protein in the liver of at most about 1.1-fold, at most about 1.2-fold, at most about 1.3-fold, at most about 1.4-fold, at most about 1.5-fold, at most about 1.6-fold, at most about 1.7-fold, at most about 1.8-fold, at most about 1.9-fold, or at most about 2-fold increased compared to expression in the liver of an untreated subject. In some embodiments, administration of the gene therapy vector results in expression of wild-type or functional LAMP-2B protein in the liver of at most 1.1-fold, at most 1.2-fold, at most 1.3-fold, at most 1.4-fold, at most 1.5-fold, at most 1.6-fold, at most 1.7-fold, at most 1.8-fold, at most 1.9-fold, or at most 2-fold increased compared to expression in the liver of an untreated subject. In some embodiments, administration of the gene therapy vector results in expression of wild-type or functional LAMP-2B protein in the liver of 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, or 2-fold increased compared to expression in the liver of an untreated subject.

In an embodiment, the disclosure provides a method of treating a disease or disorder, optionally Danon disease, in a subject in need thereof, comprising contacting cells with a gene therapy vector according to the present disclosure and administering the cells to the subject. In an embodiment, the cells are stem cells, optionally pluripotent stem cells. In an

embodiment, the stem cells are capable of differentiation into cardiac tissue. In an embodiment, the stem cells are capable of differentiation into muscle tissue, *e.g.*, cardiac muscle tissue and/or skeletal muscle tissue. In an embodiment, the stem cells are autologous. In an embodiment, the stem cells are induced pluripotent stem cells (iPSCs).

In an embodiment, the disease or disorder is an autophagy disorder. In some embodiments, the autophagy disorder is selected from the group consisting of, but not limited to, end-stage heart failure, myocardial infarction, drug toxicities, diabetes, end-stage renal failure, and aging. In an embodiment, the subject is a mammal, *e.g.*, a human. In an embodiment, the subject is exhibiting symptoms of Danon disease or another autophagy disorder. In an embodiment, the subject has been identified as having reduced or non-detectable LAMP-2 expression. In an embodiment, the subject has been identified as having a mutated LAMP-2 gene.

Subjects/patients amenable to treatment using the methods described herein include, but are not limited to, individuals at risk of a disease or disorder characterized by insufficient autophagic flux (*e.g.*, Danon disease as well as other known disorders of autophagy including, but not limited to, systolic and diastolic heart failure, myocardial infarction, drug toxicities (for example, anthracyclines chloroquine and its derivatives), diabetes, end-stage renal disease, and aging) but not showing symptoms, as well as subjects presently showing symptoms. Such subject may have been identified as having a mutated LAMP-2 gene or as having reduced or non-detectable levels of LAMP-2 expression.

In some embodiments, the patient is a human. In some embodiments, the patient is a pediatric, adolescent, or adult human. In some embodiments, the patient is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years old, or more than 20 years old. In some embodiments, the patient is 20 to 50 years old. In some embodiments, the patient is 50 to 65 years old. In some embodiments, the patient is 1 to 5, 2 to 6, 3 to 7, 4 to 8, 5 to 9, 6 to 10, 7 to 11, 8 to 12, 9 to 13, 10 to 14, 11 to 15, 12 to 16, 13 to 17, 14 to 18, 15 to 19, or 16 to 20 years old. In some embodiments, the patient is 5 to 6, 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12, 12 to 13, 13 to 14, 14 to 15, 15 to 16, 16 to 17, 17 to 18, 18 to 19, 19 to 20, or 20 to 21 years old. In a particular embodiment, the patient is 15 to 16 years old.

In some embodiments, the patient is a human male. In some embodiments, the patient is a pediatric, adolescent, or adult human male. In some embodiments, the patient is a 1, 2, 3,

4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years old male, or a more than 20 years old male. In some embodiments, the patient is a 20 to 50 years old male. In some embodiments, the patient is a 50 to 65 years old male. In some embodiments, the patient is a 1 to 5, 2 to 6, 3 to 7, 4 to 8, 5 to 9, 6 to 10, 7 to 11, 8 to 12, 9 to 13, 10 to 14, 11 to 15, 12 to 16, 13 to 17, 14 to 18, 15 to 19, or 16 to 20 years old male. In some embodiments, the patient is a 5 to 6, 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12, 12 to 13, 13 to 14, 14 to 15, 15 to 16, 16 to 17, 17 to 18, 18 to 19, 19 to 20, or 20 to 21 year old male. In a particular embodiment, the patient is 15 to 16 years old.

In some embodiments, the patient is a human female. In some embodiments, the patient is a pediatric, adolescent, or adult human female. In some embodiments, the patient is a 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years old female, or a more than 20 years old female. In some embodiments, the patient is a 20 to 50 years old female. In some embodiments, the patient is a 50 to 65 years old female.

In some embodiments, the subject is exhibiting symptoms of a disease or disorder characterized by insufficient autophagic flux (*e.g.*, Danon disease as well as other known disorders of autophagy including, but not limited to, systolic and diastolic heart failure, myocardial infarction, drug toxicities, diabetes, end-stage renal disease, and aging). The symptoms may be actively manifesting, or may be suppressed or controlled (*e.g.*, by medication) or in remission. The subject may or may not have been diagnosed with the disorder, *e.g.*, by a qualified physician.

In some embodiments, the viral vector (*e.g.* AAV vector), or a pharmaceutical composition comprising that vector, is effective when administered systemically. For example, the viral vectors of the disclosure, in some cases, demonstrate efficacy when administered intravenously to subject (*e.g.,* a primate, such as a non-human primate or a human). In some embodiments, the viral vectors of the disclosure are capable of inducing expression of LAMP-2B in various tissues when administered systemically (*e.g.*, in heart, muscle, and/or lung). In particular embodiments, administration of an AAV9 vector comprising a transgene substantially identical to, or identical to, SEQ ID NO: 2 to a subject intravenously results in detectable expression of LAMP-2B in heart tissue. In some embodiments, expression of LAMP-2B is detectable in one or more, or all, of the left ventricle, the right ventricle, the left atrium, and the right atrium of the heart of the subject. In

some embodiments, expression of LAMP-2B is detectable in sub-region 1 and/or sub-region 2 of the left ventricle of the heart of the subject.

"Detectable expression" typically refers to transgene expression at least 5%, 10%, 15%, 20% or more compared to a control subject or tissue not treated with the viral vector. In some embodiments, detectable expression means expression at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, or at least 100-fold greater than a no-vector control. Transgene expression can be determined as the increase over expression of the wild-type or endogenous gene in the cell (accounting for the potential that expression of the transgene may influence expression of the endogenous gene). Transgene expression can also be determined by RT-PCR detection of sequences that are present on the transgene mRNA transcript but not on the mRNA transcript of the endogenous gene. For example, the 3′ UTR of the transcript may be used to determine the expression of the transgene independent of the expression of the endogenous gene (which may have a different 3′ UTR). Expression of the polypeptide encoded by the transgene can be assessed by western blot or enzyme-linked immunosorbent assay (ELISA), as described in the examples that follow, or other methods known in the art. Antibodies cross-reactive to the wild-type and exogenous copies of the protein may be used. In some cases, an antibody specific to the exogenous protein can be identified and used to determine transgene expression. Those of skill in the art can design appropriate detection methodologies taking into account the target cell or tissue. In some cases, expression is measured quantitatively using a standard curve. Standard curves can be generated using purified LAMP-2 protein, by methods described in the examples or known in the art. Alternatively, expression of the transgene can be assessed by quantification of the corresponding mRNA.

As used herein, the terms "vector genome" and "genome copies" refer, interchangeably, to the number of single-stranded AAV genome polynucleotides in a sample. Vector genome copies can be measured using quantitative polymerase chain reaction (qPCR) or digital droplet polymerase chain reaction (ddPCR) using primers specific to the recombinant AAV genome, such as primers flanking the WPRE sequence of the genome. Quantification may be made with respect to a standard curve generated with a reference sample, such as a sample containing plasmid DNA bearing the target amplicon for the primers used. Methods of ddPCR and qPCR are well known in the art. The dose units for preclinical studies are expressed in vector genomes (vg) per kg body weight. The clinical

doses are expressed as vector genome copies (GC) per kg. Both of unit terminologies (GC/kg and vg/kg) are intended to describe the same entity and are used interchangeably in the present disclosure.

In some embodiments, detectable expression of LAMP-2B in heart tissue occurs at doses, in vector genomes (vg) per kilogram weight of subject (kg), of $5x10^{14}$ vg/kg or less, 3×10^{14} vg/kg or less, 2×10^{14} vg/kg or less, 1×10^{14} vg/kg or less, 9×10^{13} vg/kg or less, 8×10^{13} vg/kg or less, 7×10^{13} vg/kg or less, 6×10^{13} vg/kg or less, 5×10^{13} vg/kg or less, 4×10^{13} vg/kg or less, 3×10^{13} vg/kg or less, 2×10^{13} vg/kg or less, or 1×10^{13} vg/kg or less.

In some embodiments, detectable expression of LAMP-2B in heart tissue occurs at doses, in vector genomes (vg) per kilogram weight of subject (kg), of 1×10^{13} vg/kg to 2×10^{13} vg/kg, 2×10^{13} vg/kg to 3×10^{13} vg/kg, 3×10^{13} vg/kg to 4×10^{13} vg/kg, 4×10^{13} vg/kg to 5×10^{13} vg/kg, 5×10^{13} vg/kg to 6×10^{13} vg/kg, 6×10^{13} vg/kg to 7×10^{13} vg/kg, 7×10^{13} vg/kg to 8×10^{13} vg/kg, 8×10^{13} vg/kg to 9×10^{13} vg/kg, 9×10^{13} vg/kg to 1×10^{14} vg/kg, 1×10^{14} vg/kg to 2×10^{14} vg/kg, 2×10^{14} vg/kg to 3×10^{14} vg/kg, or 3×10^{14} vg/kg to $5x10^{14}$ vg/kg.

In some embodiments, detectable expression of LAMP-2B in heart tissue occurs at doses, in vector genomes (vg) per kilogram weight of subject (kg), of 1×10^{13} vg/kg to 3×10^{13} vg/kg, 3×10^{13} vg/kg to 5×10^{13} vg/kg, 5×10^{13} vg/kg to 7×10^{13} vg/kg, 7×10^{13} vg/kg to 9×10^{13} vg/kg, 9×10^{13} vg/kg to 2×10^{14} vg/kg, or 2×10^{14} vg/kg to $5x10^{14}$ vg/kg. In some embodiments, detectable expression of LAMP-2B in heart tissue occurs at doses, in vector genomes (vg) per kilogram weight of subject (kg), of 1×10^{13} vg/kg to 5×10^{13} vg/kg, 5×10^{13} vg/kg to 9×10^{13} vg/kg, 9×10^{13} vg/kg or to $5x10^{14}$ vg/kg. In some embodiments, detectable expression of LAMP-2B in heart tissue occurs at doses, in vector genomes (vg) per kilogram weight of subject (kg), of 1×10^{13} vg/kg to 9×10^{13} vg/kg, or 9×10^{13} vg/kg or to $5x10^{14}$ vg/kg.

In some embodiments, detectable expression of LAMP-2B in heart tissue occurs at doses, in vector genomes (vg) per kilogram weight of subject (kg), of 1×10^{13} vg/kg to 5×10^{13} vg/kg, 5×10^{13} vg/kg to 1×10^{14} vg/kg, or 1×10^{14} vg/kg to $5x10^{14}$ vg/kg.

In some embodiments, detectable expression of LAMP-2B in heart tissue occurs at doses, in vector genomes (vg) per kilogram weight of subject (kg), of 1×10^{13} vg/kg to $5x10^{14}$ vg/kg. In some embodiments, detectable expression of LAMP-2B in heart tissue occurs at doses, in vector genomes (vg) per kilogram weight of subject (kg), of 1×10^{13} vg/kg to 1×10^{14}.

Co-Administration

Safety and/or efficacy may be increase, in some cases, by co-administration of one or more secondary agents, including but not limited to immunomodulatory agents.

In some embodiments, the method comprises administering to the subject an effective amount of corticosteroid, including without limitation dexamethasone, methylprednisolone, or prednisone. Appropriate dosages and dose regimens of corticosteroid for administration of AAV therapy are known in the art. *See* Diehl et al. Cell. & Mol. Immunol. 14, 146–179 (2017).

In some embodiments, the method comprises administering to the subject an effective amount of tacrolimus, cyclosporine, rapamycin, sirolimus, or a derivative thereof. In some embodiments, the method further comprises administering to the subject an effective amount of corticosteroids prior to administering the effective amount of tacrolimus. As disclosed herein, tacrolimus may in some cases permit more rapid taper of corticosteroid levels after administrating to the subject of the AAV. Tacrolimus may be administered 7-21 days prior to the AAV, *e.g.* 21 days, 14 days, 10 days, 7 days, 5 days, 2 days, or 1 day before the AAV, preferably 1 day before. Tacrolimus administration may be continued after administration of the AAV. Tacrolimus may be administered for the duration of 120 days subsequent to the AAV, *e.g.* 1 day, 5 days, 10 days, 20 days, 30 days, 40 days, 50 days, 60 days, 70 days, 80 days, 90 days, 100 days, 110 days, or for 120 days after the AAV, preferably for 90 days after. *See* Tardieu et al. *Hum. Gen. Ther.* 25(6):506-516 (2014).

Tacrolimus may be administered at a dose of 0.01 mg/kg, 0.05 mg/kg, 0.1 mg.kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, or 0.6 mg/kg. Tacrolimus may be administered concurrently with 700 mg/m2, 800 mg/m2, 900 mg/m2, 1000 mg/m2, 1100 mg/m2, 1200 mg/m2, 1300 mg/m2, 1400 mg/m2, 1500 mg/m2, 1600 mg/m2, or 1700 mg/m2 mycophenolate mofetil.

In some embodiments, the method comprises administering to the subject 0.2 mg/kg tacrolimus concurrently with 1200 mg/m2 mycophenolate mofetil.

Tacrolimus may be administered per oral daily in 2 divided doses. Tacrolimus may be administered at an effective amount to maintain trough serum levels of 2 ng/mL, 2.5 ng/mL, 3 ng/mL, 3.5 ng/ml, 4 ng/mL, 4.5 ng/mL, or 5 ng/mL. Alternatives to tacrolimus include,

without limitation mycophenolate, cyclosporine, cyclosporine modified, sirolimus, everolimus, or belatacept.

In some embodiments, the method comprises administering to the subject an effective amount of rituximab. As disclosed herein, rituximab may in some cases reduce and/or prevent an immune response to the AAV. Rituximab may be administered 7-21 days prior to the AAV, *e.g.* 21 days, 14 days, 10 days, 7 days, 5 days, 2 days, or 1 day before the AAV, preferably 1 day before. Rituximab may be administered 7-21 days after to the AAV, *e.g.* 21 days, 14 days, 10 days, 7 days, 5 days, 2 days, or 1 day after the AAV, preferably 1 day after. Rituximab may be administration 1, 2, 3, or more times to the subject. *See* Corti et al. Hum. Gene Ther. Clin. Dev. 28(4):208-218 (2017) and Corti et al. Mol. Ther. Meth. Clin. Dev. 1, 14033 (2014).

Rituximab may be administered at a dose of 300 mg/m2, 400 mg/m2, 500 mg/m2, 600 mg/m2, 700 mg/m2, 750 mg/m2, 800 mg/m2, 900 mg/m2, 1000 mg/m2, 1100 mg/m2, or 1200 mg/m2, preferably 750 mg/m2. Alternatives to ritixumab include, without limitation obinutuzumab, Tuxima (rituximab-abbs), or lenalidomide.

Tacrolimus may be administered before rituximab. Tacrolimus may be administered concurrently with rituximab. Tacrolimus may be administered after rituximab. Rituximab administration may be discontinued while tacrolimus administration is continued.

In some embodiments, rituximab is administered on Days -14 and -7 prior to administration of AAV and tacrolimus is administered beginning Day -7 prior to administration of AVV through 3 months following administration of AAV.

In some embodiments, the method comprises administering to the subject an effective amount of eculizumab, ravulizumab, or another complement inhibitor. Methods of treatment with complement inhibitors are known in the art. *See* Zipfel et al. Front. Immunol. (2019). Eculizumab is approved for treatment of atypical hemolytic-uremic syndrome (aHUS).

In some embodiments, the subject is at risk for sequelae of complement activation, such as atypical hemolytic-uremic syndrome (aHUS), optionally aHUS resulting in reversible thrombocytopenia and/or acute kidney injury (AKI).

In some embodiments, the method further comprises administering to the subject an effective amount of rituximab; administering to the subject an effective amount of tacrolimus; and/or administering to the subject an effective amount of eculizumab.

Various corticosteroids known in the art may be used. In some embodiments, the method comprises administering to the subject an effective amount of dexamethasone, methylprednisolone, bethamethasone, prednisone, prednisolone, triamcinolone, hydrocortisone, cortisone, fludrocortisone, or a combination thereof.

Pharmaceutical Compositions and Dosages

In another aspect, the disclosure provides pharmaceutical compositions. In various embodiments, the pharmaceutical compositions contain vehicles (*e.g.*, carriers, diluents and excipients) that are pharmaceutically acceptable for a formulation capable of being injected. Exemplary excipients include a poloxamer. Formulation buffers for viral vectors (including AAV) general contains salts to prevent aggregation and other excipients (*e.g.* poloxamer) to reduce stickiness of the vector. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. Advantageously, the formulation is stable for storage and use when frozen (*e.g.* at less than 0°C, about -60°C, or about -72°C).

In some embodiments, the pharmaceutical compositions comprises a buffer (*e.g.*, a phosphate buffer) at a suitable concentration (*e.g.*, 200 mM) and pH (*e.g.*, pH 7.2 ±0.1) for administration to a subject. The pharmaceutical composition may include Poloxamer at a suitable concentration (*e.g.*, 0.01%.). The pharmaceutical composition may be provided at the site of treatment0 as a frozen product. The final volume of the unit dose of the AAV may be determined, in whole or in part, on the patient weight, *e.g.*, in kilograms (kg), and the calculated or experimentally determined level of vector genome (vg) copies of the AAV per volume, *e.g.*, milliliter (mL), or the pharmaceutical composition. The pharmaceutical composition may be diluted as necessary to obtain a desired concentration or volume for injection.

In some embodiments, the pharmaceutical composition comprises 200 mM NaCl, 10 mM NaH_2PO4, 1% (w/v) sucrose, 0.01% Poloxamer 188, pH 7.2 ±0.1.

In some embodiments, the AAV vector is administered at a dose of between about 1×10^{12} and about 5×10^{14} vector genomes (vg) of the AAV vector per kilogram (vg) of total body mass of the subject (vg/kg). In some embodiments, the AAV vector is administered at a dose of between about 1×10^{13} and about 5×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of between about 5×10^{13} and about 3×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of between about 5×10^{13} and about 1×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of less than about 1×10^{12} vg/kg, less than about 3×10^{12} vg/kg, less than about 5×10^{12} vg/kg, less than about 7×10^{12} vg/kg, less than about 1×10^{13} vg/kg, less than about 3×10^{13} vg/kg, less than about 5×10^{13} vg/kg, less than about 7×10^{13} vg/kg, less than about 1×10^{14} vg/kg, less than about 3×10^{14} vg/kg, or less than about 5×10^{14} vg/kg.

In some embodiments, the AAV vector is administered at a dose of between about 6.7×10^{13} and 2×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of between about 6.7×10^{13} and about 1.1×10^{14} vg/kg.

In some embodiments, the AAV vector is administered at a dose of about 1×10^{13} vg/kg, about 3×10^{13} vg/kg, about 5×10^{13} vg/kg, about 7×10^{13} vg/kg, about 1×10^{14} vg/kg, about 3×10^{14} vg/kg, or about 5×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of about 6.7×10^{13} vg/kg, about 1.1×10^{13} vg/kg, or about 2.0×10^{13} vg/kg.

In some embodiments, the AAV vector is administered at a dose of 1×10^{12} vg/kg, 3×10^{12} vg/kg, 5×10^{12} vg/kg, 7×10^{12} vg/kg, 1×10^{13} vg/kg, 3×10^{13} vg/kg, 5×10^{13} vg/kg, 7×10^{13} vg/kg, 1×10^{14} vg/kg, 3×10^{14} vg/kg, 5×10^{14} vg/kg, 7×10^{14} vg/kg, 1×10^{15} vg/kg, 3×10^{15} vg/kg, 5×10^{15} vg/kg, or 7×10^{15} vg/kg. In some embodiments, the AAV vector is administered at a dose of 6.7×10^{13} vg/kg, 1.1×10^{13} vg/kg, or 2.0×10^{13} vg/kg.

In some embodiments, the AAV vector is administered systemically at a dose of between about 1×10^{13} and 5×10^{14} vector genomes (vg) of the AAV vector per kilogram (vg) of total body mass of the subject (vg/kg). In some embodiments, the AAV vector is administered systemically at a dose of between about 1×10^{13} and 5×10^{14} vg/kg. In some

embodiments, the AAV vector is administered systemically at a dose of between about 5×10^{13} and 3×10^{14} vg/kg. In some embodiments, the AAV vector is administered systemically at a dose of between about 5×10^{13} and 1×10^{14} vg/kg. In some embodiments, the AAV vector is administered systemically at a dose of less than about less than about 1×10^{13} vg/kg, less than about 3×10^{13} vg/kg, less than about 5×10^{13} vg/kg, less than about 7×10^{13} vg/kg, less than about 1×10^{14} vg/kg, less than about 3×10^{14} vg/kg, or less than about 5×10^{14} vg/kg.

In some embodiments, the AAV vector is administered systemically at a dose of between about 6.7×10^{13} and 2×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of between about 6.7×10^{13} and about 1.1×10^{14} vg/kg.

In some embodiments, the AAV vector is administered systemically at a dose of about 1×10^{13} vg/kg, about 3×10^{13} vg/kg, about 5×10^{13} vg/kg, about 7×10^{13} vg/kg, about 1×10^{14} vg/kg, about 3×10^{14} vg/kg, or about 5×10^{14} vg/kg. In some embodiments, the AAV vector is systemically administered at a dose of about 6.7×10^{13} vg/kg, about 1.1×10^{13} vg/kg, or about 2.0×10^{13} vg/kg.

In some embodiments, the AAV vector is administered systemically at a dose of 1×10^{13} vg/kg, 3×10^{13} vg/kg, 5×10^{13} vg/kg, 7×10^{13} vg/kg, 1×10^{14} vg/kg, 3×10^{14} vg/kg, or 5×10^{14} vg/kg. In some embodiments, the AAV vector is systemically administered at a dose of 6.7×10^{13} vg/kg, 1.1×10^{13} vg/kg, or 2.0×10^{13} vg/kg.

In some embodiments, the AAV vector is administered intravenously at a dose of between about 1×10^{13} and 5×10^{14} vector genomes (vg) of the AAV vector per kilogram (vg) of total body mass of the subject (vg/kg). In some embodiments, the AAV vector is administered intravenously at a dose of between about 1×10^{13} and 5×10^{14} vg/kg. In some embodiments, the AAV vector is administered intravenously at a dose of between about 5×10^{13} and 3×10^{14} vg/kg. In some embodiments, the AAV vector is administered intravenously at a dose of between about 1×10^{13} and 1×10^{14} vg/kg. In some embodiments, the AAV vector is administered intravenously at a dose of less than about less than about 1×10^{13} vg/kg, less than about 3×10^{13} vg/kg, less than about 5×10^{13} vg/kg, less than about 7×10^{13} vg/kg, less than about 1×10^{14} vg/kg, less than about 3×10^{14} vg/kg, or less than about 5×10^{14} vg/kg.

In some embodiments, the AAV vector is administered intravenously at a dose of between about 6.7×10^{13} and 2×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of between about 6.7×10^{13} and about 1.1×10^{14} vg/kg.

In some embodiments, the AAV vector is administered intravenously at a dose of about 1×10^{13} vg/kg, about 3×10^{13} vg/kg, about 5×10^{13} vg/kg, about 7×10^{13} vg/kg, about 1×10^{14} vg/kg, about 3×10^{14} vg/kg, or about 5×10^{14} vg/kg. In some embodiments, the AAV vector is intravenously administered at a dose of about 6.7×10^{13} vg/kg, about 1.1×10^{13} vg/kg, or about 2.0×10^{13} vg/kg.

In some embodiments, the AAV vector is administered intravenously at a dose of 1×10^{13} vg/kg, 3×10^{13} vg/kg, 5×10^{13} vg/kg, 7×10^{13} vg/kg, 1×10^{14} vg/kg, 3×10^{14} vg/kg, or 5×10^{14} vg/kg. In some embodiments, the AAV vector is intravenously administered at a dose of 6.7×10^{13} vg/kg, 1.1×10^{13} vg/kg, or 2.0×10^{13} vg/kg.

In some embodiments, a therapeutically effective amount of the AAV virion is between about 1×10^{12} and about 5×10^{14} vector genomes (vg) of the AAV virion per kilogram (vg) of total body mass of the subject (vg/kg). In some embodiments, the therapeutically effective amount is between about 1×10^{13} and about 5×10^{14} vg/kg. In some embodiments, the therapeutically effective amount is between about 5×10^{13} and about 3×10^{14} vg/kg. In some embodiments, the therapeutically effective amount is between about 5×10^{13} and about 1×10^{14} vg/kg. In some embodiments, the therapeutically effective amount is less than about 1×10^{12} vg/kg, less than about 3×10^{12} vg/kg, less than about 5×10^{12} vg/kg, less than about 7×10^{12} vg/kg, less than about 1×10^{13} vg/kg, less than about 3×10^{13} vg/kg, less than about 5×10^{13} vg/kg, less than about 7×10^{13} vg/kg, less than about 1×10^{14} vg/kg, less than about 3×10^{14} vg/kg, or less than about 5×10^{14} vg/kg.

In some embodiments, the therapeutically effective amount of the AAV virion is between about 6.7×10^{13} and 2×10^{14} vg/kg. In some embodiments, the AAV virion is administered at a dose of between about 6.7×10^{13} and about 1.1×10^{14} vg/kg.

In some embodiments, the therapeutically effective amount of the AAV virion is about 1×10^{13} vg/kg, about 3×10^{13} vg/kg, about 5×10^{13} vg/kg, about 7×10^{13} vg/kg, about 1×10^{14} vg/kg, about 3×10^{14} vg/kg, or about 5×10^{14} vg/kg. In some embodiments, the

therapeutically effective amount is about 6.7×10^{13} vg/kg, about 1.1×10^{13} vg/kg, or about 2.0×10^{13} vg/kg.

In some embodiments, the therapeutically effective amount of the AAV virion is 1×10^{12} vg/kg, 3×10^{12} vg/kg, 5×10^{12} vg/kg, 7×10^{12} vg/kg, 1×10^{13} vg/kg, 3×10^{13} vg/kg, 5×10^{13} vg/kg, 7×10^{13} vg/kg, 1×10^{14} vg/kg, 3×10^{14} vg/kg, 5×10^{14} vg/kg, 7×10^{14} vg/kg, 1×10^{15} vg/kg, 3×10^{15} vg/kg, 5×10^{15} vg/kg, or 7×10^{15} vg/kg. In some embodiments, the therapeutically effective amount is 6.7×10^{13} vg/kg, 1.1×10^{13} vg/kg, or 2.0×10^{13} vg/kg.

Definitions

The terms "lysosome-associated membrane protein 2" and "LAMP-2" interchangeably refer to nucleic acids and polypeptide polymorphic variants, alleles, mutants, and interspecies homologs that: (1) have an amino acid sequence that has greater than about 90% amino acid sequence identity, for example, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200, 300, 400, or more amino acids, or over the full-length, to an amino acid sequence encoded by a LAMP-2 nucleic acid (*see, e.g.*, GenBank Accession Nos. NM_002294.2 (isoform A). NM_013995.2 (isoform B), NM_001122606.1 (isoform C)) or to an amino acid sequence of a LAMP-2 polypeptide (*see e.g.*, GenBank Accession Nos. NP_002285.1 (isoform A), NP_054701.1 (isoform B), NP_001116078.1 (isoform C)); (2) bind to antibodies, *e.g.*, polyclonal antibodies, raised against an immunogen comprising an amino acid sequence of a LAMP-2 polypeptide (*e.g.*, LAMP-2 polypeptides described herein); or an amino acid sequence encoded by a LAMP-2 nucleic acid (*e.g.*, LAMP-2 polynucleotides described herein), and conservatively modified variants thereof; (3) specifically hybridize under stringent hybridization conditions to an anti-sense strand corresponding to a nucleic acid sequence encoding a LAMP-2 protein, and conservatively modified variants thereof; (4) have a nucleic acid sequence that has greater than about 90%, preferably greater than about 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher nucleotide sequence identity, preferably over a region of at least about 25, 50, 100, 200, 500, 1000, 2000 or more nucleotides, or over the full-length, to a LAMP-2 nucleic acid (*e.g.*, LAMP-2 polynucleotides, as described herein, and LAMP-2 polynucleotides that encode LAMP-2 polypeptides, as described herein).

The terms "lysosome-associated membrane protein 2B" and "LAMP-2B" interchangeably refer to nucleic acids and polypeptide polymorphic variants, alleles, mutants, and interspecies homologs that: (1) have an amino acid sequence that has greater than about 90% amino acid sequence identity, for example, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200, 300, 400, or more amino acids, or over the full-length, to an amino acid sequence encoded by a LAMP-2B nucleic acid (*see e.g.,* NM_013995.2) or to an amino acid sequence of a LAMP-2B polypeptide (*see e.g.*, NP_054701.1); (2) bind to antibodies, *e.g.*, polyclonal antibodies, raised against an immunogen comprising an amino acid sequence of a LAMP-2B polypeptide (*e.g.*, LAMP-2B polypeptides described herein); or an amino acid sequence encoded by a LAMP-2B nucleic acid (*e.g.*, LAMP-2B polynucleotides described herein), and conservatively modified variants thereof; (3) specifically hybridize under stringent hybridization conditions to an anti-sense strand corresponding to a nucleic acid sequence encoding a LAMP-2B protein, and conservatively modified variants thereof; (4) have a nucleic acid sequence that has greater than about 90%, preferably greater than about 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher nucleotide sequence identity, preferably over a region of at least about 25, 50, 100, 200, 500, 1000, 2000 or more nucleotides, or over the full-length, to a LAMP-2B nucleic acid (*e.g.*, LAMP-2B polynucleotides, as described herein, and LAMP-2B polynucleotides that encode LAMP-2B polypeptides, as described herein).

The term "functional variant" in respect to a protein (*e.g.* a LAMP-2B) refers to a polypeptide sequence, or a fragment of a polypeptide sequence having at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, or at least about 80 amino acid resides, that retains one or more functional attributes of the protein. For example, a functional variant of LAMP-2B is a LAMP-2B (as defined herein) that retains one or more functions such as: (1) regulating human cardiomyocyte function (Chi et al. (2019) *PNAS USA* 116 (2) 556-565); (2) improving metabolic and physiological function in Danon disease (Adler et al. (2019) *J. Am. College Cardiology* S0735-1097(19)31295-1); and/or (3) autophagy (Rowland et al. (2016) *J. Cell Sci.* (2016) 129, 2135-2143).

LAMP-2B has a lumenal domain (residues 29-375), a transmembrane domain (residues 376-399), and a cytoplasmic domain (residues 400-410), *see* UniProt Accession No. P13473. LAMP-2B functions in include chaperone-mediated autophagy, a process that

mediates lysosomal degradation of proteins in response to various stresses and as part of the normal turnover of proteins with a long biological half-live (Cuervo et al. *Science* 273:501-503(1996), Cuervo et al. *J. Cell Sci.* 113:4441-4450(2000), Bandyopadhyay et al. *Mol. Cell. Biol.* 28:5747-5763(2008), Li et al. *Exp. Cell Res.* 327:48-56(2014), Hubert et al. *Biol. Open* 5:1516-1529(2016)). LAMP-2B may target GAPDH and MLLT11 for lysosomal degradation. LAMP-2B may be required for the fusion of autophagosomes with lysosomes during autophagy. It has been suggested that cells that lack LAMP2 express normal levels of VAMP8, but fail to accumulate STX17 on autophagosomes, which is the most likely explanation for the lack of fusion between autophagosomes and lysosomes. LAMP-2B may be required for normal degradation of the contents of autophagosomes. LAMP-2B may be required for efficient MHCII-mediated presentation of exogenous antigens via its function in lysosomal protein degradation; antigenic peptides generated by proteases in the endosomal/lysosomal compartment are captured by nascent MHCII subunits (Crotzer et al. *Immunology* 131:318-330(2010)).

Functional variants of LAMP-2B therefore include fragments of LAMP-2B that are capable of mediating any of the foregoing functions. In some embodiments, the function fragment of LAMP-2B includes one or more of the lumenal, transmembrane, and cytoplasmic domains. In some embodiments, the functional variant of LAMP-2B comprises one or more C-terminal or N-terminal deletions with respect to native LAMP-2B. In some embodiments, the functional variant of LAMP-2B comprises one or more internal deletions with respect to native LAMP-2B.

The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.* , share at least about 80% identity, for example, at least about 85%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity over a specified region to a reference sequence, *e.g.*, LAMP-2 polynucleotide or polypeptide sequence as described herein, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be “substantially identical.” This definition also refers to the compliment of a test sequence. Preferably, the identity exists over a region that is at least about 25 amino acids or nucleotides in length, for

example, over a region that is 50, 100, 200, 300, 400 amino acids or nucleotides in length, or over the full-length of a reference sequence.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. For sequence comparison of nucleic acids and proteins to LAMP-2 nucleic acids and proteins, the BLAST and BLAST 2.0 algorithms and the default parameters are used.

A "comparison window," as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wl), or by manual alignment and visual inspection (see, *e.g.*, Ausubel et al., eds., Current Protocols in Molecular Biology (1995 supplement)). Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., J. Mol. Biol. 215:403-410 (1990) and Altschul et al., Nucleic Acids Res. 25:3389-3402 (1977), respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (on the worldwide web at ncbi.nlm.nih.gov/).

An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross

reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

As used herein, "administering" refers to local and systemic administration, *e.g.*, including enteral, parenteral, pulmonary, and topical/transdermal administration. Routes of administration for compounds (*e.g.*, polynucleotide encoding one or more LAMP- 2 isoforms) that find use in the methods described herein include, *e.g.*, oral (per os (P.O.)) administration, nasal or inhalation administration, administration as a suppository, topical contact, transdermal delivery (*e.g.*, via a transdermal patch), intrathecal (IT) administration, intravenous ("iv") administration, intraperitoneal ("ip") administration, intramuscular ("im") administration, intralesional administration, or subcutaneous ("sc") administration, or the implantation of a slow-release device *e.g.*, a mini-osmotic pump, a depot formulation, etc. , to a subject. Administration can be by any route including parenteral and transmucosal (*e.g.*, oral, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.*, intravenous, intramuscular, intraarterial, intrarenal, intraurethral, intracardiac, intracoronary, intramyocardial, intradermal, epidural, subcutaneous, intraperitoneal, intraventricular, ionophoretic and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc.

The term "correction" refers to a change in a clinical parameter relative to a baseline level in the subject that causes the parameter normalize to a level equal to or approximately equal to the level of that parameter observed in a person that does not have Danon disease.

The term "improvement" refers to a change in a clinical parameter relative to a baseline level in the subject that causes the parameter increase (or decrease) to a level substantially greater than (or less than) the level of that parameter observed in the subject prior to administration of treatment. For example, an improvement may include reduction in size or number of autophagic vacuoles in the heart of the subject.

The terms “systemic administration” and “systemically administered” refer to a method of administering a compound or composition to a mammal so that the compound or composition is delivered to sites in the body, including the targeted site of pharmaceutical action, via the circulatory system. Systemic administration includes, but is not limited to, oral, intranasal, rectal and parenteral (*e.g.*, other than through the alimentary tract, such as intramuscular, intravenous, intra-arterial, transdermal and subcutaneous) administration.

The term “co-administering” or “concurrent administration”, when used, for example with respect to the compounds (*e.g.*, LAMP-2 polynucleotides) and/or analogs thereof and another active agent, refers to administration of the compound and/or analogs and the active agent such that both can simultaneously achieve a physiological effect. The two agents, however, need not be administered together. In certain embodiments, administration of one agent can precede administration of the other. Simultaneous physiological effect need not necessarily require presence of both agents in the circulation at the same time. However, in certain embodiments, co-administering typically results in both agents being simultaneously present in the body (*e.g.*, in the plasma) at a significant fraction (*e.g.*, 20% or greater, *e.g.*, 30% or 40% or greater, *e.g.*, 50% or 60% or greater, *e.g.*, 70% or 80% or 90% or greater) of their maximum serum concentration for any given dose.

The term “therapeutically effective amount” refers to the amount and/or dosage, and/or dosage regime of a gene therapy vector necessary to bring about the desired result *e.g.*, increased expression of one or more LAMP-2 isoforms in an amount sufficient to reduce the ultimate severity of a disease characterized by impaired or deficient autophagy (*e.g.*, Danon disease).

The term “effective amount” refers to the amount and/or dosage, and/or dosage regime of a gene therapy vector necessary to bring about the desired result, *e.g.*, the immunosuppressive effect of an immunosuppresive drug.

The phrase “cause to be administered” refers to the actions taken by a medical professional (*e.g.*, a physician), or a person controlling medical care of a subject, that control and/or permit the administration of the agent(s)/compound(s) at issue to the subject. Causing to be administered can involve diagnosis and/or determination of an appropriate therapeutic or prophylactic regimen, and/or prescribing particular agent(s)/compounds for a subject. Such

prescribing can include, for example, drafting a prescription form, annotating a medical record, and the like.

As used herein, the terms "treating" and "treatment" refer to delaying the onset of, retarding or reversing the progress of, reducing the severity of, or alleviating or preventing either the disease or condition to which the term applies, or one or more symptoms of such disease or condition. The terms "treating" and "treatment" also include preventing, mitigating, ameliorating, reducing, inhibiting, eliminating and/or reversing one or more symptoms of the disease or condition.

The term "mitigating" refers to reduction or elimination of one or more symptoms of that pathology or disease, and/or a reduction in the rate or delay of onset or severity of one or more symptoms of that pathology or disease, and/or the prevention of that pathology or disease. In certain embodiments, the reduction or elimination of one or more symptoms of pathology or disease can include, *e.g.*, measurable and sustained increase in the expression levels of one or more isoforms of LAMP-2.

As used herein, the phrase "consisting essentially of refers to the genera or species of active pharmaceutical agents recited in a method or composition, and further can include other agents that, on their own do not have substantial activity for the recited indication or purpose.

The terms "subject," "individual," and "patient" interchangeably refer to a human subject.

The terms "gene transfer" or "gene delivery" refer to methods or systems for reliably inserting foreign DNA into host cells. Such methods can result in transient expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (*e.g.* episomes), or integration of transferred genetic material into the genomic DNA of host cells.

The term "vector" is used herein (when appearing alone) to refer to a nucleic acid molecule capable transferring or transporting another nucleic acid molecule. The transferred nucleic acid is generally linked to, *e.g.*, inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication or reverse transcription in a cell, or may include sequences sufficient to allow integration into host cell DNA. "Vectors"

include gene therapy vectors. As used herein, the term "gene therapy vector" refers to a vector (such as an AAV virion) capable of use in performing gene therapy, *e.g.*, delivering a polynucleotide sequence encoding a therapeutic polypeptide to a subject. Gene therapy vectors may comprise a nucleic acid molecule ("transgene") encoding a therapeutically active polypeptide, *e.g.*, a LAMP-2B or other gene useful for replacement gene therapy when introduced into a subject. Useful vectors include, but are not limited to, viral vectors. The terms "AAV vector" and "AAV virion" are used interchangeably herein to refer to a vector genome packaged into an AAV capsid.

As used herein, the term "expression cassette" refers to a DNA segment that is capable in an appropriate setting of driving the expression of a polynucleotide (a "transgene") encoding a therapeutically active polypeptide (*e.g.*, LAMP-2B) that is incorporated in said expression cassette. When introduced into a host cell, an expression cassette *inter alia* is capable of directing the cell's machinery to transcribe the transgene into RNA, which is then usually further processed and finally translated into the therapeutically active polypeptide. The expression cassette can be comprised in a gene therapy vector. Generally, the term expression cassette excludes polynucleotide sequences 5′ to the 5′ ITR and 3′ to the 3′ ITR.

All patents, patent publications, and other publications referenced and identified in the present specification are individually and expressly incorporated herein by reference in their entirety for all purposes.

EXAMPLES

EXAMPLE 1: Recombinant AAV9 Vector Expressing LAMP2B

Recombinant AAV9 vector expressing LAMP2B was generated through a 3-plasmid, helper virus-free system. Transient transfection of pAAV-LAMP2B transfer plasmid, pAAV-2/9 packaging plasmid, and pAd-Helper adenovirus helper plasmid into HEK293T producer cells generated recombinant AAV particles containing serotype 9 capsid proteins and AAV2 ITRs flanking a human LAMP2B expression cassette (AAV9.LAMP2B).

The structure of the AAV cis transfer plasmid (pAAV-LAMP2B) contains the transgene expression cassette flanked by viral ITR regions derived from AAV2 as depicted in **FIG. 1**. The expression cassette contains the human LAMP2B coding sequence driven by a chimeric promoter containing the CMV IE enhancer (CMV IEE), chicken β-actin (CBA)

promoter, chimeric chicken β-actin and rabbit globin intron. The expression cassette also includes a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) and is terminated by the rabbit globin polyA signal (RGpA).

EXAMPLE 2: Preclinical In Vitro and In Vivo Efficacy Studies

In vitro studies were conducted in iPSC-derived cardiomyocytes from a patient with Danon Disease (DD) indicated a dose-dependent increase in LAMP2B expression and a beneficial effect on mitochondrial membrane potential, a key cellular feature of cardiomyocytes in DD. After demonstration of *in vitro* phenotypic correction, *in vivo* studies of LAMP-2B gene therapy were performed in a clinically relevant calorically-restricted LAMP2 knockout (KO) mouse model.

Lamp2 KO mice were intravenously injected with phosphate buffered saline (PBS) or AAV9.LAMP2B at doses of 1×10^{13}, 5×10^{13}, and 1×10^{14} vg/kg at 2 months of age and subjected to 6-weeks alternate fasting prior to their evaluation at 3 months post-treatment. As shown in **FIGs. 2** and **3**, dose-dependent increases in human LAMP2B and reductions in LC3-II (a marker of autophagic flux) were observed in organs particularly affected in DD including the heart, liver, and skeletal muscle of the AAV9-treated KO mice.

As shown in **FIG. 4**, AAV9.LAMP2B administration resulted in improved cardiac ultrastructure, including fewer visible autophagic vacuoles in Lamp2 KO mice treated at doses of 5×10^{13} and 1×10^{14} vg/kg 3 months after treatment. Cardiac ultrastructure from the Lamp2 KO mice cohort dosed at 1×10^{14} vg/kg was significantly improved and similar to the control WT animals. Conversely, KO mice that received only PBS showed an increased number of vacuoles in their cardiac tissue. To examine cardiac function, invasive hemodynamics were performed prior to study termination. As shown in FIG. 11, contractility was evaluated by invasive left intraventricular pressure (dP/dt max and dP/dt min) and found to be significantly decreased in untreated PBS-control Lamp2 KO mice compared to WT controls. Average dP/dt max and dP/dt min values in WT mice were 7050 and -5550 mmHg/s, respectively. In LAMP2 KO mice, average dP/dt max and dP/dt min values were: 3656 and -3265 mmHg/s for PBS (n=7), and 4197 and -3542 mmHg/s for 1×10^{13} vg/kg (n=7), 5073 and -3905 mmHg/s for 5×10^{13} vg/kg (n=12), and 4729 and -3765 mmHg/s for 1×10^{14} vg/kg (n=11) AAV9.LAMP2B treated groups.

As shown in FIG. 12, AAV9.LAMP2B significantly improved the ultrastructure in hearts of adult Lamp2 KO mice. As shown in FIG. 13, a significant improvement in cardiac function was demonstrated by invasive hemodynamics at the 3-month time-point, and improvement was dose-dependent through the highest dose tested of 2×10^{14} vg/kg. At this highest dose, cardiac hemodynamics were comparable to the wild-type phenotype, thereby suggesting maximum possible efficacy and likely optimal efficacious dose. A significant reduction in hepatic enzymes suggested improved liver abnormality was also observed in the AAV9-dose animals. Results from this study indicated that AAV9.LAMP2B mediated gene therapy was safe and was successful at reversing the established disease phenotype by improving cardiac physiology, autophagic flux, and liver abnormality in older mice at an efficacious dose of 2×10^{14} vg/kg.

EXAMPLE 3: Preclinical Pharmacology

Safety measurements including clinical pathology, histopathology, and immune response to LAMP-2B gene therapy were performed in Lamp2 KO mice. A serum chemistry panel showed no adverse outcomes in mice that were either PBS controls or dosed with AAV9.LAMP2B vector. A significant increase in serum potassium (K+) levels was noted in the untreated PBS control KO mice relative to WT animals. These K+ levels were significantly reduced, returning to the expected range in mice treated with 5×10^{13} vg/kg, 1×10^{14} vg/kg and 2×10^{14} vg/kg of AAV9.LAMP2B compared to PBS-injected Lamp2 KO control mice. Elevated serum potassium in the setting of normal serum creatine and blood urea nitrogen levels may result from excessive muscle breakdown (Lehnhardt 2011). The observed reduction in serum potassium with AAV9.LAMP2B administration therefore shows a reduced myopathy in LAMP-2B gene therapy treated mice, an important feature of DD. Histopathology of the heart, liver, and skeletal muscle tissue showed no noteworthy changes to the AAV9.LAMP2B-treated samples. Biodistribution of vector was examined by qPCR, which revealed significant distribution among systemic organs with highest levels of vector genomes (vg) noted in liver with 10-fold less vg in heart and skeletal muscle and 100-fold less in brain compared to liver. The spleen and gonads exhibited the lowest amount of vector genome distribution. Quantitation of transgene expression by mRNA estimation was examined in a subset of tissues using RT-qPCR in mice dosed with 1×10^{14} vg/kg and 2×10^{14} vg/kg of AAV9.LAMP2B. On average, heart had the highest level of human LAMP2B mRNA followed closely by levels in liver and skeletal muscle. Brain, lungs, kidneys, and gonads also showed moderate levels of human

LAMP2B mRNA, and very low levels were detected in the spleen at the 6-month timepoint post dosing.

The preclinical studies described herein were performed according to good laboratory practice (GLP) showed no unexpected mortality, physical, behavioral, morphologic, hematologic or biochemical abnormalities associated with doses up to 3×10^{14} vg/kg. Results from this study showed that AAV9.LAMP2B-mediated gene therapy demonstrated safety and durable benefit in the DD phenotype in Lamp2 KO mice. This study demonstrates a safe and effective profile for IV (intravenous) administration of LAMP-2B gene therapy and indicated minimal efficacious dose of AAV9.LAMP2B to be in the range of 5×10^{13} to 1×10^{14} vg/kg, demonstrating LAMP-2B gene therapy's clinical potential in humans for the treatment of Danon disease.

EXAMPLE 4: Preclinical Toxicology

Additionally, a 102-day non-GLP study was performed in 2-year-old non-human primates (NHPs) (cynomolgus monkeys) treated with the therapeutic vector at the highest dose level tested in the GLP murine toxicology study (3×10^{14} vg/kg). Animals were pre-screened for AAV9 neutralizing antibodies prior to shipment. All animals assigned to treatment and vehicle control groups demonstrated complete seronegativity (no virus neutralization at 1:5, 1:20, 1:80 dilutions). Two monkeys were assigned to the therapeutic vector group (3×10^{14} vg/kg) and 2 monkeys were assigned to the vehicle control group. Following dosing (intravenous injection into the saphenous vein), animals were evaluated at baseline and Days 3, 7, 15, 21, 30 42, 50, 60, 91, and 102. There was no unexpected mortality or significant changes in body weight. Blood samples were obtained from both cohorts at multiple timepoints for clinical pathology. A mild, transient transaminase increase at Day 7 and a concomitant transient decrease in platelets at Day 7 (within normal range) were observed in the AAV9.LAMP2B-injected group without any pathologic sequelae. Mild elevations (but within normal range) in creatinine kinase and lactate dehydrogenase at study Day 50 were also noted, with no accompanying clinical signs of toxicity. No other significant treatment-related effects were noted for any of the other hematologic or biochemical assessments performed.

EXAMPLE 5: LAMP-2B Gene Therapy Clinical Studies

An initial dose of 6.7×10^{13} GC/kg was selected for clinical study. The approach for dose escalation involves evaluation of intermediate doses between 6.7×10^{13} GC/kg and 2.0×10^{14} GC/kg.

The dose units for preclinical studies evaluating AAV9.LAMP2B are expressed in vector genomes (vg) per kg body weight. The clinical doses of AAV9.LAMP2B administered in this study are expressed as vector genome copies (GC) per kg, as this nomenclature is considered an optimal description of the investigational material as quantified in the manufacturing process. Both of these unit terminologies (GC/kg and vg/kg) are intended to describe the same entity with respect to transgene quantity.

The study will exclude subjects who have high pre-existing anti-AAV9 serum neutralizing antibody titers (Anti-AAV9 neutralizing antibody titer >1:40). Patients with evidence of synthetic or cholestatic hepatic dysfunction (PT/INR >1.5×ULN; bilirubin >1.5×ULN) will also be excluded (transaminase elevations up to 10×ULN or GGT up to 2×ULN are permitted because this is a prominent component of DD and are believed to predominantly reflect muscle aberrancies). Systemic corticosteroid therapy will be administered one day prior to AAV infusion, continued during the weeks following administration, and tapered to discontinuation between 8 and 12 weeks subsequent to infusion.

Safety monitoring included frequent testing of liver enzymes (including transaminases, bilirubin, ALP and coagulation parameters). Platelets and comprehensive coagulation profile (including PT/aPTT/fibrinogen/D-Dimer) will also be evaluated, as will complement pathway components. Serum and whole blood will also be obtained for evaluation of potential humoral and cell-mediated immune response against both viral capsid components and LAMP-2B.

Objectives and Endpoints

Primary Objectives

- To characterize the safety and toxicity associated with infusion of a rAAV9 capsid containing the human LAMP2B transgene (investigational product).

- To evaluate a range of single IV doses of investigational product with respect to safety, toxicity, and preliminary efficacy.
- To determine if infusion of investigational product results in cardiomyocyte (and skeletal muscle) transduction and gene expression (as determined by assessment of myocardial LAMP2B DNA, RNA, and protein) via endomyocardial biopsy, to determine if there is correction of disease-associated histologic abnormalities (autophagic vacuoles, myofibrillar disarray), and to enable preliminary characterization of the extent of cardiomyocyte molecular and histologic correction.
- To enable preliminary assessment of clinical stabilization, as determined by medical, radiographic, and cardiopulmonary exercise/physiologic parameters at approximately 8–12 weeks following investigational product infusion (similar evaluations at subsequent timepoints will be conducted to evaluate secondary objectives, as described subsequently).

Secondary Objectives

- To determine the percentage of patients for whom infusion of investigational product results in a sustained (6-month through 3-year following investigational product) improvement, stabilization (or reduced rate of deterioration versus historical controls) in cardiovascular pathophysiology, as determined by medical evaluation, radiographic evaluation of cardiac structure and function, and cardiopulmonary exercise/physiologic parameters.
- To determine the percentage of patients in whom cardiomyocytes contain the corrected LAMP2B gene and/or protein and improvement in DD-associated histologic abnormalities, and when feasible to quantify the extent of genetic and histologic correction in the myocardium.
- To determine and characterize immunologic response to investigational product (immunogenicity), including evaluation of humoral (antibody) and cellular (T-lymphocyte) anti-AAV9 and anti-LAMP-2B protein activity.
- To evaluate the percentage of patients receiving investigational product who require and/or receive subsequent cardiac transplantation, LVAD, implantable cardioverter-defibrillator or pacemaker placement, electrophysiologic ablative procedure for cardiac conduction aberrancy, or subsequent hospitalizations for heart failure.

- To evaluate overall survival in patients receiving investigational product, including 1- and 3-year overall survival assessment of all patients and specifically patients receiving investigational product at the dose selected for subsequent evaluation.

Exploratory Objectives

- To evaluate potential correlations between evidence of molecular and histologic correction in cardiomyocytes, with parameters of clinical stabilization or improvement, and to evaluate potential correlations between cardiomyocyte molecular/histologic correction and evidence of *LAMP2B* gene/protein in skeletal muscle and blood.
- To evaluate serologic markers of muscle injury (including CPK and transaminases) and of congestive heart failure (including BNP, high-sensitivity troponin) and ascertain if earlier (i.e., 8–12 week) improvement in these blood markers may be potential surrogates of clinical, structural, and histologic modification of DD.
- To evaluate patient-reported outcomes/quality-of-life (PRO/QOL) in patients receiving investigational product, as evaluated by means of the Kansas City Cardiomyopathy Questionnaire (KCCQ-12) and PedsQL.
- To evaluate the presence and extent of improvement, stabilization (or reduced rate of deterioration versus historical controls) in non-cardiovascular aspects of DD, including assessment of neuromuscular and ophthalmologic function.

Endpoints

Safety Endpoints

The safety and tolerability endpoints are:

- Incidence of treatment-emergent adverse events (TEAEs) and SAEs, overall and by intensity.
- Incidence of TEAEs and SAEs, overall and by intensity, considered by the Investigator to be at least possibly related to AAV9.LAMP2B.
- Proportion of patients requiring cardiac interventions, including cardiac transplant, implantable cardioverter-defibrillator, or pacemaker placement, electrophysiologic ablative procedure for cardiac conduction aberrancy, or subsequent hospitalizations for heart failure.

- Characterization of immune response to AAV9.LAMP2B, as evidenced by antibodies or T-lymphocytes reactive to AAV-9 or to LAMP-2B protein.
- Evidence of hepatotoxicity, based on changes from baseline in liver transaminases (AST and ALT), GGT, bilirubin, and ALP.
- Evidence of coagulopathy, based on changes from baseline in platelet count, prothrombin time (PT, or International Normalized Ratio (INR)), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer, thrombin-antithrombin complex (TAT), and complement components (complement 3 (C3), complement 4 (C4), and serum membrane attack complex (sC5b-9).
- Changes from baseline in:
    -Vital signs measurements.
    - Safety laboratory test results.
    - Physical examination findings.

Efficacy Endpoints

The efficacy endpoints include assessments of clinical improvement, stabilization (or reduced rate of deterioration versus historical controls) in cardiovascular pathophysiology, as determined by medical evaluation, radiographic evaluation of cardiac structure and function, and cardiopulmonary exercise/physiologic parameters. Preliminary assessments of efficacy endpoints will occur during the initial safety-focused follow-up (initial 8–12 weeks following investigational product infusion) and during a more sustained (6-month through 3-year) follow-up period.

Study Design

This is a non-randomized, open-label, Phase 1 study in patients with DD.

During this Phase 1 study, approximately 11–23 patients will receive a single IV infusion of investigational product (IP), with cohorts of patients receiving AAV9.LAMP2B at sequentially higher dose-levels according to the guidelines detailed below. Study sites that are not administering IP may participate in the trial. These sites would perform the initial screening and visits subsequent to IP dosing. The IP administration and immediate subsequent study visits are to be performed at the study site performing the IP administration. This is intended to reduce the burden of extensive travel for patients and their families.

Three dose levels are planned to be investigated in 6 distinct cohorts. Dosing of a cohort evaluating a given dose in a pediatric population (ages 8–14) will be feasible only when it is determined that fewer than 33% of patients within the comparable dose adult cohort have experienced dose-limiting toxicity (DLT). Dosing of cohorts in which adult or pediatric patients receive higher doses will be feasible only when it is determined that fewer than 33% of patients within a prior lower-dose cohort have experienced DLT. To be evaluable for DLT assessment, a patient must have received the intended dose of IP and remained available for follow-up during the 8 weeks subsequent to infusion of IP (with the exception of patients with fatal AEs considered related to investigational product during the initial 8 weeks subsequent to infusion).

Pediatric patients (ages 8–14) at a given dose level will commence only pending determination of safety of the dose level in the older (adults and ages 15–17) cohort. **FIG. 8** depicts the overall sequence of planned enrollment in the cohorts, according to a scenario in which DLT is not identified. **FIG. 9** depicts the sequence of enrollment within any given cohort. Decisions regarding expansion of cohorts, opening of a subsequent cohort (involving an increased or otherwise modified investigational product dose), and recommendation of the dose(s) to be evaluated in subsequent clinical development will be made by the IDSMC based on the safety profile identified in prior cohorts and the prospect of direct benefit. Additional regularly scheduled reviews of investigational product safety by the IDSMC will occur subsequent to the initial 8-week DLT-evaluation periods for patients in each study cohort.

Investigational Product

The Investigational Product (AAV9.LAMP2B) is gene therapy product consisting of an AAV9 capsid containing the human *LAMP2B* transgene with ITR elements, CAG promoter comprising CMV IEE, CBA promoter, CBA and rabbit globin introns, WPRE, and RGpA as shown in **FIG. 1** and described in ***Example 1***.

Dosage Form

The composition of AAV9.LAMP2B consists of the active ingredient (recombinant AAV9.LAMP2B viral particles at a concentration of [3.0–6.0×10^{13} vg/mL] and capable of transducing target cells to express the therapeutic protein LAMP2B) formulated in buffer (200 mM NaCl, 10 mM NaH_2PO4, 1% (w/v) sucrose, 0.01% Poloxamer 188, pH 7.2 ±0.1)

suitable for infusion. AAV9.LAMP2B is provided to the clinical site as a frozen product and the final volume of the dose of AAV9.LAMP2B is predicated on the patient weight in kilograms (kg) and the calculated vector genome (vg) copies of AAV9 per milliliter (mL).

Doses to be Investigated

During this Phase 1 study, 11–23 patients will receive a single IV infusion of investigational product, with up to 3 specific cohorts of adult and pediatric patients receiving AAV9.LAMP2B at dose-levels according to the guidelines below. Activation of a cohort evaluating a given dose in a pediatric population (8–14) is only feasible when it is determined that fewer than 33% of patients within the comparable dose adult cohort have experienced a dose-limiting toxicity (DLT). Three AAV9.LAMP2B dose levels will be investigated according to a dose escalation design, as follows:

- Cohort 1: Adult and age 15–17: 6.7×10^{13} GC/kg (n=3)*
- Cohort 2: Adult and age 15–17: 1.1×10^{14} GC/kg (n=2–4)
- Cohort 3: Adult and age 15–17: 2.0×10^{14} GC/kg (n=2–4)
- Cohort 1A: Pediatric age 8–14: 6.7×10^{13} GC/kg (n=2–4)
- Cohort 2A: Pediatric age 8–14: 1.1×10^{14} GC/kg (n=2–4)
- Cohort 3A: Pediatric age 8–14: 2.0×10^{14} GC/kg (n=2–4)
- Cohort 1 enrolled 3 patients per previous protocol version

Evaluation of AAV9.LAMP2B in pediatric patients (age 8–14) at a given dose level will commence only pending determination of safety of the dose level in the older (adults and those aged 15–17 and generally capable of providing assent) population. AAV9.LAMP2B will be administered at doses based on total body weight. If the patient is obese (body mass index (BMI) >85%, per the Centers for Disease Control and Prevention (CDC) growth chart, the IP may be administered at doses based on lean body mass (LBM) using the Hume formula below. $LBM = (0.32810 \times W) + (0.33929 \times H) - 29.5336$

The study excluded patients with pre-existing anti-AAV9 serum neutralizing antibody titers (>1:40). Patients received prophylaxis for anti-AAV immunogenic response with rituximab, tacrolimus, and corticosteroids. Rituxumab was administered prior to IP infusion. Tacrolimus was administered for 3 months and started prior to IP administration. Corticosteroids started the day prior to administration of the therapeutic vector and then daily

through Week 8 post-treatment, followed by a 4-week taper prior to discontinuation. The incorporation of tacrolimus and rituximab as part of the immunosuppressive regimen enables a reduced overall corticosteroid administration.

Clinical Safety Profile for Cohort 1

A cohort of three patients 15 years and older (Cohort 1) received LAMP-2B gene therapy at a dose of 6.7×10^{13} GC/kg with concomitant corticosteroids as per protocol. Subject characteristics are shown in Table 1. The second and third patients also received tacrolimus. Treatment regimen and LAMP2B relative expression are shown in Table 2. No significant anti-drug antibody (ADA) response was noted to LAMP2B.

The patients showed some constitutional symptoms (such as nausea, vomiting, abdominal pain, and low grade fever) in the days after receiving IP. As expected, the patients developed an immune response subsequent to IP administration. This immune response was associated with decreased blood cell counts (platelets, white blood cells), elevated transaminases, elevated skeletal muscle- and heart-related enzymes and peptide levels. The decrease in platelet count that occurred approximately 1 to 2 weeks after treatment was associated with a corresponding increase in D-dimer and decreases in C3 and C4 (complement moieties). Decreases in platelet count have been observed in other AAV gene therapy programs and are consistent with an acute complement mediated immune reaction against the AAV9 capsid. The patients treated to date with LAMP-2B gene therapy were maintained on corticosteroids and were not treated with eculizumab. The observed changes in platelets, D-dimer and C3/C4 levels improved after several days.

Increases in aspartate transaminase (AST) and alanine aminotransferase (ALT) have been demonstrated in other AAV gene therapy programs. The AST/ALT increases observed were not associated with increases in bilirubin above normal range and with clinical signs/symptoms of hepatobiliary disease. Elevations in GGT (a liver enzyme that is not affected by injury to skeletal muscle, unlike AST/ALT) were more modest, and did not exceed levels greater than 5X baseline. The EliSpot assay has been negative for both the AAV9 capsid and the LAMP2B transgene for the first three patients in Cohort 1. Because the GGT increases from baseline have been proportionally lower than the AST and ALT increases from baseline, these increases in AST and ALT are predominantly skeletal muscle in origin. This is also seen with the associated elevations of CPK. Two of the three patients in

Cohort 1 developed a skeletal muscle weakness consistent with a steroid induced myopathy. The skeletal muscle weakness recovered as the corticosteroids were tapered to discontinuation. At 6 months, 9 months, and 12 months post-treatment, the patients are stable at home.

Table 1

Patient ID	**Age at Treatment**	**Dosing Weight**	**Cohort Dose**	**Total Dose**
A501-005-1001	17-years	52.2 kg	6.7 x 1013 GC/kg	3.25 x 1015 GC
A501-005-1002	20-years	89.1 kg	6.7 x 1013 GC/kg	5.97 x 1015 GC
A501-005-1005	18-years	97.8 kg	6.7 x 1013 GC/kg	6.08 x 1015 GC

Table 2

Subject		LAMP2B Relative Expression vs. Normal*			
	Regimen	**Baseline**	**Week 8**	**Month 6**	**Month 12**
Subject 1001	Steroids only (limited compliance)	1.0%	7.8%	9.4%	2.6%
Subject 1002	Steroids only (local monitoring)	8.9%	36.9%	ND	67.8%
Subject 1005	Steroids → Tacrolimus	3.3%	17.6%	92.4%**	Jan 2021

*Percent area of cell staining was quantitated using software in a blinded fashion and expression compared to normal heart tissue. Values represent average of 3-14 sections.
**Sample taken at Month 9

Gene Expression and Efficacy Endpoints of Cohort 1

A cohort of three patients 15 years and older (Cohort 1) received LAMP-2B gene therapy at a dose of 6.7×10^{13} GC/kg with concomitant corticosteroids as per protocol. Following LAMP-2B gene therapy treatment, the vector DNA copy numbers were analyzed, as shown in **FIG. 5**. As shown in Table 2, all three patients demonstrated evidence of cardiac LAMP2B expression by Western Blot and immunohistochemistry, including the first patient whose compliance with the immunosuppressive regimen was limited. Patients 1002 and 1005 who had good compliance with the immunosuppressive regimen demonstrated high levels of cardiac *LAMP2B* expression along with clinical biomarker improvements. In cardiac biopsies of patients treated at a systemic dose of $6.7\text{x}10^{13}$ gc/kg, *LAMP2B* gene expression was demonstrated to be present in 68-92% of cells versus normal as determined by immunohistochemistry (IHC) at 9 and 12-months as well as up to 61% of normal LAMP2B

protein expression that was measured by Western blot assessment in one patient. As shown in **FIG. 6**, patient 1002 demonstrated robust cardiac expression of LAMP2 following LAMP-2B gene therapy treatment. Patients 1002 and 1005 showed a consistent increase in percentage and level of IHC staining at the later time points.

As shown in **FIG. 7**, two of the three patients demonstrated key clinical biomarker improvements consistent with improved cardiac function. Brain natriuretic peptide (BNP), a key marker of heart failure, improved (*i.e.* decreased) in all three patients, including by greater than 50% in patients 1002 and 1005 (**FIGs. 7B** and **7C**) with confirmed immunosuppressive regimen compliance. Creatine kinase myocardial band (CPK-MB) either improved or was stabilized in patients 1002 and 1005. Notably there were visible improvements in autophagic vacuoles, a hallmark of Danon pathology, as assessed by electron microscopy. As shown in Table 3, patients 1002 and 1005 with confirmed immunosuppressive regimen compliance and follow-up at 9 and 12-months demonstrated improvement in cardiac output as measured by invasive hemodynamics. Because patients with Danon Disease do not improve independently, the benefits observed in all three patients in the present studies are clinically meaningful and offer a transformative gene therapy approach to this otherwise devastating disease.

Table 3

Cardiac Output (L/min)a	**Baseline**	**Week 8**	**Month 6**	**Month 12**
Subject 1001	5.2	6.2	6.1	4.12
Subject 1002	3.58	5	ND	5.8 (1.62x increase)
Subject 1005	4.5	3.7	6.08* (1.35x increase)	ND

*Month 9

Definition of Dose-limiting Toxicity

Dose-limiting Toxicity (DLT) is defined as any occurrence of AE(s) occurring within 8 weeks after investigational product administration, as follows:

- National Cancer Institute Common Terminology Criteria for AEs (NCI CTCAE), version 5.0, Grade 3 or higher infusion-related reactions.
- Grade 4 transaminase elevations persisting for more than 2 weeks associated with liver injury despite supportive therapy.
- Grade 3 transaminase elevations that do not improve or resolve with supportive regimens.
- Other Grade 3 or higher AE, with the exception of Grade 3 nausea/vomiting, diarrhea, constipation, fever, fatigue, or skin rash that resolve to Grade <3 within 72 hours, or Grade 3 laboratory abnormalities not associated with clinical symptoms that resolve to Grade <3 within 2 weeks.

DLTs will be determined irrespective of Investigator-attributed causality. In settings in which there is a likely etiology not related to study therapy (for example Grade 3 pain secondary to motor vehicle accident), a determination that an event does not represent a DLT may be made but will require evaluation by the IDSMC. The baseline values for each patient will be based on available clinical data within 6 months prior to IP administration.

Dose Escalation Procedure

Enrollment within a cohort will be staggered such that the initial patient must be followed for at least 8 weeks before subsequent patients in a cohort may receive IP. The addition of rituximab and tacrolimus is anticipated to reduce the immune response following IP administration. After the suppression of the immune response has been demonstrated, the duration of 8 weeks between subsequent patients in a cohort may be reduced with IDSMC agreement. Each cohort will consist of at least 2 patients. If 1 out of the first 2 patients in a cohort experiences a DLT, an additional 2 patients will be enrolled in the cohort. In order for activation of a subsequent (higher-dose or pediatric) cohort to commence, all patients in a prior cohort must have been followed for at least 8 weeks subsequent to infusion of IP and there has been evidence of DLT resolution or stabilization. Pediatric patients (ages 8–14) at a given dose level will commence only pending determination of safety of the dose level in cohorts 1, 2, and 3 (adults and ages 15–17) cohort.

If it is determined that administration of 6.7×10^{13} GC/kg (Low Dose) results in sub-therapeutic LAMP-2B expression with acceptable safety in an initial 2 or 3 patients in Cohort 1, Cohort 2 at an intermediate dose of 1.1×10^{14} GC/kg will be activated. Cohort 1A (Pediatric age 8–14) at the 6.7×10^{13} GC/kg dose will not be performed if the 6.7×10^{13} GC/kg is considered sub-therapeutic. Cohort 2A (Pediatric age 8–14) at an intermediate dose of 1.1×10^{14} GC/kg will commence enrollment only after completion of Cohort 2 (Adult and age 15– 17) at an intermediate dose of 1.1×10^{14} GC/kg, and pending review of safety with IDSMC. Refer to **FIG. 8**.

Investigational Product Administration

Patients will receive AAV9.LAMP2B gene therapy product via IV infusion on Day 0; it is intended that the IP will be infused as a single dose to the patient. Patients will receive investigational product in an inpatient (hospitalized) setting in a facility experienced with

investigational therapeutics for cardiovascular disorders. Patients will remain hospitalized for 48–72 hours and may remain hospitalized for up to 14 days subsequent to investigational therapy infusion, and may be discharged thereafter at the discretion of the treating Study Investigator. Daily assessments will continue through Day 7 and may be extended at the discretion of the Study Investigator.

Pre-Treatment Medications and Concomitant Treatment

Prophylaxis for anti-AAV immunogenic response will be administered prior to and following infusion of investigational product. In addition to corticosteroids, rituximab and tacrolimus will be administered to suppress the immune response to IP.

Pre-Treatment Medications

Pre-medications prior to Rituximab infusion:

- Acetaminophen – 15 mg/kg PO (maximum 1 g)
- Diphenhydramine – 12.5 to 50mg PO, or as per product insert
- Rituximab: Patients will receive rituximab at a dose of 750 mg/m2 on Days -14 and -7 prior to IP infusion.
- Corticosteroids: Patients will receive prednisone (oral or IV) at a dose of 1 mg/kg body weight starting the day prior to investigational product infusion (Day -1) and then daily through Week 8 post-treatment, followed thereafter by a 4-week taper prior to discontinuation by Month 3; in settings in which enhanced immunosuppressive therapy (i.e. rituximab, tacrolimus) is administered as prophylaxis, the corticosteroid taper may commence at an earlier juncture, including within the initial 4 weeks following IP administration, at the discretion of the Study Investigator with Sponsor's Medical Monitor.
    - In settings in which prednisone results in unacceptable AEs or is unavailable, methylprednisolone or dexamethasone may be substituted at equivalent doses (0.8 mg/kg methylprednisolone; 0.15 mg/kg dexamethasone).
    - The corticosteroid taper may extend over a period longer than 4 weeks in settings of increased liver enzymes (bilirubin or transaminases) over baseline or other AEs attributable to investigational product.

- The corticosteroid taper may commence earlier than Week 8 in settings of intolerable corticosteroid-related AEs or in settings of enhanced prophylactic immunosuppression as noted above, at the discretion of the Study Investigator in conjunction with the Sponsor's Medical Monitor.
- Medications to be co-administered to prevent corticosteroid complications may be given in accordance with institutional standards and should include prophylaxis for *Pneumocystis jirovecii* pneumonia (PCP), gastrointestinal ulcers, and constipation. If no institutional guidelines exist, then these may include and not limited to atovaquone or trimethoprim/sulfamethoxazole 3 times per week, either an H2-antagonist (i.e., ranitidine) or a proton-pump inhibitor (i.e., omeprazole), and a stool softener (i.e., docusate) with or without a laxative, administered daily at age/weight-appropriate doses.

- Tacrolimus: Patients will receive tacrolimus per oral daily in 2 divided doses. Dosage will be adjusted as needed based on tacrolimus trough serum levels to maintain a level of 2–5 ng/mL, with administration starting on Day -7 through 3 months. Tacrolimus may be discontinued at the discretion of the Study Investigator.
- Supportive Medications during corticosteroid course: As per institutional practice, patients will receive prophylaxis for Pneumocystis jiroveci pneumonia (PJP/PCP) prophylaxis, gastrointestinal ulcers, and constipation.
- Additional pre-treatment medication(s): not required prior to infusion of investigational product but may be considered if required by institutional guidelines for infusion of AAV investigational agents; such agents may include acetaminophen (paracetamol) and/or histaminergic H1- or H2-antagonists.

<u>Concomitant (supportive care) medications:</u>

Additional supportive therapies may be administered, prior to or following IP administration, to prevent or treat adverse effects at the discretion of the Study Investigator. These may include and are not limited to:

- Corticosteroids
- Eculizumab in settings of complement activation. Prior to eculizumab administration the patient must have received meningococcal vaccination as recommended by Centers for Disease Control as appropriate for age and health condition. The

meningococcal vaccination(s) may be administered by the study site prior to receiving investigational product;

- Transfusion of platelets or plasma products in settings of coagulopathy;
- Anti-emetics (such as ondansetron);
- Growth factors (such as G-CSF [Neupogen®]) in settings of neutropenia;
- Analgesic and anti-inflammatory agents in settings of infusion-related reactions

Patient Inclusion Criteria

Patients must meet all the following criteria (and none of the exclusion criteria) to be eligible for study participation:

1. DD diagnosis with a confirmed *LAMP2* mutation(s).
2. Cardiac involvement as documented by at least one abnormal finding on ECG, echocardiogram, gadolinium-enhanced cardiac MRI, or electrophysiology study.
3. Age ≥15 years for cohorts 1, 2, and 3; 8–14 years for cohorts 1A, 2A, and 3A.
4. Male gender.
5. NYHA Class II or III. Patients with NYHA Class I are eligible if unable to walk ≥450 meters during the 6MWT.
6. Adequate hematologic function as defined by:
   a. Hemoglobin ≥10 g/dL (6.2 mmol/L; Grade 1 anemia, per NCI CTCAE version 5.0).
   b. Absolute neutrophil count ≥1,500/mm3 (1.5×109/L; Grade ≤1 neutropenia).
   c. Platelet count ≥75,000/mm3 (75×109/L; Grade ≤1 thrombocytopenia).
7. Hepatic function as defined by:
   a. AST and ALT ≤10.0×ULN or GGT ≤2×ULN (transaminase elevations in DD are considered extensively to result from muscle injury; hence the relatively high upper limit for these transaminases and consideration of GGT levels, and the presence of additional hepatic eligibility markers of bilirubin and PT/INR).
   b. Serum bilirubin ≤1.5×ULN (*i.e.*, Grade bilirubin increase).
   c. PT/INR ≤1.5×ULN (in the absence of anticoagulation)
   d. Absence of cirrhosis on liver ultrasound
8. Renal function as follows: creatinine ≤1.5×ULN; (if creatinine is >1.5 ULN, then creatinine clearance ≥50 mL/min/1.73m^2 is required, as calculated by Modification of Diet in Renal Disease (MDRD) equation (Stevens 2006), the revised Schwartz formula (for patients under 18 years old) (Schwartz 2009), or 24-hour urine collection).

9. Ability to provide informed consent (for adult patients and parents/legal guardians of pediatric patients) and assent (for patients age 15–17).

10. Ability to comply with study procedures including investigational therapy and follow-up evaluations.

11. Able to walk >150 meters unassisted during the 6MWT.

12. Patient has received meningococcal vaccination recommended by Centers for Disease Control as appropriate for age and health condition.

Patient Exclusion Criteria

Patients meeting any of the following criteria are excluded from study participation:

1. IV therapy with positive inotropes, vasodilators or diuretics within the 30 days prior to enrollment (i.e., patient must be stable on oral medical therapy).

2. Prior cardiac transplantation or prior transplant of other organ (lung, liver, other).

3. Cardiac surgery, percutaneous cardiac intervention, or valvuloplasty within 30 days prior to enrollment.

4. Presence or requirement of a LVAD.

5. Myocardial infarction, unstable angina, stroke, or TIA within 90 days prior to enrollment.

6. Significant (greater than moderate) valvular stenosis or regurgitation on echocardiogram.

7. Requires mechanical ventilation.

8. Anti-AAV9 neutralizing antibody titer >1:40.

9. Concurrent enrollment in any other clinical investigation involving use of an investigational agent for the treatment of CHF or cardiomyopathy.

10. Active hepatitis B or C infection (including patients with positive HBsAg, HBeAg, or detectable HBV or HCV viral load). Patients with previous, adequately resolved HBV or HCV are eligible.

11. Significant medical conditions including documented HIV infection, active viral or other hepatitis, poorly-controlled hypertension or diabetes, poorly controlled cardiac arrhythmia, or uncontrolled viral, bacterial, or fungal infection.

12. Any concomitant medical or psychiatric condition that in the opinion of the Investigator renders the patient unfit for study participation or at higher than acceptable risk for study participation.

13. Active hematologic or solid organ malignancy, not including non-melanoma skin cancer or other carcinoma *in situ*. Patients with previously resected solid organ malignancies or definitively treated hematologic malignancies may be eligible if there has been no evidence of active malignancy during the prior 3 years.

14. Any contraindication to use of tacrolimus which includes hypersensitivity to tacrolimus or HC-60 (polyoxyl 60 hydrogenated castor oil).

Study Duration

Patients will be screened and have screening assessments performed within approximately 60 days before investigational product administration on Day 0. All patients are planned to be followed for 36 months after investigational product administration under the auspices of this protocol. Overall survival will be assessed and patients who elect to discontinue other components of follow-up (strongly discouraged unless in the context of severe, prohibitive deterioration in health status); such patients will be provided the option of maintaining contact with study personnel for evaluation of overall health status and survival. After the end of the follow-up period, patients will enter a Long-Term Follow-Up (LTFU) study enabling follow-up for an additional 2 to 5 years post-IP administration.

Long-Term Follow-Up

Following assessment of Phase 1 safety endpoints (initial 8–12 weeks post-infusion) and subsequent follow-up (initial 3 years post-infusion); ongoing follow-up for safety and toxicity (i.e., AEs), overall survival, overall health status including requirement for cardiac transplant and other adverse health outcomes will be conducted at regular intervals for an additional 2–5 years (5–8 years total, inclusive of the 3-year follow-up stipulated for the study). Long-Term Follow-Up will be planned for 5 years but will be re-evaluated if no serious AEs attributable to the investigational therapy are identified during the first 2 years.

If it is determined that no serious adverse drug reactions are identified during the study, then Long- Term Follow-Up will be planned for an additional 2 years, with longer follow-up (up to 5 years) depending on the extent, severity and resolution of AEs related to investigational product.

Schedule of Events and Study Procedures

Schedule of Events

The investigational product will be administered via IV infusion on Day 0. After investigational product infusion, patients will remain hospitalized or in-patient in a dedicated research facility for at least 48–72 hours and up to 14 days after infusion at the discretion of the Study Investigator's clinical judgement. Post-IP infusion, patients should attend study visits at frequencies outlined in **FIGs. 10A-10D**. Assessments that are performed daily for 7 days following investigational product. Daily evaluations may continue post-Day 7.

Follow-up visits will involve a series of clinical, clinical-laboratory, cellular and genetic, cardiac imaging and cardio-physiologic evaluations occurring in the days, weeks, months and years following infusion of investigational product. An endomyocardial biopsy (performed via central venous access catheterization of the right ventricle) and a skeletal muscle biopsy will also be required prior to, and at selected time points subsequent to investigational product infusion.

Follow-up regarding evidence *LAMP2B* gene and protein in cardiomyocytes (and histologic evaluation of DD histology including quantification of autophagic vacuoles) will occur via endomyocardial biopsy 1at Week 8 and Months 6, 12, and 36 post-infusion. As detailed subsequently, additional less invasive assessments, including skeletal muscle biopsy, evaluation of *LAMP2B* blood levels (plasma (LAMP-2B protein) and mononuclear cells (*LAMP2B* DNA) and other serologic and radiographic parameters of cardiomyopathy and CHF, will be assessed concomitantly with the exploratory intent of identifying potential surrogate markers of molecular and histologic improvement in myocardium. The detailed Schedule of Events is presented in **FIGs. 10A-10D**, footnotes to which are as follows:

1. Screening visit assessments will be performed over a 60-day period. Assessments performed as part of standard of care prior to enrollment may be used for the study as screening assessments and do not need to be repeated (based on Study Investigator judgement in consultation with the Sponsor).

2. Baseline visit assessments may be performed after patient has completed the screening visit and have met all inclusion and exclusion criteria. All assessments must be completed by Day -1 and prior to the administration of investigational product. Baseline visit

assessments do not need to be completed before rituximab and tacrolimus treatment has started.

3. During the screening visit, a comprehensive medical history will include any relevant cardiovascular and other potential disease-related prior events, including hospitalizations and cardiac interventions; family history of disease will also be recorded.

4. Concomitant medications and procedures during the screening visit and Day -1 should include any medications & procedures during the prior 30 days. During the follow-up visits, this should include any medications and procedures subsequent to the most recent prior evaluations.

5. Height will be collected once at the screening visit and annually thereafter.

6. Quality of Life/Patient Reported Outcomes questionnaire will include the KCCQ-12 and PedsQL.

7. Daily laboratory assessments Day 1 through Day 7.8. Laboratory assessments will be performed up to 3 times per week.

9. Serum membrane attack complex (sC5b-9) is required at the protocol specified time points post-administration of the investigational product. The test may be performed at additional timepoints or more frequently depending on the clinical status of the patient, and at the discretion of the Investigator.

10. Viral serology during the screening visit should include HIV, HBV and HCV viral load, and comprehensive HBV antigen/antibody panel including HBsAg, HBeAg, and HBsAb.

11. Cardiac serology will include potential markers of CHF including BNP, CK-MB, and Troponin-I levels.

12. Immunologic response to the investigational product (AAV9 and LAMP-2B) will include evaluations in serum (anti-drug antibody (ADA); IgG, IgM) and in peripheral blood T lymphocytes (ELISpot).

13. Whole blood will be collected, processed for serum, plasma and PBMCs (for screening assessment only) and stored for potential exploratory assays, including evaluation

of LAMP2 levels in serum and blood cells. The study ICF will provide patients the opportunity to provide or withhold consent that stored samples may be utilized for potential additional future research not specified in the protocol.

14. A screening saliva, urine, feces, and blood (plasma) sample will be collected subsequent to investigational product infusion for the evaluation of vector particle shedding. Evaluation of each bodily fluid/substance will continue as indicated until there are two negative evaluations (or when the vector levels have plateaued at low or negligible levels) for a given fluid/substance, at which point no subsequent evaluations are required.

15. Gene sequencing to confirm and define LAMP2 mutations will be conducted by means of an Arrhythmia and Cardiomyopathy Panel via either saliva or blood (mononuclear cells). If the LAMP2 genotype is not defined as pathogenic, a protein assessment for LAMP2 will be performed from isolated peripheral blood mononuclear cells or biopsy tissue to confirm LAMP2 deficiency.

16. MRI with gadolinium will be performed when not contraindicated by presence of implanted pacemakers, defibrillators, other indwelling devices or medical conditions (i.e., renal dysfunction precluding the use of gadolinium contrast, per institutional guidelines). In settings of renal dysfunction, non-gadolinium contrast agents may be utilized at the discretion of the consulting radiologist or in accordance with institutional guidelines. In settings where MRI is contraindicated, CT scan may be used.

17. When feasible, 6-minute walk should be performed at a similar time during the day for the initial and subsequent evaluations. During follow-up assessments, the 6-minute walk and cardiopulmonary exercise tests should, whenever possible, not be performed on the same day.

18. Rituximab pre-medications, acetaminophen and diphenhydramine, will be given 30–60 minutes prior to Rituximab infusion. Refer to the Pharmacy Manual for details.

19. Corticosteroids will be administered daily starting on Day -1 at a dose of prednisone (IV or PO) 1 mg/kg (or methylprednisolone or dexamethasone equivalent) through W8. Corticosteroid taper will commence on W8 (Day 56) following investigational product, with discontinuation (d/c) planned by 3 months following investigational product. A more protracted taper, or initiation of taper at a time point prior to or subsequent to Day 56 is

permitted at the discretion of the treating Investigator in conjunction with the study medical monitor. Supportive medications for corticosteroid administration may be provided per institutional standards.

Neutralizing Anti-AAV9 Antibody Titers in Serum

A blood sample for determination of neutralizing anti-AAV9 antibody titer in serum is to be collected at screening; Day -1, Week 2, Week 4, Week 8, Month 3, Month 6, Month 12, Month 24, Month 36 as per Schedule of Events (**FIGs. 10A-10D**). Patients with anti-AAV9 neutralizing antibody titers >1:40 are not eligible for study participation.

Hepatic Ultrasound

In addition to laboratory assessment of bilirubin, PT/INR and transaminase levels, an ultrasound of the liver will be performed at screening to evaluate findings consistent with cirrhosis or other hepatic compromise. Subsequent (post-infusion) ultrasounds may be performed if clinically indicated.

Safety Assessments

Vital Signs

Vital signs to be measured include systolic/diastolic blood pressure, pulse, respiration rate, pulse oximetry, and temperature, and will be performed in accordance with institutional standards. Vital signs will be measured at every study visit as per the Schedule of Events (**FIGs. 10A-10D**).

Height and Weight

Height (cm) will be measured at screening and annually thereafter. Weight (kg) will be measured at screening; Day -1; once a week from Week 1 through Week 8; once every 3 months (Month 3–Month 12); and once every 6 months (Month 12–Month 36) as per the Schedule of Events (**FIGs. 10A-10D**). The weight measurement taken on Day -1 will be used in the calculation of the patient's dose.

Clinical and Physical Examination

A complete physical examination (including performance status, general appearance; head eyes, ears, nose, and throat; cardiovascular; dermatologic, abdominal; genitourinary; lymph nodes; hepatic; musculoskeletal; respiratory; and neurological) is to be conducted screening; Day -14 and Day -7; Day -1; Day 0; daily Day 1 through Day 7; once a week from Week 2 through Week 8; once every 3 months (Month 3–Month 12); and once every 6 months (Month 12–Month 36) as per the Schedule of Events (**FIGs. 10A-10D**).

Hematology, Coagulation Studies, and Chemistry

Blood samples for clinical laboratory tests, including CBC and differential, coagulation studies, and chemistry, will be performed as specified below, and in the Schedule of Events (**FIGs. 10A-10D**). Clinical laboratory tests are to be performed and reviewed by the Investigator or qualified designee (e.g., physician's assistant, nurse practitioner). The following clinical laboratory parameters are to be determined:

- Hematology: Hemoglobin, hematocrit, erythrocyte (RBC) count, mean cell volume (MCV), mean cell hemoglobin concentration (MVHC), platelets, white blood cell (WBC) count and differential including neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

- Chemistry: Sodium, potassium, chloride, carbon dioxide (or bicarbonate), blood urea nitrogen (BUN), creatinine, glucose, ALP, ALT, AST, bilirubin, GGT, calcium, magnesium, and phosphorus.

Hematology and chemistry evaluations will be performed at screening; Day -1; daily Day 1 through Day 7; up to 3× per week from Week 2 through Week 4 (hematology), through Week 8 (chemistry); once every 3 months (Month 3–Month 12); and once every 6 months (Month 12–Month 36). During the screening visit if serum creatinine is >1.5×ULN, then creatinine clearance (CrCl) may be calculated, and the patient will be considered eligible if CrCl >50mL/min/1.73m^2, as calculated by MDRD equation.

- Coagulation Studies: PT (and/or INR), aPTT, D-dimer, thrombin-antithrombin complex (TAT), and fibrinogen. Evaluations will be performed at screening; Day -1, Day 2, Day 4, Day 7; once a week from Week 2 through Week 8; once every 3 months (Month 3–Month 12); and once every 6 months (Month 12–Month 36).

- Complement: C3, C4, and sC5b-9 membrane attack complex.

C3 and C4 evaluations will be performed at screening; Day -1, Day 1, Day 3, Day 5, Day 7; up to 3× per week from Week 2 through Week 8; once every 3 months (Month 3–Month 12); and once every 6 months (Month 12–Month 36). sC5b-9 evaluations will be performed at screening; Day -1; daily Day 1 through Day 7; up to 3× per week during Week 2; once a day from Week 3 through Week 8; Month 3 and Month 6. Additional clinical laboratory tests may be performed at the Study Investigator's discretion.

Cardiac Serology

Blood samples for cardiac serology are to be collected per the Schedule of Events (**FIGs. 10A-10D**). Parameters to be measured include:

- BNP collected at screening; Day -1, once during Week 2, Week 4, Week 6, Week 8; once every 3 months (Month 3–Month 12); and once every 6 months (Month 12–Month 36)
- CK-MB collected at screening; Day -1, Day 7, once a week from Week 2 through Week 8; once every 3 months (Month 3–Month 12); and once every 6 months (Month 12–Month 36)
- High-sensitivity troponin levels collected at screening; Day -1, Day 2, Day 4, Day 7; once a week from Week 2 through Week 8; once every 3 months (Month 3–Month 12); and once every 6 months (Month 12–Month 36)

Urinalysis

Urine sample for specific gravity, pH, protein, glucose, ketones, blood, urine leukocyte esterase will be collected at screening; Day 7, Week 4, Week 8; once during Month 12, Month 24, and Month 36 as per the Schedule of Events (**FIGs. 10A-10D**).

Immunogenicity

Blood samples for immunogenicity are to be collected for determination of humoral (antibody) and cellular (T-lymphocyte) anti-AAV9 and anti-LAMP-2B protein activity in whole blood and serum; IgG and IgM will also be collected as per the Schedule of Events (**FIGs. 10A-10D**). Blood samples for antibody evaluation will be collected at screening; Day 7; once during Week 2, Week 4, Week 8, Month 3, Month 6, Month 12, Month 24, and

Month 36. Blood samples for cellular evaluation will be collected at screening; once during Week 4, Week 8, Month 3, Month 6, Month 12, Month 24, and Month 36. Blood samples for IgG and IgM evaluation will be collected at screening; Day -1, Day 2, Day 4, Day 7; once during Week 4, Week 8, Month 3, and Month 6.

Vector Shedding

Blood (plasma), saliva, urine, and fecal samples will be collected at screening; Day 3, once during Week 2, Week 4, Week 8, Month 3, Month 6, and Month 9 according to the Schedule of Events (**FIGs. 10A-10D**) for evaluation of vector particle shedding. Evaluation of each bodily fluid/substance will continue as indicated until there are two negative evaluations or when the vector levels have plateaued at low or negligible levels for a given fluid/substance, at which point no subsequent evaluations are required.

Adverse Events

All AEs occurring from provision of informed consent and, if applicable, assent will be recorded. This includes AEs the patients report spontaneously, those observed by the Investigator, and those elicited by the Investigator in response to open-ended questions during scheduled study center visits. Information to be systematically recorded incudes the type of AE, dates of onset and resolution, severity, and perceived relationship to experimental therapy. The severity of each AE will be rated based on the NCI CTCAE, version 5.0.

Efficacy Assessments

Electrocardiogram

Twelve-lead ECGs are to be performed at screening; Day -1; Day 1; once a week from Week 2 through Week 8; once every 3 months (Month 3–Month 12); and once every 6 months (Month 12– Month 36) as per the Schedule of Events (**FIGs. 10A-10D**).

Echocardiogram

Echocardiography is to be performed at screening; Week 4, Week 8; once every 3 months (Month 3- Month 12); and once every 6 months (Month 12–Month 36) as per the Schedule of Events (**FIGs. 10A-10D**). Components of the echocardiogram include assessment of Left Ventricular Ejection Fraction (LVEF), Left and Right Ventricular

Dimensions (for example, LV end-systolic and end-diastolic dimensions, volumes and indices, septal and posterior wall thickness and LV outflow tract dimension), assessments of concentric hypertrophy and diastolic patterns, assessments of valvular stenosis and regurgitation, assessments of wall motion abnormalities, pulmonary pressures, IVC size and changes with respiration, pericardium, LV mass, Left Atrial (LA) diameter and volume, Isovolumetric relaxation time, Doppler velocity measurements, global longitudinal strain, and Left Ventricular Outflow Tract (LVOT) grading.

New York Heart Association (NYHA) Classification

The NYHA classification provides a simple way of classifying the extent of heart failure according to the severity of symptoms, as shown in Table 4. It places patients in one of the four categories based on how much they are limited during physical activity. NYHA evaluation will be performed at every study visit, with the exception of the baseline visit as per the Schedule of Events (**FIGs. 10A-10D**).

Table 4

Classification	Patient Symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitations, dyspnea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitations, dyspnea (shortness of breath).
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

Cardiac Magnetic Resonance Imaging

MRI with gadolinium will be performed when not contraindicated by presence of implanted pacemakers, defibrillators, other indwelling devices or medical conditions (i.e., renal dysfunction precluding the use of gadolinium contrast, per institutional guidelines). In

settings of renal dysfunction, non-gadolinium contrast agents (i.e., ferumoxytol) may be utilized at the discretion of the consulting radiologist or in accordance with institutional guidelines. Evaluations will be performed during screening; Week 8, Month 6, Month 12, Month 18, Month 24, Month 30, and Month 36 as per the Schedule of Events (**FIGs. 10A-10D**).

Cardiac MRI will involve injection of IV gadolinium and acquisition of multiple sequences over an estimated 30–40-minute total scan time. Assessments will include LVEF, LV mass indexed for body surface area (BSA), Maximal LV wall thickness, *z* Score for maximal wall thickness, LV end-diastolic and end-systolic volumes, LA diameter, volume and volume index, assessment of Late Gadolinium Enhancement (LGE), and LGE patterns (Raja 2018). Additional assessments will include resting perfusion myocardial blood flow (MBF), extracellular volume (T1 map pre- and post-gadolinium), and Right Ventricular Ejection Fraction (RVEF). In settings where MRI is contraindicated, cardiac CT scan with intravenous contrast may be used instead of MRI.

Six-Minute Walk Test

The 6MWT is a practical and simple test that requires a 100-ft hallway but no exercise equipment or advanced training for technicians. This test measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes, and thereby is a quantitative assessment of an important day-to-day activity that is progressively compromised in patients with DD. During the screening visit, patients must be able to walk >150 meters unassisted during the 6MWT to be eligible for study participation. Additionally, Ross Class I patients will be considered eligible if they are unable to walk at least 450 meters unassisted during the 6MWT.

The 6MWT is to be completed at screening; Week 8, Month 3, Month 6, Month 12, Month 18, Month 24, Month 30, and Month 36 as per the Schedule of Events (**FIGs. 10A-10D**). Whenever possible should be performed at the same time of the day at each timepoint evaluated during study follow- up. Identical instructions at specified intervals during the 6MWT will be given to each patient at each timepoint when the evaluation is conducted. The 6MWT should be performed on a different day than the CPET at timepoints when both evaluations are stipulated. This assessment will be performed twice at the time of each study visit, approximately 24 hours apart.

Cardiopulmonary Exercise Testing

Cardiopulmonary Exercise Testing (CPET), including assessment of oxygen consumption (VO_2), is to be performed at baseline; Week 8; Month 6, Month 12, Month 18, Month 24, Month 30, and Month 36 as per the Schedule of Events (**FIGs. 10A-10D**). CPET involves evaluations on a cycle ergometer or treadmill including resting, unloaded exercise and incremental ramp exercise designed to yield 8–12 minutes of total exercise duration with measurement of expired gases for determination of oxygen consumption and carbon dioxide production. Measurements include vital signs, pulmonary indices (including maximum voluntary ventilation), ventilatory threshold measurements (including respiratory exchange ratio and the ratio of minute ventilation to carbon dioxide production), peak exercise measurements (including peak vital signs and peak VO_2), anaerobic threshold measurements (including VO_2) and recovery assessments. The CPET should be performed on a different day than the 6MWT at each timepoint when both evaluations are stipulated.

Pulmonary Function Testing

Pulmonary Function Testing (PFTs) will be evaluated at baseline; Month 12, Month 24, Month 36 as per the Schedule of Events (**FIGs. 10A-10D**) to enable assessment of both pulmonary and diaphragmatic muscle capacity. PFT evaluations will include measurements of flow volume (including FVC, FEV_1), vital capacity, and diffusion capacity (including $DLCO_2$). Maximal inspiratory and expiratory pressures will also be evaluated.

Right Heart Catheterization and Endomyocardial Biopsy

Right heart catheterization and endomyocardial biopsy will be performed at baseline; Week 8, Month 6, Month 12, and Month 36 as per the Schedule of Events (**FIGs. 10A-10D**). The catheterization and biopsy will be performed by an interventional cardiology team with expertise in this procedure. The use of anesthesia during the right heart catheterization and endomyocardial biopsy procedure will be based on Institutional guidelines and per the Study Investigator's clinical judgement. Risks associated with anesthesia are described in product package inserts.

Right Heart Catheterization with Hemodynamic Assessment

A right heart catheterization will be performed to enable assessment of cardiopulmonary hemodynamic parameters. Hemodynamic parameters assessed will include right atrial and pulmonary artery pressures, pulmonary artery wedge pressure, mixed venous oxygen saturation, and assessments of cardiac output and cardiac index (Fick formula), pulmonary capillary wedge pressure, pulmonary vascular resistance, and pulmonary hypertension.

Endomyocardial Biopsy

The endomyocardial biopsy will be performed via central venous access catheterization of the right ventricle and catheter-mediated biopsy of the intraventricular septum, involving approximately 3– 5 samples per procedure.

The biopsy will enable evaluation of any therapy-related alterations in *LAMP2B* gene/protein expression and changes in DD-related myocardial histology (i.e., autophagic vacuoles, myofibrillar disarray). The recommendations for right heart catheterization and endomyocardial biopsy during subsequent investigations will be evaluated based on the extent of observed histologic changes and LAMP-2B expression during Phase 1 and including potential correlation of myocardial molecular and histologic changes with improvements in parameters from less invasive assessments, including LAMP-2B expression from skeletal muscle biopsy, LAMP-2B levels in blood, and other serologic and radiographic parameters of cardiomyopathy and CHF.

Skeletal Muscle Biopsy

Skeletal muscle biopsies will be performed to evaluate *LAMP2B* gene/protein expression in skeletal muscle, both in order to ascertain the potential of the investigational product to prevent or reverse the musculoskeletal components of DD, and to enable assessment as to whether a *LAMP2* skeletal muscle is a potential viable surrogate of *LAMP2* myocardial genetic correction and protein expression. An open biopsy of the Vastus Lateralis Muscle will be performed at baseline; Week 8, Month 6, Month 12, and Month 36 to enable assessment of the parameters detailed above. Biopsies at sequential assessments (i.e., baseline and 8 weeks post-therapy) will alternate between contralateral muscles (i.e., right leg at baseline, left leg at post-therapy assessment) in order to minimize potential procedure-

associated side-effects. In subsequent (Phase 2) studies, the requirement for muscle biopsy will be evaluated based on the extent of observed histologic changes and LAMP-2B expression during Phase 1 study, and correlation between endomyocardial and skeletal muscle gene/protein expression. Consideration will also be given to utilization of a needle biopsy during subsequent studies. The use of anesthesia during skeletal muscle biopsy procedures will be based on Institutional guidelines and per the Study Investigator's clinical judgement. Risks associated with anesthesia are described in product package inserts. The skeletal muscle biopsy may be performed in conjunction with the endomyocardial biopsy to limit anesthesia or sedation if needed.

Neurocognitive Evaluation

For patients aged ≥16 years, and considered to possess normal or near-normal (mildly limited) cognitive function, the neurocognitive evaluation will include the following components:

- Wechsler Adult Intelligence Scale (WAIS-IV).
- Vineland Adaptive Behavior Scale, Third Edition (Vineland-3).

Patients aged ≥16 years with more limited cognitive function will not be evaluated by means of the WAIS-IV. These patients will be evaluated by the following components:

- Vineland Adaptive Behavior Scale, Third Edition (Vineland-3).
- Differential Ability Scales (DAS-II).

The determination of the most appropriate evaluation will be made by the evaluating psychologist/neurocognitive assessment expert, in conjunction with the Study Investigator.

For patients aged <18 years, the neurocognitive evaluation will include the following components:

- WAIS-IV (if age 16 or 17 and considered of normal or near-normal cognitive function).
- Vineland-3 Parent/Caregiver assessment.
- Differential Ability Scales (DAS-II).

Neurocognitive assessments are evaluated at baseline; Month 12, Month 24, and Month 36 as per the Schedule of Events (**FIGs. 10A-10D**). These should be undertaken in the same sequence at each assessment whenever possible. Brief descriptions of each instrument are provided below.

Wechsler Adult Intelligence Scale (WAIS-IV)

The WAIS-IV provides a brief, reliable measure of cognitive ability. Contains sets of standardized questions and tasks for assessing an individual's potential for purposeful and useful behavior. Designed to measure major mental abilities. This test yields standardized scores of an overall estimate of general cognitive ability, verbal comprehension, and nonverbal abilities.

Vineland Adaptive Behavior Scale, Third Edition (Vineland-3)

The Vineland-3 is a standardized measure of adaptive behavior – the things that people do to function in their everyday lives. Whereas ability measures focus on what the examinee can do in a testing situation, the Vineland-3 focuses on what he or she actually does in daily life. Because it is a norm-based instrument, the examinee's adaptive functioning is compared to that of others his or her age. This is the leading instrument for supporting the diagnosis of intellectual and developmental disabilities. The Vineland-3 Parent/Caregiver Interview Form measures adaptive behavior functioning across 4 domains: communication, daily living, socialization, and motor functioning in individuals (birth through 90 years).

Differential Ability Scales (DAS-II)

The DAS–II is a comprehensive, individually administered, clinical instrument for assessing the cognitive abilities that are important to learning. The test may be administered to children ages 2 years 6 months (2:6) through 17 years 11 months (17:11) across a broad range of developmental levels. This test yields standardized scores for overall general conceptual ability, verbal ability (verbal concepts and knowledge), nonverbal ability (complex, nonverbal, inductive reasoning requiring mental processing), and spatial ability (complex visual processing).

Neuromuscular Evaluation

The neuromuscular evaluation will be performed at baseline; Week 8, Month 6, Month 12, Month 24, and Month 36. It will include timed tests of essential neuromuscular activities, including the following:

- Rise from floor,
- 4-stair climb and descend,
- 10-meter walk/run,
- Timed up-and-go (TUG), and
- North-Star Ambulatory Assessment Test.

Ophthalmologic Examination

Ophthalmologic examinations will be evaluated at baseline; Month 12, Month 24, and Month 36 as per the Schedule of Events (**FIGs. 10A-10D**). It will include retinal evaluation by direct ophthalmoscopy/fundoscopy, fundus photography, optical coherence, tomography, autofluorescence testing, and electroretinography.

Patient-Reported Outcomes/Quality-of-Life (PRO/QOL)

PRO/QOL measures to be employed in this study include the KCCQ-12 and PedsQL. Assessments will be collected at baseline; Week 8, once every 3 months (Month 3–Month 12); and once every 6 months (Month 12–Month 36) as per the Schedule of Events (**FIGs. 10A-10D**).

Efficacy Assessment

Assessments for clinical efficacy endpoints will include the following assessments performed as per the Schedule of Events (**FIGs. 10A-10D**) during the initial months and year following investigational product administration:

- CPET, including assessment of VO2.
- 6MWT (distance) assessment.
- Endomyocardial biopsy (performed via central venous access catheterization of the right ventricle) for evaluation of DD-associated histologic abnormalities and presence of LAMP2B gene/RNA/protein in cardiomyocytes.

- Gadolinium-enhanced cardiac MRI (when not contraindicated by presence of implanted pacemakers, defibrillators, other indwelling devices, or medical conditions).
- Echocardiogram.
- Overall survival will be assessed and patients who elect to discontinue other components of follow-up (strongly discouraged unless in the context of severe, prohibitive deterioration in health status); such patients will be provided the option of maintaining contact with study personnel for evaluation of overall health status and survival.
- Evaluation of requirement for subsequent cardiac transplantation, LVAD, implantable cardioverter-defibrillator or pacemaker placement, electrophysiologic ablative procedure for cardiac conduction aberrancy or hospitalizations for CHF.

Statistical Methods

A sample size of up to 3 patients for a dosing cohort, with expansion up to 6 patients (in the event of a DLT in 1 of 3 patients) is considered a standard and safe approach regarding dose-evaluation of a novel investigational therapeutic. Assuming a true DLT rate of 5% or less, there would be a 3% chance that dose escalation would be halted based on a given cohort (i.e., observation of 2 or more patients with DLT). If a true DLT rate of 50% is assumed, then there would be an 83% chance that dose escalation would be halted based on a given cohort.

Number of Patients Planned to be Enrolled

11 - 23 (Cohort 1 enrolled 3 patients per previous protocol version)

Study Populations

Study populations evaluated will include the overall study population, the study populations will receive a range of investigational product. Additional evaluated populations will be adult patients (both age 18 and over and including patients age 15–17) and pediatric populations including the population age 8–14.

EXAMPLE 6: Phase 1 Study Results

Phase 1 study results have demonstrated strong trends in many key clinical

biomarkers and endpoints.

DD is a genetically inherited cardiomyopathy, the features and progression of disease are distinct from those of a typical adult cardiomyopathy. The majority of patients are well compensated with respect to functional impairment until late in the disease course; measurements such as LVEF and even 6MWT may be normal or only mildly abnormal until the cardiomyopathy has progressed. Mild improvement or stabilization of disease-related abnormalities are desirable in this patient population and are likely to represent an improvement over the emerging natural history; these may be accompanied by stabilization and/or improvement in clinical biomarkers that have been shown to correlate with progression in natural history studies.

The clinical data in both low and high dose adult and adolescent cohorts ages >15 yo ($6.7x10^{13}$ GC/kg and $1.1x10^{14}$ GC/kg) have demonstrated efficacy of treatment. The data include:

1. Confirmation of durable protein expression of *LAMP2* in the primary target organ.
2. Demonstration at the cellular level of improvement in morphologic DD hallmarks.
3. Improvement and/or stabilization of key cardiac parameters including BNP (decreases in each of the 4 patients with long term follow-up, including 75%-79% decreases from baseline in the 2 low dose patients for whom there was confirmed compliance with the immunosuppressive regimen).
4. Improvement in the NYHA Class in 3 of 4 adults with confirmed immunosuppression in the low and high dose cohort and stabilization in the other low dose patient, and stabilization to mild improvements in 6MWT in each of the 4 patients
5. Self-reported improvement in quality of life and function in all low dose-treated subjects.

Stable *LAMP2B* expression data (FIG. 14) has been accompanied by demonstrable and favorable changes in the myocardial architecture, and resolution of the pathologic DD hallmarks, as assessed via electron microscopy of endomyocardial biopsy samples from pre- and post-treatment timepoints. These are illustrated FIG. 14 depicting myocardial tissue from Subject 1005. Resemblant of the findings in the murine knockout model, the pre-treatment biopsy indicates numerous and widespread autophagic vacuoles and profound derangement

of the cardiac myofibrils such that distinct muscle elements are minimally discernable. A similar endomyocardial biopsy at 8 weeks post-treatment demonstrates marked diminution of the autophagic vacuoles, and restoration of myofibrillar architecture with widespread evident striation. These findings are confirmed at the later 9 month timepoint, suggesting that the molecular and histologic resolution is sustained.

Further data regarding relevant cardiac clinical markers in the low dose cohort also demonstrate either improvement or stabilization of important markers of cardiac function including B-type natriuretic peptide (BNP) (Table 5), creatine kinase- myocardial band(MB), and cardiac output as measured by invasive hemodynamics Importantly, the observed improvements in BNP from baseline (1002, 79% and 1005, 75% at 18 and 15 months respectively) were considered highly relevant and promising by experts in the field as natriuretic peptides strongly correlate with prognosis in heart failure (Januzzi et al. *J Investig Med*. 2013 Aug; 61(6): 950–955).

One of the most meaningful parameters of clinical benefit is a patient's level of function, and specifically within the context of heart failure, ability to tolerate physical activity as part of daily life. Endpoints such as New York Heart Association(NYHA) classification are therefore considered a strong measure of clinical improvement that is linked to global patient functional status (Russel et al. *Am Heart J*. 2009 Oct; 158(4 Suppl): S24–S30.). It is significant and highly inconsistent with the natural history of DD that 3 of the 4 subjects with long term follow up in the low and high dose cohort have demonstrated improvement in NYHA classification from II to I (Table 5) and the fourth subject has remained at a stable level of II.

As of the most recent long-term assessment in November 2021, all three patients have demonstrated stabilization and/or slight improvement in their 6MWT results (Table 5). In addition, as reported from the PI interview at the most recent visits, all subjects report feeling generally well and do not report having limitations in their activities. Subjects 1002 and 1005 also have reported that they have not had any firing of their implantable cardiac defibrillators

since receiving RP-A501.

Table 5. Phase 1 Clinical Endpoints in the Low and High Dose Cohort

Cohort	Patient ID	Variable	Baseline	Most Recent Follow-up	Time of Follow-up
Adult - Low Dose	**1001***	**NYHA class**	**II**	**II**	**24 months**
		BNP (pg/mL)	**70**	**30**	
		6 MWT (meters)	**443**	**467**	
	1002	**NYHA class**	**II**	**I**	**18 months**
		BNP (pg/mL)	**942**	**200**	
		6 MWT (meters)	**405**	**410**	
	1005	**NYHA class**	**II**	**I**	**15 months**
		BNP (pg/mL)	**176**	**44**	
		6 MWT (meters)	**427**	**435**	
Adult - High Dose	**1006**	**NYHA class**	**II**	**I**	**12 months**
		BNP (pg/mL)	**123**	**41**	
		6 MWT (meters)	**436**	**492**	

A hallmark of Danon cardiomyopathy in males especially is increased wall thickness resulting from hypertrophy as well as accompanying diastolic dysfunction. As illustrated in FIGs. 15A-15B, 4 of the 5 evaluable patients in the high and low dose either demonstrated stability or decrease in wall thickness as measured by serial echocardiography. In some subjects the decrease in wall thickness was accompanied by mild improvement or stability of their ejection fraction, which is a late manifestation of Danon disease (FIG 15B). In addition to echo-based parameters, invasive hemodynamics enables measurement of pulmonary capillary wedge pressures which are a measure of diastolic dysfunction and left-sided filling pressures. Consistent with the other cardiac parameters the serial wedge pressures and the cardiac output/stroke volume in treated patients demonstrated either an improvement or stabilization (FIGs 15C-15D). Given the natural history of the disease, this is in contrast to the normal progression of these patients.

Table 6 shows RP-A501 demonstrated stable cardiac vector copy numbers (VCN).

Table 6

Cohort	Patient ID	Cardiac VCN	
		Week 8	Month 12
Adult - Low Dose	1001*	0.5	0.6
	1002	6.5	1.5
	1005	2.5	1.9[1]
Adult - High Dose	1006	3.9	1.1
	1007	5.9	6.8 (RV)[2] 9.2 (LV)[2]

* Patient 1001 was only locally monitored for compliance for two weeks; longer compliance monitoring initiated after 100. VCN=Vector Copies per diploid nucleus. [1]Month 9 data. [2]Explanted heart samples at Month 5.

Table 7 shows endomyocardial LAMP2B protein expression by

immunohistochemistry (IHC).

Table 7

Cohort	Patient ID	LAMP2B Protein Expression (by IHC)**	
		Week 8	Month 12
Adult - Low Dose	1001*	7.3%	2.5% (Previously <15%)[1]
	1002	36.9%	67.8%
	1005	17.6%	92.4%[2]
Adult - High Dose	1006	5.0%	100%
	1007	6.9%	100%[3]

* Patient 1001 was only locally monitored for compliance for two weeks; longer compliance monitoring initiated after 1001.

** Endomyocardial biopsies stained for LAMP2 compared to normal control samples. Percent area of cell staining was quantitated using software in a blinded fashion from 2 to 14 sections. Qualitative assessment reported for samples with high variance.

[1] Previously disclosed as a range due to high variance, now clarified. [2] Month 9 data.
[3] Explant sample at Month 5

Table 8 shows endomyocardial LAMP2B Western Blot protein expression.

Table 8

Cohort	Patient ID	LAMP2B Protein Expression (by Western Blot)	
		Week 8	Month 5-18
Adult - Low Dose	1001	20.7%	17.9%[1]

	1002	27.3%	21.2%[2]
	1005	42.8%	61.1%[3]
Adult – High Dose	1006	14.6%	18.2%[1]
	1007	25.0%	RV: 45.1%[4] LV: 44.0%[4]

[1] Month 6 data; inadequate sample at Month 12. [2] Month 18 data; inadequate sample at Month 12. [3] Month 9 data. [4] Explanted heart; Month 5 data.

As detailed above for the low and high dose cohorts from the Phase 1 study, relevant cardiac assessments, including serum heart failure markers, invasive hemodynamic output measurements, echocardiographic assessment and overall functional assessments, all demonstrate improvement in a disease in which even stabilization would represent a positive outcome compared to the natural history of progressive and fatal cardiomyopathy with median mortality of 19 years. These clinical evaluations are accompanied by evidence of *LAMP2* expression in heart muscle and histologic improvement in the vacuolar pathology and myofibrillar disarray that are hallmarks of DD. For these reasons, the totality of the evidence - including both nonclinical and especially clinical data - overwhelmingly demonstrates the prospect of direct benefit of RP-A501.

RP-A501 was generally well tolerated at the low and high dose levels. All observed adverse effects were reversible with no lasting sequelae. Early transaminase and creatinine kinase elevations as well as platelet and hemoglobin decreases returned to baseline or eventually improved. RP-A501 r-AAV dose-dependent toxicity was seen in one of the two patients treated at the high dose level. The affected patient, who received the largest total dose, developed thrombotic microangiopathy (TMA) that fully resolved with supportive treatment including transient hemodialysis. Across both dose levels, the adverse events were reversible and largely resolved at 3 months following treatment with a tailored immune suppressive regimen.

* * *

All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

CLAIMS

What is claimed is:

1. Use of a recombinant adeno-associated virus (rAAV) virion comprising a capsid and a vector genome in the manufacture of a medicament for treating Danon disease in a subject identified as suffering from or at risk for Danon disease and/or having an inactivating mutation in one or more isoforms of the LAMP-2 gene,

wherein the rAAV virion is to be administered at a therapeutically effective amount of less than about 5×10^{14} vector genomes (vg) per kilogram (kg) of the subject's body weight,

wherein the vector genome comprises a polynucleotide sequence encoding a LAMP-2 protein, preferably a LAMP-2B protein.

2. The use of claim 1, wherein the therapeutically effective amount is less than about 2×10^{14} vector genomes (vg) per kilogram (kg) of the subject's body weight.

3. The use of claim 1, wherein the therapeutically effective amount is less than about 1.5×10^{14} vg/kg of the subject's body weight.

4. The use of claim 1, wherein the therapeutically effective amount is less than about 1×10^{14} vg/kg of the subject's body weight.

5. The use of any one of claims 1-4, wherein the therapeutically effective amount is at least about 1×10^{13} vg/kg of the subject's body weight.

6. The use of claim 1, wherein the therapeutically effective amount is about 6.7×10^{13} vg/kg of the subject's body weight.

7. The use of claim 1, wherein the therapeutically effective amount is about 1.1×10^{14} vg/kg of the subject's body weight.

8. The use of claim 1, wherein the therapeutically effective amount is about 2.0×10^{14} vg/kg of the subject's body weight.

9. The use of any one of claims 1-8, wherein an effective amount of tacrolimus, an effective amount of rituximab, and/or an effective amount of eculizumab is/are to be further administered to the subject.

10. The use of claim 9, wherein the subject is at risk for atypical hemolytic-uremic syndrome (aHUS), optionally aHUS resulting in reversible thrombocytopenia and/or acute kidney injury (AKI).

11. The use of any one of claims 1-10, wherein an effective amount of corticosteroids is to be further administered to the subject, optionally prior to administration of an effective amount of tacrolimus to the subject.

12. The use of any one of claims 1-11, wherein the subject is a juvenile subject, optionally having an age of 8-14 years old and/or 15-17 years old, or a pediatric subject, optionally having an age of 0-8 years old.

13. The use of any one of claims 1-12, wherein the therapeutically effective amount of the AAV is to be administered intravenously, by direct cardiac injection, by intraperitoneal injection.

14. The use of any one of claims 1-13, wherein the use results in one or more of:

a) transduction of cardiomyocyte and/or skeletal muscle by the AAV;

b) expression of exogenous ribonucleic acid polynucleotide encoding LAMP-2B and/or expression of exogenous LAMP-2B protein, optionally in cardiomyocyte and/or skeletal muscle;

c) correction or improvement of one or more Danon disease-associated histologic abnormalities, optionally autophagic vacuoles or myofibrillar disarray;

d) correction or improvement of cardiomyocyte molecular marker expression;

e) correction or improvement of cardiomyocyte histology;

f) sustained improvement or stabilization in cardiovascular pathophysiology, radiographic evaluation, and/or cardiopulmonary exercise/physiologic parameters;

g) correction of LAMP2B gene and/or protein expresision;

h) improvement in Danon disease-associated histologic abnormalities;

i) tolerable immunologic response to the AAV;

j) low incidence and/or intensity of treatment-emergent adverse events (TEAEs) and SAEs;

k) low incidence of cardiac interventions, including cardiac transplant, implantable cardioverter-defibrillator, or pacemaker placement, electrophysiologic ablative procedure for cardiac conduction aberrancy, or subsequent hospitalizations for heart failure;

l) low immune response to the AAV, optionally antibodies or T-lymphocytes reactive to AAV and/or to LAMP-2B protein;

m) low incidence and/or intensity of hepatotoxicity, optionally low changes in liver transaminases (AST and ALT), GGT, bilirubin, and ALP; and/or

n) low incidence and/or intensity of h coagulopathy, based on changes from baseline in platelet count, prothrombin time (PT, or International Normalized Ratio (INR)), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer, thrombin-antithrombin complex (TAT), and complement components (complement 3 (C3), complement 4 (C4), and serum membrane attack complex (sC5b-9).

15. The use of any one of claims 1-14, wherein the AAV comprises an expression cassette comprising the polynucleotide sequence encoding the LAMP-2B protein operatively linked to a promoter, and wherein the polynucleotide sequence shares at least 90% identity to SEQ ID NO: 2 and/or the LAMP-2B protein shares at least 90% identity to SEQ ID NO: 1.

16. The use of any one of claims 1-15, wherein the promoter is a CAG promoter, optionally wherein the promoter comprises an enhancer/promoter region that shares at least 95% identity to SEQ ID NO: 22.

17. The use of claim 15, wherein the expression cassette comprises, in 5′ to 3′ order:

(a) an enhancer/promoter region that comprises a sequence having at least 90% identity to SEQ ID NO: 22;

(b) the polynucleotide sequence that encodes the LAMP-2B protein, wherein the polynucleotide sequence comprises a sequence having at least 90% identity to SEQ ID NO: 3;

(c) a 3′ UTR sequence comprising a sequence having at least 90% identity to SEQ ID NO: 27; and/or

(d) a poly-adenylation sequence comprising a sequence having at least 90% identity to SEQ ID NO: 7.

18. The use of claim 15, wherein the capsid is an AAV9 capsid, optionally wherein the AAV9 capsid comprises one or more capsid proteins that comprise a sequence having at least 90% identity to amino acids 1 to 736 of SEQ ID NO: 28, a sequence having at least 90% identity to amino acids 138 to 736 of SEQ ID NO: 28, or a sequence having at least 90% identity to amino acids 203 to 736 of SEQ ID NO: 28.

19. A unit dose, pharmaceutical composition, or composition for use in treating Danon disease, comprising a therapeutically effective amount of an adeno-associated virus (AAV) comprising a polynucleotide sequence encoding a LAMP-2 protein, preferably a LAMP-2B protein, wherein the therapeutically effective amount is less than about 5×10^{14} vector genomes (vg) per kilogram (kg) of the subject's body weight.

20. The unit dose, pharmaceutical composition, or composition for use of claim 19, wherein the therapeutically effective amount is less than about 2×10^{14} vector genomes (vg) per kilogram (kg) of the subject's body weight, optionally wherein the therapeutically effective amount is less than about 1.5×10^{14} vg/kg or less than about 1×10^{14} vg/kg and is at least about 1×10^{13} vg/kg.

21. The unit dose, pharmaceutical composition, or composition for use of any one of claims 19-20, wherein the therapeutically effective amount is about 6.7×10^{13} vg/kg, about 1.1×10^{14} vg/kg, about 1.1×10^{14} vg/kg, or about 2.0×10^{14} vg/kg.

22. The unit dose, pharmaceutical composition, or composition for use of any one of claims 19-21, wherein the AAV comprises an expression cassette comprising the polynucleotide sequence encoding the LAMP-2B protein operatively linked to a promoter, and wherein the polynucleotide sequence shares at least 90% identity to SEQ ID NO: 2 and/or the LAMP-2B protein shares at least 90% identity to SEQ ID NO: 1.

23. The unit dose, pharmaceutical composition, or composition for use of any one of claims 19-22, wherein the promoter is a CAG promoter, optionally wherein the promoter comprises an enhancer/promoter region that shares at least 95% identity to SEQ ID NO: 22.

24. The unit dose, pharmaceutical composition, or composition for use of any one of claims 19-23, wherein the expression cassette comprises, in 5′ to 3′ order:

(a) an enhancer/promoter region that comprises a sequence having at least 90% identity to SEQ ID NO: 22;

(b) the polynucleotide sequence that encodes the LAMP-2B protein, wherein the polynucleotide sequence comprises a sequence having at least 90% identity to SEQ ID NO: 3;

(c) a 3′ UTR sequence comprising a sequence having at least 90% identity to SEQ ID NO: 27; and/or

(d) a poly-adenylation sequence comprising a sequence having at least 90% identity to SEQ ID NO: 7.

25. The unit dose, pharmaceutical composition, or composition for use of any one of claims 19-24, wherein the capsid is an AAV9 capsid, optionally wherein the AAV9 capsid comprises one or more capsid proteins that comprise a sequence having at least 90% identity to amino acids 1 to 736 of SEQ ID NO: 28, a sequence having at least 90% identity to amino acids 138 to 736 of SEQ ID NO: 28, or a sequence having at least 90% identity to amino acids 203 to 736 of SEQ ID NO: 28.

26. A kit comprising the unit dose, pharmaceutical composition, or composition for use of any one of claims 19-25; and instructions for use in treating Danon disease, optionally wherein

the kit further comprises one or more unit dose, pharmaceutical composition, or composition comprising one or more of: rituximab; tacrolimus; eculizumab; and a corticosteroid.