TREATMENT OF MUCOPOLYSACCHARIDOSIS IVA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/967,499, filed January 29, 2020, the content of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] This application incorporates by reference a Sequence Listing submitted with this application as text file entitled "Sequence_Listing_12656-130-228.txt" created on January 21, 2020 and having a size of 47,601 bytes.

1. FIELD

[0003] The field relates to the treatment of mucopolysaccharidosis type IVA (MPS IVA). Provided herein are methods and compositions for treatment of MPS IVA involving recombinant adeno-associated viruses (rAAVs).

2. BACKGROUND

[0004] Mucopolysaccharidosis type IVA (MPS IVA; Morquio A Syndrome) is an autosomal recessive lysosomal storage disorder caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS) (Khan, *et al.*, Mol Genet Metab., 2017; 120(1-2):78-95). Deficiency of the enzyme results in a progressive accumulation of the glycosaminoglycans (GAGs), chondroitin 6-sulfate (C6S), and keratan sulfate (KS) leading to a systemic and unique skeletal dysplasia with incomplete ossification and successive imbalance of growth resulting in a short neck and trunk, cervical spinal cord compression, tracheal obstruction, pectus carinatum, laxity of joints, kyphoscoliosis, coxa valga, and genu valgum. Other clinical maaanifestations of the disease can include hearing loss, heart valve involvement, and corneal opacity. Over 200 different mutations have been identified in patients and the prevalence in the United States is approximately 1 in 250,000.

[0005] Patients with a severe type die of airway compromise, cervical spinal cord complications or heart valve disease in their 20s or 30s if untreated (Khan, et al., Mol Genet Metab., 2017; 120(1-2):78-95); Tomatsu, S., et al. Mol. Genet. Metab. 2016; 117, 150-156;

Montaño, A.M., et al. J. Inherit. Metab. Dis. 2007; 30, 165–174; Tomatsu, S., et al. Res. Rep. Endocr. Disord. 2012; 2012, 65–77; Pizarro, C., et al. Ann. Thorac. Surg. 2016; 102, e329-331). Enzyme replacement therapy (ERT), hematopoietic stem cell transplantation (HSCT), and various surgical intervention are currently available as supportive therapy for patients with MPS IVA in clinical practice. In February of 2014, the FDA approved the use of an ERT (elosulfase-alpha) (Hendriksz, *et al.*, J Inherit Metab Dis., 2014; 37(6): 979–990). ERT, the current standard of care, results in partial improvement in soft tissue pathology and activity of daily living (ADL) of patients with MPS IVA, however, these therapies provide very limited impact in bone and cartilage due to the avascular character of these lesions. Current limitations of ERT include: i) weekly injections for 5-6 hours are required, ii) drug is rapidly cleared from the circulation, iii) the treatment cost is very expensive ($500,000 per year per patient), and v) the drug shows limited penetration to bone (Algahim and Almassi, Ther Clin Risk Manag., 2013;9:45–53; Tomatsu *et al.*, Curr Pharm Biotechnol., 2011;12:931–945). For MPS IVA, weekly administration of recombinant human N-acetylgalactosamine-6-sulfate sulfatase (rhGALNS: Vimizim™, elosulfase alfa) currently provides no impact on bone and cartilage lesions of patients with MPS IVA. While HSCT may provide a better impact than ERT on bone, this cell-based therapy may not be applicable to all patients because of limited matched donors, the age-limit for effective treatment, a lack of well-trained facilities, the mortality risk of the procedure such as graft-versus-host disease (GVHD), infection, and other complications (Tomatsu *et al.*, Drug Des Devel Ther., 2015; 9: 1937–1953). In this sense, a novel drug for MPS IVA, in particular a novel drug for treating skeletal dysplasia in patients with MPS IVA, is urgently required.

[0006] Gene therapy has the potential to be a one-time permanent therapy. Many preclinical studies of gene transfer using viral and non-viral vectors showed the therapeutic potential of this therapy in MPS diseases. Adeno-associated virus (AAV) vector is an attractive vehicle to deliver a therapeutic gene into target organs since vectors provide a long-term expression of transgene product and a low risk of immunogenicity. Because of these advantages, clinical trials of AAV-mediated gene therapy are either ongoing or scheduled for MPS I, II, IIIA, IIIB, and VI (ClinicalTrials.gov; Sawamoto *et al.*, Expert Opin. Orphan Drugs, 2016; 4, 941-951). Delivery of the sufficient enzyme into the cartilage lesions and growth plate region has the potential to resolve the skeletal dysplasia in MPS IVA patients. Our previous study showed that GALNS

gene transfer using AAV2 vector provided therapeutic enzyme level in tissues (Alméciga-Díaz, C.J., et al. Pediatr. Res. 2018; *84*, 545-551); however, until now there has been no study demonstrating that AAV-mediated gene therapy corrects skeletal lesions of MPS IVA mouse model.

[0007] Dvorak-Ewell and colleagues showed that 10 mg/kg rhGALNS conjugated Alexa-488 fluorophore injected intravenously into wild-type mice five times every other day, resulted in the detection of the enzyme in the growth plate and articular cartilage (Dvorak-Ewell, M., et al. PLoS One. 2010; 5, e12194). This finding indicates that a high level of circulating enzyme can provide enzyme penetration into cartilage lesions. AAV8 vectors efficient in transducing liver, and a 10-100-fold greater efficiency in liver gene transfer was shown with the recombinant AAV8 vector, compared to the early generation of the AAV2 vector (Gao, G.P., et al. Proc. Natl. Acad. Sci. U S A. 2002; 99, 11854-11859). The use of liver-specific promoters exhibited a significantly reduced host immune response since liver-directed AAV gene therapy has been reported to induce immune tolerance to the transgene product, compared to ubiquitous promoters (Mingozzi, F., et al. J. Clin. Invest. 2003; 111, 1347-1356; Ziegler, R.J., et al. Mol. Ther. 2004; 9, 231-240; Dobrzynski, E., et al. Proc. Natl. Acad. Sci. U S A. 2006; 103, 4592-4597; Cao, O., et al. Blood 2007; 110, 1132–1140; Mingozzi, F., et al. Blood 2007; 110, 2334-2341). This suppressed immune response can provide a long-term expression of the transgene product (Wang, L., et al. Mol. Ther. 2000; 1, 154-158; Sondhi, D., et al. Gene Ther. 2005; 12, 1618-1632). The previous study demonstrated that the recombinant AAV8 vector in combination with liver-specific promoter provided greater impact on skeletal lesions of mouse and feline model in MPS VI (Tessitore, A., et al. Mol. Ther. 2008; 16, 30-37; Cotugno, G., et al. Mol. Ther. 2011; 19, 461-469).

[0008] Patients with MPS IVA show the most severe skeletal abnormalities in all types of MPS (Melbouci, M., et al. Mol. Genet. Metab. 2018; 124, 1-10), and a bone-targeting strategy could supply sufficient enzyme to penetrate the cartilage region. We have previously demonstrated enhanced bone targeting by attaching a short acidic amino acid tag to the N- or C-terminus of several enzymes (Montaño, A.M., et al Mol. Genet. Metab. 2008; 94, 178-189; Tomatsu, S., et al. Mol. Ther. 2010;18, 1094-1102). Hydroxyapatite (HA) is the major inorganic component in bone and has a positively charged surface that contains calcium ion. Bone sialoprotein and osteopontin bind to HA and these phosphorylated acidic glycoproteins have

repeated sequences of negatively charged acidic amino acids (Asp and Glu), which can be the potential target for bone-targeting strategy (Oldberg, A., et al. J. Biol. Chem. 1988; 263, 19430–19432; Kasugai, S., et al. J. Bone Miner. Res. 2000; 15, 936–943).

[0009] Due to its safety profile, versatility, and ability to be engineered for specific functions, rAAVs can be used in a wide range of gene therapy applications in many diseases (*see*, *e.g.*, Naso *et al.*, BioDrugs. 2017; 31(4): 317–334). Clinical trials using AAV gene therapy have been performed for a wide range of genetic diseases including neuromuscular, ocular, and immunological diseases (*see*, *e.g.*, Kumar *et al.*, Molecular Therapy-Methods & Clinical Development, 2016, 3:16034).

[0010] Citation of a reference herein shall not be construed as an admission that such is prior art to the present disclosure.

3. SUMMARY

[0011] Provided herein are gene therapy methods for the treatment of mucopolysaccharidosis type IVA (MPS IVA) involving the use of recombinant adeno-associated viruses (rAAVs) to deliver human N-acetylgalactosamine-6-sulfate sulfatase (hGALNS) to the bone of a human subject diagnosed with MPS IVA. Also provided herein are rAAVs that can be used in the gene therapy methods, methods of making such rAAVs, as well as polynucleotides, plasmids, and cells that can be used for making such rAAVs.

[0012] In one aspect, provided herein is a recombinant adeno-associated virus (rAAV) comprising: (a) an AAV capsid (for example, AAV8 capsid or AAV9 capsid); and (b) a recombinant AAV genome comprising a human N-acetylgalactosamine-6-sulfate sulfatase (hGALNS) expression cassette flanked by AAV-inverted terminal repeats (ITRs) (for example, AAV2-ITRs, AAV8-ITRs, or AAV9-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a transgene, such as the transgene encoding a fusion protein that is hGALNS fused to an acidic oligopeptide (for example, D8). In a specific embodiment, the hGALNS expression cassette further comprises a nucleotide sequence encoding a liver-specific promoter, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding the fusion protein. In a further specific embodiment, the liver-specific promoter is a thyroxine binding globulin (TBG) promoter.

[0013] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 capsid or AAV9 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs (for example, AAV2-ITRs, AAV8-ITRs, or AAV9-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a liver-specific promoter and a nucleotide sequence encoding hGALNS, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding hGALNS. In a specific embodiment, the liver-specific promoter is a TBG promoter.

[0014] In another aspect, provided herein is a recombinant adeno-associated virus (rAAV) comprising: (a) an AAV capsid (for example, AAV8 capsid); and (b) a recombinant AAV genome comprising a human N-acetylgalactosamine-6-sulfate sulfatase (hGALNS) expression cassette flanked by AAV-inverted terminal repeats (ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene.

[0015] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[0016] In a specific embodiment, the bone-specific promoter is a Sp7/Osx promoter. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:23. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:23.

[0017] In a specific embodiment, the bone-specific promoter is a minimal Sp7/Osx promoter. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:24. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:24.

[0018] In a specific embodiment, the liver-specific promoter is an hAAT (ΔATG) promoter. In a specific embodiment, the hAAT (ΔATG) promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:22. In a specific embodiment, the hAAT (ΔATG) promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:22.

[0019] In a specific embodiment, the bone-liver tandem promoter further comprises a nucleotide sequence encoding an ApoE enhancer. In a specific embodiment, the bone-liver tandem promoter further comprises a nucleotide sequence encoding a hepatic control region comprising an ApoE enhancer. In a specific embodiment, the ApoE enhancer comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:20. In a specific embodiment, the ApoE enhancer promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:20. In a specific embodiment, the hepatic control region comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:19. In a specific embodiment, the hepatic control region comprises a nucleotide sequence that is 100% identical to SEQ ID NO:19.

[0020] In a specific embodiment, the bone-liver tandem promoter is an LBTP1 promoter. In a specific embodiment, the LBTP1 promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:17. In a specific embodiment, the LBTP1 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:17.

[0021] In a specific embodiment, the bone-liver tandem promoter is an LBTP2 promoter. In a specific embodiment, the LBTP2 promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:18. In a specific embodiment, the LBTP2 promoter is comprises a nucleotide sequence that 100% identical to SEQ ID NO:18.

[0022] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

[0023] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[0024] In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:23. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:23.

[0025] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

[0026] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic

oligopeptide, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[0027] In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:24. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:24.

[0028] In various embodiments of the aspects and embodiments of the rAAV described herein, the hGALNS expression cassette further comprises a nucleotide sequence encoding an intron. In a specific embodiment, the intron is a chimeric intron. In a specific embodiment, the chimeric intron is a β-globin/Ig intron. In a specific embodiment, the β-globin/Ig intron comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:10. In a specific embodiment, the β-globin/Ig intron comprises a nucleotide sequence that is 100% identical to SEQ ID NO:10.

[0029] In various embodiments of the aspects and embodiments of the rAAV described herein, the nucleotide sequence encoding the transgene is codon-optimized. In various embodiments of the aspects and embodiments of the rAAV described herein, the nucleotide sequence encoding the transgene has CpG sites depleted. In a specific embodiment, the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:12. In a specific embodiment, the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that is 100% identical to SEQ ID NO: 12.

[0030] In various embodiments of the aspects and embodiments of the rAAV described herein, the nucleotide sequence encoding the transgene comprises a polyadenylation signal. In a specific embodiment, the polyadenylation signal is a β-globin polyadenylation signal. In a specific embodiment, the β-globin polyadenylation signal comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:25. In a specific embodiment, the β-globin polyadenylation signal comprises a nucleotide sequence that is 100% identical to SEQ ID NO:25. In a specific embodiment, the polyadenylation signal is a rabbit globin poly A site. In a specific embodiment, the rabbit globin poly A site comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at

least 95%, at least 98% or 100% identical to SEQ ID NO:9. In a specific embodiment, the rabbit globin poly A site comprises a nucleotide sequence that is 100% identical to SEQ ID NO:9.

[0031] In various embodiments of the aspects and embodiments of the rAAV described herein, the AAV is AAV8. In various embodiments of the aspects and embodiments of the rAAV described herein, the AAV is AAV9.

[0032] In another aspect, provided herein is a pharmaceutical composition comprising an rAAV provided herein and a pharmaceutically acceptable carrier.

[0033] In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (for example, AAV2-ITRs, AAV8-ITRs, or AAV9-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a transgene, such as the transgene encoding a fusion protein that is hGALNS fused to an acidic oligopeptide (for example, D8). In a specific embodiment, the hGALNS expression cassette further comprises a nucleotide sequence encoding a liver-specific promoter, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding the fusion protein. In a further specific embodiment, the liver-specific promoter is a TBG promoter.

[0034] In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (for example, AAV2-ITRs, AAV8-ITRs, or AAV9-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a liver-specific promoter and a nucleotide sequence encoding hGALNS, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding hGALNS. In a specific embodiment, the liver-specific promoter is a TBG promoter.

[0035] In another aspect, provided herein is a polynucleotide comprising a hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene.

[0036] In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a

nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[0037] In a specific embodiment, the bone-specific promoter is a Sp7/Osx promoter. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:23. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:23.

[0038] In a specific embodiment, the bone-specific promoter is a minimal Sp7/Osx promoter. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:24. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:24.

[0039] In a specific embodiment, the liver-specific promoter is an hAAT (ΔATG) promoter. In a specific embodiment, the hAAT (ΔATG) promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:22. In a specific embodiment, the hAAT (ΔATG) promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:22.

[0040] In a specific embodiment, the bone-liver tandem promoter further comprises a nucleotide sequence encoding an ApoE enhancer. In a specific embodiment, the bone-liver tandem promoter further comprises a nucleotide sequence encoding a hepatic control region comprising an ApoE enhancer. In a specific embodiment, the ApoE enhancer comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:20. In a specific embodiment, the ApoE enhancer promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:20. In a specific embodiment, the hepatic control region comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:19. In a

specific embodiment, the hepatic control region comprises a nucleotide sequence that is 100% identical to SEQ ID NO:19.

[0041] In a specific embodiment, the bone-liver tandem promoter is an LBTP1 promoter. In a specific embodiment, the LBTP1 promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:17. In a specific embodiment, the LBTP1 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:17.

[0042] In a specific embodiment, the bone-liver tandem promoter is an LBTP2 promoter. In a specific embodiment, the LBTP2 promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:18. In a specific embodiment, the LBTP2 promoter is comprises a nucleotide sequence that 100% identical to SEQ ID NO:18.

[0043] In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

[0044] In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[0045] In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:23. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:23.

[0046] In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a

nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

[0047] In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[0048] In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:24. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:24.

[0049] In various embodiments of the aspects and embodiments of the polynucleotide described herein, the hGALNS expression cassette further comprises a nucleotide sequence encoding an intron. In a specific embodiment, the intron is a chimeric intron. In a specific embodiment, the chimeric intron is a β-globin/Ig intron. In a specific embodiment, the β-globin/Ig intron comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:10. In a specific embodiment, the β-globin/Ig intron comprises a nucleotide sequence that is 100% identical to SEQ ID NO:10.

[0050] In various embodiments of the aspects and embodiments of the polynucleotide described herein, the nucleotide sequence encoding the transgene is codon-optimized. In various embodiments of the aspects and embodiments of the polynucleotide described herein, the nucleotide sequence encoding the transgene has CpG sites depleted. In a specific embodiment, the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:12. In a specific embodiment, the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that is 100% identical to SEQ ID NO: 12.

[0051] In various embodiments of the aspects and embodiments of the polynucleotide described herein, the nucleotide sequence encoding the transgene comprises a polyadenylation signal. In a specific embodiment, the polyadenylation signal is a β-globin polyadenylation signal. In a specific embodiment, the β-globin polyadenylation signal comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:25. In a specific embodiment, the β-globin polyadenylation signal comprises a nucleotide sequence that is 100% identical to SEQ ID NO:25. In a specific embodiment, the polyadenylation signal is a rabbit globin poly A site. In a specific embodiment, the rabbit globin poly A site comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:9. In a specific embodiment, the rabbit globin poly A site comprises a nucleotide sequence that is 100% identical to SEQ ID NO:9.

[0052] In various embodiments of the aspects and embodiments of the polynucleotide described herein, the AAV is AAV8. In various embodiments of the aspects and embodiments of the polynucleotide described herein, the AAV is AAV9.

[0053] In another aspect, provided herein is an rAAV plasmid comprising a polynucleotide provided herein.

[0054] In another aspect, provided herein is an *ex vivo* cell comprising a polynucleotide provided herein or an rAAV plasmid provided herein.

[0055] In another aspect, provided herein is a method of making an rAAV comprising transfecting an *ex vivo* cell with an rAAV plasmid provided herein and one or more helper plasmids collectively comprising the nucleotide sequences of AAV genes Rep, Cap, VA, E2a and E4.

[0056] In another aspect, provided herein is a method for treating a human subject diagnosed with mucopolysaccharidosis type IVA (MPS IVA), which comprises administering to the human subject an rAAV provided herein or a pharmaceutical composition provided herein.

[0057] In another aspect, provided herein is a method for treating a human subject diagnosed with MPS IVA, comprising delivering to the bone and liver of the human subject a therapeutically effective amount of hGALNS or a fusion protein that is hGALNS fused to an acidic oligopeptide (as the case may be), by administering to the human subject an rAAV

provided herein. In a specific embodiment, the hGALNS or the fusion protein is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell.

[0058] In another aspect, provided herein is a method for treating a human subject diagnosed with MPS IVA, comprising delivering to the bone of the human subject a therapeutically effective amount of hGALNS or a fusion protein that is hGALNS fused to an acidic oligopeptide (as the case may be), by administering to the human subject an rAAV provided herein.

[0059] In another aspect, provided herein is a method for treating a human subject diagnosed with MPS IVA, which comprises delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve of the human subject a therapeutically effective amount of a transgene, such as the transgene encoding a fusion protein that is hGALNS fused to an acidic oligopeptide (for example, D8), by administering to the human subject an rAAV provided herein. In a specific embodiment, the hGALNS is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell.

[0060] In another aspect, provided herein is a method for treating a human subject diagnosed with MPS IVA, which comprises delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve of the human subject a therapeutically effective amount of hGALNS that is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell, by administering to the human subject an rAAV provided herein.

[0061] In another aspect, provided herein is a method for treating a human subject diagnosed with MPS IVA, which comprises delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve of the human subject a therapeutically effective amount of a fusion protein that is hGALNS fused to an acidic oligopeptide (for example, D8), wherein the fusion protein is produced from an rAAV genome (for example, a recombinant AAV8 genome (*i.e.*, a recombinant genome comprising the backbone of an AAV8 genome)).

[0062] In another aspect, provided herein is a method for treating a human subject diagnosed with MPS IVA, which comprises delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve of the human subject a therapeutically effective amount of a transgene encoding a transgene, such as the transgene

encoding a fusion protein that is hGALNS fused to an acidic oligopeptide (for example, D8), wherein the fusion protein is produced from an rAAV genome (for example, a recombinant AAV8 genome (*i.e.*, a recombinant genome comprising the backbone of an AAV8 genome)) and is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell.

[0063] In another aspect, provided herein is a method for treating a human subject diagnosed with MPS IVA, which comprises delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve of the human subject a therapeutically effective amount of hGALNS that is produced from an rAAV genome (for example, a recombinant AAV8 genome (*i.e.*, a recombinant genome comprising the backbone of an AAV8 genome)) and is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell.

[0064] In certain aspects and embodiments of the method of treating a human subject diagnosed with MPS IVA that comprises delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve of the human subject, the step of delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve is a step of delivering to the bone and/or cartilage.

[0065] In certain aspects and embodiments of the method of treating a human subject diagnosed with MPS IVA that comprises delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve of the human subject, the step of delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve is a step of delivering to (a) the bone and/or cartilage, and (b) ligament, meniscus, growth plate, liver, spleen, lung, heart muscle, and/or heart valve

[0066] In one aspect, provided herein is a recombinant adeno-associated virus (rAAV) comprising: (a) an AAV capsid (for example, AAV8 capsid or AAV9 capsid); and (b) a recombinant AAV genome comprising a human N-acetylgalactosamine-6-sulfate sulfatase (hGALNS) expression cassette flanked by AAV-inverted terminal repeats (ITRs) (for example, AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes

hGALNS, and wherein the nucleotide sequence encoding the promoter is operably linked to the nucleotide sequence encoding the transgene.

[0067] In one aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a promoter and a nucleotide sequence encoding a transgene, and wherein the nucleotide sequence encoding the promoter is operably linked to the nucleotide sequence encoding the transgene.

[0068] In some aspects, the promoter is TBG. In some aspects, the nucleotide sequence encoding the promoter comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 6; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 6; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 6; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 6; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 6; or a nucleotide sequence that is 100% identical to SEQ ID NO: 6.

[0069] In some aspects, the promoter is CAG. In some aspects, the nucleotide sequence encoding the promoter comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 28; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 28; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 28; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 28; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 28; or a nucleotide sequence that is 100% identical to SEQ ID NO: 28.

[0070] In some aspects, the promoter is LSPX1. In some aspects, the nucleotide sequence encoding the promoter comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 13; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 13; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 13; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 13; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 13; or a nucleotide sequence that is 100% identical to SEQ ID NO: 13.

[0071] In some aspects, the promoter is LMTP6. In some aspects, the nucleotide sequence encoding the promoter comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 16; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 16; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 16; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 16; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 16; or a nucleotide sequence that is 100% identical to SEQ ID NO: 16.

[0072] In some aspects, the promoter is LBTP2. In some aspects, the nucleotide sequence encoding the promoter comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 18; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 18; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 18; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 18; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 18; or a nucleotide sequence that is 100% identical to SEQ ID NO: 18.

[0073] In some aspects, the nucleotide sequence encoding the transgene comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 27; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 27; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 27; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 27; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 27; or a nucleotide sequence that is 100% identical to SEQ ID NO: 27.

[0074] In some aspects, the nucleotide sequence encoding the transgene comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 3; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 3; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 3; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 3; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 3; or a nucleotide sequence that is 100% identical to SEQ ID NO: 3.

[0075] In some aspects, the nucleotide sequence encoding the transgene comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 12; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 12; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 12; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 12; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 12; or a nucleotide sequence that is 100% identical to SEQ ID NO: 12.

[0076] In some aspects, the nucleotide sequence encoding the transgene comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 2; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 2; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 2; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 2; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 2; or a nucleotide sequence that is 100% identical to SEQ ID NO: 2.

[0077] In one aspect, provided herein is a recombinant adeno-associated virus (rAAV) comprising: (a) an AAV capsid (for example, AAV8 capsid or AAV9 capsid); and (b) a recombinant AAV genome comprising a human N-acetylgalactosamine-6-sulfate sulfatase (hGALNS) expression cassette flanked by AAV-inverted terminal repeats (ITRs) (for example, AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, wherein the nucleotide sequence encoding the promoter is operably linked to the nucleotide sequence encoding the transgene, and wherein the nucleotide sequence encoding the transgene comprises: a nucleotide sequence that is at least 80% identical to SEQ ID NO: 27; a nucleotide sequence that is at least 85% identical to SEQ ID NO: 27; a nucleotide sequence that is at least 90% identical to SEQ ID NO: 27; a nucleotide sequence that is at least 95% identical to SEQ ID NO: 27; a nucleotide sequence that is at least 98% identical to SEQ ID NO: 27; or a nucleotide sequence that is 100% identical to SEQ ID NO: 27.

[0078] In some aspects, provided herein is a pharmaceutical composition comprising one or more rAAV of the disclosure and a pharmaceutically acceptable carrier. In some aspects, provided herein is a method for treating a human subject diagnosed with mucopolysaccharidosis type IVA (MPS IVA), comprising administering to the human subject one or more rAAV of the disclosure.

3.1 Illustrative Embodiments

1. A recombinant adeno-associated virus (rAAV) comprising:
   (a) an AAV capsid; and
   (b) a recombinant AAV genome comprising a human N-acetylgalactosamine-6-sulfate sulfatase (hGALNS) expression cassette flanked by AAV-inverted terminal repeats (ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene.
2. An rAAV comprising:

(a) an AAV capsid; and

(b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene.

3. The rAAV of claim 2, wherein the acidic oligopeptide is D8.
4. The rAAV of any one of paragraphs 1-3, wherein the bone-specific promoter is a Sp7/Osx promoter.
5. The rAAV of paragraph 4, wherein the Sp7/Osx promoter:
   (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 23;
   (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 23;
   (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 23;
   (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 23;
   (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 23; or
   (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 23.
6. The rAAV of paragraph 4, wherein the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 23.
7. The rAAV of any one of paragraphs 1-3, wherein the bone-specific promoter is a minimal Sp7/Osx promoter.
8. The rAAV of paragraph 7,wherein the minimal Sp7/Osx promoter:
   (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 24;
   (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 24;
   (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 24;
   (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 24;
   (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 24; or
   (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 24.
9. The rAAV of paragraph 7, wherein the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 24.

10. The rAAV of any one of paragraphs 1-9, wherein the liver-specific promoter is an hAAT (ΔATG) promoter.
11. The rAAV of paragraph 10, wherein the hAAT (ΔATG) promoter:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 22;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 22;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 22;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 22;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 22; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 22.
12. The rAAV of paragraph 10, wherein the hAAT (ΔATG) promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 22.
13. The rAAV of any one of paragraphs 1-12, wherein the bone-liver tandem promoter further comprises a nucleotide sequence encoding an ApoE enhancer.
14. The rAAV of any one of paragraphs 1-12, wherein the bone-liver tandem promoter further comprises a nucleotide sequence encoding a hepatic control region comprising an ApoE enhancer.
15. The rAAV of paragraph 13 or 14, wherein the ApoE enhancer:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 20;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 20;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 20;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 20;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 20; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 20.
16. The rAAV of paragraph 13 or 14, wherein the ApoE enhancer comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 20.
17. The rAAV of paragraph 14, wherein the hepatic control region:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 19;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 19;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 19;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 19;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 19; or

(f) comprises a nucleotide sequence that is at least 100% identical to SEQ ID NO: 19.

18. The rAAV of paragraph 14, wherein the hepatic control region comprises a nucleotide sequence that is at least 100% identical to SEQ ID NO: 19.
19. The rAAV of any one of paragraphs 1-3, wherein the bone-liver tandem promoter is an LBTP1 promoter.
20. The rAAV of paragraph 19, wherein the LBTP1 promoter:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 17;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 17;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 17;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 17;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 17; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 17.
21. The rAAV of paragraph 19, wherein the LBTP1 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 17.
22. The rAAV of any one of paragraphs 1-3, wherein the bone-liver tandem promoter is an LBTP2 promoter.
23. The rAAV of paragraph 22, wherein the LBTP2 promoter:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 18;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 18;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 18;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 18;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 18; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 18.
24. The rAAV of paragraph 22, wherein the LBTP2 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 18.
25. An rAAV comprising:
    (a) an AAV capsid; and
    (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the

transgene encodes hGALNS, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

26. An rAAV comprising:

(a) an AAV capsid; and

(b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

27. The rAAV of paragraph 26, wherein the acidic oligopeptide is D8.

28. The rAAV of any one of paragraphs 25-27,wherein the Sp7/Osx promoter:

(a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 23;

(b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 23;

(c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 23;

(d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 23;

(e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 23; or

(f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 23.

29. The rAAV of any one of paragraphs 25-27, wherein the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 23.

30. An rAAV comprising:

(a) an AAV capsid; and

(b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

31. An rAAV comprising:

(a) an AAV capsid; and

(b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

32. The rAAV of paragraph 31, wherein the acidic oligopeptide is D8.
33. The rAAV of any one of paragraphs 30-32,wherein the minimal Sp7/Osx promoter:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 24;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 24;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 24;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 24;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 24; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 24.
34. The rAAV of any one of paragraphs 30-32, wherein the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 24.
35. The rAAV of any one of paragraphs 1-34, wherein the hGALNS expression cassette further comprises a nucleotide sequence encoding an intron.
36. The rAAV of paragraph 35, wherein the intron is a chimeric intron.
37. The rAAV of paragraph 36, wherein the chimeric intron is a β-globin/Ig intron.
38. The rAAV of paragraph 37, wherein the β-globin/Ig intron:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 10;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 10;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 10;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 10;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 10; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 10.
39. The rAAV of paragraph 37, wherein the β-globin/Ig intron comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 10.
40. The rAAV of any one of paragraphs 1-39, wherein the nucleotide sequence encoding the transgene is codon-optimized.

41. The rAAV of any one of paragraphs 1-40, wherein the nucleotide sequence encoding the transgene has CpG sites depleted.
42. The rAAV of any one of paragraphs 1-41, wherein the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that:
    (a) is at least 80% identical to SEQ ID NO: 12;
    (b) is at least 85% identical to SEQ ID NO: 12;
    (c) is at least 90% identical to SEQ ID NO: 12;
    (d) is at least 95% identical to SEQ ID NO: 12;
    (e) is at least 98% identical to SEQ ID NO: 12; or
    (f) is 100% identical to SEQ ID NO: 12.
43. The rAAV of any one of paragraphs 1-41, wherein the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that is 100% identical to SEQ ID NO: 12.
44. The rAAV of any one of paragraphs 1-43, wherein the nucleotide sequence encoding the transgene comprises a polyadenylation signal.
45. The rAAV of paragraph 44, wherein the polyadenylation signal is a β-globin polyadenylation signal.
46. The rAAV of paragraph 45, wherein the β-globin polyadenylation signal:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 25;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 25;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 25;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 25;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 25; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 25.
47. The rAAV of paragraph 45, wherein the β-globin polyadenylation signal comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 25.
48. The rAAV of paragraph 44, wherein the polyadenylation signal is a rabbit globin poly A site.
49. The rAAV of paragraph 48, wherein the rabbit globin poly A site:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 9;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 9;

(c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 9;

(d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 9;

(e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 9; or

(f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 9.

50. The rAAV of paragraph 48, wherein the rabbit globin poly A site comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 9.

51. The rAAV of any one of paragraphs 1-50, wherein the AAV is AAV8.

52. The rAAV of any one of paragraphs 1-50, wherein the AAV is AAV9.

53. A pharmaceutical composition comprising the rAAV of any one of paragraphs 1-52 and a pharmaceutically acceptable carrier.

54. A polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene.

55. A polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene.

56. The polynucleotide of paragraph 55, wherein the acidic oligopeptide is D8.

57. The polynucleotide of any one of paragraphs 54-56, wherein the bone-specific promoter is a Sp7/Osx promoter.

58. The polynucleotide of paragraph 57, wherein the Sp7/Osx promoter:

(a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 23;

(b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 23;

(c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 23;

(d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 23;

(e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 23; or

(f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 23.

59. The polynucleotide of paragraph 57, wherein the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 23.

60. The polynucleotide of any one of paragraphs 54-56, wherein the bone-specific promoter is a minimal Sp7/Osx promoter.

61. The polynucleotide of paragraph 60,wherein the minimal Sp7/Osx promoter:

(a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 24;

(b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 24;

(c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 24;

(d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 24;

(e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 24; or

(f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 24.

62. The polynucleotide of paragraph 60, wherein the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 24.

63. The polynucleotide of any one of paragraphs 54-62, wherein the liver-specific promoter is an hAAT (ΔATG) promoter.

64. The polynucleotide of paragraph 63, wherein the hAAT (ΔATG) promoter:

(a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 22;

(b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 22;

(c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 22;

(d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 22;

(e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 22; or

(f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 22.

65. The polynucleotide of paragraph 63, wherein the hAAT (ΔATG) promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 22.

66. The polynucleotide of any one of paragraphs 54-65, wherein the bone-liver tandem promoter further comprises a nucleotide sequence encoding an ApoE enhancer.

67. The polynucleotide of any one of paragraphs 54-65, wherein the bone-liver tandem promoter further comprises a nucleotide sequence encoding a hepatic control region comprising an ApoE enhancer.
68. The polynucleotide of paragraph 66 or 67, wherein the ApoE enhancer:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 20;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 20;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 20;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 20;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 20; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 20.
69. The polynucleotide of paragraph 66 or 67, wherein the ApoE enhancer comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 20.
70. The polynucleotide of paragraph 67, wherein the hepatic control region:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 19;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 19;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 19;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 19;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 19; or
    (f) comprises a nucleotide sequence that is at least 100% identical to SEQ ID NO: 19.
71. The polynucleotide of paragraph 67, wherein the hepatic control region comprises a nucleotide sequence that is at least 100% identical to SEQ ID NO: 19.
72. The polynucleotide of any one of paragraphs 54-56, wherein the bone-liver tandem promoter is an LBTP1 promoter.
73. The polynucleotide of paragraph 72, wherein the LBTP1 promoter:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 17;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 17;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 17;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 17;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 17; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 17.

74. The polynucleotide of paragraph 72, wherein the LBTP1 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 17.
75. The polynucleotide of any one of paragraphs 54-56, wherein the bone-liver tandem promoter is an LBTP2 promoter.
76. The polynucleotide of paragraph 75, wherein the LBTP2 promoter:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 18;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 18;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 18;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 18;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 18; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 18.
77. The polynucleotide of paragraph 75, wherein the LBTP2 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 18.
78. A polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.
79. A polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.
80. The polynucleotide of paragraph 79, wherein the acidic oligopeptide is D8.
81. The polynucleotide of any one of paragraphs 78-80,wherein the Sp7/Osx promoter:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 23;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 23;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 23;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 23;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 23; or

(f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 23.

82. The polynucleotide of any one of paragraphs 78-80, wherein the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 23.
83. A polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.
84. A polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.
85. The polynucleotide of paragraph 84, wherein the acidic oligopeptide is D8.
86. The polynucleotide of any one of paragraphs 83-85,wherein the minimal Sp7/Osx promoter:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 24;
    (b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 24;
    (c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 24;
    (d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 24;
    (e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 24; or
    (f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 24.
87. The polynucleotide of any one of paragraphs 83-85, wherein the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 24.
88. The polynucleotide of any one of paragraphs 54-87, wherein the hGALNS expression cassette further comprises a nucleotide sequence encoding an intron.
89. The polynucleotide of paragraph 88, wherein the intron is a chimeric intron.
90. The polynucleotide of paragraph 89, wherein the chimeric intron is a β-globin/Ig intron.
91. The polynucleotide of paragraph 90, wherein the β-globin/Ig intron:
    (a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 10;

(b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 10;

(c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 10;

(d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 10;

(e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 10; or

(f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 10.

92. The polynucleotide of paragraph 90, wherein the β-globin/Ig intron comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 10.

93. The polynucleotide of any one of paragraphs 54-92, wherein the nucleotide sequence encoding the transgene is codon-optimized.

94. The polynucleotide of any one of paragraphs 54-93, wherein the nucleotide sequence encoding the transgene has CpG sites depleted.

95. The polynucleotide of any one of paragraphs 54-94, wherein the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that:

(a) is at least 80% identical to SEQ ID NO: 12;

(b) is at least 85% identical to SEQ ID NO: 12;

(c) is at least 90% identical to SEQ ID NO: 12;

(d) is at least 95% identical to SEQ ID NO: 12;

(e) is at least 98% identical to SEQ ID NO: 12; or

(f) is 100% identical to SEQ ID NO: 12.

96. The polynucleotide of any one of paragraphs 54-94, wherein the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that is 100% identical to SEQ ID NO: 12.

97. The polynucleotide of any one of paragraphs 54-96, wherein the nucleotide sequence encoding the transgene comprises a polyadenylation signal.

98. The polynucleotide of paragraph 97, wherein the polyadenylation signal is a β-globin polyadenylation signal.

99. The polynucleotide of paragraph 98, wherein the β-globin polyadenylation signal:

(a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 25;

(b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 25;

(c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 25;

(d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 25;

(e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 25; or

(f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 25.

100. The polynucleotide of paragraph 98, wherein the β-globin polyadenylation signal comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 25.

101. The polynucleotide of paragraph 97, wherein the polyadenylation signal is a rabbit globin poly A site.

102. The polynucleotide of paragraph 101, wherein the rabbit globin poly A site:

(a) comprises a nucleotide sequence that is at least 80% identical to SEQ ID NO: 9;

(b) comprises a nucleotide sequence that is at least 85% identical to SEQ ID NO: 9;

(c) comprises a nucleotide sequence that is at least 90% identical to SEQ ID NO: 9;

(d) comprises a nucleotide sequence that is at least 95% identical to SEQ ID NO: 9;

(e) comprises a nucleotide sequence that is at least 98% identical to SEQ ID NO: 9; or

(f) comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 9.

103. The polynucleotide of paragraph 101, wherein the rabbit globin poly A site comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 9.

104. The polynucleotide of any one of paragraphs 54-103, wherein the AAV is AAV8.

105. The polynucleotide of any one of paragraphs 54-103, wherein the AAV is AAV9.

106. An rAAV plasmid comprising the polynucleotide of any one of paragraphs 54-105.

107. An *ex vivo* cell comprising the polynucleotide of any one of paragraphs 54-105 or the rAAV plasmid of paragraph 106.

108. A method of making an rAAV comprising transfecting an *ex vivo* cell with the rAAV plasmid of paragraph 106 and one or more helper plasmids collectively comprising the nucleotide sequences of AAV genes Rep, Cap, VA, E2a and E4.

109. A method for treating a human subject diagnosed with mucopolysaccharidosis type IVA (MPS IVA), comprising administering to the human subject the rAAV of any one of paragraphs 1-52 or the pharmaceutical composition of paragraph 53.

110. A method for treating a human subject diagnosed with MPS IVA, comprising delivering to the bone and liver of the human subject a therapeutically effective amount of hGALNS or a fusion protein that is hGALNS fused to an acidic oligopeptide, by administering to the human subject an rAAV of any one of paragraphs 1-24, or of any

one of paragraphs 35-52 when dependent directly or indirectly on any one of paragraphs 1-24.

111. The method of paragraph 110, wherein the hGALNS or the fusion protein is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell.

112. A method for treating a human subject diagnosed with MPS IVA, comprising delivering to the bone of the human subject a therapeutically effective amount of hGALNS or a fusion protein that is hGALNS fused to an acidic oligopeptide, by administering to the human subject an rAAV of any one of paragraphs 25-34, or of any one of paragraphs 35-52 when dependent directly or indirectly on any one of paragraphs 25-34.

113. An rAAV comprising:

(a) an AAV capsid; and

(b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, wherein the nucleotide sequence encoding the promoter is operably linked to the nucleotide sequence encoding the transgene, and wherein the nucleotide sequence encoding the transgene comprises:

(a) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 27;

(b) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 27;

(c) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 27;

(d) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 27;

(e) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 27; or

(f) a nucleotide sequence that is 100% identical to SEQ ID NO: 27.

114. The rAAV of paragraph 13, wherein the AAV capsid is an AAV8 capsid or a variant thereof.

115. The rAAV of paragraph 13, wherein the AAV capsid is an AAV9 capsid or a variant thereof.

116. A polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a

promoter and a nucleotide sequence encoding a transgene, and wherein the nucleotide sequence encoding the promoter is operably linked to the nucleotide sequence encoding the transgene.

117. The rAAV of any one of paragraphs 113-115 or the polynucletide of paragraph 116, wherein the promoter is TBG.

118. The rAAV of any one of paragraphs 113-115 or 117 or the polynucletide of any one of paragraphs 116-117, wherein the nucleotide sequence encoding the promoter comprises:

(a) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 6;

(b) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 6;

(c) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 6;

(d) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 6;

(e) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 6; or

(f) a nucleotide sequence that is 100% identical to SEQ ID NO: 6.

119. The rAAV of any one of paragraphs 113-115 or the polynucletide of paragraph 116, wherein the promoter is CAG.

120. The rAAV of any one of paragraphs 113-115 or 119 or the polynucletide of any one of paragraphs 116 or 119, wherein the nucleotide sequence encoding the promoter comprises:

(a) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 28;

(b) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 28;

(c) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 28;

(d) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 28;

(e) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 28; or

(f) a nucleotide sequence that is 100% identical to SEQ ID NO: 28.

121. The rAAV of any one of paragraphs 113-115 or the polynucletide of paragraph 116, wherein the promoter is LSPX1.

122. The rAAV of any one of paragraphs 113-115 or 121 or the polynucletide of any one of paragraphs 116 or 121, wherein the nucleotide sequence encoding the promoter comprises:

(a) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 13;

(b) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 13;

(c) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 13;

(d) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 13;

(e) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 13; or

(f) a nucleotide sequence that is 100% identical to SEQ ID NO: 13.

123. The rAAV of any one of paragraphs 113-115 or the polynucletide of paragraph 116, wherein the promoter is LMTP6.

124. The rAAV of any one of paragraphs 113-115 or 123 or the polynucletide of any one of paragraphs 116 or 124, wherein the nucleotide sequence encoding the promoter comprises:

(a) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 16;

(b) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 16;

(c) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 16;

(d) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 16;

(e) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 16; or

(f) a nucleotide sequence that is 100% identical to SEQ ID NO: 16.

125. The rAAV of any one of paragraphs 113-115 or the polynucletide of paragraph 116, wherein the promoter is LBTP2.

126. The rAAV of any one of paragraphs 113-115 or 125 or the polynucletide of any one of paragraphs 116 or 125, wherein the nucleotide sequence encoding the promoter comprises:

(a) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 18;

(b) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 18;

(c) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 18;

(d) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 18;

(e) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 18; or

(f) a nucleotide sequence that is 100% identical to SEQ ID NO: 18.

127. The rAAV of any one of claims 113-115 or 117-126 or the polynucletide of any one of claims 116-126, wherein the AAV-ITRs is AAV2-ITRs.

128. A pharmaceutical composition comprising the rAAV of any one of claims 113-115 or 117-127 and a pharmaceutically acceptable carrier.

129. A method for treating a human subject diagnosed with mucopolysaccharidosis type IVA (MPS IVA), comprising administering to the human subject the rAAV of any one of claims 113-115 or 117-127 or the pharmaceutical composition of claim 128.

4. ABBREVIATIONS

MPS IVA	mucopolysaccharidosis type IVA
GALNS	N-acetylgalactosamine-6-sulfate sulfatase
hGALNS	human N-acetylgalactosamine-6-sulfate sulfatase
GAG	glycosaminoglycan
C6S	chondroitin 6-sulfate
KS	keratan sulfate
ERT	enzyme replacement therapy
HSCT	hematopoietic stem cell transplantation
AAV	adeno-associated virus
TBG	thyroxine binding globulin
ITR	inverted terminal repeats
D8	aspartic acid octapeptide
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
HS	heparan sulfate
IS	internal standard
LC-MS/MS	liquid chromatography/tandem mass spectrometry
OD	optical density
PBS	phosphate buffered saline
RBG pA	rabbit beta-globin poly A
KO	knockout
Sp7/Osx	Osterix promoter

5. BRIEF DESCRIPTION OF THE FIGURES

[0079] The foregoing and other objects, features and advantages will be apparent from the following description of particular embodiments of the invention, as illustrated in the

accompanying drawings. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of various embodiments of the invention.

[0080] **FIG. 1.** Schematics of rAAV genomes.

[0081] **FIGS. 2A-2D.** (A) Intracellular enzyme activity was determined in HuH-7 cells after transfection with either the TBG-hGALNS plasmid, TBG-hGALNS-CoOpt plasmid, TBG-D8-hGALNS plasmid, or TBG-D8-hGALNS-CoOpt plasmid (n=2). (B) Depiction of individual runs of the intracellular enzyme activity determined in HuH-7 cells. (C) Enzyme activity in the media was determined after HuH-7 cells were transfected with the TBG-hGALNS plasmid, TBG-hGALNS-CoOpt plasmid, TBG-D8-hGALNS plasmid, or TBG-D8-hGALNS-CoOpt plasmid (n=2). (D) Depiction of individual runs of the enzyme activity in media determined in HuH-7 cells.

[0082] **FIG. 3.** Intracellular enzyme activity was determined in HepG2 cells after transfection with either the TBG-hGALNS plasmid, TBG-hGALNS-CoOpt plasmid, TBG-D8-hGALNS plasmid, or TBG-D8-hGALNS-CoOpt plasmid.

[0083] **FIG. 4.** Schedule of the *in vivo* study in which AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS were administered to 4-week-old MPS IVA KO mice (galns -/-) and immune tolerant mice ($Galns^{tm(hC79S.mC76S)slu}$, Mtol). The schedule of enzyme assay and KS assay in blood is shown. When describing dosage, vector copies per kilogram (vc/kg) and gene copies per kilogram (GC/kg) are used interchangeably.

[0084] **FIGS. 5A-5B.** hGALNS enzyme activity over time measured in (A) white blood cells (WBCs) and (B) plasma of MPS IVA KO mice (galns -/-) after administration with AAV8-TBG-hGALNS r or AAV-TBG-D8-hGALNS.

[0085] **FIG. 6.** hGALNS enzyme activity over time measured in plasma of Mtol mice after administration with AAV8-TBG-hGALNS (n=4) or AAV8-TBG-D8-hGALNS (n=4).

[0086] **FIGS. 7A-7D.** hGALNS enzyme activity measured in (A) the liver of MPS IVA KO mice (galns -/-), (B) the liver of Mtol mice, (C) the heart of MPS IVA KO mice (galns -/-) and the heart of Mtol mice, and (D) the bone of MPS IVA KO mice (galns -/-) and the bone of Mtol mice.

[0087] **FIG. 8.** Mono-sulfated KS levels in the plasma of MPS IVA KO mice (galns -/-) treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS.

[0088] **FIGS. 9A-9B.** (A) Mono-sulfated KS levels in the plasma of Mtol mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS over time as compared to untreated Mtol and WT mice. (B) Mono-sulfated KS levels in the plasma of Mtol mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS were significantly less as compared to untreated Mtol mouse levels at 16 weeks of age (n = 4-5; mean ± SD; *p <0.05 vs. WT; #p < 0.05 vs. Untreated; one-way ANOVA).

[0089] **FIG. 10.** Blood diHS-0S levels measured over time in MPS IVA KO mice (galns -/-) treated with AAV8-TBG-hGALNS, treated with AAV8-TBG-D8-hGALNS, untreated, or WT mice.

[0090] **FIGS. 11A-11P.** (A) Graphical depiction of bone pathology scores. Bone pathology was evaluated by histopathological analysis 12 weeks after administration of vectors AAV8-hGALNS or AAV8-D8-hGALNS in MPS IVA KO mice (galns -/-). Histopathology of (B) knee joints (Lig-Ligament; M-meniscus; F-Femur; T-Tibia), (C-F) femur articular cartilage (40x magnification), (G-J) femur growth plate (40x magnification), (K) meniscus (40x magnification), (L) ligament (tibia side, 40x magnification), (M, N) base of the heart valve (40x magnification), and (O, P) heart valve (40x magnification).

[0091] **FIGS. 12A-12C.** (A) hGALNS enzyme activity levels measured in the liver of MPS IVA KO mice (galns -/-) and Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD). (B) hGALNS enzyme activity levels measured in the spleen of MPS IVA KO mice (galns -/-) and the spleen of Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD). (C) hGALNS enzyme activity levels measured in the lung of MPS IVA KO mice (galns -/-) and the lung of Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD).

[0092] **FIGS. 13A-13B.** (A) hGALNS enzyme activity levels measured in the bone of MPS IVA KO mice (galns -/-) and the bone of Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD). (B) hGALNS

enzyme activity levels measured in the heart of MPS IVA KO mice (galns -/-) and the heart of Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD).

[0093] **FIG. 14.** Mono-sulfated KS levels in the plasma of MPS IVA KO mice (galns -/-) treated with AAV8-TBG-D8-hGALNS as compared to untreated MPS IVA KO mice and untreated wild type mice (n=4-8; mean ± SD).

[0094] **FIGS. 15A-15B.** (A) Mono-sulfated KS levels in the plasma of Mtol mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS over time as compared to untreated Mtol and WT mice. (B) Mono-sulfated KS levels in the plasma of Mtol mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS were significantly less as compared to untreated Mtol mouse levels at 16 weeks of age (n = 4-5; mean ± SD; *p <0.05 vs. WT; #p < 0.05 vs. Untreated; one-way ANOVA).

[0095] **FIGS. 16A-16C.** (A) Mono-sulfated KS levels in the liver of MPS IVA KO mice (galns -/-) treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS as compared to untreated MPS IVA KO mice and untreated wild type mice. (B) Mono-sulfated KS levels in the lung of MPS IVA KO mice (galns -/-) treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS as compared to untreated MPS IVA KO mice and untreated wild type mice. (C) Mono-sulfated KS levels in the liver of Mtol mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS as compared to untreated Mtol mice and untreated wild type mice. For (A)-(C), n = 3-8; mean ± SD; *p <0.05 vs. WT; #p < 0.05 vs. Untreated; one-way ANOVA.

[0096] **FIGS. 17A-17E.** Histopathology of femur growth plate (40x magnification) in (A) wild type mice (all chondrocytes were non-vacuolated and column structure was well organized), (B) untreated MPS IVA KO mice (galns -/-) (all chondrocytes were vacuolated and column structure was largely disorganized and distorted), (C) untreated Mtol mice (all chondrocytes were vacuolated and column structure was largely disorganized and distorted), (D) AAV8-TBG-hGALNS treated Mtol mice (chondrocytes were moderately vacuolated but column structure was better), and (E) AAV8-TBG-D8-hGALNS treated Mtol mice (chondrocytes were moderately vacuolated but column structure was partially recovered).

[0097] **FIGS. 18A-18D.** (A) Chondrocyte cell size measured in the femur growth plate of untreated wild type mice, untreated MPS IVA KO mice (galns -/-), AAV8-TBG-hGALNS

treated MPS IVA KO mice (galns -/-), or AAV8-TBG-D8-hGALNS treated MPS IVA KO mice (galns -/-). (B) Chondrocyte cell size measured in the femur growth plate of untreated wild type mice, untreated Mtol mice, AAV8-TBG-hGALNS treated Mtol mice, or AAV8-TBG-D8-hGALNS treated Mtol mice. (C) Chondrocyte cell size measured in the tibia growth plate of untreated wild type mice, untreated MPS IVA KO mice (galns -/-), AAV8-TBG-hGALNS treated MPS IVA KO mice (galns -/-), or AAV8-TBG-D8-hGALNS treated MPS IVA KO mice (galns -/-). (D) Chondrocyte cell size measured in the tibia growth plate of untreated wild type mice, untreated Mtol mice, AAV8-TBG-hGALNS treated Mtol mice, or AAV8-TBG-D8-hGALNS treated Mtol mice. For (A)-(D), n=4-6; mean ± SD; *p <0.05 vs. WT; #p < 0.05 vs. untreated; one-way ANOVA.

[0098] **FIG. 19.** Histopathology of heart valve (40x magnification) in MPS IVA KO mice (galns -/-) and Mtol mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated mice.

[0099] **FIG. 20.** Histopathology of heart muscle (40x magnification) in Mtol mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated Mtol mice.

[00100] **FIGS. 21A-21D.** (A) Pathology score of the heart valve tissue of untreated wild type mice, untreated MPS IVA KO(galns -/-) mice, MPS IVA KO(galns -/-) mice treated with AAV8-TBG-hGALNS, or MPS IVA KO(galns -/-) mice treated with AAV8-TBG-D8-hGALNS. (B) Pathology score of the heart valve tissue of untreated wild type mice, untreated Mtol mice, Mtol mice treated with AAV8-TBG-hGALNS, or Mtol mice treated with AAV8-TBG-D8-hGALNS. (C) Pathology score of the heart muscle tissue of untreated wild type mice, untreated MPS IVA KO(galns -/-) mice, MPS IVA KO(galns -/-) mice treated with AAV8-TBG-hGALNS, or MPS IVA KO(galns -/-) mice treated with AAV8-TBG-D8-hGALNS. (D) Pathology score of the heart muscle tissue for untreated wild type mice, untreated Mtol mice, Mtol mice treated with AAV8-TBG-hGALNS, or Mtol mice treated with AAV8-TBG-D8-hGALNS. (For FIGS. 21A-21D, n = 4-6; mean ± SD; *p <0.05 vs. WT; #p < 0.05 vs. Untreated; one-way ANOVA).

[00101] **FIG. 22.** hGALNS enzyme activity over time measured in plasma of MPS IVA KO mice (galns -/-) after administration with AAV8-TBG-hGALNS or AAV-TBG-D8-hGALNS (n=4-7; mean + SD).

[00102] **FIG. 23.** hGALNS enzyme activity over time measured in plasma of Mtol mice after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS (n=4-5; mean + SD).

[00103] FIGS. 24A-24K. Blood and tissue human N-acetylgalactosamine-6-sulfate sulfatase (hGALNS) enzyme activity in MPS IVA mice treated with AAV8 vectors. (A) Schematic structure of AAV8-TBG-hGALNS and AAV8-TBG-D8-hGALNS viral vector genome. A blood sample was collected from MPS IVA mice every other week until 16 weeks of age, and plasma hGALNS enzyme activity was measured in (B) knock-out (KO), and (C) tolerant (MTOL) mice. n = 4-7. The tissue sample was collected from MPS IVA mice 12 weeks post-injection of AAV vectors with or without bone-targeting signal. hGALNS enzyme activity in tissues including (D) liver, (E) spleen, (F) lung, (G) kidney, (H) heart and (I) bone (leg) was measured in KO and MTOL mice (n = 3-8; statistics were analyzed by one-way ANOVA with the Bonferroni's post-hoc test and data are presented as mean ± SD. *$p < 0.05$). The levels of hGALNS activity in the liver, spleen, lung, kidney, heart, and bond in MPS IVA KO mice 12 weeks after IV delivery of AAV vectors are shown in (J). The levels of hGALNS activity in the liver, spleen, lung, kidney, heart, and bond in MTOL mice 12 weeks after IV delivery of AAV vectors are shown in (K).

[00104] FIGS. 25A-25D. Blood and tissue glycosaminoglycan (GAG) level in MPS IVA mice treated with AAV8 vectors. A blood sample was collected from MPS IVA mice every other week until 16 weeks of age, and plasma mono-sulfated KS level was measured in (A) knock-out (KO), and (B) tolerant (MTOL) mice. n = 4-8. The tissue sample was collected from MPS IVA mice 12 weeks post-injection of AAV vectors with or without bone-targeting signal. The amount of KS in tissues including (C) liver and (D) lung was measured in KO and MTOL mice. n = 4-8. Statistics were analyzed by one-way ANOVA with the Bonferroni's post-hoc test. Data are presented as mean ± SD. *$p < 0.05$.

[00105] FIGS. 26A-26C. Correction of bone pathology in MPS IVA mice treated with AAV8 vectors. Correction of chondrocytes vacuolization was assessed by toluidine blue staining analysis using light microscopy of (A) growth plate and (B) articular disc in the knee joint of MPS IVA mice treated with AAV8 vectors. Bone pathology in knock-out (KO), and tolerant (MTOL) mice were compared with wild-type, untreated MPS IVA and treated MPS IVA with AAV8 vectors with or without bone-targeting signal. Scale bars = 25 μm. (C) Chondrocyte cell size in growth plate lesions of femur or tibia was quantified by Image J software. Data expressed fold-change from wild-type group. n = 4-7. Statistics were analyzed by one-way ANOVA with the Bonferroni's post-hoc test. Data are presented as mean ± SD. *$p < 0.05$.

[00106] **FIGS. 27A-27B.** Correction of heart pathology in MPS IVA mice treated with AAV8 vectors. Correction of vacuolization was assessed by toluidine blue staining analysis using light microscopy of (A) heart valve and (B) heart muscle of MPS IVA mice treated with AAV8 vectors. Heart pathology in knock-out (KO) and tolerant (MTOL) mice were compared with wild-type, untreated MPS IVA, and treated MPS IVA with AAV8 vectors with or without bone-targeting signal. The arrows indicate the location of disease-related vacuoles. Scale bars = 25 μm.

[00107] **FIGS. 28.** Circulating of anti-hGALNS antibody titers in MPS IVA mice treated with AAV8 vectors. Plasma was collected from MPS IVA mice 12 weeks post-injection of AAV8 vectors with or without bone targeting signal. Circulating snit-hGALNS antibody titers were detected by indirect ELISA assay. OD 405 values were measured in a microplate spectrophotometer. n=4-8. Statistics were analyzed by one-way ANOVA with the Bonferroni's post-hoc test. Data are presented as mean ± SD. *$p < 0.05$.

[00108] **FIGS. 29A-29B.** Evaluation of optimized hGALNS with or without bone targeting signal. Huh-7 cells were transfected with AAV8 vector plasmid expressing hGALNS or codon-optimized hGALNS with or without bone targeting signal, respectively. After 48 hr transfection, cell pellet and medium were collected, and hGALNS activity was measured. (A) Intracellular enzyme activity was determined in HuH-7 cells after transfection with either the TBG-hGALNS plasmid, TBG-hGALNS-CoOpt plasmid, TBG-D8-hGALNS plasmid, or TBG-D8-hGALNS-CoOpt plasmid. (B) Enzyme activity in the media was determined after HuH-7 cells were transfected with the TBG-hGALNS plasmid, TBG-hGALNS-CoOpt plasmid, TBG-D8-hGALNS plasmid, or TBG-D8-hGALNS-CoOpt plasmid. Data are presented as mean. n = 2.

[00109] **FIG. 30.** Blood heparan sulfate (HS) level in MPS IVA mice treated with AAV8 vectors. A blood sample was collected from MPS IVA mice and plasma diHS-0S level was measured in knock-out (KO), and tolerant (MTOL) mice at 16 weeks of age. Data are presented as mean ± SD. AAV: adeno-associated virus, TBG: thyroxin-binding globulin, hGALNS: N-acetylgalactosamine-6-sulfate sulfatase.

[00110] **FIG. 31.** Tissue heparin sulfate (HS) level in MPS IVA mice treated with AAV8 vectors. The tissue sample was collected from MPS IVA mice 12 weeks post-injection of AAV vectors with or without bone-targeting signal. The amount of diHS-0S in tissues including liver,

spleen, lung and kidney was measured in KO and MTOL mice. Data are presented as mean ± SD.

[00111] FIGS. 32A-32B. Correction of bone pathology in MPS IVA mice treated with AAV8 vectors. Correction of chondrocytes vacuolization was assessed by toluidine blue staining analysis using light microscopy of (A) ligament and (B) meniscus in the knee joint of MPS IVA mice treated with AAV8 vectors. Bone pathology in knock-out (KO), and tolerant mice (MTOL) were compared with wild-type, untreated MPS IVA and treated MPS IVA with AAV8 vectors with or without bone-targeting signal. The arrows indicate the location of disease-related vacuoles (scale bars = 25 μm).

[00112] FIG. 33. A LBTP1 promoter map showing a minimal Sp7/Osx promoter fragment (Lu, X., et al. JBC 281, 6297-6306, January 12, 2006) (SEQ ID NO:24) flanked 5' by a liver-specific ApoE enhancer/hepatic control region, and 3' by a hAAT promoter depleted of ATG trinucleotides (hAAT(ΔATG)) to drive hepatocyte-specific expression. A chimeric β-globin/Ig intron was placed downstream (3') of the promoter sequence, i.e. downstream of the hAAT(ΔATG). The minimal Sp7/Osx promoter fragment drives osteoblast-specific expression and was determined to be a transcriptionally active fragment of the full-length Sp7/Osx promoter (Lu, X., et al. JBC 281, 6297-6306, January 12, 2006).

[00113] FIG. 34. A LBTP2 promoter map showing a full-length Sp7/Osx promoter (Lu, X., et al. JBC 281, 6297-6306, January 12, 2006) (SEQ ID NO:23) flanked 5' by a liver-specific ApoE enhancer/hepatic control region, and 3' by a hAAT promoter depleted of ATG trinucleotides (hAAT(ΔATG)) to drive hepatocyte-specific expression.

[00114] FIG. 35. A schematic diagram of a full-length Sp7/Osx promoter-driving hGALNS-expressing AAV construct.

[00115] FIG. 36. A schematic diagram of a minimal Sp7/Osx promoter-driving hGALANS-expressing AAV construct.

[00116] FIG. 37. A schematic diagram of a LBTP1 promoter-driving hGALNS-expressing AAV construct for hepatocyte and osteoblast-specific expression. The construct comprises: a minimal Sp7/Osx promoter fragment driving osteoblast-specific expression. The minimal Sp7 promoter fragment is flanked by 5' liver-specific ApoE enhancer and 3' hAAT promoter depleted of ATG trinucleotides (hAATΔ) that drives hepatocyte-specific expression, a chimeric intron, and a codon optimized and CpG-negative GALNS protein coding sequence.

[00117] **FIG. 38.** A schematic diagram of a LBTP2 promoter-driving hGALNS-expressing AAV construct for hepatocyte and osteoblast-specific expression. The construct comprises: a full length Sp7/Osx promoter fragment driving osteoblast-specific expression. The full-length Sp7/Osx promoter fragment is flanked by 5' liver-specific ApoE enhancer and 3' hAAT promoter depleted of ATG trinucleotides (hAATΔ) that drives hepatocyte-specific expression, a chimeric intron, and a codon optimized and CpG-negative GALNS protein coding sequence

[00118] **FIG. 39.** Volumes of interest of trabecular bone shown in white regions (trabecular bone on left, cortical bone on right).

6. DETAILED DESCRIPTION

[00119] The present invention is at least partially based on a surprising finding that administration of recombinant adeno-associated viruses (rAAVs) comprising certain hGALNS expression cassettes in animal models of mucopolysaccharidosis type IVA (MPS IVA) maintained high levels of hGALNS enzymatic activity throughout the monitoring period and resulted in improvement in tissues including the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and heart valve, exhibiting an improvement over what has been achieved by enzyme replacement therapy (ERT).

[00120] Described herein are rAAVs for use in the treatment of MPS IVA in a human subject in need of treatment. These rAAVs comprise a recombinant AAV genome encoding for hGALNS. The rAAV can be administered to an MPS IVA patient resulting in the synthesis of hGALNS and the delivery of hGALNS to the affected tissues, such as bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve, thereby improving pathology, and preventing the progression of the disease.

[00121] Provided is a recombinant adeno-associated virus (rAAV) comprising an AAV capsid and a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-inverted terminal repeats (ITRs). In certain embodiments, the rAAV capsid is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to the serotype AAV8 capsid. In certain embodiments, the amino acid sequence of the rAAV capsid is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID: NO. 1. In certain embodiments, the amino acid sequence of the rAAV capsid is 80-85%, 85-90%, 90-95%, 95-99% or 99-99.9% identical to SEQ ID: NO. 1. In certain embodiments, the amino acid sequence of the rAAV capsid is

identical to SEQ ID NO: 1. In certain embodiments, the amino acid sequence of the rAAV capsid comprises SEQ ID NO: 1. In certain embodiments, the rAAV capsid is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to the serotype AAV9 capsid. In certain embodiments, the amino acid sequence of the rAAV capsid is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID: NO. 26. In certain embodiments, the amino acid sequence of the rAAV capsid is 80-85%, 85-90%, 90-95%, 95-99% or 99-99.9% identical to SEQ ID: NO. 26. In certain embodiments, the amino acid sequence of the rAAV capsid is identical to SEQ ID NO: 26. In certain embodiments, the amino acid sequence of the rAAV capsid comprises SEQ ID NO: 26. For more detail regarding rAAV capsids, see Section 6.1.1. In some embodiments, the hGALNS expression cassette comprises a nucleotide sequence encoding a fusion protein that is hGALNS fused to an acidic oligopeptide. In certain embodiments, the acidic oligopeptide is D8. In certain embodiments, the hGALNS expression cassette further comprises a nucleotide sequence encoding a liver-specific promoter (for example, a thyroxine binding globulin (TBG) promoter). In certain embodiments, the hGALNS expression cassette further comprises a nucleotide sequence encoding a bone-liver tandem promoter (for example, a LBTP1 or LBTP2 promoter), wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter. In certain embodiments, the bone-specific promoter is a Sp7/Osx promoter or a minimal Sp7/Osx promoter. In certain embodiments, the liver-specific promoter is an hAAT promoter or preferably an hAAT (ΔATG) promoter. In certain embodiments, the hGALNS expression cassette additionally comprises a nucleotide sequence encoding a poly A site. In other embodiments, the hGALNS expression cassette comprises a nucleotide sequence encoding a liver-specific promoter (for example, a TBG promoter) and a nucleotide sequence encoding hGALNS, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding hGALNS. In certain embodiments, the hGALNS expression cassette additionally comprises a nucleotide sequence encoding a poly A site.

[00122] Also provided herein are polynucleotides comprising an hGALNS expression cassette as described herein. Further provided are plasmids and cells (*e.g., ex vivo* host cells) comprising a polynucleotide provided herein for making the rAAVs for use with the methods and compositions provided herein.

[00123] Further provided herein are methods for making an rAAV described herein.

[00124] Also provided herein are methods for treating a human subject diagnosed with mucopolysaccharidosis type IVA (MPS IVA). In one aspect, the method comprises administering an rAAV described herein to the human subject. In another aspect, the method comprises delivering glycosylated hGALNS (for example, hGALNS that is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell) to the affected tissue(s). In another aspect, the method comprises delivering a fusion protein that is hGALNS fused to an acidic oligopeptide to the affected tissue(s). The fusion protein can be glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell.

[00125] Further provided herein are pharmaceutical compositions and kits comprising an rAAV described herein.

[00126] The rAAVs provided herein are described in Section 6.1, which includes a description of rAAV capsids in Section 6.1.1 and a description of the hGALNS expression cassette in Section 6.1.2. Methods of making an rAAV provided herein as well as polynucleotides, plasmids and cells that can be used in such methods are described in Section 6.2. Methods for treating a human subject diagnosed with MPS IVA, including target patient populations, routes of administration and dosage regimens are described in Section 6.3. Combination therapies are described in Section 6.4. Disease markers and methods to assess clinical outcomes are described in Section 6.5. Non-limiting illustrative examples are provided in Section 7.

[00127] Without being bound by theory, the manufacture, composition, and method of use of the rAAVs can be modified such that it still results in delivery of the hGALNS enzyme to the bone, cartilage, ligament, meniscus, and/or heart valve of a human subject as a treatment for MPS IVA.

6.1 RECOMBINANT ADENO-ASSOCIATED VIRUSES (rAAVs)

[00128] Provided herein are rAAVs useful for the treatment of MPS IVA in a human subject in need thereof, which rAAVs comprise an AAV capsid and a recombinant AAV genome comprising an hGALNS expression cassette.

[00129] In one aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 or AAV9 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a transgene, such as the transgene encoding a fusion

protein that is hGALNS fused to an acidic oligopeptide. The hGALNS expression cassette may further comprise a nucleotide sequence encoding a liver-specific promoter, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding the fusion protein.

[00130] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 or AAV9 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a liver-specific promoter and a nucleotide sequence encoding hGALNS, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding hGALNS.

[00131] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 or AAV9 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV- ITRs, the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene.

[00132] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 or AAV9 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[00133] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 or AAV9 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence

encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

[00134] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 or AAV9 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[00135] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 or AAV9 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

[00136] In another aspect, provided herein is an rAAV comprising: (a) an AAV capsid (for example, AAV8 or AAV9 capsid); and (b) a recombinant AAV genome comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[00137] In specific embodiments, provided herein is an rAAV comprising: (a) an AAV9 capsid; and (b) a recombinant AAV genome comprising an hGALNScoV2 expression cassette flanked by AAV2- ITRs, the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a CAG promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS (or hGALNScoV2), and wherein the nucleotide sequence

encoding the CAG promoter is operably linked to the nucleotide sequence encoding the transgene. In some embodiments, this construct is further referred to as AAV9-CAG-hGALNScoV2, e.g., as disclosed in Example 12 of the disclosure.

[00138] In specific embodiments, provided herein is an rAAV comprising: (a) an AAV9 capsid; and (b) a recombinant AAV genome comprising an hGALNScoV2 expression cassette flanked by AAV2- ITRs, the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a LMTP6 promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS (or hGALNScoV2), and wherein the nucleotide sequence encoding the LMTP6 promoter is operably linked to the nucleotide sequence encoding the transgene. In some embodiments, this construct is further referred to as AAV9-LMTP6-hGALNScoV2, e.g., as disclosed in Example 12 of the disclosure.

[00139] In specific embodiments, provided herein is an rAAV comprising: (a) an AAV9 capsid; and (b) a recombinant AAV genome comprising an hGALNScoV2 expression cassette flanked by AAV2- ITRs, the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a LBTP2 promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS (or hGALNScoV2), and wherein the nucleotide sequence encoding the LBTP2 promoter is operably linked to the nucleotide sequence encoding the transgene. In some embodiments, this construct is further referred to as AAV9-LBTP2-hGALNScoV2, e.g., as disclosed in Example 12 of the disclosure.

[00140] In specific embodiments, provided herein is an rAAV comprising: (a) an AAV8 capsid; and (b) a recombinant AAV genome comprising an hGALNScoV2 expression cassette flanked by AAV2- ITRs, the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a CAG promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS (or hGALNScoV2), and wherein the nucleotide sequence encoding the CAG promoter is operably linked to the nucleotide sequence encoding the transgene. In some embodiments, this construct is further referred to as AAV8-CAG-hGALNScoV2, e.g., as disclosed in Example 12 of the disclosure.

[00141] In specific embodiments, provided herein is an rAAV comprising: (a) an AAV8 capsid; and (b) a recombinant AAV genome comprising an hGALNScoV2 expression cassette flanked by AAV2- ITRs, the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a LMTP6 promoter and a nucleotide sequence encoding a transgene, wherein

the transgene encodes hGALNS (or hGALNScoV2), and wherein the nucleotide sequence encoding the LMTP6 promoter is operably linked to the nucleotide sequence encoding the transgene. In some embodiments, this construct is further referred to as AAV8-LMTP6-hGALNScoV2, e.g., as disclosed in Example 12 of the disclosure.

[00142] In specific embodiments, provided herein is an rAAV comprising: (a) an AAV8 capsid; and (b) a recombinant AAV genome comprising an hGALNScoV2 expression cassette flanked by AAV2- ITRs, the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a LBTP2 promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS (or hGALNScoV2), and wherein the nucleotide sequence encoding the LBTP2 promoter is operably linked to the nucleotide sequence encoding the transgene. In some embodiments, this construct is further referred to as AAV8-LBTP2-hGALNScoV2, e.g., as disclosed in Example 12 of the disclosure.

[00143] In specific embodiments, provided herein is an rAAV comprising: (a) an AAV8 capsid; and (b) a recombinant AAV genome comprising an hGALNScoV2 expression cassette flanked by AAV2- ITRs, the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a TBG promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS (or hGALNScoV2), and wherein the nucleotide sequence encoding the TBG promoter is operably linked to the nucleotide sequence encoding the transgene. In some embodiments, this construct is further referred to as AAV8-TBG-hGALNScoV2, e.g., as disclosed in Example 12 of the disclosure.

[00144] In specific embodiments, provided herein is an rAAV comprising: (a) an AAV8 capsid; and (b) a recombinant AAV genome comprising an hGALNScoV2 expression cassette flanked by AAV2- ITRs, the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a LSPX1 promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS (or hGALNScoV2), and wherein the nucleotide sequence encoding the LSPX1 promoter is operably linked to the nucleotide sequence encoding the transgene. In some embodiments, this construct is further referred to as AAV8-LSPX1-hGALNScoV2, e.g., as disclosed in Example 12 of the disclosure.

[00145] In specific embodiments, provided herein is an rAAV comprising: (a) an AAV8 capsid; and (b) a recombinant AAV genome comprising an hGALNSco expression cassette flanked by AAV2- ITRs, the hGALNSco expression cassette comprising a nucleotide sequence

encoding a TBG promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS (or hGALNSco), and wherein the nucleotide sequence encoding the TBG promoter is operably linked to the nucleotide sequence encoding the transgene. In some embodiments, this construct is further referred to as AAV8-TBG-hGALNSco, e.g., as disclosed in Example 12 of the disclosure.

[00146] In various embodiments of the aspects and embodiments described herein, the AAV is AAV8. In various embodiments of the aspects and embodiments of the rAAV described herein, the AAV is AAV9.

[00147] In certain embodiments, the hGALNS expression cassette comprises a nucleotide sequence encoding a liver-specific promoter, such that the hGALNS protein is expressed in the liver, which hGALNS protein, once secreted from liver cells, is translocated to other tissues, including, but are not limited to, the severely affected organs, such as the bone, cartilage and associated tissue, and heart valve.

[00148] The different components of rAAVs provided herein are described in detail below.

6.1.1 Capsid

[00149] The capsid is the protein shell of a virus that packages and protects the viral genome while interacting with the host environment. According to the invention, an rAAV provided herein comprises an AAV capsid. In a specific embodiment, an AAV capsid is the capsid of a naturally found AAV (for example, the capsid of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, or AAV11). In another specific embodiment, an AAV capsid is derived from the capsid of a naturally found AAV (for example, the capsid of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, or AAV11), for example, by having an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.9%, or 100% identical to the amino acid sequence of the capsid of the naturally found AAV.

[00150] In certain embodiments, AAV variant capsids that can be used according to the invention described herein include Anc80 or Anc80L65, as described in Zinn et al., 2015, Cell Rep. 12(6): 1056-1068, which is incorporated by reference in its entirety. In certain embodiments, AAV variant capsids that can be used according to the invention described herein comprise one of the following amino acid insertions: LGETTRP or LALGETTRP, as described

in United States Patent Nos. 9,193,956; 9,458,517; and 9,587,282 and US patent application publication no. 2016/0376323, each of which is incorporated herein by reference in its entirety. In certain embodiments, AAV variant capsids that can be used according to the invention described herein include AAV.7m8, as described in United States Patent Nos. 9,193,956; 9,458,517; and 9,587,282 and US patent application publication no. 2016/0376323, each of which is incorporated herein by reference in its entirety. In certain embodiments, AAV variant capsids that can be used according to the invention described herein include any AAV disclosed in United States Patent No. 9,585,971, such as AAV-PHP.B. In certain embodiments, AAV variant capsids that can be used according to the invention include, but are not limited to, those disclosed in any of the following patents and patent applications, each of which is incorporated herein by reference in its entirety: United States Patent Nos. 7,282,199; 7,906,111; 8,524,446; 8,906,675; 8,999,678; 8,628,966; 8,927,514; 8,734,809; US 9,284,357; 9,409,953; 9,169,299; 9,193,956; 9458517; 9,587,282; 9,737,618; 9,840,719; US patent application publication nos. 2015/0374803; 2015/0126588; 2017/0067908; 2013/0224836; 2016/0215024; 2017/0051257; and International Patent Application Nos. PCT/US2002/033630; PCT/US2004/028817; PCT/2002/033629; PCT/US2006/013375; PCT/US2015/034799; PCT/EP2015/053335; PCT/US2016/042472; PCT/US2017/027392.

[00151] In certain embodiments, a single-stranded AAV (ssAAV) may be used *supra*. In certain embodiments, a self-complementary vector, *e.g.*, scAAV, may be used (*see*, *e.g.*, Wu, 2007, Human Gene Therapy, 18(2):171-82, McCarty et al, 2001, Gene Therapy, Vol 8, Number 16, Pages 1248-1254; and U.S. Patent Nos. 6,596,535; 7,125,717; and 7,456,683, each of which is incorporated herein by reference in its entirety).

[00152] In specific embodiments, the AAV capsid contained in the rAAV is the capsid of AAV8 or derived from the capsid of AAV8. AAV8 has greater liver transduction efficiency than other serotypes and low reactivity to antibodies against human AAVs. Importantly, specific regions of the AAV8 capsid contribute to the high liver transduction by mediating nuclear entry and capsid uncoating (Tenney *et al.*, Virology, 2014, 454–455: 227-236; Nam *et al.*, J Virol., 2007 81(22): 12260–12271). As a result, AAV8 has a tropism for hepatocytes (Sands, M., Methods Mol Biol., 2011;807:141–157). In certain embodiments, the amino acid sequence of the AAV capsid contained in the rAAV is identical to the amino acid sequence of the AAV8 capsid (SEQ ID NO: 1). In certain embodiments, the amino acid sequence of the AAV capsid

contained in the rAAV is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.9% identical to the amino acid sequence of the AAV8 capsid (SEQ ID NO: 1), while retaining the ability of the AAV8 capsid to package a viral genome and preferably also the ability of the AAV8 capsid to transduce liver cells at a high efficiency. In certain embodiments, the amino acid sequence of the AAV capsid contained in the rAAV is identical to the amino acid sequence of the AAV8 capsid (SEQ ID NO: 1) except for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acid residues, while retaining the ability of the AAV8 capsid to package a viral genome and preferably also the ability of the AAV8 capsid to transduce liver cells at a high efficiency. In a specific embodiment of the treatment method described herein, AAV8 is used for targeted liver expression of the hGALNS protein.

[00153] In specific embodiments, the AAV capsid contained in the rAAV is the capsid of AAV9 or derived from the capsid of AAV9. In certain embodiments, the amino acid sequence of the AAV capsid contained in the rAAV is identical to the amino acid sequence of the AAV9 capsid (SEQ ID NO: 26). In certain embodiments, the amino acid sequence of the AAV capsid contained in the rAAV is at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.9% identical to the amino acid sequence of the AAV9 capsid (SEQ ID NO: 26), while retaining the ability of the AAV9 capsid to package a viral genome and preferably also the ability of the AAV9 capsid to transduce liver cells at a high efficiency. In certain embodiments, the amino acid sequence of the AAV capsid contained in the rAAV is identical to the amino acid sequence of the AAV9 capsid (SEQ ID NO: 26) except for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acid residues, while retaining the ability of the AAV9 capsid to package a viral genome and preferably also the ability of the AAV9 capsid to transduce liver cells at a high efficiency. In a specific embodiment of the treatment method described herein, AAV9 is used for targeted liver expression of the hGALNS protein.

6.1.2 **hGALNS Expression Cassette**

[00154] AAV has a linear single-stranded DNA (ssDNA) genome that contains two inverted terminal repeats (ITRs) at the termini. AAV enters into cells by endocytosis (Meier and Greber, J Gene Med., 2004;6 Suppl 1:S152-63). Upon capsid breakdown, the ssDNA genome is released

and converted to double-stranded DNA (dsNDA), from which genes encoded by the viral genome can be expressed (Ding *et al.*, 2005, Gene Ther., 12: 873-880).

[00155] According to the invention, an rAAV provided herein comprises a recombinant AAV genome. The recombinant AAV genome can comprise the backbone of an AAV genome or its variant (for example, the backbone of an AAV1, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, or AAV11 genome or its variant). In some embodiments, the recombinant AAV genome can comprise the backbone of an AAV8 genome or its variant. In some embodiments, the recombinant AAV genome can comprise the backbone of an AAV9 genome or its variant. In a specific embodiment, the recombinant AAV genome comprises the backbone of an AAV8 genome or its variant. In a specific embodiment, the recombinant AAV genome comprises the backbone of an AAV9 genome or its variant.

[00156] According to the invention, the recombinant AAV genome comprises an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs). In some embodiments, the hGALNS expression cassette is hGALNSco. In some embodiments, the hGALNS expression cassette is hGALNScoV2. In some embodiments, the recombinant AAV genome comprises the backbone of an AAV8 genome or its variant and comprises hGALNScoV2 expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs). In some embodiments, the recombinant AAV genome comprises the backbone of an AAV9 genome or its variant and comprises hGALNScoV2 expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs). In some embodiments, the recombinant AAV genome comprises the backbone of an AAV8 genome or its variant and comprises hGALNSco expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs). In some embodiments, the recombinant AAV genome comprises the backbone of an AAV9 genome or its variant and comprises hGALNSco expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs). In some embodiments, the hGALNS expression cassette comprises a nucleotide sequence encoding a fusion protein that is hGALNS fused to an acidic oligopeptide. The hGALNS expression cassette may further comprise a nucleotide sequence encoding a liver-specific promoter, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding the fusion protein. In other embodiments, the hGALNS expression cassette comprises a nucleotide sequence encoding a liver-specific promoter and a nucleotide sequence encoding hGALNS, wherein the nucleotide sequence

encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding hGALNS.

(a) **hGALNS**

[00157] In some embodiments, hGALNS is one or more of hGALNS, hGALNSco, D8-hGALNS, D8-GALNSco, or hGALNScoV2. In some embodments, hGALNS is hGALNScoV2. In some embodments, hGALNS is hGALNSco. In some embodments, hGALNS is hGALNS. In some embodments, hGALNS is D8-GALNSco. In some embodments, hGALNS is D8-GALNS.

[00158] In certain embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein comprises the sequence of SEQ ID NO: 2, 3, or 27. In certain embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the sequence set forth in SEQ ID NO: 2, 3, or 27.

[00159] In specific embodiments, the nucleotide sequence encoding hGALNS is hGALNScoV2. In certain embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein is hGALNScoV2. In some embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein comprises the sequence of SEQ ID NO: 27. In some embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the sequence set forth in SEQ ID NO: 27.

[00160] In specific embodiments, the nucleotide sequence encoding hGALNS is hGALNSco. In certain embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein is hGALNSco. In some embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein comprises the sequence of SEQ ID NO: 3. In some embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the sequence set forth in SEQ ID NO: 3.

[00161] In certain embodiments, the nucleotide sequence encoding the fusion protein comprises the sequence of SEQ ID NO: 4 or 5. In certain embodiments, the nucleotide sequence encoding the fusion protein is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the sequence set forth in SEQ ID NO: 4 or 5.

[00162] In certain embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein comprises the cDNA sequence of hGALNS (e.g., hGALNS, hGALNSco, D8-hGALNS, D8-GALNSco, hGALNScoV2). In certain embodiments, the nucleotide sequence encoding hGALNS or the hGALNS portion of the fusion protein is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the cDNA sequence of hGALNS.

[00163] In certain embodiments, the nucleotide sequence encoding the fusion protein comprises the cDNA sequence of the fusion protein. In certain embodiments, the nucleotide sequence encoding the fusion protein is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the cDNA sequence of the fusion protein.

[00164] In certain embodiments, the nucleotide sequence encoding hGALNS or the nucleotide sequence encoding the fusion protein is codon-optimized, for example, via any codon-optimization technique known to one of skill in the art (*see*, *e.g.*, review by Quax *et al.*, 2015, Mol Cell 59:149-161).

[00165] In certain embodiments, CpG sites are depleted in the nucleotide sequence encoding hGALNS or the nucleotide sequence encoding the fusion protein.

[00166] In various embodiments of the aspects and embodiments described herein, the nucleotide sequence encoding the transgene is codon-optimized. In various embodiments of the aspects and embodiments of the rAAV described herein, the nucleotide sequence encoding the transgene has CpG sites depleted. In a specific embodiment, the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:12. In a specific

embodiment, the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that is 100% identical to SEQ ID NO: 12.

(b) **Acidic Oligopeptide**

[00167] Acidic oligopeptides have high binding affinities for hydroxyapatite, a major component of bones and cartilages. The term "acid oligopeptide" as used herein refers to an oligopeptide with a repeating amino acid sequence of glutamic acid (E) and/or aspartic acid (D) residues. The number of amino acid residues in an acidic oligopeptide may be, for example, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In specific embodiments, the number of amino acid residues in an acidic oligopeptide is 4-8. In specific embodiments, the number of amino acid residues in an acidic oligopeptide is 6-8. In a specific embodiment, the number of amino acid residues in an acidic oligopeptide is 6. In another specific embodiment, the number of amino acid residues in an acidic oligopeptide is 8.

[00168] In a specific embodiment, the acidic oligopeptide is D8 (*i.e.*, an oligopeptide with an amino acid sequence of eight aspartic acid residues. In another embodiment, the acidic oligopeptide is E6 (*i.e.*, an oligopeptide with an amino acid sequence of six glutamic acid residues. The E6 sequence is described in Tomatsu *et al.*, 2010, Molecular Therapy, 18(6):11094-1102, which is incorporated by reference herein in its entirety.

[00169] In a specific embodiment, the acidic oligopeptide is fused to the N-terminus of hGALNS. In another embodiment, the acidic oligopeptide is fused to the C-terminus of hGALNS.

[00170] In a specific embodiment, the acidic oligopeptide is fused directly to hGALNS, with no intervening amino acid sequence. In another specific embodiment, the acidic oligopeptide is fused to hGALNS via a linker amino acid sequence (*e.g.*, an amino acid sequence that is 1-10, 2-8, or 4-6 amino acid residues in length).

[00171] In certain embodiments, the hGALNS enzyme can be delivered to the lysosomes in the bone and cartilage area to improve bone and cartilage pathology.

(c) **Promoters and Modifiers of Gene Expression:**

[00172] In certain embodiments, the hGALNS expression cassette described herein comprises components that modulate gene delivery or gene expression (*e.g.*, "expression control elements"). In certain embodiments, the hGALNS expression cassette described herein comprises components that modulate gene expression. In certain embodiments, the hGALNS

expression cassette described herein comprises components that influence binding or targeting to cells. In certain embodiments, the hGALNS expression cassette described herein comprises components that influence the localization of the hGALNS within the cell after uptake. In certain embodiments, the hGALNS expression cassette described herein comprises components that can be used as detectable or selectable markers, *e.g.*, to detect or select for cells that have taken up the hGALNS expression cassette. In certain embodiments, the hGALNS expression cassette described herein comprises nucleotide sequence(s) encoding one or more promoters, at least one of which is operably linked to the nucleotide sequence encoding hGALNS or the fusion protein that is hGALNS fused to an acidic oligopeptide. In certain embodiments, the promoter can be a constitutive promoter. In alternate embodiments, the promoter can be an inducible promoter.

[00173] In certain embodiments, the promoter is a CAG promoter. In some embodiments, the CAG promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 28. In some embodiments, the CAG promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 28. In some embodiments, the CAG promoter comprises a nucleotide sequence of SEQ ID NO: 28 or a portion of SEQ ID NO:28.

[00174] In some embodiments, a portion is about or at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical e.g., to a promoter of the disclosure. For example, in some embodiments, a CAG promoter comprising a nucleotide sequence of a portion of SEQ ID NO: 28 is a CAG promoter comprising about or at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of SEQ ID NO: 28.

[00175] In certain embodiments, the promoter is a liver-specific promoter.

[00176] The liver-specific promoter can be, but is not limited to, a thyroxine binding globulin (TBG) promoter (*see, e.g.*, Yan *et al.*, 2012, Gene, 506(2):289-94, incorporated by reference herein in its entirety). In certain embodiments, the TBG promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:6. In certain embodiments, the TBG promoter comprises a nucleotide

sequence that is 100% identical to SEQ ID NO:6. In certain embodiments, the TBG promoter comprises a nucleotide sequence of SEQ ID NO:6 or a portion of SEQ ID NO:6.

[00177] In certain embodiments, the liver-specific promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:13. In certain embodiments, the liver-specific promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:14. In certain embodiments, the liver-specific promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:15. In certain embodiments, the liver-specific promoter is SEQ ID NO:13. In certain embodiments, the liver-specific promoter is SEQ ID NO:14. In certain embodiments, the liver-specific promoter is SEQ ID NO:15. In certain embodiments, the liver-specific promoter comprises SEQ ID NO:13 or a portion of SEQ ID NO:13. In certain embodiments, the liver-specific promoter comprises SEQ ID NO:14 or a portion of SEQ ID NO:15. In certain embodiments, the liver-specific promoter comprises SEQ ID NO:15 or a portion of SEQ ID NO:15.

[00178] In certain embodiments, the promoter is a liver- and muscle- specific promoter.

[00179] In certain embodiments, the liver- and muscle- promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:16. In certain embodiments, the liver- and muscle-promoter is SEQ ID NO:16. In certain embodiments, the liver- and muscle- promoter comprises SEQ ID NO:16 or a portion of SEQ ID NO:16.

[00180] In certain embodiments, the promoter comprises or is a bone-liver tandem promoter that comprises a bone-specific promoter and a liver-specific promoter.

[00181] In a specific embodiment, the bone-specific promoter is a Sp7/Osx promoter. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:23. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:23. In a specific embodiment, the Sp7/Osx promoter comprises SEQ ID NO:23 or a portion of SEQ ID NO:23.

[00182] In a specific embodiment, the bone-specific promoter is a minimal Sp7/Osx promoter. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:24. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:24. In a specific embodiment, the minimal Sp7/Osx promoter comprises SEQ ID NO:24 or a portion of SEQ ID NO:24.

[00183] In a specific embodiment, the liver-specific promoter is an hAAT (ΔATG) promoter. In a specific embodiment, the hAAT (ΔATG) promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:22. In a specific embodiment, the hAAT (ΔATG) promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:22. In a specific embodiment, the hAAT (ΔATG) promoter comprises SEQ ID NO:22 or a portion of SEQ ID NO:22.

[00184] In a specific embodiment, the liver-specific promoter is an hAAT promoter. In a specific embodiment, the hAAT promoter comprises a nucleotide sequence that is at least 80%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to SEQ ID NO:21. In a specific embodiment, the hAAT promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:21. In a specific embodiment, the hAAT promoter comprises SEQ ID NO:21 or a portion of SEQ ID NO:21.

[00185] In a specific embodiment, the bone-liver tandem promoter further comprises a nucleotide sequence encoding an enhancer. In a specific embodiment, the enhancer is a liver-specific enhancer. In a specific embodiment, the liver-specific embodiment is an ApoE enhancer. In a specific embodiment, the bone-liver tandem promoter further comprises a nucleotide sequence encoding a hepatic control region comprising a liver-specific enhancer (for example, an ApoE enhancer). In a specific embodiment, the ApoE enhancer comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:20. In a specific embodiment, the ApoE enhancer promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:20. . In a specific

embodiment, the ApoE enhancer promoter comprises SEQ ID NO:20 or a portion of SEQ ID NO:20. In a specific embodiment, the hepatic control region comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:19. In a specific embodiment, the hepatic control region comprises a nucleotide sequence that is 100% identical to SEQ ID NO:19. In a specific embodiment, the hepatic control region comprises SEQ ID NO:19 or a portion of SEQ ID NO:19. In a specific embodiment, the enhancer (for example, the ApoE enhancer) and the hepatic control region are upstream of the bone-specific promoter.

[00186] In a specific embodiment, the bone-liver tandem promoter is an LBTP1 promoter (see, *e.g.* FIGS. 33 and 37). In a specific embodiment, the LBTP1 promoter comprises a nucleotide sequence that at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:17. In a specific embodiment, the LBTP1 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:17. In a specific embodiment, the LBTP1 promoter comprises SEQ ID NO:17 or a portion of SEQ ID NO:17.

[00187] In a specific embodiment, the bone-liver tandem promoter is an LBTP2 promoter (see, *e.g.* FIGS. 34 and 38). In a specific embodiment, the LBTP2 promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:18. In a specific embodiment, the LBTP2 promoter is comprises a nucleotide sequence that 100% identical to SEQ ID NO:18. In a specific embodiment, the LBTP2 promoter comprises SEQ ID NO:18 or a portion of SEQ ID NO:18.

[00188] In certain embodiments, the promoter comprises or is a bone-specific promoter.

[00189] In a specific embodiment, the bone-specific promoter is a Sp7/Osx promoter (see, *e.g.* Lee et al., 2019, Mol Therapy: Methods & Clinical Development 15:101-111; Lu et al., 2006, J. Biological Chemistry, 281(10) :6297-6306; and Nishio et al., 2006, Gene 372 :62-70; which are incorporated by reference herein in their entireties). In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:23. In a specific embodiment, the Sp7/Osx

promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:23 (see, *e.g.* FIG. 35). In a specific embodiment, the Sp7/Osx promoter comprises SEQ ID NO:23 or a portion of SEQ ID NO:23.

[00190] In a specific embodiment, the bone-specific promoter is a minimal Sp7/Osx promoter. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% identical to SEQ ID NO:24. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:24 (see, *e.g.* FIG. 36). In a specific embodiment, the minimal Sp7/Osx promoter comprises SEQ ID NO:24 or a portion of SEQ ID NO:24.

[00191] In certain embodiments, the promoter comprises or is a liver-specific promoter.

[00192] In a specific embodiment, the liver-specific promoter is an hAAT promoter. In a specific embodiment, the hAAT promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:21. In a specific embodiment, the hAAT promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:21. In a specific embodiment, the hAAT promoter comprises SEQ ID NO:21 or a portion of SEQ ID NO:21.

[00193] In a specific embodiment, the liver-specific promoter is an hAAT(ΔATG) promoter. In a specific embodiment, the hAAT(ΔATG) promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:22. In a specific embodiment, the hAAT(ΔATG) promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:22. In a specific embodiment, the hAAT(ΔATG) promoter comprises SEQ ID NO:22 or a portion of SEQ ID NO:21. SEQ ID NO: 22 comprises an ATG to GTG modification, which is shown in underline in Section 8 (Table of Sequences).

[00194] In certain embodiments, the promoter comprises one or more elements that enhance the expression of hGALNS or the fusion protein. In certain embodiments, the promoter comprises a TATA box.

[00195] In certain embodiments, the one or more promoter elements can be inverted or moved relative to one another. In certain embodiments, the elements of the promoter can be positioned to function cooperatively. In certain embodiments, the elements of the promoter can be

positioned to function independently. In certain embodiments, the hGALNS expression cassette described herein comprises one or more promoters selected from the group consisting of the liver-specific TBG promoter, the human CMV immediate early gene promoter, the SV40 early promoter, the Rous sarcoma virus (RS) long terminal repeat, and rat insulin promoter. In certain embodiments, the hGALNS expression cassette provided herein comprise one or more tissue specific promoters. In a specific embodiment, the tissue-specific promoter is a liver-specific promoter. In a specific embodiment, the TBG promoter has the nucleotide sequence of SEQ ID NO. 6.

[00196] In certain embodiments, the hGALNS expression cassette comprises one or more additional expression control elements, which can include a nucleotide sequence encoding an enhancer (*e.g.*, an alpha mic/bik enhancer), a repressor, a nucleotide sequence encoding an intron or a chimeric intron (*e.g.*, first intron of the chicken beta-actin gene), and/or a nucleotide sequence encoding a poly A site (*e.g.*, a rabbit globin poly A site). In a specific embodiment, the nucleotide sequence encoding the rabbit globin poly A site has the sequence of SEQ ID NO: 9. In a specific embodiment, the nucleotide sequence encoding the intron has the sequence of SEQ ID NO: 10. In a specific embodiment, the nucleotide sequence encoding the alpha mic/bik enhancer has the sequence of SEQ ID NO: 11.

[00197] In a specific embodiment, the hGALNS expression cassette comprises an alpha mic/bik enhancer, a nucleotide sequence encoding an intron, a nucleotide sequence encoding a TBG promoter, a nucleotide sequence encoding hGALNS or a fusion protein that is hGALNS fused to an acidic oliopeptide (preferably, D8), and a nucleotide sequence encoding a rabbit globin poly A site. In a specific embodiment, the nucleotide sequence encoding the rabbit globin poly A site has the sequence of SEQ ID NO: 9. In a specific embodiment, the nucleotide sequence encoding the intron has the sequence of SEQ ID NO: 10. In a specific embodiment, the nucleotide sequence encoding the alpha mic/bik enhancer has the sequence of SEQ ID NO: 11.

[00198] In certain embodiments, the hGALNS expression cassette comprises one or more additional expression control elements, which can include a nucleotide sequence encoding an enhancer (*e.g.*, an ApoE enhancer as described herein), and/or a nucleotide sequence encoding a poly A site (*e.g.*, β-globin polyadenylation signal as described herein, or a rabbit globin poly A

site as described herein). In a specific embodiment, the nucleotide sequence encoding the rabbit globin poly A site has the sequence of SEQ ID NO: 9.

[00199] In various embodiments of the aspects and embodiments of the rAAV described herein, the hGALNS expression cassette further comprises a nucleotide sequence encoding an intron. In a specific embodiment, the intron is a chimeric intron. In a specific embodiment, the chimeric intron is a β-globin/Ig intron. In a specific embodiment, the β-globin/Ig intron comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:10. In a specific embodiment, the β-globin/Ig intron comprises a nucleotide sequence that is 100% identical to SEQ ID NO:10.

[00200] In various embodiments of the aspects and embodiments of the rAAV described herein, the nucleotide sequence encoding the transgene comprises a polyadenylation signal. In a specific embodiment, the polyadenylation signal is a β-globin polyadenylation signal. In a specific embodiment, the β-globin polyadenylation signal comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:25. In a specific embodiment, the β-globin polyadenylation signal comprises a nucleotide sequence that is 100% identical to SEQ ID NO:25. In a specific embodiment, the polyadenylation signal is a rabbit globin poly A site. In a specific embodiment, the rabbit globin poly A site comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:9. In a specific embodiment, the rabbit globin poly A site comprises a nucleotide sequence that is 100% identical to SEQ ID NO:9.

(d) **Inverted Terminal Repeats**

[00201] In some embodiments of the invention, the hGALNS expression cassette described herein (e.g., hGALNScoV2, hGALNSco, D8-GALNSco, hGALNS, or D8-hGALNS) is flanked by two AAV-inverted terminal repeats (ITRs). In specific embodiments, the hGALNS expression cassette is hGALNSco. In specific embodiments, the hGALNS expression cassette is hGALNScoV2. ITR sequences may be used for packaging a recombinant gene expression cassette into the virion (*see, e.g.*, Yan *et al.*, 2005, J. Virol., 79(1):364-379; United States Patent No. 7,282,199 B2, United States Patent No. 7,790,449 B2, United States Patent No. 8,318,480

B2, United States Patent No. 8,962,332 B2 and International Patent Application No. PCT/EP2014/076466, each of which is incorporated herein by reference in its entirety). In some embodiments, the flanking ITRs are AAV2 ITRs. In some embodiments, the flanking ITRs are AAV9 ITRs. In a specific embodiment, the flanking ITRs are AAV8 ITRs. In a specific embodiment, the ITR sequence can have a sequence of SEQ ID NO.: 7. In a specific embodiment, the ITR sequence can have a sequence of SEQ ID NO.: 8. In a specific embodiment, the 5' ITR can have a sequence of SEQ ID NO.: 7. In a specific embodiment, the 3' ITR can have a sequence of SEQ ID NO.: 8.

(e) **Untranslated Regions**

[00202] In certain embodiments, the hGALNS expression cassette described herein comprises one or more untranslated regions (UTRs), *e.g.*, 3' and/or 5' UTRs. In certain embodiments, the UTRs are optimized for the desired level of protein expression. In certain embodiments, the UTRs are optimized for the mRNA half life of the hGALNS. In certain embodiments, the UTRs are optimized for the stability of the mRNA of the hGALNS. In certain embodiments, the UTRs are optimized for the secondary structure of the mRNA of the hGALNS.

6.1.3 Pharmaceutical Compositions and Kits

[00203] In certain embodiments, provided herein are pharmaceutical compositions comprising an rAAV provided herein and a pharmaceutically acceptable carrier. The pharmaceutical composition may be prepared as individual, single unit dosage forms. The pharmaceutical compositions provided herein can be formulated for, for example, parenteral, subcutaneous, intramuscular, intravenous, intraperitoneal, intranasal, intrathecal, or transdermal administration. In a specific embodiment, the pharmaceutical composition is formulated for intravenous administration. A suitable pharmaceutically acceptable carrier (*e.g.*, for intravenous administration and transduction in liver cells) would be readily selected by one of skill in the art.

[00204] Provided herein are kits comprising a pharmaceutical composition described herein, contained in one or more containers. The containers that the pharmaceutical composition can be packaged in can include, but are not limited to, bottles, packets, ampoules, tubes, inhalers, bags, vials, and containers. In certain embodiments, the kit comprises instructions for administering the pharmaceutical administration. In certain embodiments, the kit comprises devices that can be

used to administer the pharmaceutical composition, including, but not limited to, syringes, needle-less injectors, drip bags, patches and inhalers.

[00205] Also provided are devices and blood circulation systems that can be utilized when treating MPS IVA using an rAAV described herein by gene therapy. Such devices and systems would be readily selected by one of skill in the art.

6.2 MANUFACTURE OF rAAVS

[00206] Also provided herein are polynucleotides comprising an hGALNS expression cassette as described herein, plasmids and cells that can be used to generate an rAAV provided herein, and methods of making an rAAV provided herein.

6.2.1 Polynucleotides, Plasmids and Cells

[00207] Provided herein are polynucleotides comprising an hGALNS expression cassette.

[00208] In one aspect, provide herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a transgene, such as the transgene encoding a fusion protein that is hGALNS fused to an acidic oligopeptide (for example, D8). The hGALNS expression cassette may further comprise a nucleotide sequence encoding a liver-specific promoter (for example, a TBG promoter), wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding the fusion protein. In some embodiments, the hGALNS expression cassette may further comprise a nucleotide sequence encoding a CAG promoter. In some embodiments, the nucleotide sequence encoding the CAG promoter is operably linked to the nucleotide sequence encoding the fusion protein. In certain embodiments, the CAG promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:28. In certain embodiments, the CAG promoter is SEQ ID NO:28. In certain embodiments, the liver-specific promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:13. In certain embodiments, the liver-specific promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:14. In certain embodiments, the liver-specific promoter comprises a nucleotide

sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:15. In certain embodiments, the liver-specific promoter is SEQ ID NO:13. In certain embodiments, the liver-specific promoter is SEQ ID NO:14. In certain embodiments, the liver-specific promoter is SEQ ID NO:15.

[00209] In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a liver-specific promoter (for example, a TBG promoter) and a nucleotide sequence encoding hGALNS, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to the nucleotide sequence encoding hGALNS.

[00210] In one aspect, provide herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a transgene, such as the transgene encoding a fusion protein that is hGALNS fused to an acidic oligopeptide (for example, D8). The hGALNS expression cassette may further comprise a nucleotide sequence encoding a promoter, wherein the nucleotide sequence encoding the promoter is operably linked to the nucleotide sequence encoding the fusion protein. In certain embodiments, the promoter is a CAG promoter.

[00211] In one aspect, provide herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a transgene, such as the transgene encoding a fusion protein that is hGALNS fused to an acidic oligopeptide (for example, D8). The hGALNS expression cassette may further comprise a nucleotide sequence encoding a liver- and muscle specific- promoter, wherein the nucleotide sequence encoding the liver- and muscle specific- promoter is operably linked to the nucleotide sequence encoding the fusion protein. In certain embodiments, the liver- and muscle specific- promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:16. In certain embodiments, the promoter is SEQ ID NO:16.

[00212] In another aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a promoter and a nucleotide sequence encoding hGALNS, wherein the nucleotide sequence encoding the promoter is operably linked to the

nucleotide sequence encoding hGALNS. In certain embodiments, the promoter is a CAG promoter. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:28. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:13. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:14. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:15. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:16. In certain embodiments, the promoter is SEQ ID NO:28. In certain embodiments, the promoter is SEQ ID NO:13. In certain embodiments, the promoter is SEQ ID NO:14. In certain embodiments, the promoter is SEQ ID NO:15. In certain embodiments, the promoter is SEQ ID NO:16. In certain embodiments, the promoter comprises SEQ ID NO:28. In certain embodiments, the promoter comprises SEQ ID NO:13. In certain embodiments, the promoter comprises SEQ ID NO:14. In certain embodiments, the promoter comprises SEQ ID NO:15. In certain embodiments, the promoter is SEQ ID NO:16. The hGALNS expression cassette can be as described in Section 6.1.2.

[00213] In a specific embodiment, provided herein is a polynucleotide comprising an hGALNScoV2 expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a TBG promoter and a nucleotide sequence encoding hGALNScoV2 or hGALNS, wherein the nucleotide sequence encoding the TBG promoter is operably linked to the nucleotide sequence encoding hGALNScoV2 or hGALNS. In a specific embodiment, the nucleotide sequence encoding the TBG promoter is 100% identical to SEQ ID NO: 6. In a specific embodiment, the nucleotide sequence encoding hGALNScoV2 is 100% identical to SEQ ID NO: 27. In a specific embodiment, the nucleotide sequence encoding hGALNS is 100% identical to SEQ ID NO: 12. In certain embodiments, the nucleotide sequence encoding the TBG promoter has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 6. In certain

embodiments, the nucleotide sequence encoding hGALNScoV2 has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 27. In certain embodiments, the nucleotide sequence encoding hGALNS has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 12.

[00214] In a specific embodiment, provided herein is a polynucleotide comprising an hGALNSco expression cassette flanked by AAV-ITRs, the hGALNSco expression cassette comprising a nucleotide sequence encoding a TBG promoter and a nucleotide sequence encoding hGALNSco or hGALNS, wherein the nucleotide sequence encoding the TBG promoter is operably linked to the nucleotide sequence encoding hGALNSco or hGALNS. In a specific embodiment, the nucleotide sequence encoding the TBG promoter is 100% identical to SEQ ID NO: 6. In a specific embodiment, the nucleotide sequence encoding hGALNSco is 100% identical to SEQ ID NO: 3. In certain embodiments, the nucleotide sequence encoding the TBG promoter has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 6. In certain embodiments, the nucleotide sequence encoding hGALNScoV2 has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 3. In a specific embodiment, the nucleotide sequence encoding hGALNS is 100% identical to SEQ ID NO: 12. In certain embodiments, the nucleotide sequence encoding hGALNS has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 12.

[00215] In a specific embodiment, provided herein is a polynucleotide comprising an hGALNScoV2 expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a CAG promoter and a nucleotide sequence encoding hGALNScoV2 or hGALNS, wherein the nucleotide sequence encoding the CAG promoter is operably linked to the nucleotide sequence encoding hGALNScoV2 or hGALNS. In a specific embodiment, the nucleotide sequence encoding the CAG promoter is 100% identical to SEQ ID NO: 28. In a specific embodiment, the nucleotide sequence encoding hGALNScoV2 is 100% identical to SEQ ID NO: 27. In certain embodiments, the nucleotide sequence encoding the CAG promoter has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 28. In certain embodiments, the nucleotide sequence encoding hGALNScoV2 has at least 80%,

85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 27. In a specific embodiment, the nucleotide sequence encoding hGALNS is 100% identical to SEQ ID NO: 12. In certain embodiments, the nucleotide sequence encoding hGALNS has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 12.

[00216] In a specific embodiment, provided herein is a polynucleotide comprising an hGALNScoV2 expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a LSPX1 promoter and a nucleotide sequence encoding hGALNScoV2 or hGALNS, wherein the nucleotide sequence encoding the LSPX1 promoter is operably linked to the nucleotide sequence encoding hGALNScoV2 or hGALNS. In a specific embodiment, the nucleotide sequence encoding the LSPX1 promoter is 100% identical to SEQ ID NO: 13. In a specific embodiment, the nucleotide sequence encoding hGALNScoV2 is 100% identical to SEQ ID NO: 27. In certain embodiments, the nucleotide sequence encoding the LSPX1 promoter has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 13. In certain embodiments, the nucleotide sequence encoding hGALNScoV2 has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 27. In a specific embodiment, the nucleotide sequence encoding hGALNS is 100% identical to SEQ ID NO: 12. In certain embodiments, the nucleotide sequence encoding hGALNS has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 12.

[00217] In a specific embodiment, provided herein is a polynucleotide comprising an hGALNScoV2 expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a LMTP6 promoter and a nucleotide sequence encoding hGALNScoV2 or hGALNS, wherein the nucleotide sequence encoding the LMTP6 promoter is operably linked to the nucleotide sequence encoding hGALNScoV2 or hGALNS. In a specific embodiment, the nucleotide sequence encoding the LMTP6 promoter is 100% identical to SEQ ID NO: 16. In a specific embodiment, the nucleotide sequence encoding hGALNScoV2 is 100% identical to SEQ ID NO: 27. In certain embodiments, the nucleotide sequence encoding the LMTP6 promoter has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID

NO: 16. In certain embodiments, the nucleotide sequence encoding hGALNScoV2 has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 27. In a specific embodiment, the nucleotide sequence encoding hGALNS is 100% identical to SEQ ID NO: 12. In certain embodiments, the nucleotide sequence encoding hGALNS has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 12.

[00218] In a specific embodiment, provided herein is a polynucleotide comprising an hGALNScoV2 expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNScoV2 expression cassette comprising a nucleotide sequence encoding a LBTP2 promoter and a nucleotide sequence encoding hGALNScoV2 or hGALNS, wherein the nucleotide sequence encoding the LBTP2 promoter is operably linked to the nucleotide sequence encoding hGALNScoV2 or hGALNS. In a specific embodiment, the nucleotide sequence encoding the LBTP2 promoter is 100% identical to SEQ ID NO: 18. In a specific embodiment, the nucleotide sequence encoding hGALNScoV2 is 100% identical to SEQ ID NO: 27. In certain embodiments, the nucleotide sequence encoding the LBTP2 promoter has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 18. In certain embodiments, the nucleotide sequence encoding hGALNScoV2 has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 27. In a specific embodiment, the nucleotide sequence encoding hGALNS is 100% identical to SEQ ID NO: 12. In certain embodiments, the nucleotide sequence encoding hGALNS has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 12.

[00219] In a specific aspect, provided herein is a polynucleotide comprising a hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene.

[00220] In another specific aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS

expression cassette comprising a nucleotide sequence encoding a bone-liver tandem promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, wherein the bone-liver tandem promoter comprises a bone-specific promoter and a liver-specific promoter, and wherein the nucleotide sequence encoding the bone-liver tandem promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[00221] In a specific embodiment, the bone-specific promoter is a Sp7/Osx promoter. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:23. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:23.

[00222] In a specific embodiment, the bone-specific promoter is a minimal Sp7/Osx promoter. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% to SEQ ID NO:24. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:24.

[00223] In a specific embodiment, the liver-specific promoter is an hAAT (ΔATG) promoter. In a specific embodiment, the hAAT (ΔATG) promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% to SEQ ID NO:22. In a specific embodiment, the hAAT (ΔATG) promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:22.

[00224] In a specific embodiment, the bone-liver tandem promoter further comprises a nucleotide sequence encoding an ApoE enhancer. In a specific embodiment, the bone-liver tandem promoter further comprises a nucleotide sequence encoding a hepatic control region comprising an ApoE enhancer. In a specific embodiment, the ApoE enhancer comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% to SEQ ID NO:20. In a specific embodiment, the ApoE enhancer promoter comprises a

nucleotide sequence that is 100% identical to SEQ ID NO:20. In a specific embodiment, the hepatic control region comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:19. In a specific embodiment, the hepatic control region comprises a nucleotide sequence that is 100% identical to SEQ ID NO:19.

[00225] In a specific embodiment, the bone-liver tandem promoter is an LBTP1 promoter. In a specific embodiment, the LBTP1 promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% to SEQ ID NO:17. In a specific embodiment, the LBTP1 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:17.

[00226] In a specific embodiment, the bone-liver tandem promoter is an LBTP2 promoter. In a specific embodiment, the LBTP2 promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:18. In a specific embodiment, the LBTP2 promoter is comprises a nucleotide sequence that 100% identical to SEQ ID NO:18.

[00227] In another specific aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

[00228] In another specific aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[00229] In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:23. In a specific embodiment, the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:23.

[00230] In another specific aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene.

[00231] In another specific aspect, provided herein is a polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs (e.g., AAV2-ITRs), the hGALNS expression cassette comprising a nucleotide sequence encoding a minimal Sp7/Osx promoter and a nucleotide sequence encoding a transgene, wherein the transgene encodes a fusion protein that is hGALNS fused to an acidic oligopeptide, and wherein the nucleotide sequence encoding the minimal Sp7/Osx promoter is operably linked to the nucleotide sequence encoding the transgene. In a specific embodiment, the acidic oligopeptide is D8.

[00232] In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:24. In a specific embodiment, the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO:24.

[00233] In various embodiments of the aspects and embodiments of the polynucleotide described herein, the hGALNS expression cassette further comprises a nucleotide sequence encoding an intron. In a specific embodiment, the intron is a chimeric intron. In a specific embodiment, the chimeric intron is a β-globin/Ig intron. In a specific embodiment, the β-globin/Ig intron comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:10. In a specific embodiment, the β-globin/Ig intron comprises a nucleotide sequence that is 100% identical to SEQ ID NO:10.

[00234] In various embodiments of the aspects and embodiments of the polynucleotide described herein, the nucleotide sequence encoding the transgene is codon-optimized. In various embodiments of the aspects and embodiments of the polynucleotide described herein, the nucleotide sequence encoding the transgene has CpG sites depleted. In a specific embodiment, the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:12. In a specific embodiment, the nucleotide sequence encoding the transgene comprises a nucleotide sequence encoding hGALNS that is 100% identical to SEQ ID NO: 12.

[00235] In various embodiments of the aspects and embodiments of the polynucleotide described herein, the nucleotide sequence encoding the transgene comprises a polyadenylation signal. In a specific embodiment, the polyadenylation signal is a β-globin polyadenylation signal. In a specific embodiment, the β-globin polyadenylation signal comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:25. In a specific embodiment, the β-globin polyadenylation signal comprises a nucleotide sequence that is 100% identical to SEQ ID NO:25. In a specific embodiment, the polyadenylation signal is a rabbit globin poly A site. In a specific embodiment, the rabbit globin poly A site comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:9. In a specific embodiment, the rabbit globin poly A site comprises a nucleotide sequence that is 100% identical to SEQ ID NO:9.

[00236] In various embodiments of the aspects and embodiments of the polynucleotide described herein, the AAV is AAV8. In various embodiments of the aspects and embodiments of the polynucleotide described herein, the AAV is AAV9.

[00237] In a specific embodiment, the polynucleotide is in the form of a ssDNA. In another specific embodiment, the polynucleotide is in the form of a dsDNA.

[00238] Also provided herein are plasmids comprising a polynucleotide provided herein (hereinafter "rAAV plasmids"). In a specific embodiment, the rAAV plasmid is a ssDNA plasmid. In another specific embodiment, the rAAV plasmid is a dsDNA plasmid. In some

embodiments, the rAAV plasmid is in a circular form. In other embodiments, the rAAV plasmid is in a linear form.

[00239] In a certain embodiment, the constructs described herein comprise the following components (LSPX1): (1) AAV inverted terminal repeats (ITRs) that flanks the expression cassette; (2) control elements, which include a) two tandem Mik/BikE enhancers, b) ApoE enhancer, c) human AAT (hAAT) promoter, d) a poly A signal, and e) optionally an intron; (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2. In a specific embodiment, the constructs described herein comprise the following components: (1) AAV2 inverted terminal repeats that flank the expression cassette; (2) control elements, which include a) two tandem Mik/BikE enhancers, b) ApoE enhancer, c) hAAT promoter, d) a rabbit β-globin poly A signal and e) optionally a chimeric intron derived from human β-globin and Ig heavy chain; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2.

[00240] In a certain embodiment, the constructs described herein comprise the following components (LSPX2): (1) AAV inverted terminal repeats (ITRs) that flanks the expression cassette; (2) control elements, which include a) two tandem ApoE enhancers, b) hAAT promoter, c) a poly A signal; and d) optionally an intron; and (3) nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2. In a specific embodiment, the constructs described herein comprise the following components: (1) AAV2 inverted terminal repeats that flank the expression cassette; (2) control elements, which include a) two tandem ApoE enhancers, b) hAAT promoter, c) a poly A signal; and d) optionally a chimeric intron derived from human β-globin and Ig heavy chain; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2.

[00241] In a certain embodiment, the constructs described herein comprise the following components (LTP1): (1) AAV inverted terminal repeats (ITRs) that flanks the expression cassette; (2) control elements, which include a) two tandem Mik/BikE enhancers, b) TBG promoter, c) hAAT (ΔATG) promoter, d) a poly A signal; and e) optionally an intron; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2. In a specific embodiment, the constructs described herein comprise the following components: (1) AAV2 inverted terminal repeats that flank the expression cassette; (2) control elements, which include a) two tandem Mik/BikE enhancers, b) TBG promoter, c) hAAT (ΔATG) promoter, d) a poly A

signal; and e) optionally a chimeric intron derived from human β-globin and Ig heavy chain; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2.

[00242] In a certain embodiment, the constructs described herein comprise the following components (LTP2): (1) AAV inverted terminal repeats (ITRs) that flanks the expression cassette; (2) control elements, which include a) ApoE enhancer, b) two tandem Mik/BikE enhancers, c) TBG promoter, d) hAAT (ΔATG) promoter, e) a poly A signal; and f) optionally an intron; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2. In a specific embodiment, the constructs described herein comprise the following components: (1) AAV2 inverted terminal repeats that flank the expression cassette; (2) control elements, which include a) ApoE enhancer, b) two tandem MckE enhancers, c) TBG promoter, d) hAAT (ΔATG) promoter, e) a poly A signal; and f) optionally a chimeric intron derived from human β-globin and Ig heavy chain; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2.

[00243] In a certain embodiment, the constructs described herein comprise the following components (LMTP6): (1) AAV inverted terminal repeats (ITRs) that flanks the expression cassette; (2) control elements, which include a) ApoE enhancer, b) three tandem MckE enhancers, c) CK promoter, d) hAAT (ΔATG) promoter, e) a poly A signal; and f) optionally an intron; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2. In a specific embodiment, the constructs described herein comprise the following components: (1) AAV2 inverted terminal repeats that flank the expression cassette; (2) control elements, which include a) ApoE enhancer, b) three tandem MckE enhancers, c) CK promoter, d) hAAT (ΔATG) promoter, e) a poly A signal; and f) optionally a chimeric intron derived from human β-globin and Ig heavy chain; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2.

[00244] In a certain embodiment, the constructs described herein comprise the following components (liver-bone tandem promoter 1 (LBTP1)): (1) AAV inverted terminal repeats (ITRs) that flanks the expression cassette; (2) control elements, which include a) an ApoE enhancer, b) an hAAT(ΔATG) promoter, c) a poly A signal, d) optionally an intron, and e) a Sp7/Osx promotor or minimal Sp7/Osx promotor; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2. In a specific embodiment, the constructs described herein comprise the following components: (1) AAV2 inverted terminal repeats that flank the

expression cassette; (2) control elements, which include a) an ApoE enhancer, b) an hAAT(ΔATG) promoter, c) a rabbit β-globin poly A signal or β-globin polyadenylation signal, d) optionally a chimeric intron derived from human β-globin and Ig heavy chain; and e) a minimal Sp7/Osx promotor; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2. In a specific embodiment, the nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2 is fused to an acidic oligopeptide (such as an acidic oligopeptide described in Section 6.1.2 (b), for example, D8). In a specific embodiment, the nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2 has the CpG sites depleted.

[00245] In a certain embodiment, the constructs described herein comprise the following components (liver-bone tandem promoter 2 (LBTP2)): (1) AAV inverted terminal repeats (ITRs) that flanks the expression cassette; (2) control elements, which include a) an ApoE enhancer, b) an hAAT(ΔATG) promoter, c) a poly A signal, d) optionally an intron, and e) a Sp7/Osx promotor; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2. In a specific embodiment, the constructs described herein comprise the following components: (1) AAV2 inverted terminal repeats that flank the expression cassette; (2) control elements, which include a) an ApoE enhancer, b) an hAAT(ΔATG) promoter, c) a rabbit β-globin poly A signal or β-globin polyadenylation signal, d) optionally a chimeric intron derived from human β-globin and Ig heavy chain; and (e) a Sp7/Osx promotor; and (3) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2. In a specific embodiment, the nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2 is fused to an acidic oligopeptide (such as an acidic oligopeptide described in Section 6.1.2 (b), for example, D8). In a specific embodiment, the nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2 has the CpG sites depleted.

[00246] In a certain embodiment, the constructs described herein comprise the following components (LBTP1) in the following order: (1) AAV inverted terminal repeat (ITR), (2) an ApoE enhancer, 3) a minimal Sp7/Osx promotor 4) an hAAT(ΔATG) promotor, 5) optionally an intron, (6) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2, (7) a poly A signal, and (8) AAV inverted terminal repeat (ITR). In a specific embodiment, the constructs described herein comprise the following components (LBTP1) in the following order: (1) AAV2 inverted terminal repeat (ITR), (2) an ApoE enhancer, 3) a minimal Sp7/Osx promotor 4) an hAAT(ΔATG) promotor, 5) optionally a chimeric intron derived from human β-globin and

Ig heavy chain, (6) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2, (7) a rabbit β-globin poly A signal or β-globin polyadenylation signal, and (8) an AAV2 inverted terminal repeat (ITR). In a specific embodiment, the nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2 is fused to an acidic oligopeptide (such as an acidic oligopeptide described in Section 6.1.2 (b), for example, D8). In a specific embodiment, the nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2 has the CpG sites depleted.

[00247] In a certain embodiment, the constructs described herein comprise the following components (LBTP2) in the following order: (1) an AAV inverted terminal repeat (ITR), (2) an ApoE enhancer, 3) a Sp7/Osx promotor, 4) an hAAT(ΔATG) promotor, 5) optionally an intron, (6) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2, (7) a poly A signal, and (8) an AAV inverted terminal repeat (ITR). In a specific embodiment, the constructs described herein comprise the following components (LBTP2) in the following order: (1) an AAV2 inverted terminal repeat (ITR), (2) an ApoE enhancer, 3) a Sp7/Osx promotor, 4) an hAAT(ΔATG) promotor, 5) optionally a chimeric intron derived from human β-globin and Ig heavy chain, (6) a nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2, (7) a rabbit β-globin poly A signal or β-globin polyadenylation signal, and (8) an AAV2 inverted terminal repeat (ITR). In a specific embodiment, the nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2 is fused to an acidic oligopeptide (such as an acidic oligopeptide described in Section 6.1.2 (b), for example, D8). In a specific embodiment, the nucleotide sequence encoding hGALNS, hGALNSco, or hGALNScoV2 has the CpG sites depleted.

[00248] Further provided herein are cells (preferably *ex vivo* cells) expressing (*e.g.,* recombinantly) an rAAV provided herein. In certain embodiments, the cell (preferably *ex vivo* cell) comprises a polynucleotide provided herein or an rAAV plasmid provided herein. In certain embodiments, the cell (preferably *ex vivo* cell) further comprises helper polynucleotide(s) or helper plasmids providing the AAV Rep, Cap, and Ad5 functions. The cell (preferably *ex vivo* cells) can by a mammalian host cell, for example, HEK293, HEK293-T, A549 , WEHI, 10T1/2, BHK, MDCK, COS1, COS7, BSC 1, BSC 40, BMT 10, VERO, W138, HeLa, 293, Saos, C2C12, L, HT1080, HepG2, primary fibroblast, hepatocyte, and myoblast cells. The mammalian host cell can be derived from, for example, human, monkey, mouse, rat, rabbit, or

hamster. In a specific embodiment, the mammalian host cell is a human embryonic kidney 293 (HEK293) cell or HEK293-T cell.

6.2.2 Methods of Making rAAVs

[00249] Provided are methods of making an rAAV provided herein. In certain embodiments, the method comprises transfecting a cell (preferably an *ex vivo* cell) with an rAAV plasmid provided in Section 6.2.1 and one or more helper plasmids collectively providing the AAV Rep, Cap, and Ad5 functions. In certain embodiments, the one or more helper plasmids collectively comprising the nucleotide sequences of AAV genes Rep, Cap, VA, E2a and E4.

[00250] The manufacture of an rAAV provided herein for gene therapy applications can use methods known in the art, for example, as described in Clement *et al.*, 2016, Molecular Therapy-Methods & Clinical Development, 27:16002, which is incorporated by reference herein in its entirety. In certain embodiments, transfection of the plasmid DNA is performed using calcium phosphate plasmid precipitation on human embryonic kidney 293 cells (HEK293) or HEK293-T with the rAAV plasmid and the helper plasmid(s) that provide the AAV Rep and Cap functions as well as the Ad5 genes (VA RNAs, E2a, and E4) as is described in the art. In certain embodiments, the Rep, Cap, and Ad5 genes can be on the same helper plasmid. In certain embodiments, a two-helper method (or triple transfection) is utilized where AAV Rep, Cap, and Ad5 functions are provided from separate plasmids. In certain embodiments, the HEK293 cells can be adapted to grow in suspension in an animal component and antibiotic-free media.

[00251] In certain embodiments, rAAV can be manufactured using packaging and producer cell lines. The rAAV provided herein may be manufactured using mammalian host cells, for example, A549 , WEHI, 10T1/2, BHK, MDCK, COS1, COS7, BSC 1, BSC 40, BMT 10, VERO, W138, HeLa, HEK293, HEK293-T, Saos, C2C12, L, HT1080, HepG2, primary fibroblast, hepatocyte, and myoblast cells. The rAAV provided herein may be manufactured using host cells from human, monkey, mouse, rat, rabbit, or hamster. In certain embodiments, stable cell lines can be engineered by introducing the means of producing viruses in the host cells, for example, the replication and capsid genes (*e.g.*, the rep and cap genes of AAV) and the rAAV plasmid provided herein. In a specific embodiment, the rAAV can be manufactured using HEK293 cells. In certain embodiments, rAAV can be produced in Sf9 insect cells by coinfecting

three recombinant baculovirus plasmids with genes encoding the rep gene, the cap gene, and the rAAV genome.

[00252] The cells can be cultured, transfected, and harvested according to appropriate protocols which would be readily selected by one of skill in the art. In certain embodiments, the cells can be cultured in standard Dulbecco's modified Eagle medium (DMEM), including, but not limited to, fetal calf serum, glucose, penicillin, streptomycin, and 1-glutamine (McClure *et al.*, J Vis Exp. 2011, (57): 3348; Shin *et al.*, Methods Mol Biol. 2012, 798: 267–284). Cells can be transfected in components which would be readily selected by one of skill in the art. In certain embodiments, transfection can take place in media solutions including, but not limited to, DMEM and Iscove's modified Dulbecco's medium (IMDM). In certain embodiments, the transfection time can take 46 hr, 47 hr, 48 hr, 49 hr, 50 hr, 51 hr, 52 hr, 53 hr, 54 hr, 55 hr, 56 hr, 57 hr, 58 hr, 59 hr, 60 hr, 61 hr, 62 hr, 63 hr, 64 hr, 65 hr, 66 hr, 67 hr, 68 hr, 69 hr, 70 hr, 50-55 hr, 55-60 hr, 60-65 hr, or 65-70 hr. After transfection, the cells can be harvested by scraping cells to remove them from the culture wells and washing the wells to collect all of the transfected cells.

[00253] For a method of producing rAAV comprising AAV8 capsids, *see* Section IV of the Detailed Description of U.S. Patent No. 7,282,199 B2, which is incorporated herein by reference in its entirety. Genome copy titers of the vectors may be determined, for example, by TAQMAN® analysis. Virions may be recovered, for example, by $CsCl_2$ sedimentation. In a specific embodiment, the rAAV described herein is an isolated or purified rAAV.

[00254] Multiple AAV serotypes have been identified. In certain embodiments, rAAVs or polynucleotides provided herein comprise one or more components derived from one or more serotypes of AAV. In certain embodiments, rAAVs or polynucleotides provided herein comprise one or more components derived from one or more of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, or AAV11. In a certain embodiment, rAAVs or polynucleotides provided herein can comprise one or more components from one or more of AAV8, AAV9, AAV10, or AAV11 serotypes. In a specific embodiment, rAAVs or polynucleotides provided herein can comprise one or more components from the AAV8 serotype. In some embodiments, rAAVs or polynucleotides provided herein can comprise one or more components from the AAV9 serotype. Nucleic acid sequences of AAV components and methods of making recombinant AAV and AAV capsids are described, for example, in United

States Patent No. 7,282,199 B2, United States Patent No. 7,790,449 B2, United States Patent No. 8,318,480 B2, United States Patent No. 8,962,332 B2 and International Patent Application No. PCT/EP2014/076466, each of which is incorporated herein by reference in its entirety. In specific embodiments, provided herein are rAAV8s which encode hGALNS.

[00255] Described in certain embodiments are rAAV8s comprising (i) a recombinant genome comprising an expression cassette containing the hGALNS or the fusion protein that is hGALNS fused to an acidic oligopeptide under the control of regulatory elements and flanked by ITRs; and (ii) a viral capsid that has the amino acid sequence of the AAV8 capsid protein or is at least 95%, 96%, 97%, 98%, 99% or 99.9% identical to the amino acid sequence of the AAV8 capsid protein (SEQ ID NO: 1) while retaining the ability of the AAV8 capsid to package a viral genome and preferably also the ability of the AAV8 capsid to transduce liver cells at a high efficiency. In certain embodiments, the AAV8 capsid has the sequence of SEQ ID NO: 1 with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acid substitutions and retaining the ability of the AAV8 capsid to package a viral genome and preferably also the ability of the AAV8 capsid to transduce liver cells at a high efficiency.

[00256] Described in certain embodiments are rAAV9s comprising (i) a recombinant genome comprising an expression cassette containing the hGALNS or the fusion protein that is hGALNS fused to an acidic oligopeptide under the control of regulatory elements and flanked by ITRs; and (ii) a viral capsid that has the amino acid sequence of the AAV9 capsid protein or is at least 95%, 96%, 97%, 98%, 99% or 99.9% identical to the amino acid sequence of the AAV9 capsid protein (SEQ ID NO: 26) while retaining the ability of the AAV9 capsid to package a viral genome and preferably also the ability of the AAV9 capsid to transduce liver cells at a high efficiency. In certain embodiments, the AAV9 capsid has the sequence of SEQ ID NO: 26 with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 amino acid substitutions and retaining the ability of the AAV9 capsid to package a viral genome and preferably also the ability of the AAV9 capsid to transduce liver cells at a high efficiency.

6.2.3 Assessment of Efficacy

[00257] In vitro assays, *e.g.*, cell culture assays, can be used to measure hGALNS expression from an rAAV described herein, thus indicating, *e.g.*, potency of the rAAV. Cells utilized for the assay can include, but are not limited to, A549 , WEHI, 10T1/2, BHK, MDCK, COS1, COS7, BSC 1, BSC 40, BMT 10, VERO, W138, HeLa, HEK293, HEK293-T, HuH7, Saos, C2C12, L, HT1080, HepG2, primary fibroblast, hepatocyte, and myoblast cells. In a specific embodiment, the cells utilized in the cell culture assay comprise HuH7 cells. In certain embodiments, cells transfected with the rAAV can be analyzed for hGALNS enzyme activity.

[00258] Animal models may also be used to assess the expression of hGALNS from an rAAV described herein and its efficacy. Mouse models for MPS IVA have been described (*see, e.g.*, Tomatsu *et al.*, 2003, Hum Mol Genet 12(24):3349-3358). The mouse model for MPS IVA has a targeted disruption of Exon 2 of mouse GALNS. These mice have no detectable GALNS enzyme activity and increased levels of GAGs are detected in the urine. At 2 months old, increased storage of GAGs is seen in the reticuloendothelial cells, Kupffer cells, and the sinusoidal cells which line the spleen. At 12 months old, vacuolar change is observed in the visceral epithelial cells of glomeruli and cells at the base of heart valves but it is not present in parenchymal cells such as hepatocytes and renal tubular epithelial cells. Lysosomal storage of GAGs is seen in hippocampal and neocortical neurons, meningeal cells. Keratan sulfate (KS) and chondroitin-6-sulfate (C6S) is increased in the corneal epithelial cells of this mouse model compared to wild type, however no skeletal indications become evident in the mouse model. Additionaly, a mouse model for MPS IVA which is tolerant to human GALNS has also been described (*see, e.g.,* Tomatsu *et al.*, 2005, Hum Mol Genet 14(22):3321-3335). *See* Examples in Section 7 for exemplary assays to assess the hGALNS expression from an rAAV described herein and its efficacy.

[00259] According to some embodiments, the methods include gene therapy vectors, *e.g.* the combination of regulatory elements and transgenes that provide increased expression of a functional hGALNS protein. Such expression may be measured 1) by several protein (hGALNS) determination assays known to the skilled person, not limited to sandwich ELISA, Western Blot, histological staining, and liquid chromatography tandem mass spectrometry (LC-MS/MS); 2) by several protein activity assays, such as enzymatic assays or functional assays; and/or 3) by several substrate detection assays, not limited to keratan sulfate (KS), glycosaminoglycans

(CAG), and/or chondroitin-6-sulfate (C6S) detection, and be determined as efficacious and suitable for human treatment (Hintze, J.P. et al, *Biomarker Insights* 2011:6 69–78). Assessment of the quantitative and functional properties of hGALNS using such *in vitro* and *in vivo* cellular, blood and tissue studies have been shown to correlate to the efficacy of certain therapies (Hintze, J.P. et al, 2011, *supra*), and were utilized to evaluate response to gene therapy treatment of MPS IVA with the vectors described herein.

[00260] The invention thus provides methods and gene therapy vectors that increase intracellular hGALNS enzyme activity in tissue cells, e.g. including hepatic, muscle, white blood cells, kidney, lung, spleen cardiac, bone, or cartilage cells of the subject to levels compared to wild-type levels, or that increase intracellular hGALNS enzyme activity to about 2-fold wild-type hGALNS activity levels, or about 5-fold wild-type hGALNS activity levels, about 10-fold wild-type hGALNS activity levels, about 25-fold wild-type hGALNS activity levels, about 40-fold wild-type hGALNS activity levels, about 50-fold wild-type hGALNS activity levels, about 60-fold wild-type hGALNS activity levels, about 70-fold wild-type hGALNS activity levels, about 75-fold wild-type hGALNS activity levels, about 80-fold wild-type hGALNS activity levels, about 85-fold wild-type hGALNS activity levels, about 90-fold wild-type hGALNS activity levels, about 95-fold wild-type hGALNS activity levels, or about 100-fold wild-type hGALNS activity levels, as measured by a hGALNS enzymatic activity assay, e.g. using an assay format as described in Examples 2, 3 and 8 herein, or a substantially similar assay. In some embodiments, the gene therapy provides a method of increasing hGALNS activity levels in the subject two weeks after administration of the gene therapy as compared to the levels prior administration or the average levels in the untreated subjects. In some embodiments, the gene therapy provides a method of increasing hGALNS activity levels in the subject two weeks after administration of the gene therapy. In some embodiments, the gene therapy provides a method of increasing hGALNS activity levels in blood or tissues, for example liver, muscle, kidney, lung, spleen, heart, bone, or cartilage of the subject two weeks after administration of the gene therapy. In some embodiments, the increase in hGALNS activity levels in the subject is measured ten weeks after administration of the gene therapy.

[00261] The invention also provides methods and gene therapy vectors that reduce blood (*e.g.* plasma or serum) levels or tissue levels of KS in the subject to levels compared to the levels of KS in untreated wild-type subjects, or that reduce KS levels to about 1.1-fold wild-type KS

levels, or about 1.2-fold wild-type KS levels, about 1.3-fold wild-type KS levels, about 1.4-fold wild-type KS levels, about 1.5-fold wild-type KS levels, about 1.6-fold wild-type KS levels, about 1.7-fold wild-type KS levels, about 1.8-fold wild-type KS levels, about 1.9-fold wild-type KS levels, about 2-fold wild-type KS levels, about 2.5-fold wild-type KS levels, about 3-fold wild-type KS levels, about 3.5-fold wild-type KS levels, or about 4-fold wild-type KS levels, as measured by a KS assay, e.g. using an assay format as described in Examples 2, 3 and 8 herein, or a substantially similar assay. In some embodiments, the gene therapy provides a method of reducing KS levels in the subject two weeks after administration of the gene therapy. In some embodiments, the gene therapy provides a method of reducing tissue levels of KS in the subject two weeks after administration of the gene therapy. In some embodiments, the KS assay comprises measurement of mono-sulfated KS in blood or tissue, and the gene therapy provides a method of reducing mono-sulfated KS levels in the subject two weeks after administration of the gene therapy.

6.3 METHODS FOR TREATMENT

[00262] Provided herein are methods for treating a human subject diagnosed with MPS IVA.

[00263] In one aspect, the method comprises administering to the human subject an rAAV described herein or a pharmaceutical composition described herein.

[00264] In another aspect, provided herein is a method for treating a human subject diagnosed with MPS IVA, comprising delivering to the bone and liver of the human subject a therapeutically effective amount of hGALNS or a fusion protein that is hGALNS fused to an acidic oligopeptide (as the case may be), by administering to the human subject an rAAV provided herein. In a specific embodiment, the hGALNS or the fusion protein is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell.

[00265] In another aspect, provided herein is a method for treating a human subject diagnosed with MPS IVA, comprising delivering to the bone of the human subject a therapeutically effective amount of hGALNS or a fusion protein that is hGALNS fused to an acidic oligopeptide (as the case may be), by administering to the human subject an rAAV provided herein.

[00266] In another aspect, the method comprises delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve (*e.g.*, delivering to the bone and/or cartilage) of the human subject a therapeutically effective

amount of a transgene, such as the transgene encoding a fusion protein that is hGALNS fused to an acidic oligopeptide, by administering to the human subject an rAAV provided herein. In a specific embodiment, the hGALNS is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell.

[00267] In another aspect, the method comprises delivering to the bone, cartilage, ligament, growth plate, meniscus, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve (*e.g.*, delivering to the bone and/or cartilage) of the human subject a therapeutically effective amount of hGALNS that is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell, by administering to the human subject an rAAV provided herein.

[00268] In another aspect, the method comprises delivering to the bone, cartilage, ligament, growth plate, meniscus, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve (*e.g.*, delivering to the bone and/or cartilage) of the human subject a therapeutically effective amount of a fusion protein that is hGALNS fused to an acidic oligopeptide (such as an acidic oligopeptide described in Section 6.1.2 (b), for example, D8), wherein the fusion protein is produced from an rAAV genome. The rAAV genome may comprise an hGALNS expression cassette as described in Section 6.1.2.

[00269] In another aspect, the method comprises delivering to the bone, cartilage, ligament, growth plate, meniscus, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve (*e.g.*, delivering to the bone and/or cartilage) of the human subject a therapeutically effective amount of a fusion protein that is hGALNS fused to an acidic oligopeptide (such as an acidic oligopeptide described in Section 6.1.2 (b), for example, D8), wherein the fusion protein is produced from an rAAV genome and is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell. The rAAV genome may comprise an hGALNS expression cassette as described in Section 6.1.2. In some embodiments, the rAAV genome comprises a nucleotide sequence encoding a CAG promoter, wherein the nucleotide sequence encoding the CAG promoter is operably linked to a nucleotide sequence encoding the fusion protein. In certain embodiments, the CAG promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:28. In a specific embodiment, the rAAV genome comprises a nucleotide sequence encoding a liver-specific promoter, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to a nucleotide sequence encoding the fusion protein.

In a specific embodiment, the liver-specific promoter is a TBG promoter. In certain embodiments, the liver-specific promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:13. In certain embodiments, the liver-specific promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:14. In certain embodiments, the liver-specific promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:15. In certain embodiments, the CAG promoter is SEQ ID NO:28. In certain embodiments, the liver-specific promoter is SEQ ID NO:13. In certain embodiments, the liver-specific promoter is SEQ ID NO:14. In certain embodiments, the liver-specific promoter is SEQ ID NO:15.

[00270] In another aspect, the method comprises delivering to the bone, cartilage, ligament, growth plate, meniscus, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve (*e.g.*, delivering to the bone and/or cartilage) of the human subject a therapeutically effective amount of a fusion protein that is hGALNS fused to an acidic oligopeptide (such as an acidic oligopeptide described in Section 6.1.2 (b), for example, D8), wherein the fusion protein is produced from an rAAV genome and is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell. The rAAV genome may comprise an hGALNS expression cassette as described in Section 6.1.2. In a specific embodiment, the rAAV genome comprises a nucleotide sequence encoding a liver- and muscle- specific promoter, wherein the nucleotide sequence encoding the liver- and muscle- specific promoter is operably linked to a nucleotide sequence encoding the fusion protein. In certain embodiments, the liver- and muscle-promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:16. In certain embodiments, the promoter is SEQ ID NO:16.

[00271] In another aspect, the method comprises delivering to the bone, cartilage, ligament, growth plate, meniscus, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve (*e.g.*, delivering to the bone and/or cartilage) of the human subject a therapeutically effective amount of a fusion protein that is hGALNS fused to an acidic oligopeptide (such as an acidic oligopeptide described in Section 6.1.2 (b), for example, D8), wherein the fusion protein is produced from an rAAV genome and is glycosylated with mannose-6-phosphate by having been

produced in and secreted from a liver cell. The rAAV genome may comprise an hGALNS expression cassette as described in Section 6.1.2. In a specific embodiment, the rAAV genome comprises a nucleotide sequence encoding a promoter, wherein the nucleotide sequence encoding the promoter is operably linked to a nucleotide sequence encoding the fusion protein. In certain embodiments, the promoter is a CAG promoter.

[00272] In another aspect, the method comprises delivering to the bone, cartilage, ligament, growth plate, meniscus, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve (*e.g.*, delivering to the bone and/or cartilage) of the human subject a therapeutically effective amount of hGALNS that is produced from an rAAV genome and is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell. The rAAV genome may comprise an hGALNS expression cassette as described in Section 6.1.2. In a specific embodiment, the rAAV genome comprises a nucleotide sequence encoding a liver-specific promoter, wherein the nucleotide sequence encoding the liver-specific promoter is operably linked to a nucleotide sequence encoding hGALNS. In a specific embodiment, the liver-specific promoter is a TBG promoter.

[00273] In another aspect, the method comprises delivering to the bone, cartilage, ligament, growth plate, meniscus, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve (*e.g.*, delivering to the bone and/or cartilage) of the human subject a therapeutically effective amount of hGALNS that is produced from an rAAV genome and is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell. The rAAV genome may comprise an hGALNS expression cassette as described in Section 6.1.2. In a specific embodiment, the rAAV genome comprises a nucleotide sequence encoding a promoter, wherein the nucleotide sequence encoding the promoter is operably linked to a nucleotide sequence encoding hGALNS. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:28. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:13. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:14. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:15. In certain embodiments, the promoter comprises a nucleotide sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO:16. In certain embodiments, the promoter is SEQ ID NO:28. In certain embodiments, the promoter is SEQ ID NO:13. In certain embodiments, the promoter is SEQ ID NO:14. In certain embodiments, the promoter is SEQ ID NO:15. In certain embodiments, the promoter is SEQ ID NO:16.

[00274] In various embodiments of the methods of treating described herein, the rAAV or rAAV genome comprises one or more components derived from one or more serotypes of AAV. In certain embodiments, the rAAV or rAAV genome comprises one or more components derived from one or more of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, or AAV11. In a certain embodiment, the rAAV or rAAV genome comprises one or more components from one or more of AAV8, AAV9, AAV10, or AAV11 serotypes. In a specific embodiment, the rAAV or rAAV genome comprises one or more components from the AAV8 serotype. In some embodiments, the rAAV or rAAV genome comprises one or more components from the AAV9 serotype. Nucleic acid sequences of AAV components and methods of making recombinant AAV and AAV capsids are described, for example, in United States Patent No. 7,282,199 B2, United States Patent No. 7,790,449 B2, United States Patent No. 8,318,480 B2, United States Patent No. 8,962,332 B2 and International Patent Application No. PCT/EP2014/076466, each of which is incorporated herein by reference in its entirety.

[00275] In various embodiments of the methods of treating described herein, the step of delivering to the bone, cartilage, ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve is a step of delivering to (a) the bone and/or cartilage, and (b) ligament, meniscus, growth plate, liver, spleen, lung, kidney, trachea, heart muscle, and/or heart valve.

6.3.1 **Target Patient Populations**

[00276] According to the invention, the human subject or patient is an individual who has been diagnosed with MPS IVA (Morquio A syndrome). In specific embodiments, the patient has one or more of the following symptoms of MPS IVA: abnormal heart valve morphology, carious teeth, cervical myelopathy, cervical subluxation, chondroitin sulfate excretion in urine, coarse

facial features, constricted iliac wings, coxa valga, disproportionate short-trunk, short stature, epiphyseal deformities of tubular bones, flaring of rib cage, genu valgum, grayish enamel, hearing impairment, hepatomegaly, hyperlordosis, hypoplasia of the odontoid process, inguinal hernia, joint laxity, juvenile onset, keratin sulfate excretion in urine, kyphosis, large elbow, mandibular prognathia, metaphyseal widening, opacification of the corneal stroma, osteoporosis, ovoid vertebral bodies, platyspondyly, pointed proximal second through fifth metacarpals, prominent stermum, recurrent upper respiratory tract infection, restrictive ventilator defect, scoliosis, ulnar deviation of the wrist, wide mouth, and widely spaced teeth.

[00277] In certain embodiments, the patient has been identified as responsive to treatment with hGALNS.

[00278] In a specific embodiment, the patient has a severe and rapidly progressive, early-onset form of MPS IVA. In another specific embodiment, the patient has a slowly progressive, later-onset form of MPS IVA.

[00279] In a specific embodiment, the patient is an adult (at least age 16). In another specific embodiment, the patient is an adolescent (age 12-15). In another specific embodiment, the patient is a child (under age 12).

[00280] In a specific embodiment, the patient is under age 6.

6.3.2 **Administration and Dosage**

[00281] The route of administration of an rAAV described herein and the amount of rAAV to be administered to the human patient can be determined based on the severity of the disease, condition of the human patient and the knowledge of the treating physician.

(a) Therapeutic Dosage

[00282] In specific embodiments, the amount of rAAV administered to a human subject is sufficient to supply a therapeutically effective amount of hGALNS to the affected tissue (bone, cartilage, ligament, meniscus, and/or heart valve).

[00283] In certain embodiments, dosages are measured by the number of genome copies administered to the human subject via rAAVs provided herein. In a specific embodiment, 1×10^{10} to 1×10^{16} genome copies are administered. In another specific embodiment, 1×10^{10} to 1×10^{11} genome copies are administered. In another specific embodiment, 1×10^{11} to 1×10^{12} genome copies are administered. In another specific embodiment, 1×10^{12} to 1×10^{13} genome

copies are administered. In another specific embodiment, 1 x 10^{13} to 1 x 10^{14} genome copies are administered. In another specific embodiment, 1 x 10^{14} to 1 x 10^{15} genome copies are administered. In another specific embodiment, 1 x 10^{15} to 1 x 10^{16} genome copies are administered.

[00284] Without being bound by theory, at least 10% of the rAAV administered infects the liver of the human subject to which is was administered. In certain embodiments, 10-15%, 15-20%, 20-25%, 25-35%, 30-40%, 35-45%, 40-50%, 45-55%, 50-60%, 55-65%, 60-70%, 65-75%, 70-80%, 75-85%, 80-90%, 85-95%, or 90-100% of the rAAV administered infects the liver of the human subject.

[00285] Without being bound by theory, at least 10% of the hGALNS enzyme expressed from the rAAV viral genome is expressed in liver cells. In certain embodiments, 10-15%, 15-20%, 20-25%, 25-35%, 30-40%, 35-45%, 40-50%, 45-55%, 50-60%, 55-65%, 60-70%, 65-75%, 70-80%, 75-85%, 80-90%, 85-95%, or 90-100% of the hGALNS enzyme expressed from the rAAV viral genome is expressed in liver cells.

[00286] Without being bound by theory, at least 10% of the hGALNS enzyme expressed from the rAAV viral genome reaches the affected tissue (*e.g.*, bone) of the human subject. In certain embodiments, 10-15%, 15-20%, 20-25%, 25-35%, 30-40%, 35-45%, 40-50%, 45-55%, 50-60%, 55-65%, 60-70%, 65-75%, 70-80%, 75-85%, 80-90%, 85-95%, or 90-100% of the hGALNS enzyme expressed from the rAAV viral genome reaches the affected tissue (*e.g.*, bone) of the human subject.

[00287] Without being bound by theory, at least 10% of the hGALNS enzyme expressed from the rAAV viral genome is glycosylated by having been expressed in and secreted from the liver cells. In certain embodiments, 10-15%, 15-20%, 20-25%, 25-35%, 30-40%, 35-45%, 40-50%, 45-55%, 50-60%, 55-65%, 60-70%, 65-75%, 70-80%, 75-85%, 80-90%, 85-95%, or 90-100% of the hGALNS enzyme expressed from the rAAV viral genome is glycosylated by having been expressed in and secreted from the liver cells.

[00288] Without being bound by theory, at least 10% of the liver-cell glycosylated hGALNS enzyme can reach the affected tissue (*e.g.*, bone) of the human subject. In certain embodiments, 10-15%, 15-20%, 20-25%, 25-35%, 30-40%, 35-45%, 40-50%, 45-55%, 50-60%, 55-65%, 60-70%, 65-75%, 70-80%, 75-85%, 80-90%, 85-95%, or 90-100% of the liver-cell glycosylated hGALNS enzyme can reach the affected tissue (*e.g.*, bone) of the human subject.

(b) Routes of administration

[00289] In a specific embodiment, the rAAV can be present in a pharmaceutical composition in order to be administered to the human subject (see Section 6.1.3).

[00290] The rAAV can be administered, for example, by parenteral, subcutaneous, intramuscular, intravenous, intraperitoneal, intranasal, intrathecal, or transdermal administration. In a specific embodiment, the rAAV is administered by intravenous administration.

6.4 COMBINATION THERAPIES

6.4.1 Co-therapy with Immune Suppression

[00291] While the delivery of rAAV should minimize immune reactions, the clearest potential source of toxicity related to gene therapy is generating immunity against the expressed hGALNS protein in human subjects who are genetically deficient for hGALNS and, therefore, potentially not tolerant of the enzyme or the rAAV. Thus, in a certain embodiment, it is advisable to co-treat the patient with immune suppression therapy -- especially when treating patients with severe disease who have close to zero levels of hGALNS. Immune suppression therapies involving a regimen of tacrolimus or rapamycin (sirolimus) in combination with mycophenolic acid, or other immune suppression regimens used in tissue transplantation procedures can be employed. Such immune suppression treatment may be administered during the course of gene therapy, and in certain embodiments, pre-treatment with immune suppression therapy may be preferred. Immune suppression therapy can be continued subsequent to the gene therapy treatment, based on the judgment of the treating physician, and may thereafter be withdrawn when immune tolerance is induced; *e.g.,* after 180 days.

[00292] In certain embodiments, the methods of treatment provided herein further comprise administering to the human patient an immune suppression regimen comprising prednisolone, mycophenolic acid, and tacrolimus. In certain embodiments, the methods of treatment provided herein further comprise administering to the human patient an immune suppression regimen comprising prednisolone, mycophenolic acid, and rapamycin (sirolimus). In certain embodiments, the methods of treatment provided herein further comprise administering to the human patient an immune suppression regimen that does not comprise tacrolimus. In certain embodiments, the methods of treatment provided herein further comprise administering to the human patient an immune suppression regimen comprising one or more corticosteroids such as

methylprednisolone and/or prednisolone, as well as tacrolimus and/or sirolimus. In certain embodiments, the immune suppression therapy comprises administering a combination of (a) tacrolimus and mycophenolic acid, or (b) rapamycin and mycophenolic acid to the subject before or concurrently with the hGALNS treatment and continuing thereafter. In certain embodiments, the immune suppression therapy is withdrawn after 180 days. In certain embodiments, the immune suppression therapy is withdrawn after 30, 60, 90, 120, 150, or 180 days.

6.4.2 Co-therapy with Other Treatments

[00293] Combination therapy involving administration of the rAAV as described herein to the human subject accompanied by administration of other available treatments are encompassed by the methods of the described embodiment. The additional treatments may be administered before, concurrently or subsequent to the gene therapy treatment. Available treatments for MPS IVA that could be combined with the gene therapy of the invention include but are not limited to enzyme replacement therapy (ERT) and/or HSCT therapy. In a specific embodiment, ERT can be performed using the D8-hGALNS enzyme produced in human cell lines by recombinant DNA technology. Human cell lines that can be used for such enzyme production include but are not limited to HT-22, SK-N-MC, HCN-1A, HCN-2, NT2, SH-SY5y, hNSC11, ReNcell VM, human embryonic kidney 293 cells (HEK293), HEK293-T, fibrosarcoma HT-1080, HKB-11, CAP, HuH-7, and retinal cell lines, PER.C6, or RPE (see, e.g., Dumont *et al.*, 2016, Critical Rev in Biotech 36(6):1110-1122 "Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives" which is incorporated by reference in its entirety.

6.5 DISEASE MARKERS AND TREATMENT ASSESSMENT

[00294] In certain embodiments, efficacy of a treatment method as described herein may be monitored by measuring reductions in biomarkers of disease (such as GAG, KS, and C6S storage) and/or increase in hGALNS enzyme activity in bone, cartilage, ligament, meniscus, heart valve, urine, and/or serum. Signs of inflammation and other safety events may also be monitored.

[00295] In certain embodiments, efficacy of a treatment method as described herein is monitored by measuring the level of a disease biomarker in the patient. In certain embodiments, the level of the disease biomarker is measured in the serum of the patient. In certain

embodiments, the level of the disease biomarker is measured in the urine of the patient. In certain embodiments, the disease biomarker is GAG. In certain embodiments, the disease biomarker is KS. In certain embodiments, the disease biomarker is C6S. In certain embodiments, the disease biomarker is hGALNS enzyme activity.

[00296] In certain embodiments, efficacy of a treatment method as described herein can be monitored by measuring physical characteristics associated with lysosomal storage deficiency in the patient. In certain embodiments, the physical characteristics can be storage lesions. In certain embodiments, the physical characteristic can be short neck and trunk. In certain embodiments, the physical characteristic can be pectus carinatum. In certain embodiments, the physical characteristic can be laxity of joints. In certain embodiments, the physical characteristic can be kyphoscoliosis. In certain embodiments, the physical characteristic can be tracheal obstruction. In certain embodiments, the physical characteristic can be spinal cord compression. In certain embodiments, the physical characteristic can be hearing impairment. In certain embodiments, the physical characteristic can be corneal opacity. In certain embodiments, the physical characteristics can be bone and joint deformities. In certain embodiments, the physical characteristic can be cardiac valve disease. In certain embodiments, the physical characteristics can be restrictive/obstructive airway. Such physical characteristics may be measured by any method known to one of skill in the art.

7. EXAMPLES

[00297] Certain embodiments provided herein are illustrated by the following non-limiting examples.

7.1 Example 1. Design of plasmids encoding rAAV genomes and *in vitro* transfection assays

[00298] To generate recombinant AAV genomes containing an hGALNS expression cassette, which were to be packaged in AAV8 capsids, plasmids encoding the recombinant AAV genomes were designed. Four plasmids were designed and generated: TBG-hGALNS (the hGALNS expression cassette contains a nucleotide sequence encoding hGALNS, whose expression is under the regulation of the liver-specific TBG promoter), TBG-hGALNS CoOpt (the hGALNS expression cassette contains a codon optimized nucleotide sequence encoding hGALNS, whose expression is under the regulation of the liver-specific TBG promoter), TBG-D8-hGALNS (the

hGALNS expression cassette contains a nucleotide sequence encoding a fusion protein that is hGALNS fused to D8, whose regulation is under the regulation of the liver-specific TBG promoter), or TBG-D8-hGALNS CoOpt (the hGALNS expression cassette contains a codon optimized nucleotide sequence encoding a fusion protein that is hGALNS fused to D8, whose regulation is under the regulation of the liver-specific TBG promoter). The resulting rAAVs fall into two categories: (a) rAAVs comprising a recombinant AAV genome that contains an hGALNS expression cassette flanked by AAV-inverted terminal repeats (ITRs), wherein the hGALNS expression cassette comprises an hGALNS cDNA sequence operably linked to the liver-specific TBG promoter sequence and a nucleotide sequence encoding a poly A site; and (b) rAAVs comprising a recombinant AAV genome that contains an hGALNS expression cassette flanked by AAV-inverted terminal repeats (ITRs), wherein the hGALNS expression cassette comprises a D8-hGALNS cDNA sequence operably linked to the liver-specific TBG promoter sequence and a nucleotide sequence encoding a poly A site (FIG. 1). D8 is a bone-targeting aspartic acid octapeptide.

[00299] Next, human hepatocellular carcinoma (HuH7) cells were transfected with one of the four plasmids using Lipofectamine-3000 protocol to test expression of hGALNS *in vitro*. After a 48 hour incubation, the transfected HuH7 cells and the supernatant were collected and analyzed for GALNS enzyme activity in cell pellets and media. Huh7 cells transfected with a GFP plasmid were used as a control. Intracellular hGALNS enzyme activity was increased equally by transfection with the TBG-hGALNS or TBG-hGALNS CoOpt plasmid (FIGS. 2A and 2B). Intracellular enzyme activity was also increased after transfection with the TBG-D8-hGALNS or TBG-D8-hGALNS CoOpt plasmid, although to a lesser extent than transfection with the TBG-hGALNS or TBG-hGALNS CoOpt plasmid (FIGS. 2A and 2B). Enzyme activity detected in the cell media was increased by the transfection of any of the plasmids (FIGS. 2C and 2D).

[00300] Similarly, human liver carcinoma cells (HepG2) were transfected with one of the four plasmids using Lipofectamine-3000 protocol to test expression of hGALNS *in vitro* (FIG. 3). After a 72 hour incubation, the transfected HepG2 cells were collected and analyzed for hGALNS enzyme activity in cell pellets. Intracellular hGALNS enzyme activity was increased by transfection with the TBG-hGALNS or TBG-hGALNS CoOpt plasmid as compared to transfection with the control plasmid. Transfection with the TBG-D8-hGALNS or TBG-D8-

hGALNS CoOpt plasmid did not lead to increased hGALNS activity as compared to the control plasmid.

7.2 Example 2. *In vivo* administration of rAAV to MPS IVA knock out (galns -/-) mice

[00301] rAAV8 were generated that comprise viral genomes capable of expressing native human GALNS (hGALNS) under the liver-specific promoter TBG (AAV8-TBG-hGALNS, also labeled as AAV8-hGALNS in some figures) or hGALNS with an aspartic acid octapeptide (D8) under the liver-specific promoter (AAV8-TBG-D8-hGALNS, also labeled as AAV8-D8-hGALNS in some figures). The TBG-hGALNS CoOpt and TBG-D8-hGALNS CoOpt plasmids were used to generate the viral genomes respectively. The two types of viruses were each administered intravenously to 4-week-old MPS IVA knock-out (KO) mice and Mtol immunotolerant mice at a dose of $5x10^{13}$ GC/kg body weight. KO mice have a targeted disruption of Exon 2 of mGALNS and have no detectable GALNS enzyme activity. Mtol mice are tolerized to human GALNS protein. Untreated KO mice and wild-type (WT) mice served as controls. These mice were monitored for 14 weeks post-injection. Blood was collected biweekly and assayed for hGALNS activity and keratan sulfate (KS). The schedule of rAAV administration, blood collection, GALNS enzyme assay, and KS assay is shown in FIG. 4. At necropsy, bone pathology was evaluated by histopathological analysis.

[00302] As seen in FIG. 5A, hGALNS enzyme activity increased in the white blood cells (WBCs) of rAAV-treated mice, reaching close to WT mice-levels 10 weeks after treatment. Two weeks after administration of rAAV, hGALNS enzyme activity in the plasma of all rAAV-treated mice was elevated on average 20-fold compared to levels in WT mice (ranging from 5-100 fold compared to levels in WT mice) (FIG. 5B). This increase was maintained throughout the 14 weeks monitoring period (FIG. 5B). Similar data is shown in FIG. 22. Plasma enzyme activity levels in Mtol mice treated with AAV8-TBG-D8-hGALNS were significantly higher than the levels in the mice treated with AAV8-TBG-hGALNS (FIG. 6), but enzyme activity levels of both treated groups were elevated above WT levels. Similar data is shown in FIG. 23.

[00303] hGALNS activity measured in the liver of KO (galns -/-) mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS was compared to the liver hGALNS activity of WT mice (FIG. 7A). The mice treated with AAV8-TBG-hGALNS had 40 times greater levels of hGALNS activity in the liver compared to WT levels, while mice treated with AAV8-TBG-D8-

hGALNS had 8 times higher liver hGALNS activity than WT levels. hGALNS activities in liver of Mtol mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS were elevated over untreated Mtol mice (PBS-treated) (FIG. 7B). Mice treated with AAV8-TBG-hGALNS had 50 times greater levels of hGALNS activity in the liver compared with untreated mice, while mice treated with AAV8-TBG-D8-hGALNS had 8 times higher liver hGALNS activity than untreated Mtol mice. *See* FIG. 12A for more data regarding hGALNS activity levels measured in the liver of MPS IVA KO mice (galns -/-) and Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD).

[00304] hGALNS activity was also measured in the heart of (a) WT mice, (b) untreated MPS IVA KO (galns -/-) mice, (c) MPS IVA KO (galns -/-) mice treated with AAV8-TBG-hGALNS, (d) MPS IVA KO (galns -/-) mice treated with AAV8-TBG-D8-hGALNS, (e) untreated Mtol mice, (f) Mtol mice treated with AAV8-TBG-hGALNS, and (g) Mtol mice treated with AAV8-TBG-D8-hGALNS (FIG. 7C). For both MPS IVA KO (galns -/-) mice and Mtol mice, mice treated with AAV8-TBG-hGALNS and mice treated with AAV8-TBG-D8-hGALNS both had greater levels of hGALNS activity in the heart compared with untreated mice. *See* FIG. 13B for more data regarding hGALNS activity levels measured in the heart of MPS IVA KO mice (galns -/-) and Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD).

[00305] Similarly, hGALNS activity was measured in the bone of (a) WT mice, (b) untreated MPS IVA KO (galns -/-) mice, (c) MPS IVA KO (galns -/-) mice treated with AAV8-TBG-hGALNS, (d) MPS IVA KO (galns -/-) mice treated with AAV8-TBG-D8-hGALNS, (e) untreated Mtol mice, (f) Mtol mice treated with AAV8-TBG-hGALNS, and (g) Mtol mice treated with AAV8-TBG-D8-hGALNS (FIG. 7D). For both MPS IVA KO (galns -/-) mice and Mtol mice, mice treated with AAV8-TBG-hGALNS and mice treated with AAV8-TBG-D8-hGALNS both had greater levels of hGALNS activity in the bone compared with untreated mice. *See* FIG. 13A for more data regarding hGALNS activity levels measured in the bone of MPS IVA KO mice (galns -/-) and Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD).

[00306] In both MPS IVA KO (galns -/-) mice and Mtol mice, hGALNS activity levels in liver, heart and bone of treated mice were elevated after the delivery of AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS vectors. In addition, there was a direct correlation between hGALNS enzyme activities in blood and bone.

[00307] hGALNS enzyme activity levels were also measured in the spleen of MPS IVA KO mice (galns -/-) and the spleen of Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD) (FIG. 12B). For both MPS IVA KO (galns -/-) mice and Mtol mice, mice treated with AAV8-TBG-hGALNS and mice treated with AAV8-TBG-D8-hGALNS both had greater levels of hGALNS activity in the spleen compared with untreated mice.

[00308] In addition, hGALNS enzyme activity levels were also measured in the lung of MPS IVA KO mice (galns -/-) and the lung of Mtol mice, respectively, after administration with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice (galns -/-), untreated Mtol mice and wild type mice (n= 3-8; mean ± SD) (FIG. 12C). For both MPS IVA KO (galns -/-) mice and Mtol mice, mice treated with AAV8-TBG-hGALNS and mice treated with AAV8-TBG-D8-hGALNS both had greater levels of hGALNS activity in the lung compared with untreated mice.

[00309] Blood keratan sulfate (KS) levels were measured. In the KO (galns -/-) mice, rAAV treatment in both groups resulted in a reduction of mono-sulfated keratan sulfate (KS) levels in the plasma to WT levels two weeks after administration (FIG. 8 and FIG. 14). These reduced levels in the plasma of both rAAV-treated groups were maintained until necropsy at 12 weeks post-injection. In contrast, the KS levels in the plasma of the untreated KO mice did not decrease and remained elevated over the monitored time period. Administration of either of the rAAV in Mtol mice resulted in a reduction of mono-sulfated keratan sulfate (KS) levels in the plasma as compared to WT levels two weeks after treatment and the KS levels in the plasma were significantly reduced as compared to untreated Mtol mice (FIGS. 9A-9B and FIGS. 15A-15B). Blood diHS-0S levels, however, did not differ between the untreated, AAV8-TBG-hGALNS-treated, AAV8-TBG-D8-hGALNS-treated, and WT mice groups (FIG. 10).

[00310] Mono-sulfated KS levels were measured in the liver of MPS IVA KO mice (galns -/-), the liver of Mtol mice, and the lung of MPS IVA KO mice (galns -/-), respectively, treated

with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compared to untreated MPS IVA KO mice and untreated wild type mice (FIGS. 16A-16C). Administration of either of the rAAV resulted in a significant reduction of mono-sulfated KS in the liver and a significant reduction of mono-sulfated KS in the lung as compared to untreated mice. KS levels in liver and lung of MPS IVA KO mice (galns -/-) and Mtol mice were almost normalized after AAV vector administration.

[00311] Histo-pathological evaluation of the liver from the treated KO mice showed complete clearance of GAG storage in sinus lining cells and Kupffer cells.

[00312] Administration of AAV8-TBG-hGALNS and AAV8-TBG-D8-hGALNS maintained high levels of enzymatic activity in the plasma KO and Mtol mouse models throughout the monitoring period. This continuous presence of circulating enzyme reduced KS in plasma to WT levels which is a significant improvement over what has been achieved by ERT (Tomatsu *et al.*, Human Molecular Genetics, 2008, 17(6): 815-824). While KS levels in the plasma were normalized two weeks post rAAV administration in both mouse models and for both AAV8-TBG-hGALNS and AAV8-TBG-D8-hGALNS, in previous studies where the KO mice were treated with ERT, the KS levels in the plasma were not normalized even after 12 weeks of weekly infusions (Tomatsu *et al.*, Human Molecular Genetics, 2008, 17(6): 815-824). Additionally, the high enzyme levels combined with longer circulation time increased the penetration into bone and cartilage therapy thereby improving storage in these regions.

[00313] Mice were euthanized 12 weeks after rAAV treatment and tissues were collected and assessed for the storage of glycosaminoglycans (GAGs). Tissues were stained with toluidine blue. Bone pathology was evaluated by histopathological analysis and the pathology scores are presented in a graphical depiction for MPS IVA KO (galns -/-) mice (FIG. 11A). FIG. 11B shows staining images of the knee joints (MPS IVA KO (galns -/-) mice).

[00314] FIG. 11C shows 40x magnified staining images of femur articular cartilage for MPS IVA KO (galns -/-) mice. In the untreated mice (left panel), the superficial cells were disorganized and the chondrocytes were ballooned and vacuolated. Further, the column structure was distorted and disorganized. In contrast, the tissue of the MPS IVA KO (galns -/-) mice treated with either AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS showed organized superficial cells, less vacuolated chondrocytes, and the maintenance of the column structure (right two panels). These aspects are shown in greater detail in FIGS. 11D-11F.

[00315] FIG. 11G shows 40x magnified staining images of femur growth plate of untreated or rAAV-treated MPS IVA KO (galns -/-) mice. In the untreated mice (left panel), the chondrocytes were vacuolated and the column structure was largely disorganized and distorted. The chondrocytes in mice treated with AAV8-TBG-hGALNS (middle panel) were also vacuolated but the column structure was only moderately distorted. In contrast, for the tissue of the MPS IVA KO (galns -/-) mice treated with AAV8-TBG-D8-hGALNS (right panel), the chondrocytes were moderately vacuolated and the column structure was more organized. These aspects are shown in greater detail in FIGS. 11H-11J. FIGS. 17A-17E also show 40x magnified staining images of femur growth plate in (A) wild type mice (all chondrocytes were non-vacuolated and column structure was well organized), (B) untreated MPS IVA KO mice (galns -/-) (all chondrocytes were vacuolated and column structure was largely disorganized and distorted), (C) untreated Mtol mice (all chondrocytes were vacuolated and column structure was largely disorganized and distorted), (D) AAV8-TBG-hGALNS treated Mtol mice (chondrocytes were moderately vacuolated but column structure was better), and (E) AAV8-TBG-D8-hGALNS treated Mtol mice (chondrocytes were moderately vacuolated but column structure was partially recovered).

[00316] FIG. 18A shows the chondrocyte cell size measured in the femur and tibia growth plate of untreated wild type mice, untreated MPS IVA KO mice (galns -/-), AAV8-TBG-hGALNS treated MPS IVA KO mice (galns -/-), or AAV8-TBG-D8-hGALNS treated MPS IVA KO mice (galns -/-). FIG. 18B shows the chondrocyte cell size measured in the femur growth plate of untreated wild type mice, untreated Mtol mice, AAV8-TBG-hGALNS treated Mtol mice, or AAV8-TBG-D8-hGALNS treated Mtol mice. FIG. 18C shows the chondrocyte cell size measured in the tibia growth plate of untreated wild type mice, untreated MPS IVA KO mice (galns -/-), AAV8-TBG-hGALNS treated MPS IVA KO mice (galns -/-), or AAV8-TBG-D8-hGALNS treated MPS IVA KO mice (galns -/-). FIG. 18D shows the chondrocyte cell size measured in the tibia growth plate of untreated wild type mice, untreated Mtol mice, AAV8-TBG-hGALNS treated Mtol mice, or AAV8-TBG-D8-hGALNS treated Mtol mice.

[00317] Chondrocyte size and column structure in growth plate in MPS IVA KO mice and Mtol mice were both substantially improved after AAV gene transfer.

[00318] FIG. 11K shows 40x magnified staining images of the meniscus of untreated or rAAV-treated MPS IVA KO (galns -/-) mice. In the untreated mice (left panel), most of the cells

were ballooned and vacuolated. Some cells in the meniscus of mice treated with AAV8-TBG-hGALNS (middle panel) were vacuolated. Most of the cells in the tissue of mice treated with AAV8-TBG-D8-hGALNS (right panel) were not vacuolated.

[00319] FIG. 11L shows 40x magnified staining images of the ligament on the tibia side of untreated or rAAV-treated MPS IVA KO (galns -/-) mice. In the untreated mice (left panel), most of the cells were vacuolated. Some cells in the ligament of mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS (right two panels) remained vacuolated.

[00320] FIG. 11M shows 40x magnified staining images of the base of the heart valve of untreated or rAAV-treated MPS IVA KO (galns -/-) mice. Many of the cells at the base of the mitral valve in the untreated mice (left panel) were vacuolated, while no vacuolated cells were seen at the base of the mitral valve tissue of mice treated with AAV8-TBG-hGALNS (middle panel) or AAV8-TBG-D8-hGALNS (right panel). These aspects of the untreated mice tissue are shown in greater detail in FIG. 11N. Similar results were seen in the tissue of the heart valve (FIG. 11O). Similar results for the Mtol mice are shown in FIG 19 (bottom panel). Many heart valve cells in the untreated mice (left panel) were vacuolated, while no vacuolated cells were seen in heart valve tissue of mice treated with AAV8-TBG-hGALNS (middle panel) or AAV8-TBG-D8-hGALNS (right panel). These aspects of the untreated mice tissue are shown in greater detail in FIG. 11P.

[00321] FIG. 20 shows the histopathology of heart muscle (40x magnification) in Mtol mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, as compare to untreated Mtol mice.

[00322] Heart valve and heart muscle had no obvious vacuolated cells in both of MPS IVA KO (galns -/-) mice and Mtol mice after AAV gene transfer.

[00323] FIG. 21A shows the pathology score of the heart valve tissue of untreated wild type mice, untreated MPS IVA KO(galns -/-) mice, MPS IVA KO(galns -/-) mice treated with AAV8-TBG-hGALNS, or MPS IVA KO(galns -/-) mice treated with AAV8-TBG-D8-hGALNS. FIG. 21B shows the pathology score of the heart valve tissue of untreated wild type mice, untreated Mtol mice, Mtol mice treated with AAV8-TBG-hGALNS, or Mtol mice treated with AAV8-TBG-D8-hGALNS. FIG. 21C shows the pathology score of the heart muscle tissue of untreated wild type mice, untreated MPS IVA KO(galns -/-) mice, MPS IVA KO(galns -/-) mice treated with AAV8-TBG-hGALNS, or MPS IVA KO(galns -/-) mice treated with AAV8-TBG-D8-

hGALNS. FIG. 21D shows the pathology score of the heart muscle tissue for untreated wild type mice, untreated Mtol mice, Mtol mice treated with AAV8-TBG-hGALNS, or Mtol mice treated with AAV8-TBG-D8-hGALNS.

[00324] Bone pathology was evaluated by histopathological analysis for Mtol mice as well. Unpaired t-test was used to examine the differences of pathology scores between the untreated and each of the treated groups (Score: 0 (Normal) – 3 (Severe)), *see* Table 1.

[00325] **Table 1.** Pathology score in bone of Mtol mice (n=2-5, mean ±SD)

			Untreated	AAV8-TBG-hGALNS-treated	AAV8-TBG-D8-hGALNS- treated
Growth plate	Femur	Vacuolization	2.90 ± 0.10	1.38 ± 0.34 *	1.41 ± 0.21 *
		Column structure	2.93 ± 0.11	1.44 ± 0.33 *	1.47 ± 0.16 *
	Tibia	Vacuolization	2.85 ± 0.21	1.56 ± 0.26 *	1.50 ± 0.29 *
		Column structure	2.75 ± 0.31	1.63 ± 0.20 *	1.41 ± 0.33 *
Articular cartilage	Femur	Vacuolization	2.48 ± 0.34	1.38 ± 0.18 *	1.16 ± 0.33 *
		Column structure	2.35 ± 0.44	1.38 ± 0.18 *	1.22 ± 0.19 *
	Tibia	Vacuolization	2.53 ± 0.16	1.19 ± 0.14 *	1.27 ± 0.21 *
		Column structure	2.44	1.38	1.21 ± 0.26
Ligament			2.80 ± 0.26	1.72 ± 0.56 *	1.66 ± 0.26 *
Meniscus			2.73 ± 0.34	1.41 ± 0.33 *	1.34 ± 0.26 *

*p<0.05 vs untreated

[00326] Both viruses reduced GAG storage in articular cartilage, ligaments, and meniscus surrounding the articular cartilage and growth plate region in tibia and femur. The reduction of GAG storage observed in the bone and cartilage was comparatively greater for the AAV-TBG-D8-hGALNS treated mice.

[00327] Bone, growth plate, articular cartilage, meniscus, ligament, and heart tissues were substantially improved in rAAV treated mice. In addition, treated mice had almost complete remission with respect to defects in the heart valve and base of the heart valve, and no obvious vacuolated cells were seen at both the heart valve base and heart valve. The results show significant improvement over ERT since ERT-treated mice showed no clearance of vacuolated cells in growth plate, had disorganized column structure in growth plate, and had substantial vacuolated cells in heart valve (Tomatsu *et al.*, Human Molecular Genetics, 2008, 17(6): 815-824).

[00328] The fact that therapeutic effect was observed in tissues other than liver (where the hGALNS and D8-hGALNS proteins were produced) suggests that there was mannose-6-phosphate receptor mediated cross correction.

7.3 Example 3. Liver targeted AAV8 gene therapy ameliorates skeletal and cardiovascular pathology in mucopolysaccharidosis IVA murine model

[00329] This example relates to the experiments described and performed in other examples described herein, including, Examples 1-2 and presents additional data from Examples 1-2. In this example, AAV8 vectors expressing hGALNS with or without a bone-targeting signal, under the control of liver-specific thyroxin-binding globulin (TBG) promoter are evaluated and the therapeutic efficacy of these recombinant AAV8 vectors in bone and heart lesions of both mouse models of MPS IVA disease are studied. Both bone and heart are major organs affected in this disorder.

7.3.1 Results

(a) GALNS enzyme activity in blood and tissues: AAV-hGALNS delivery results in a marked increase of GALNS activity in plasma and various tissues in mouse models of MPS IVA.

[00330] Two mouse models (MPS IVA KO and MTOL) with MPS IVA recapitulate the human disease in terms of the deficiency of hGALNS activity, increased levels of KS in blood and tissues, and storage materials (vacuoles) in various tissues including chondrocytes, meniscus, ligaments, and heart muscle and valves. These biomarkers have been widely used to evaluate the severity of phenotype and the therapeutic efficacy of several approaches in these mouse models (Tomatsu, S., et al., Hum. Mol. Genet., 2008, 17, 815-824; Tomatsu, S., et al., Hum. Mol. Genet., 2003, 12, 3349-3358; Tomatsu, S., et al., Hum. Mol. Genet., 2005, 14, 3321-3335; Tomatsu, S., et al., Mol. Ther., 2010, 18, 1094-1102). For this study, we delivered AAV8-TBG-hGALNSco and AAV8-TBG-D8-hGALNSco (FIG. 24A) intravenously into 4-week-old MPS IVA KO and MTOL mice at a uniform dose of 5×10^{13} GC/kg body weight. The mice were monitored for 12 weeks post-injection and blood samples were collected every other week to analyze the enzyme activity and KS levels. Additionally, at necropsy, tissue samples were taken from different organs for enzymatic activity and KS levels as well as knee joints and heart valves for histopathology analysis.

[00331] Plasma enzyme activity in MPS IVA KO and MTOL mice are shown in FIGs. 24B-24C. No plasma hGALNS activity was detected in untreated MPS IVA mice. Two weeks post-injection, plasma hGALNS activity in MPS IVA KO mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS was significantly increased, compared to that in wild-type mice. The

enzyme activity from AAV8-TBG-D8-hGALNS was higher than that from AAV8-TBG-hGALNS 2 weeks post-injection; however, plasma hGALNS activity was not different between these two AAV vectors 12 weeks post-injection. In MTOL mice treated with both AAV vectors, plasma hGALNS activity was significantly increased compared to that in wild-type mice 2 weeks post-injection. The levels of enzyme activity from mice treated with AAV8-TBG-D8-hGALNS were higher than that from mice treated with AAV8-TBG-hGALNS throughout the entire study duration, suggesting that hGALNS with a bone-targeting signal had prolonged blood circulation, compared to native hGALNS possible due to the reduced uptake into tissues. These results demonstrated that supraphysiological levels of circulating hGALNS activity were achieved after a single injection of AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS in both MPS IVA mouse models, and the high levels of enzyme activities were maintained during the study.

[00332] The levels of hGALNS activity in the liver 12 weeks after IV delivery of AAV vectors are shown in FIG. 24J and FIG. 24K. The hGALNS activity levels in all treated MPS IVA mice were significantly higher than that in untreated MPS IVA mice. The mean enzyme activity levels in MPS IVA KO mice, treated with AAV8-TBG-hGALNS and AAV8-TBG-D8-hGALNS, were 49-, and 9-fold, respectively, higher than the levels observed in wild-type mice. In MTOL mice treated with AAV8-TBG-hGALNS and AAV8-TBG-D8-hGALNS, hGALNS activity was 60-, and 9-fold higher than levels found in wild-type mice. GALNS activities in livers of KO and MTOL mice treated with AAV8-TBG-D8-hGALNS was significantly lower than those in mice treated with AAV8-TBG-hGALNS.

[00333] The levels of tissue hGALNS activity in tissues of MPS IVA mice were examined to evaluate the potential cross-correction of hGALNS deficiency. The hGALNS activity was observed in all examined tissues including spleen, lung, kidney, bone (leg), and heart in both KO and MTOL mice after both AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS treatments (FIGs. 24J-24K). The enzyme activities were similar or higher than wild-type level in spleen, and heart, and slightly lower levels of activities were observed in the lung and kidney. Notably, 37% and 20% of wild-type enzyme activities were observed in the bone of KO mice treated with AAV8-TBG-hGALNS and AAV8-TBG-D8-hGALNS, respectively. Also, 57% and 43% of wild-type enzyme activities were observed in MTOL mice treated with these two AAV vectors. These results suggest that stable supraphysiological levels of hGALNS enzyme contributed to

the penetration of the enzyme into various tissues including the bone of MPS IVA mice after AAV gene transfer. The hGALNS activity levels in bone were no statistically different between AAV8-TBG-hGALNS and AAV8-TBG-D8-hGALNS.

(b) Levels of mono-sulfated KS in the blood and tissue decreased as a result of AAV-GALNS delivery

[00334] We measured mono-sulfated KS, which is the major component of KS, in plasma and tissues of MPS IVA mice. The levels of plasma mono-sulfated KS in KO and MTOL mice are shown in FIGs. 25A-25B**.** Before administration of AAV vectors, plasma KS levels in untreated KO mice were significantly higher than that in wild-type mice (mean: 41.8 vs. 16.3 ng/ml). Two weeks post-injection, mono-sulfated KS levels in plasma were completely normalized for both AAV vectors, and this level was maintained for at least another 10 weeks (at necropsy). Mono-sulfated KS levels were similar in wild-type mice and untreated MTOL mice at four weeks of age. The mono-sulfated KS levels in wild-type mice were maintained at a constant level throughout the study; however, the levels of mono-sulfated KS in untreated MTOL mice gradually increased with age. MTOL mice treated with either of the AAV vector maintained the normal levels throughout the entire study period. At 16 weeks of age, mono-sulfated KS levels in MTOL mice treated with AAV vectors were significantly decreased when compared with those in the untreated MTOL mice.

[00335] Mono-KS levels in tissues of MPS IVA mice are measrued. At necropsy, excessive storage of GAG was present in tissues of both KO and MTOL mice. The amount of of mono-sulfated KS in liver and lung of KO and MTOL mice were significantly decreased 12 weeks post-injection of either AAV vector (FIGs. 25C-25D). To assess the effect of these AAV vectors expressing hGALNS on other GAG levels, the levels of heparan sulfate (HS) were analyzed in blood and tissues of MPS IVS mice. Both KO and MTOL had normal levels of diHS-0S in plasma, and the levels were not affected after injection by AAV vectors (FIG. 30). Tissue diHS-0S levels in the liver and lung were also not changed between all groups 12 weeks post-injection of AAV vectors (FIG. 31).

(c) Delivery of AAV GALNS vectors improved bone and cartilage pathology in MPS IVA mice

[00336] Tissues including bone (femur and tibia) and heart (muscle and valve) were assessed from MPS IVA mice 12 weeks post-injection of AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS.

[00337] Untreated MPS IVA KO and MTOL mice at 16 weeks of age exhibited GAG storage vacuoles in the growth plate of the femur and tibia (hyaline cartilage) (FIG. 27A), articular disc (FIG. 27B), ligament surrounding knee joint (FIG. 32A), and meniscus (FIG. 32B). The growth plate also exhibited a disorganized column structure with ballooned and vacuolated chondrocytes (FIG. 27A-27B). In KO mice treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS, the growth plate, articular cartilage, ligaments, and meniscus in the knee joint had a partial reduction of storage material, and the column structure of chondrocytes was improved but remained disorganized and distorted. In MTOL mice treated with these AAV vectors, the growth plate, articular cartilage, ligaments, and meniscus in the knee joint had greater observable reduction of storage, and column structure of the growth plate and articular cartilage showed greater recovery than in untreated MTOL mice.

[00338] To objectively assess the improvement of vacuolization in cartilage cells of the growth plate, chondrocyte cell size was quantified in the growth plate lesions of KO and MTOL mice (4C). We observed a moderate reduction of chondrocyte size in these growth plate lesions, which reached statistical significance in the MTOL mice. Untreated MPS IVA mice exhibited GAG storage vacuoles in heart valves and muscle. AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS provided nearly complete clearance in these heart lesions of treated KO and MTOL mice (FIG. 27A-27B).

(d) Circulating of anti-hGALNS antibodies

[00339] Overall, improvement of bone pathology in KO mice was less remarkable when compared to that in MTOL mice 12 weeks post-injection of AAV8 vectors. To investigate the possibility of a humoral response to hGALNS, antibody titers to hGALNS were measured by enzyme-linked immunosorbent assay (ELISA). Indirect ELISA method detected anti-hGALNS antibodies in plasma by using full-length rhGALNS coated on the plate. Plasma from KO mice treated with AAV vectors showed significantly higher levels of circulating anti-hGALNS antibodies, compared to that from other groups (0.50 ± 0.38 or 0.62 ± 0.43 optical density (OD)

unit for KO treated with AAV8-TBG-hGALNS or AAV8-TBG-D8-hGALNS) (FIG. 28). Circulating anti-hGALNS antibodies were not detected in wild-type, untreated KO, and MTOL mice.

7.3.2 MATERIALS AND METHODS

(a) Developing AAV hGALNS expression cassette

[00340] To develop an AAV8 vector with hGALNS, we determined the optimized codon sequence of hGALNS. The optimized 1569 bp sequence, translated into 526 amino acids, under the control of liver-specific TBG promoter was packaged in AAV8 capsid. In the vector plasmid with the bone-targeting signal, an Aspartic Acid Octapeptide (D8) sequence was inserted after N-terminal signal peptide of hGALNS, producing bone-targeting hGALNS with high affinity for major bone matrix, hydroxyapatites (FIG. 24A). Production of GALNS by these AAV vector plasmids was confirmed after performing transfection experiment with Huh-7 cell. Intra- and extra-cellular hGALNS activity levels from the codon-optimized open reading frame were similar to that produced by native hGALNS coding sequence (FIGs. 29A-29B).

(b) Expression cassette design and AAV vector production

[00341] The expression cassettes carrying the native and D8 containing GALNS transgenes were designed for packaging into AAV8 vector (FIG. 28). The bone-targeting signal, an Aspartic Acid Octapeptide (D8) sequence was inserted after N-terminal signal peptide of hGALNS. The design included a liver-specific thyroxin-binding globulin (TBG) promoter along with a rabbit betaglobulin polyadenylation tail (polyA). We used a codon optimized hGALNS sequence for both vectors for the mouse studies. We confirmed the GALNS enzymatic activity of these expression cassette plasmids in a transfection experiment using Huh-7 cells. We determined the activity levels in both cell lysate and supernatant 48 hours post transfection (FIGs. 29A-29B). The GALNS activity levels from the codon-optimized construct were similar to that produced by native hGALNS coding sequence .

[00342] AAV8-TBG-hGALNS and AAV8-TBG-D8-hGALNS vectors were generated following a scaled down version of the proprietary GMP vector production protocols at REGENXBIO (Rockville, MD). Briefly, HEK293 cells (RGX293) were triple-transfected with the helper plasmid, AAV8 Capsid Plasmid and the transgene plasmid containing the hGALNS/D8-hGALNS plasmid. The packaged vectors were purified from the cell culture

supernatant using affinity chromatography and tittered using Digital Droplet PCR (BioRad,) method.

(c) Murine models and in vivo study design

[00343] We tested the therapeutic potential of AAV8-TBG-hGALNS and AAV8-TBG-D8-hGALNS by using two MPS IVA murine models (Tomatsu *et al.*, Hum Mol Genet 2003; 12(24):3349-3358; Tomatsu *et al.*, Hum. Mol. Genet. 2005; 14, 3321-3335). The first type is a *Galns* knock-out mouse model (KO: *Galns*-/-) with disrupt of the gene ((Tomatsu *et al.*, Hum Mol Genet 2003; 12(24):3349-3358). The second one is a murine model (MTOL: Galns $^{tm(hC79S.mC76S)slu}$) tolerant to human GALNS containing both a transgene expressing hGALNS in β-globin/Ig intron and an active site mutation (C76S) adjacent to exon 2, thereby introducing both the inactive hGALNS coding sequence with C79S active site mutation and the C76S mutation into the murine *Galns* gene by targeted mutagenesis (Tomatsu *et al.*, Hum. Mol. Genet. 2005; 14, 3321-3335). Both models had no detectable enzyme activity in blood and tissues and showed the accumulation of storage materials primarily within reticuloendothelial Kupffer cells, heart valves cardiac muscle, and chondrocytes including growth plate and articular cartilage.

[00344] We had previously described the development of two MPS IVA murine models, MPS IVA knockout mouse (*Galns*-/-) (Tomatsu *et al.*, Hum Mol Genet 2003; 12(24):3349-3358) and MPS IVA mouse tolerant to human GALNS protein (Galns$^{tm(hC79S.mC76S)slu}$) (Tomatsu *et al.*, Hum. Mol. Genet. 2005; 14, 3321-3335) in C57BL/6 background. The GALNS knock-out mouse model (KO: *Galns*-/-) was developed by targeted disruption of the GALNS gene (Tomatsu *et al.*, Hum Mol Genet 2003; 12(24):3349-3358). The mouse model tolerant to human GALNS (MTOL: Galns $^{tm(hC79S.mC76S)slu}$) contain a transgene expressing hGALNS in β-globin/Ig intron and an active site mutation (C76S) adjacent to exon 2, thereby introducing both the inactive hGALNS coding sequence with C79S active site mutation (Tomatsu *et al.*, Hum. Mol. Genet. 2005; 14, 3321-3335). Both mouse models had no detectable enzyme activity in blood and tissues and showed the accumulation of storage materials primarily within reticuloendothelial Kupffer cell, heart valves and muscle, and chondrocytes including growth plate and articular cartilage.

[00345] Genotyping for the experimental cohorts were done by PCR on day 14. Homozygous MPS IVA mice at 4 weeks of age were treated with either AAV8 vector, intravenously at a uniform dose of 5 x 10^{13} GC/kg. Another cohort of MPS IVA mice as well as

unaffected C57BL/6 littermates were administered with phosphate-buffered saline (PBS). The total dose volume administration was approximately 100 µl per mouse. All animal cares and experiments were approved by the Institutional Animal Care and Use Committee of Nemours/Alfred I. duPont Hospital for Children.

(d) Blood and tissue collection

[00346] Approximately 100 µl of blood was collected in tubes with EDTA (BD, Franklin Lakes, NJ, USA) every other week from all animals in the study. The blood was centrifuged at 8,000 rpm for 10 min and plasma separated was kept at -20°C until performing GALNS enzyme assay and GAG assay. At 16 weeks of age, mice were euthanized in a CO_2 chamber and perfused with 20 ml of 0.9% saline. Liver, kidney, lung, spleen, heart, and knee joint were collected and stored at -80°C until processing for GALNS enzyme assay and GAG assay. Additionally, various tissue samples were collected and stored in 10% neutral buffered formalin for histopathology analysis.

(e) GALNS activity assay

[00347] Blood and tissue GALNS activity was determined as described previously (Toietta, G., et al. Hum. Gene Ther. 2001; *12*, 2007-2016). Frozen tissue was homogenized with homogenization buffer consisting of 25 mmol/l Tris–HCl, pH 7.2, and 1 mmol/l phenylmethylsulfonyl fluoride by using a homogenizer. Tissue lysate or plasma, and 22 mM 4-methylumbelliferyl-β-galactopyranoside-6-sulfate (Research Products International, Mount Prospect, IL, USA) in 0.1 M NaCl, 0.1 M sodium acetate, pH 4.3 were incubated at 37 °C for 16 h. Then, 10 mg/ml β-galactosidase from Aspergillus oryzae (Sigma-Aldrich, St. Louis, MO, USA) in 0.1 M NaCl, 0.1 M sodium acetate, pH 4.3 was added to reaction sample, and additional incubation was at 37 °C for 2 hours. The sample was transferred to stop solution (1 M glycine, NaOH, pH 10.5), and the plate was read at excitation 366 nm and emission 450 nm on a Perkin Elmer Victor X4 plate reader (PerkinElmer, Waltham, MA, USA). Activity was expressed as nanomoles of 4-methylumbelliferone released per hour per microliter of plasma or milligram of protein. Protein concentration was determined by BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

(f) Extraction of GAG from tissue

[00348] GAG extraction from various mouse tissues was modified from that developed by Mochizuki et al. (Mochizuki, H., et al. J. Biol. Chem. 2008; *283*, 31237-31245). Briefly, excised tissues were frozen in liquid nitrogen and homogenized with acetone using a homogenizer. The obtained powder was dried under centrifuge vacuum. The defatted tissue powder was suspended in 0.5 M NaOH and incubated at 50 °C for 2 h to remove GAG chains from its core protein. After neutralization with 1 M HCl, NaCl was added to a final concentration of 3 M. Insoluble materials were removed by centrifugation, and the pH of the supernatant was adjusted below 1.0 with 1 M HCl. Precipitated nucleotides were removed by centrifugation, and the supernatant was neutralized with 1 M NaOH. The crude GAG was precipitated by the addition of two volumes of ethanol containing 1.3% potassium acetate. After centrifugation, the precipitate was dissolved in distilled water.

(g) GAG assay

[00349] Blood and tissue GAG level were measured by LC-MS/MS as described previously (Oguma, T., et al. Biomed. Chromatogr. 2007; *21*, 356-362; Oguma, T., et al. Anal. Biochem. 2007; *368*, 79-86; Shimada, T., et al. JIMD. Rep. 2014; *16*, 15-24; Shimada, T., et al. JIMD. Rep. 2015; *21*, 1-13; Kubaski, F., et al. J. Inherit. Metab. Dis. 2017; *40*, 151-158). Briefly, 50 mM Tris-HCl (pH 7.0) and sample were into a 96 well omega 10K filter plate (Pall Corporation, Port Washington, NY, USA) on a 96 well receiver plate. Samples centrifuged for 15 min at 2,500 g. The filter plate was transferred to a new receiver plate, and a cocktail mixture of 50 mM Tris-HCl (pH 7.0), 5 μg/mL chondrosine as internal standard (IS), 1 mU heparitinase, and 1 mU keratanase II was added to the filter plate. Samples were incubated at 37 °C water bath overnight. Then, the samples were centrifuged for 15 min at 2,500 g. The apparatus consisted of a 1290 Infinity LC system with a 6460 triple quad mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Disaccharides were separated on a Hypercarb column (2.0 mm i.d. 50 mm length; 5 μm particles; Thermo Fisher Scientific, Waltham, MA, USA), thermostated at 60 °C. The mobile phase was a gradient elution of 5 mM ammonium acetate, pH 11.0 (solution A) to 100% acetonitrile (solution B). The flow rate was 0.7 ml/min, and the gradient was as follows: 0 min 100% solution A, 1 min 70% solution A, 2 min 70% solution A, 2.20 min 0% solution A, 2.60 min 0% solution A, 2.61 min 100% solution A, 5 min 100% solution A. The mass spectrometer was operated with electrospray ionization in the negative ion mode (Agilent Jet

Stream technology). Specific precursor and product ions, m/z, were used to quantify each disaccharide respectively (IS, 354.3→193.1; mono-sulfated KS, 462→97; HS-0S 378.3→175.1). The injection volume was 10 µl with a running time of 5 min per sample.

(h) Toluidine blue staining and pathological assessment

[00350] Toluidine blue staining was performed as described previously (Tomatsu, S., et al. Mol. Genet. 2005, 14, 3321-3335). Briefly, knee joint and mitral heart valve were collected from MPS IVA and WT mice at 16-week-age to evaluate levels of storage granules by light microscopy. Tissues were fixed in 2% paraformaldehyde, 4% glutaraldehyde in PBS, and post-fixed in osmium tetroxide and embedded in Spurr's resin. Then, toluidine blue-stained 0.5-µm-thick sections were examined. To evaluate chondrocyte cell size (vacuolization) in the growth plate of femur or tibia, approximately 300 chondrocytes in the proliferative area were measured in each mouse by Image J software, and results were expressed as fold-change from wild-type group.

(i) Detection of antibodies against GALNS by enzyme-linked immunosorbent assay (ELISA)

[00351] An indirect ELISA method was used to detect antibodies against GALNS in plasma of treated and untreated mice as described previously (Tomatsu, S., et al. Hum. Mol. Genet. 2003; *12*, 961-973). Briefly, 96 well microtiter plate was coated overnight with 2 µg/ml purified rhGALNS (R&D Systems, Minneapolis, MN, USA) in 15 mM Na_2CO_3, 35 mM $NaHCO_3$, 0.02% NaN_3, pH 9.6. The wells were washed three times with TBS-T (10 mM Tris, pH 7.5, 150 mM NaCl, 0.05% TWEEN 20), and then blocked for 1 h at room temperature with 3% bovine serum albumin in PBS (pH 7.2). After washing three times with TBS-T, a 100-fold dilution of mouse plasma in TBS-T was added to the wells and incubated at 37°C for 2.5 h. The wells were washed four times with TBS-T, then TBS-T containing a 1:1,000 dilution of peroxidase conjugated goat anti-mouse IgG (Thermo Fisher Scientific, Waltham, MA, USA) was added to the wells and incubated at room temperature for 1 h. The wells were washed three times with TBS-T and twice with TBS (10 mM Tris, pH7.5, 150 mM NaCl). Peroxidase substrate (ABTS solution, Invitrogen, Carlsbad, CA, USA) was added (100 µl per well), and plates were incubated at room temperature for 30 min. The reaction was stopped with the addition of 1% SDS, and the

plates read at optical density 410 nm on a Perkin Elmer Victor X4 plate reader (PerkinElmer, Waltham, MA, USA).

(j) Statistical analysis

[00352] All data were expressed as means and standard deviations (SD). Multiple comparison tests were performed by one-way ANOVA with the Bonferroni's post-hoc test using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). The statistical significance of difference was considered as $p < 0.05$.

7.4 Example 4. Evaluate the effect of prolonged enzyme exposure on bone pathology

[00353] The following studies are conducted to evaluate the effect of prolonged enzyme exposure on bone pathology. For this study, AAV8-TBG-hGALNSco is administered into 4-week old MPSIVA KO mice at a dose of 5 x 10^{13} GC/kg body weight. Control groups are untreated MPS IVA KO mice and untreated wild type mice of the same age. Three groups of mice, 6-10 per group, are used in this study. The mice are monitored for either 24 weeks or 48 weeks post injection and blood samples are collected every other week to other week to analyze enzymatic activity and KS levels. Additionally, at necropsy, tissue samples are taken from different organs for enzymatic activity and KS levels as well as knee joints and heart valves for histopathology analysis.

[00354] Similarly, AAV8-TBG-hGALNSco is delivered into 4-week old MTOL mice at a dose of 5 x 10^{13} GC/kg body weight. Control groups include untreated MTOL mice, and untreated wild type mice of the same age. Three groups of mice, 6-10 per group, are used in this study. The mice are monitored for either 24 weeks or 48 weeks post injection and blood samples are collected every other week to other week to analyze enzymatic activity and KS levels. Additionally, at necropsy, tissue samples are taken from different organs for enzymatic activity and KS levels as well as knee joints and heart valves for histopathology analysis.

7.5 Example 5. Neonatal study: evaluate the effect of earlier intervention on bone pathology

[00355] The following studies are conducted on neonatal mice to evaluate the effect of earlier intervention on bone pathology. For this study, AAV8-TBG-hGALNSco is administered into MPSIVA KO neonatal mice at a dose of 5 x 10^{13} GC/kg body weight. Control groups include untreated MPS IVA KO mice, and untreated wild type mice of the same age. The mice are

scarified at 16 weeks of age and blood samples are collected every other week to other week to analyze enzymatic activity and KS levels. Additionally, at necropsy, tissue samples are taken from different organs for enzymatic activity and KS levels as well as knee joints and heart valves for histopathology analysis.

[00356] Similarly, we delivered AAV8-TBG-hGALNSco into neonatal MTOL mice at a dose of 5 x 10^{13} GC/kg body weight. Control groups include untreated MTOL mice, and untreated wild type mice of the same age. Three groups of mice, 6 per group, are used in this study The mice are scarified at 16 weeks of age and blood samples are collected every other week to other week to analyze enzymatic activity and KS levels. Additionally, at necropsy, tissue samples are taken from different organs for enzymatic activity and KS levels as well as knee joints and heart valves for histopathology analysis.

7.6 Example 6. New expression cassette evaluation

[00357] The following studies are conducted to evaluate optimized promoter constructs for improved efficacy. For this study, AAV8-TBG-hGALNSco, AAV8-CAG- hGALNSco, AAV8-Promoter 1-hGALNSco, AAV8-Promoter 2-hGALNSco, AVV9-Promoter 2-hGALNSco are administered into 4-weeks old MPSIVA KO mice at a dose of 1 x 10^{13} GC/kg body weight (10 mice per group). Control groups include untreated MPS IVA KO mice and untreated wild type mice of the same age. The mice are monitored for either 12 weeks or 48 weeks and blood samples are collected every other week to other week to analyze enzymatic activity and KS levels. Additionally, at necropsy, tissue samples are taken from different organs for enzymatic activity and KS levels as well as knee joints and heart valves for histopathology analysis.

7.7 Example 7. Late stage AAV gene therapy study

[00358] The following studies are conducted to evaluate late-stage AAV gene therapy efficacy. For this study, AAV-TBG-hGALNSco, AAV-CAG-hGALNSco, AAV-Promoter 1-hGALNSco, AAV-Promoter 2-hGALNSco, AVV-Promoter 2-hGALNSco are administered into 8-10 weeks old MPSIVA KO mice (5 mice per group). Untreated MPS IVA KO mice are used as control. The mice are monitored for a period of time and blood samples are collected every other week to other week to analyze enzymatic activity and KS levels. Additionally, at necropsy,

tissue samples re taken from different organs for enzymatic activity and KS levels as well as knee joints and heart valves for histopathology analysis.

[00359] Similarly, AAV-TBG-hGALNSco, AAV-CAG-hGALNSco, AAV-Promoter 1-hGALNSco, AAV-Promoter 2-hGALNSco, AVV-Promoter 2-hGALNSco are administered into 8-10 weeks old MTOL mice (5 mice per group). Untreated MTOL mice are used as control. The mice are monitored for a period of time and blood samples are collected every other week to other week to analyze enzymatic activity and KS levels. Additionally, at necropsy, tissue samples are taken from different organs for enzymatic activity and KS levels as well as knee joints and heart valves for histopathology analysis.

7.8 Example 8. Bone-liver Tandem Promoter-Driving Constructs

[0100] An LBTP1 (a bone-liver tandem promoter)-driving construct was constructed comprising the components illustrated in FIGS. 33 and 37. The LBTP1 promoter comprises a minimal Sp7/Osx promoter fragment driving osteoblast-specific expression. The minimal Sp7/Osx promoter was determined to be a transcriptionally active fragment of the Sp7/Osx promoter (Lu, X., et al. JBC 281, 6297-6306, January 12, 2006). The LBTP1 sequence contains one copy of the minimal Sp7 promoter fragment (SEQ ID NO:24) flanked 5' by a liver-specific ApoE enhancer/hepatic control region, and 3' by a hAAT promoter depleted of ATG trinucleotides (hAATΔATG) to drive hepatocyte-specific expression. A chimeric β-globin/Ig intron was placed downstream (3') of the promoter sequence, i.e., downstream of the hAATΔATG.

[0101] An LBTP2 (a bone-liver tandem promoter)-driving construct is constructed comprising the components illustrated in FIGS. 34 and 38. LBTP2 was designed and engineered, as follows: A full-length Sp7/Osx promoter (Lu, X., et al. JBC 281, 6297-6306, January 12, 2006) (SEQ ID NO:23) was flanked 5' by a liver-specific ApoE enhancer/hepatic control region, and 3' by a hAAT promoter depleted of ATG trinucleotides (hAATΔATG) to drive hepatocyte-specific expression. A chimeric β-globin/Ig intron was placed downstream (3') of the promoter sequence, i.e., downstream of the hAATΔATG.

[0102] Not wishing to be bound by theory, the tandem promoters having ATG sites eliminated from the downstream promoter allows for expression of two mRNA transcripts - one driven by each of the promoters depending on host cell transcription machinery- however,

protein translation initiation will occur at the single, intended start codon of the protein coding sequence in the cassette. This strategy should provide more efficient and robust expression compared to tandem promoters that contain superfluous ATG sites upstream of the protein initiation codon.

7.9 Example 9. Treatment of MPS IVA with Bone-liver Tandem Promoter-based Construct

[00360] A subject presenting with MPS IVA is administered AAV8 or AAV9 that comprises a construct as described in Section 7.8 Example 8, which encodes a GALNS transgene (*e.g.*, GALNS, GALNSco or GALNSco/CpG-), at a dose sufficient to produce a therapeutically effective concentration of the transgene product in bone and liver. The administration is done by parenteral, subcutaneous, intramuscular, intravenous, intraperitoneal, intranasal, intrathecal, or transdermal administration. Following treatment, the subject is evaluated for reductions in biomarkers of disease (such as GAG, KS, and C6S storage) and/or increase in hGALNS enzyme activity in bone, cartilage, ligament, meniscus, heart valve, urine, and/or serum. Signs of inflammation and other safety events is also monitored.

7.10 Example 10. Full-length and Minimal Sp7/Osx Promoter-Driving Constructs

[00361] An full length Sp7/Osx promoter (a bone-specific promoter)-driving construct was constructed comprising the components illustrated in FIG. 35.

[00362] A minimal Sp7/Osx promoter (a bone-specific promoter)-driving construct was constructed comprising the components illustrated in FIG. 36.

7.11 Example 11. Treatment of MPS IVA with Full-length or Minimal Sp7/Osx Promoter-based Construct

[00363] A subject presenting with MPS IVA is administered AAV8 or AAV9 that comprises a construct as described in Section 7.10 Example 10, which encodes a GALNS transgene (e.g. GALNS, GALNSco or GALNSco/CpG-), at a dose sufficient to produce a therapeutically effective concentration of the transgene product in bone and liver. The administration is done by parenteral, subcutaneous, intramuscular, intravenous, intraperitoneal, intranasal, intrathecal, or transdermal administration. Following treatment, the subject is evaluated for reductions in biomarkers of disease (such as GAG, KS, and C6S storage) and/or increase in hGALNS enzyme

activity in bone, cartilage, ligament, meniscus, heart valve, urine, and/or serum. Signs of inflammation and other safety events is also monitored.

7.12 Example 12. AAV Vectors Packaged with hGALNS under Different Expression Systems in MPS IVA KO mice

[00364] Objective: the objective of the study is to demonstrate optimized promoter constructs for improved efficacy of AAV Gene therapy for Morquio A syndrome.

[00365] Animals: MPS IVA KO mice including both male and female mice are used in equal numbers in all groups. Mice are about 4 weeks of age at the start of treatment with a body weight at dose administration of ~20g. The GALNS knock-out mouse model (KO: Galns-/-) was developed by targeted disruption of the GALNS gene. These mice had no detectable enzyme activity in blood and tissues and showed the accumulation of storage materials primarily within reticuloendothelial Kupffer cell, heart valves and muscle, and chondrocytes, including growth plate and articular cartilage. Genotyping for the experimental cohorts was done by PCR on day 14. Each test animal is assigned a unique number within the population making up the study. This number appears on the cage card visible on each cage. The cage card contains the study number, group, dose and sex. To facilitate dosing and/or necropsy, the animals are divided into cohorts. The unique test animal number is used for raw data records and specimens.

[00366] Source, Identification, and Storage Conditions: test articles are purified AAV vectors expressing hGALNS under the control of different promoter elements. The test articles are supplied as frozen aliquots. Test articles include: 1) vehicle; 2) vimizim® (elosulfase alfa); 3) AAV8-TBG-hGALNScoV2; 4) AAV8-CAG-hGALNScoV2; 5)AAV8-LSPX1-hGALNScoV2; 6) AAV8-LMTP6-hGALNScoV2; 7) AAV8-LBTP2-hGALNScoV2; 8) AAV9-CAG-hGALNScoV2; 9) AAV9-LMTP6-hGALNScoV2; 10) AAV9-LBTP2-hGALNScoV2; and 11) AAV8-TBG-hGALNSco.

[00367] Dosing Formulation Preparation: Test article formulations are provided as frozen stocks ready to use for dosing for each group on each dosing day. At the time of first thawing of the test article, test articles are aliquotted into small volumes that are used for multiple injections with appropriate labelling and stored frozen at -80°C. The calculations for the preparation of dosing solution is as follows: Total GC/animal= dose (GC/kg body weight) x body weight of the animal; Total volume of stock required= total GC/animal / concentration (GC/mL) of the stock=

x mL (where x is a desired injection volume). The material can be maintained at room temperature for up to four hours while preparing to dose.

[00368] **Table 2.** Experimental design

Group	Test Article	Route of Administration	Number of Animals	Concentration (GC/mL)	Dose Volume	Target Dose (GC/kg)	Target Dose (GC/animals)	Animal Numbers
1	Vehicle	IV	6	TBD		NA	NA	G- to G-
2	Vimizim	IV	6	TBD		2mg/kg weekly	NA	G- to G-
3	AAV8-TBG-V2	IV	6	1.8E13		5E13	TBD	G- to G-
4	AAV8-CAG-V2	IV	6	TBD		5E13	TBD	G- to G-
5	AAV8-LSPX1-V2	IV	6	2.1E13		5E13	TBD	G- to G-
6	AAV8-LMTP6-V2	IV	6	2E13		5E13	TBD	G- to G-
7	AAV8-LBTP2-V2	IV	6	TBD		5E13	TBD	G- to G-
8	AAV9-CAG-V2	IV	6	TBD		5E13	TBD	G- to G-
9	AAV9-LMTP6-V2	IV	6	3.2E13		5E13	TBD	G- to G-
10	AAV8-LBTP2-V2	IV	6	TBD		5E13	TBD	G- to G-
11	AAV8-TBG	IV	6	3.8E13		5E13	TBD	G- to G-

[00369] Body weight and clinical observations are recorded and blood samples are collected for bioanalytical analysis. The animals are euthanized after 12 weeks and selected tissues harvested for molecular, bioanalytical, and histopathological evaluation.

[00370] Study Procedures: animals included in this study are randomly placed in study groups. The vectors are administered as and when the KO animals are available without any bias towards the selection of the vector.

[00371] Dose Administration: all animals receive a single IV dosing of the test article specific to their assigned group. All animals are weighed on the day of dose administration to determine the number of viral particles to administer. Animals in all groups are weighed bi-weekly and observed regularly for morbidity and mortality beginning on the first day of dosing. Clinical signs are recorded regularly throughout the study period. The animals are observed for signs of clinical effects, illness, and/or death.

[00372] Sample Collection: blood samples (~100μL) are collected biweekly from a submandibular vein and plasma samples are prepared. Plasma samples are used for testing for enzyme activity as well as GAG levels to determine the efficacy of AAV Gene Therapy. Samples are maintained at -80 °C until use.

[00373] Necropsy: animals in all groups are euthanized following 12 weeks post administration of the test articles/vehicle. Any animal(s) determined moribund during the study period are sacrificed and samples are collected whenever possible. At necropsy, blood samples are collected from all animals to prepare plasma (from half the volume of an individual sample) and serum (from the other half volume of an individual sample) samples which are stored at -80°C. The samples are used to detect levels of enzyme activity as well as Keratan Sulfate. Selected tissue samples are collected in replicates to assay tissue enzyme activity, KS levels, vector copy number and gene expression. Samples are collected, flash frozen and stored at -80°C until use. Organs such as the heart, liver, skeletal muscle (biceps, gastrocnemius), lung, kidney, spleen, trachea, and kneecap are used for tissue enzyme activity analysis. Organs such as the heart, liver, lung, spleen, trachea, and kneecap are used for tissue KS levels analysis. The heart, liver, skeletal muscle (biceps, gastrocnemius), lung, kidney, spleen, armcap, any available bone/cartilage tissue, gonads, and brain are used for vector biodistribution analysis (gDNA-qPCR). The heart, liver, skeletal muscle (biceps, gastrocnemius), lung, kidney, spleen, armcap, and any available bone/cartilage tissue are used for gene expression analysis (RNA-qRT-PCR). Knee joint-1, tibia, fore-limb joint-2, heart valve and base, liver, brain, and spleen are formalin fixed for histology analysis. Femur are ethanol preserved and used for micro-CT analysis.

[00374] Analysis: Enzyme Activity Assay: frozen tissues are homogenized with homogenization buffer consisting of 25 mmol/l Tris–HCl, pH 7.2, and 1 mmol/l phenylmethylsulfonyl fluoride by using a homogenizer. Tissue lysate or plasma, and 22 mM 4-methylumbelliferyl-β-galactopyranoside-6-sulfate (Research Products International, Mount

Prospect, IL, USA) in 0.1 M NaCl, 0.1 M sodium acetate, pH 4.3 are incubated at 37°C for 16 h. 10 mg/ml β-galactosidase from Aspergillus oryzae (Sigma-Aldrich, St. Louis, MO, USA) in 0.1 M NaCl, 0.1 M sodium acetate, pH 4.3 are then added to reaction samples, and incubated at 37°C for 2 h. The samples are transferred to stop solution (1 M glycine, NaOH, pH 10.5), and the plate is read at excitation 366 nm and emission 450 nm on a Perkin Elmer Victor X4 plate reader (PerkinElmer, Waltham, MA, USA). The activity are expressed as nanomoles of 4-methylumbelliferone released per hour per microliter of plasma or milligram of protein. Protein concentration are determined by BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

[00375] Extraction of GAGs (KS) and Assaying KS levels: briefly, excised tissues are frozen in liquid nitrogen and homogenized with acetone using a homogenizer. The obtained powder are dried under a centrifuge vacuum. The defatted tissue powder are suspended in 0.5 M NaOH and incubated at 50°C for 2 h to remove GAG chains from its core protein. After neutralization with 1 M HCl, NaCl are added to a final concentration of 3 M. Insoluble materials are then removed by centrifugation, and the pH of the supernatant adjusted below 1.0 with 1 M HCl. Precipitated nucleotides are removed by centrifugation, and the supernatant are neutralized with 1 M NaOH. The crude GAG are precipitated by the addition of two volumes of ethanol containing 1.3% potassium acetate. After centrifugation, the precipitate are dissolved in distilled water

[00376] GAG assay: blood and tissue GAG level are measured by LC-MS/MS. Briefly, 50 mM Tris-HCl (pH 7.0) and sample are loaded into a 96-well omega 10K filter plate (Pall Corporation, Port Washington, NY, USA) on a 96-well receiver plate. Samples are centrifuged for 15 min at 2,500 g. The filter plate are then transferred to a new receiver plate, and a cocktail mixture of 50 mM Tris-HCl (pH 7.0), 5 μg/mL chondrosine as internal standard (IS), 1 mU heparitinase, and 1 mU keratanase II are added to the filter plate. Samples are incubated at 37°C water bath overnight and then centrifuged for 15 min at 2,500 g. The apparatus consist of a 1290 Infinity LC system with a 6460 triple quad mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Disaccharides are separated on a Hypercarb column (2.0 mm i.d. 50 mm long; 5 μm particles; Thermo Fisher Scientific, Waltham, MA, USA), thermostated at 60°C. The mobile phase is a gradient elution of 5 mM ammonium acetate, pH 11.0 (solution A) to 100% acetonitrile (solution B). The flow rate is 0.7 ml/min, and the gradient is as follows: 0 min 100% solution A, 1 min 70% solution A, 2 min 70% solution A, 2.20 min 0% solution A, 2.60 min 0%

solution A, 2.61 min 100% solution A, 5 min 100% solution A. The mass spectrometer is operated with electrospray ionization in the negative ion mode (Agilent Jet Stream technology). Specific precursor and product ions, m/z, are used to quantify each disaccharide, respectively (IS, 354.3→193.1; mono-sulfated KS, 462→97; HS-0S 378.3→175.1). The injection volume is 10 µL, with a running time of 5 min per sample.

[00377] Vector Distribution Analysis: Total genomic DNA is purified from the selected tissues by using the Qiagen Puregene kit according to the instruction manual (Germantown, MD). Digital PCR (dPCR) analysis on genomic DNA extracted from frozen samples of mice are performed using specific primers and probe sequences for rabbit b-globulin (rBG) poly A, which are as follows: forward primer, 5′- GCCAAAAATTATGGGGACAT-3′; reverse primer, 5′-ATTCCAACACACTATTGCAATG-3′; and probe, 6FAM-ATGAAGCCCCTTGAGCATCTGACTTCT-QSY. Genomic DNA is tested non-fragmented, as well as fragmented using either enzymatic digestion or an M220 Focused-ultrasonicator (Covaris, Woburn, MA). The rBG TaqMan assay (FAM labeled) (ThermoFisher Scientific, Waltham, MA) is used for quantitative dPCR analysis of the AAV vector. The Tfrc TaqMan® Copy Number Reference Assay (VIC® labeled) from Thermo Fisher Scientific is used for Tfrc dPCR. The instrument used for this analysis is QuantStudio™ 3D Digital PCR 20K Chip Kit v2: A26316(ThermoFisher Scientific). PCR amplification profile is conducted with ABI Geneamp 9700 PCR - Thermal Cycler w/ Dual Flat Blocks (Applied Biosystems, Waltham, MA) as follows: 96 °C for 10 min; 39 cycles of 60 °C for 2 min and 98 °C for 30 sec; 60 °C for 2 min; 10 °C final hold. After PCR amplification, chips are read on the QuantStudio 3D instrument to obtain the number of wells positive for the VIC and FAM channels, the number of wells without DNA and empty wells. Data analysis and chip quality assessment is performed using the QuantStudio 3D Analysis Suite. Using Tfrc results as a reference for 2 copies, the number of copies of AAV per mouse genome is calculated.

[00378] Histopathology Analysis: Knee joint and mitral heart valve collected from MPS IVA and WT mice at 16-week-age is used to evaluate levels of storage granules by light microscopy. Tissues are fixed in 2% paraformaldehyde, 4% glutaraldehyde in PBS, and post-fixed in osmium tetroxide and embedded in Spurr's resin. Toluidine blue-stained 0.5-µm-thick sections are then examined. To evaluate chondrocyte cell size (vacuolization) in the growth plate of femur or tibia,

approximately 300 chondrocytes in the proliferative area is considered in each mouse by Image J software, and results are expressed as fold-change from wild-type group.

[00379] Micro-CT Analysis: femora are pre-dissected prior to micro-CT sample preparation. Femora are then wrapped in salinated (0.9% Saline) gauze and stacked axially in a scanning vial. A maximum of three could be stacked in one vial per scan session. Due to the speed arising from the SkyScan 1275 system, the full bones are scanned and subsequent volumes of interest are cropped from the full scan data.

[00380] Scanning: scan conditions are set for all four femora samples according to the settings in Table 3. During the scan, 2-D projection images are obtained.

Table 3. Scan settings

	Eighteen Mice Femurs	**Description**
Pixel Size:	8 μm	Size of the pixels within each image slice
Camera Setting:	High (1944 x 1536)	Resolution of the camera (# of pixels)
Filter:	1 mm Aluminum	Removes low energy X-rays spectra to reduce the effects of beam hardening
Voltage:	80 kV	Voltage of the X-ray tube
Current:	125 μA	Current of the X-ray tube
Rotation Step:	0.4°	Angle the sample is rotated between each captured image
Frame Averaging:	6	Number of images taken at a given position and averaged to reduce noise artifacts
Scan Time:	01:07:08 (HH:MM:SS)	Total effective time to scan each bone

[00381] Reconstruction: factors associated with image quality are adjusted to prepare the cross-sectional images of each sample for 3-D volume rendering. Table 4 shows reconstruction correction factors.

Table 4. Reconstruction factors

	Value	Description
Image Format:	BMP – 8 bit	Output image file format
Beam Hardening:	20%	Lower energy X-rays are filtered out as they pass through the sample causing a hardening of the beam, leaving only the higher energy X-rays. This causes lightening on the edges of the sample and darkening in the center even though the sample may be of a uniform material. A filter, or a physical sheet of homogeneous metal such as aluminum, with uniform thickness can be used to prevent low energy X-rays from reaching the sample. In addition to the possible use of a physical filter, the Bruker SkyScan NRecon software corrects for beam hardening by using iterative reconstruction algorithms that averages out the attenuation throughout the sample.
Dynamic Range:	0.0 – 0.05	Conversion of float point values to 8-bit grayscale values

[00382] Quantitative Analysis: morphometric analysis was performed in Bruker CTAn software (v1.18.8.0). Volumes of interest (VOI) were kept identical for both cortical and trabecular bone, barring differences between the physical differences between the bones. Standard output variables reported fall in conjunction with the guidelines of the Journal of Bone & Mineral Research (Bouxsein et al. 2010).

[00383] Trabecular Bone VOI: the VOI of trabecular bone first identified using the distal epiphyseal plate. From this common mark, the starting point is made 250 μm proximal and then extends 2.5 mm. The region of interest per slice is defined using an automated protocol so as to

include a uniform distance away from the endosteal cortical wall (usually around 1mm). All efforts are made to avoid including growth plate in the ROI.

[00384] Cortical Bone VOI: the cortical bone volume of interest is determined based on distal epiphyseal plate start mark and the highest point in the proximal greater trochanter. Between these two anatomical landmarks, the total length of the bone is obtained. The total length of the bone is multiplied by 55% to yield a starting position slightly more than halfway down from the proximal trochanter. A total of 0.5mm below this starting point is captured as the VOI. The region of interest encompasses the entire cortical bone and post processing is performed to remove any trabeculae that may be present (FIG. 39).

Table 5. Analysis settings

	Value	**Description**
Threshold (Trabecular Bone)	85/255	Determines the gray scale value that identifies all trabecular bone within manually drawn ROI. There are 256 greyscale values that range from 0-255 where 0 is all white and 255 is all black. For this analysis, trabecular bone was identified by white with an average threshold of 85. A visual comparison check to the gray scale image was also performed to ensure threshold accuracy. This threshold was determined to be an average of a subset of samples that were assessed in the entire study
Threshold (Cortical Bone)	98/255	Similar to above.

8. TABLE OF SEQUENCES

SEQ ID NO.	DESCRIPTION	SEQUENCE
1	AAV8 capsid protein	MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKAN QQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADA AALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQ EDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPG KKRPVEPSPQRSPDSSTGIGKKGQQPARKRLNFGQTG DSESVPDPQPLGEPPAAPSGVGPNTMAAGGGAPMAD NNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWAL PTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFD FNRFHCHFSPRDWQRLINNNWGFRPKRLSFKLFNIQV KEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSA HQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYC LEYFPSQMLRTGNNFQFTYTFEDVPFHSSYAHSQSLD RLMNPLIDQYLYYLSRTQTTGGTANTQTLGFSQGGPN TMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWT AGTKYHLNGRNSLANPGIAMATHKDDEERFFPSNGIL IFGKQNAARDNADYSDVMLTSEEEIKTTNPVATEEYG IVADNLQQQNTAPQIGTVNSQGALPGMVWQNRDVY LQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKN TPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQK ENSKRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEPRP IGTRYLTRNL
2	Human GALNS (hGALNS)	atggcggcggttgtcgcggcgacgaggtggtggcagctgttgctggtgctcagcgc cgcggggatgggggcctcgggcgccccgcagccccccaacatcctgctcctgctc atggacgacatgggatggggtgacctcggggtgtatggagagccctccagagaga ccccgaatttggaccggatggctgcagaagggctgcttttcccaaacttctattctgcc aaccctctgtgctcgccatcgagggcggcactgctcacaggacggctacccatccg caatggcttctacaccaccaacgcccatgccagaaacgcctacacaccgcaggaga ttgtgggcggcatcccagactcggagcagctcctgccggagcttctgaagaaggcc ggctacgtcagcaagattgtcggcaagtggcatctgggtcacaggccccagttccac cccctgaagcacggatttgatgagtggtttggatccccaactgccactttggacctta tgacaacaaggccaggcccaacatccctgtgtacagggactgggagatggttggca gatattatgaagaatttcctattaatctgaagacgggggaagccaacctcacccagat ctacctgcaggaagccctggacttcattaagagacaggcacggcaccacccctttttc ctctactgggctgtcgacgccacgcacgcacccgtctatgcctccaaaccttcttgg gcaccagtcagcgagggcggtatggagacgccgtccgggagattgatgacagcat tgggaagatactggagctcctccaagacctgcacgtcgcggacaacaccttcgtctt cttcacgtcggacaacggcgctgccctcatttccgcccccgaacaaggtggcagca acggccccttctgtgtgggaagcagaccacgtttgaaggagggatgagggagcct

SEQ ID NO.	DESCRIPTION	SEQUENCE
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SEQ ID NO.	DESCRIPTION	SEQUENCE
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20	ApoE Enhancer	gctgtttgtgtgctgcctctgaagtccacactgaacaaacttcagcctactcatgtccctaaaat gggcaaacattgcaagcagcaaacagcaaacacacagccctccctgcctgctgaccttgga gctggggcagaggtcagagacctctctg
21	hAAT Promoter (underlined: modified from atg to gtg)	gatcttgctaccagtggaacagccactaaggattctgcagtgagagcagagggccagctaa gtggtactctcccagagactgtctgactcacgccacccctccaccttggacacaggacgct gtggtttctgagccaggtacaatgactcctttcggtaagtgcagtggaagctgtacactgccca ggcaaagcgtccgggcagcgtaggcgggcgactcagatcccagccagtggacttagcccc tgtttgctcctccgataactggggtgaccttggttaatattcaccagcagcctcccccgttgccc ctctggatccactgcttaaatacggacgaggacagggccctgtctcctcagcttcaggcacca ccactgacctgggacagt
22	hAAT(ΔATG) Promoter (underlined: modified from atg to gtg)	gatcttgctaccagtggaacagccactaaggattctgcagtgagagcagagggccagctaa gtggtactctcccagagactgtctgactcacgccacccctccaccttggacacaggacgct gtggtttctgagccaggtacagtgactcctttcggtaagtgcagtggaagctgtacactgccca ggcaaagcgtccgggcagcgtaggcgggcgactcagatcccagccagtggacttagcccc tgtttgctcctccgataactggggtgaccttggttaatattcaccagcagcctcccccgttgccc ctctggatccactgcttaaatacggacgaggacagggccctgtctcctcagcttcaggcacca ccactgacctgggacagt
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25	β-globin polyadenylation signal	aataaaggaaatttattttcattgcaatagtgtgttggaatttttgtgtctctca
26	AAV9 capsid protein	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQD NARGLVLPGYKYLGPGNGLDKGEPVNAADAAALEHDKAYD QQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ AKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIG KSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGS LTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVI TTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTP WGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNI QVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAH EGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYF PSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLI DQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIP GPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNP GPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMI TNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG ILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGM KHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVS VEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGV YSEPRPIGTRYLTRNL

SEQ ID NO.	DESCRIPTION	SEQUENCE
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9. EQUIVALENTS AND INCORPORATIONS BY REFERENCE

[00385] Although the invention is described in detail with reference to specific embodiments thereof, it will be understood that variations which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00386] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference in their entireties.