

CLAIMS:

1. A system for use in treating a genetic disorder, the system comprising:
 - (a) a gene editing vector comprising a nucleic acid sequence encoding a nuclease that targets the PCSK9 gene; and
 - (b) a donor vector comprising a transgene cassette comprising a nucleic acid sequence encoding a transgene and regulatory sequences that direct expression of the transgene in the target cell, the donor vector further comprising homology-directed recombination (HDR) arms 5' and 3' to the transgene cassette, wherein the transgene is not PCSK9.
2. The system according to claim 1, further comprising regulatory sequences that direct expression of the nuclease in a target cell comprising a PCSK9 gene
3. The system according to claim 1 or claim 2, wherein the nuclease targets PCSK9 exon 7.
4. The system according to any one of claims 1 to 3, wherein the nuclease is a meganuclease specific for PCSK9.
5. The system according to claim 4, wherein the meganuclease is the ARCUS meganuclease.
6. The system according to claim 1 or 2, wherein the gene editing vector comprises a sequence that encodes a Cas9 flanked by nuclear localization signals.
7. The system according to claim 6, wherein the gene editing vector further comprises an sgRNA comprising at least 20 nucleotides, which specifically binds to a target site in the PCSK9 gene, said target site being 5' to a protospacer-adjacent motif (PAM) that is specifically recognized by the Cas9.

8. The system according to claim 6, wherein the donor vector further comprises an sgRNA comprising an at least 20 nucleotide seed region, wherein the sgRNA specifically binds to a target site in the PCSK9 gene, said target site being 5' to a protospacer-adjacent motif (PAM) that is specifically recognized by the Cas9.
9. The system according to any one of claims 6 to 8, further comprising an RNA polymerase promoter.
10. The system according to claim 9, wherein the RNA polymerase promoter is the U6 promoter.
11. The system according to claim 10, wherein the U6 promoter is located 5' of the sgRNA.
12. The system according to any one of claims 7 to 11, wherein the seed region is 100% complementary to the target site sequence.
13. The system according to any one of claims 7 to 11, wherein the seed region is less than 100% complementary to the target site sequence.
14. The system according to any one of claims 1 to 13, wherein the transgene is OTC, PKU, CTLN1, or LDLR.
15. The system according to any one of claims 1 to 14, wherein at least one of the donor vector and gene editing vector is an adeno-associated viral (AAV) vector, and the AAV vector comprises AAV 5' ITRs and AAV 3' ITRs.
16. The system according to claim 15, wherein the ratio of gene editing AAV vector of (a) to donor AAV vector of (b) is such that donor AAV vector of (b) is in excess of gene editing vector of (a).

17. A system for use in treating a genetic disorder, the system comprising:
- (a) a gene editing AAV comprising an AAV capsid and a first vector genome comprising a 5' ITR, a sequence encoding a meganuclease that targets PCSK9 under control of regulatory sequences that direct expression of the meganuclease in a target cell comprising a PCSK9 gene, and a 3' ITR; and
 - (b) a donor AAV vector comprising an AAV capsid and a second vector genome comprising: a 5' ITR, a 5' homology directed recombination (HDR) arm, a transgene and regulatory sequences that direct expression of the transgene in the target cell, a 3' HDR arm, and a 3' ITR.
18. A system for use in treating a genetic disorder, the system comprising:
- (a) a gene editing AAV comprising an AAV capsid and a first vector genome comprising a 5' ITR, a 5' nuclear localization signal (NLS), a sequence encoding a Cas9 and regulatory sequences that direct expression of the saCas9 in a target cell comprising the PCSK9 gene, a 3' NLS, and a 3' ITR; and
 - (b) a donor AAV vector comprising an AAV capsid and a second vector genome comprising: a 5' ITR, a 5' homology directed recombination (HDR) arm, a transgene and regulatory sequences that direct expression of the transgene in the target cell, a 3' HDR arm, a U6 promoter, a sgRNA comprising at least 20 nucleotides that specifically bind to a target site in the PCSK9 gene, said target site being 5' to a protospacer-adjacent motif (PAM) that is specifically recognized by the Cas9, and a 3' ITR.
19. A system for use in treating a genetic disorder, the system comprising:
- (a) a gene editing AAV vector comprising an AAV capsid and a first vector genome comprising a 5' ITR, a U6 promoter, a sgRNA comprising at least 20 nucleotides that specifically bind to a target site in the PCSK9 gene, said target site being 5' to a protospacer-adjacent motif (PAM) that is specifically recognized by the Cas9, a 5' nuclear localization signal (NLS), a sequence encoding a Cas9 and regulatory sequences that direct expression of the Cas9 in a target cell comprising the PCSK9 gene, a 3' NLS, and a 3' ITR; and
 - (b) a donor AAV vector comprising an AAV capsid and a second vector genome comprising: a 5' ITR, a 5' homology directed recombination (HDR) arm, a transgene and

regulatory sequences that direct expression of the transgene in the target cell, a 3' HDR arm, and a 3' ITR.

20. A system for use in treating a genetic disorder, the system comprising:
- (a) a gene editing vector comprising:
 - (i) a lipid nanoparticle;
 - (ii) a sgRNA comprising at least 20 nucleotides that specifically bind to a target site in the PCSK9 gene, said target site being 5' to a protospacer-adjacent motif (PAM) that is specifically recognized by a Cas9;
 - (iii) an mRNA comprising a 5' nuclear localization signal (NLS), a sequence encoding the Cas9, a 3' NLS; and
 - (b) a donor AAV vector comprising an AAV capsid and a second vector genome comprising: a 5' ITR, a 5' homology directed recombination (HDR) arm, a transgene and regulatory sequences that direct expression of the transgene in the target cell, a 3' HDR arm, and a 3' ITR.
21. The system according to any one of claims 17 to 19, wherein the gene editing AAV vector of (a) and the donor AAV vector of (b) have the same AAV capsid.
22. The system according to claim 21, wherein the AAV capsid is selected from AAV8, AAV9, rh10, AAV6.2, AAV3B, hu37, rh79, and rh64.
23. The system according to any one of claims 6 to 18, or 18 to 22, wherein Cas9 is selected from *Staphylococcus aureus* or *Streptococcus pyogenes* Cas9.
24. The system according to any one of claims 2 to 19, wherein the nuclease is under the control of a tissue-specific promoter.
25. The system according to any one of claims 2 to 19, wherein the nuclease is under the control of a constitutive promoter.

26. The system according to claim 24, wherein the nuclease is under the control of a liver-specific promoter, optionally a human thyroxin-binding globulin (TBG) promoter, or hybrid liver promoter (HLP).

27. Use of the system according to any one of claims 1 to 26, in the preparation of a medicament for treating a disorder in humans wherein the system is to be co-administered.

28. Use of:

(a) a gene editing AAV vector comprising a sequence encoding a nuclease and regulatory sequences that direct expression of the nuclease in a target cell comprising a PCSK9 gene; and

(b) a donor AAV vector comprising a transgene and regulatory sequences that direct expression of the transgene in the target cell, the donor vector further comprising homology-directed recombination (HDR) arms 5' and 3' to the transgene cassette; in the preparation of a medicament for the treatment of a liver metabolic disorder in a neonate subject, wherein the gene editing AAV vector and the donor AAV vector are to be co-administered.

29. The use according to claim 28, wherein the gene editing AAV vector of (a) and the donor vector of (b) are delivered essentially simultaneously via the same route.

30. The use according to claim 28 or claim 29, wherein the gene editing AAV vector of (a) is suspended in a vehicle for injection at a concentration of about 2×10^{11} GC/mL to about 2×10^{12} GC/mL.

31. The use according to claim 28 or claim 29, wherein the AAV targeting vector of (a) is suspended in a vehicle for injection at a concentration of about 2×10^{12} GC/mL to about 1×10^{13} GC/mL.

32. The u according to any one of claims 28 to 31, wherein the liver metabolic disorder is ornithine transcarbamylase.

33. The use according to any one of claims 28 to 31, wherein the liver metabolic disorder is OTC, FH, citrullinemia type I (CTLN1) or phenylketonuria.
34. A system for use in treating genetic disorders, the system comprising:
(a) a lipid nanoparticle (LNP) comprising a mRNA sequence encoding a nuclease;
and
(b) a donor AAV vector comprising a transgene and regulatory sequences which direct its expression in the target cell, the donor vector further comprising a homology-directed recombination (HDR) arms 5' and 3' to the transgene.
35. The system according to claim 34, wherein the nuclease targets the PCSK9 gene.
36. The system according to claim 34, wherein the nuclease targets PCSK9 exon 7.
37. The system according to claim 34, wherein the nuclease is a meganuclease specific for PCSK9.
38. The system according to claim 37, wherein the meganuclease is the ARCUS meganuclease.
39. The system according to claim 34, wherein the nuclease is a Cas9 nuclease and wherein said LNP comprises an sgRNA.
40. The system according to claim 39, wherein the Cas9 nuclease is flanked by nuclear localization signals.
41. The system according to claim 39 or 40, wherein the sgRNA comprises at least 20 nucleotides which specifically bind to a target site in the PCSK9 gene, said target site being 5' to a protospacer-adjacent motif (PAM) that is specifically recognized by the Cas9.

42. The system according to any one of claims 34 to 41, further comprising an RNA polymerase promoter.
43. The system according to claim 43, wherein the RNA polymerase promoter is the U6 promoter.
44. The system according to claim 46, wherein the U6 promoter is located 5' of the sgRNA.
45. The system according to any one of claims 34 to 44, wherein the sgRNA is 100% complementary to the target site sequence.
46. The system according to any one of claims 34 to 44, wherein the sgRNA is less than 100% complementary to the target site sequence.
47. The system according to any one of claims 34 to 46, wherein the transgene is a liver-expressed gene.
48. The system according to any one of claims 34 to 47, wherein the transgene is selected from OTC, PKU, CTLN1 and FH.
49. A system for use in treating a genetic disorder, the system comprising:
(a) a gene editing vector comprising a nucleic acid sequence encoding a nuclease;
and
(b) a donor vector comprising a nucleic acid sequence encoding an exogenous product for expression from the PCSK9 locus, wherein the inserted nucleic acid sequence does not encode PCSK9,
wherein the system further comprises sequences that direct the nuclease to specifically targets the native PCSK9 gene locus; and
wherein the native PCSK9 in the target cell is optionally ablated or reduced post-dosing with the dual vector system.

50. Use of the system of claim 49 in the preparation of a medicament for treating a patient, wherein the patient's native PCSK9 expression levels are reduced and wherein the patient expresses the exogenous product.
51. An expression cassette comprising the engineered coding sequence of SEQ ID NO: 17, or a sequence sharing at least 90% identity therewith.
52. The expression cassette according to claim 51, further comprising AAV 5' and 3' ITRs.
53. An AAV vector comprising the expression cassette according to claim 51 or 52.