

WHAT IS CLAIMED IS:

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 bone-liver tandem promoter operably linked to a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS or 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.
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 Sp7/Osx promoter or a minimal Sp7/Osx promoter operably linked to a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS or a fusion protein that is hGALNS fused to an acidic oligopeptide.
3. The rAAV of claim 1 or 2, wherein the acidic oligopeptide is D8
4. The rAAV of claim 1, wherein the bone-specific promoter is a Sp7/Osx promoter or a minimal Sp7/Osx promoter and/or the liver-specific promoter is an hAAT (Δ ATG).
5. The rAAV of claim 2 or 4, wherein:
 - i) 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; or
 - ii) 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.
6. The rAAV of claim 4, wherein the hAAT (Δ ATG) 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.
7. The rAAV of claim 2 or 4, wherein the Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 23.
8. The rAAV of claim 2 or 4, wherein the minimal Sp7/Osx promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 24.
9. The rAAV of claim 4, wherein the hAAT (Δ ATG) promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 22.
10. The rAAV of any one of claims 1 and 3-9, wherein the bone-liver tandem promoter further comprises a nucleotide sequence encoding an ApoE enhancer or a nucleotide sequence encoding a hepatic control region comprising an ApoE enhancer, optionally wherein:
- i) 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; and/or
 - ii) 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.
11. The rAAV of claim 10, wherein the ApoE enhancer comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 20.
12. The rAAV of claim 10, wherein the hepatic control region comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 19.
13. The rAAV of any one of claims any one of claims 1 and 3-12, wherein the bone-liver tandem promoter is an LBTP1 promoter or an LBTP2 promoter, optionally wherein:
- i) 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; or
 - ii) 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.
14. The rAAV of claim 13, wherein the LBTP1 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 17.
15. The rAAV of claim 13, wherein the LBTP2 promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 18.
16. 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 promoter operably linked to a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS, 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.
- 17. The rAAV of any one of claims 1-16, wherein the hGALNS expression cassette further comprises a nucleotide sequence encoding an intron.
- 18. The rAAV of claim 17, wherein the intron is a chimeric intron.
- 19. The rAAV of claim 18, wherein the chimeric intron is a β -globin/Ig intron, optionally 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.
- 20. The rAAV of any one of claims 1-19, wherein the nucleotide sequence encoding the transgene is codon-optimized and/or has CpG sites depleted.
- 21. The rAAV of any one of claims 1-20, 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.

22. The rAAV of any one of claims 1-21, wherein the nucleotide sequence encoding the transgene comprises a polyadenylation signal.
23. The rAAV of claim 22, wherein the polyadenylation signal is a β -globin polyadenylation signal or a rabbit globin poly A site, optionally wherein:
- i) 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; or
 - ii) 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.
24. The rAAV of any one of claims 1-23, wherein the rAAV is a recombinant AAV8 or recombinant AAV9.
25. A pharmaceutical composition comprising the rAAV of any one of claims 1-24 and a pharmaceutically acceptable carrier.
26. A polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a bone-liver tandem promoter operably linked to a nucleotide sequence encoding a transgene, wherein the transgene encodes hGALNS or 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.
27. A polynucleotide comprising an hGALNS expression cassette flanked by AAV-ITRs, the hGALNS expression cassette comprising a Sp7/Osx promoter or a minimal Sp7/Osx promoter operably linked to a nucleotide sequence encoding a transgene, wherein the

transgene encodes hGALNS or a fusion protein that is hGALNS fused to an acidic oligopeptide.

28. An rAAV plasmid comprising the polynucleotide of claim 26 or 27.
29. An *ex vivo* cell comprising the polynucleotide of claim 26 or 27 or the rAAV plasmid of claim 28.
30. A method of making an rAAV comprising transfecting an *ex vivo* cell with the rAAV plasmid of claim 28 and one or more helper plasmids collectively comprising the nucleotide sequences of AAV genes Rep, Cap, VA, E2a and E4.
31. Use of the rAAV of any one of claims 1-24 or the pharmaceutical composition of claim 25 in the manufacture of medicament for treating a human subject diagnosed with mucopolysaccharidosis type IVA (MPS IVA), wherein the medicament is suitable for administration to the human subject.
32. The use of claim 31, wherein the hGALNS or the fusion protein is glycosylated with mannose-6-phosphate by having been produced in and secreted from a liver cell.
33. The use of claim 31, wherein the administration allows for delivery of a therapeutically effective amount of the hGALNS or the fusion protein that is hGALNS fused to the acidic oligopeptide to the bone and/or liver of the human subject.
34. The rAAV of any one of claims 16-24, wherein the promoter is TBG, CAG, LSPX1, LMTP6, or LBTP2.
35. The rAAV of any one of claims 16-24, wherein 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;
 - (f) a nucleotide sequence that is 100% identical to SEQ ID NO: 6;
 - (g) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 13;
 - (h) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 13;
 - (i) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 13;
 - (j) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 13;
 - (k) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 13;

- (l) a nucleotide sequence that is 100% identical to SEQ ID NO: 13;
 - (m) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 16;
 - (n) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 16;
 - (o) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 16;
 - (p) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 16;
 - (q) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 16;
 - (r) a nucleotide sequence that is 100% identical to SEQ ID NO: 16;
 - (s) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 18;
 - (t) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 18;
 - (u) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 18;
 - (v) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 18;
 - (w) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 18;
 - (x) a nucleotide sequence that is 100% identical to SEQ ID NO: 18;
 - (y) a nucleotide sequence that is at least 80% identical to SEQ ID NO: 28;
 - (z) a nucleotide sequence that is at least 85% identical to SEQ ID NO: 28;
 - (aa) a nucleotide sequence that is at least 90% identical to SEQ ID NO: 28;
 - (bb) a nucleotide sequence that is at least 95% identical to SEQ ID NO: 28;
 - (cc) a nucleotide sequence that is at least 98% identical to SEQ ID NO: 28; or
 - (dd) a nucleotide sequence that is 100% identical to SEQ ID NO: 28.
35. The rAAV of any one of claims 16-24, wherein the promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 6.
36. The rAAV of any one of claims 16-24, wherein the promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 13.
37. The rAAV of any one of claims 16-24, wherein the promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 16.
38. The rAAV of any one of claims 16-24, wherein the promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 18.
39. The rAAV of any one of claims 16-24, wherein the promoter comprises a nucleotide sequence that is 100% identical to SEQ ID NO: 28.
40. The rAAV of any one of claims 1-24 and 34-39, wherein the AAV-ITRs is AAV2-ITRs.