where the gene cassette remains almost exclusively episomal and it is the episomal copies of the therapeutic gene that give rise to the therapeutic protein. AAV does not have a mechanism to integrate its encapsulated DNA into the genome of the host cells but instead is maintained as an episome that is therefore not replicated when the host cell divides. Episomal DNA can also be subject to degradation over time. It has been demonstrated that when liver cells containing AAV episomes are induced to divide, the AAV genome is not replicated but is instead diluted. As a result, AAV based gene therapy is not expected to be effective when given to children whose livers have not yet achieved adult size. In addition, it is currently unknown how long a AAV based gene therapy will persist when given to adult humans, although animal data have demonstrated only small losses in therapeutic effect over periods as long as 10 years. Therefore, there is a critical need for developing new effective and permeant treatments for HemA.

SUMMARY

[0005a] In one aspect, the present disclosure refers to a system comprising: a deoxyribonucleic acid (DNA) endonuclease or nucleic acid encoding said DNA endonuclease; a guide RNA (gRNA) comprising a spacer comprising the nucleotide sequence of SEQ ID NO: 22; and a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein.

[0005b] In another aspect, the present disclosure refers to an *ex vivo* method of editing a genome in a cell, the method comprising: providing the following to the cell: (a) a gRNA comprising a spacer sequence of SEQ ID NO: 22; (b) a DNA endonuclease or nucleic acid encoding said DNA endonuclease; and
(c) a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein.

[0005c] In another aspect, the present disclosure refers to the use of the following: (a) a gRNA comprising a spacer sequence of SEQ ID NO: 22; (b) a DNA endonuclease or nucleic acid encoding said DNA endonuclease; and (c) a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein in the manufacture of a medicament for combination therapy to treat Hemophilia A in a subject.

[0005d] In another aspect, the present disclosure refers to the use of the genetically modified cell as described herein in the manufacture of a medicament for treating Hemophilia A, wherein said medicament is to be administered to the subject.

[0152] In some examples, the insertion is in the first intron of the albumin gene in the genome of the cell.

[0153] In some examples, the insertion is at least 37 bp downstream of the end of the first exon of the human albumin gene in the genome and at least 330 bp upstream of the start of the second exon of the human albumin gene in the genome.

[0154] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed under the control of the endogenous albumin promoter.

[0155] In some examples, the cell is a hepatocyte.

[0156] In some examples, the nucleic acid sequence encoding a Factor VIII (FVIII) protein or functional derivative is expressed in the liver of the subject.

[0157] In another aspect, provided herein is a kit comprising the elements of a system described above, and further comprising instructions for use.

WHAT IS CLAIMED IS:

1. A system comprising:

a deoxyribonucleic acid (DNA) endonuclease or nucleic acid encoding said DNA endonuclease;

a guide RNA (gRNA) comprising a spacer comprising the nucleotide sequence of SEQ ID NO: 22; and

a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein.

2. The system of claim 1, wherein said DNA endonuclease is Cas9.

3. The system of claim 1 or claim 2, wherein the nucleic acid encoding said DNA endonuclease is codon optimized for expression in a host cell.

4. The system of any one of claims 1-3, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein is codon optimized for expression in a host cell.

5. The system of any one of claims 1-4, wherein the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

6. The system of any one of claims 1-4, wherein the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA).

7. The system of claim 6, wherein the RNA encoding said DNA endonuclease is an mRNA.

8. The system of any one of claims 1-7, wherein the donor template is encoded in an Adeno Associated Virus (AAV) vector.

9. The system of claim 8, wherein the donor template comprises a donor cassette comprising the nucleic acid sequence encoding a Factor VIII (FVIII) protein, and wherein the donor cassette is flanked on one or both sides by a gRNA target site.

10. The system of claim 9, wherein the donor cassette is flanked on both sides by a gRNA target site.

11. The system of claim 9 or 10, wherein the gRNA target site is a target site for a gRNA in the system.

12. The system of claim 11, wherein the gRNA target site of the donor template is the reverse complement of a genomic gRNA target site for a gRNA in the system.

13. The system of any one of claims 1-12, wherein said DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

14. The system of claim 13, wherein said liposome or lipid nanoparticle also comprises the gRNA.

15. The system of any one of claims 1-14, comprising the DNA endonuclease precomplexed with the gRNA, forming a Ribonucleoprotein (RNP) complex.

16. An *ex vivo* method of editing a genome in a cell, the method comprising: providing the following to the cell:

(a) a gRNA comprising a spacer sequence of SEQ ID NO: 22;

(b) a DNA endonuclease or nucleic acid encoding said DNA endonuclease; and

(c) a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein.

17. The method of claim 16, wherein said DNA endonuclease is Cas9.

18. The method of claim 16 or claim 17, wherein the nucleic acid encoding said DNA endonuclease is codon optimized for expression in the cell.

19. The method of any one of claims 16-18, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein is codon optimized for expression in the cell.

20. The method of any one of claims 16-19, wherein the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

21. The method of any one of claims 16-19, wherein the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA).

22. The method of claim 21, wherein the RNA encoding said DNA endonuclease is an mRNA.

23. The method of any one of claims 16-22, wherein the donor template is encoded in an Adeno Associated Virus (AAV) vector.

24. The method of any one of claims 16-23, wherein the donor template comprises a donor cassette comprising the nucleic acid sequence encoding a Factor VIII (FVIII) protein, and wherein the donor cassette is flanked on one or both sides by a gRNA target site.

25. The method of claim 24, wherein the donor cassette is flanked on both sides by a gRNA target site.

26. The method of claim 24 or 25, wherein the gRNA target site is a target site for the gRNA of (a).

27. The method of claim 26, wherein the gRNA target site of the donor template is the reverse complement of a gRNA target site in the cell genome for the gRNA of (a).

28. The method of any one of claims 16-27, wherein said DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

29. The method of claim 28, wherein said liposome or lipid nanoparticle also comprises the gRNA.

30. The method of any one of claims 16-29, comprising providing to the cell the DNA endonuclease precomplexed with the gRNA, forming a Ribonucleoprotein (RNP) complex.

31. The method of any one of claims 16-30, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell more than 4 days after the donor template of (c) is provided to the cell.

32. The method of any one of claims 16-31, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell at least 14 days after (c) is provided to the cell.

33. The method of claim 31 or 32, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b).

34. The method of claim 33, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) until a target level of targeted integration of the nucleic acid sequence encoding a Factor VIII (FVIII) protein and/or a target level of expression of the nucleic acid sequence encoding a Factor VIII (FVIII) protein is achieved.

35. The method of any one of claims 16-34, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein is expressed under the control of the endogenous albumin promoter.

36. The method of any one of claims 16-35, wherein said cell is a hepatocyte.

37. A genetically modified cell in which the genome of the cell is edited by the method of any one of claims 16-36.

38. The genetically modified cell of claim 37, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein is expressed under the control of the endogenous albumin promoter.

39. The genetically modified cell of claim 37 or 38, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein is codon optimized for expression in the cell.

40. The genetically modified cell of any one of claims 37-39, wherein said cell is a hepatocyte.

41. Use of the following:

(a) a gRNA comprising a spacer sequence of SEQ ID NO: 22;

(b) a DNA endonuclease or nucleic acid encoding said DNA endonuclease; and

(c) a donor template comprising a nucleic acid sequence encoding a Factor VIII (FVIII) protein

in the manufacture of a medicament for combination therapy to treat Hemophilia A in a subject.

42. The use of claim 41, wherein said subject is a patient having or is suspected of having Hemophilia A.

43. The use of claim 40 or 41, wherein said subject is diagnosed with a risk of Hemophilia A.

44. The use of any one of claims 41-43, wherein said DNA endonuclease is Cas9.

45. The use of any one of claims 41-44, wherein the nucleic acid encoding said DNA endonuclease is codon optimized for expression in the cell.

46. The use of any one of claims 41-45, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein is codon optimized for expression in the cell.

47. The use of any one of claims 41-46, wherein the nucleic acid encoding said DNA endonuclease is a deoxyribonucleic acid (DNA).

48. The use of any one of claims 41-46, wherein the nucleic acid encoding said DNA endonuclease is a ribonucleic acid (RNA).

49. The use of claim 48, wherein the RNA encoding said DNA endonuclease is an mRNA.

50. The use of any one of claims 41-49, wherein one or more of the gRNA of (a), the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b), and the donor template of (c) are formulated in a liposome or lipid nanoparticle.

51. The use of any one of claims 41-50, wherein the donor template is encoded in an Adeno Associated Virus (AAV) vector.

52. The use of any one of claims 41-51, wherein the donor template comprises a donor cassette comprising the nucleic acid sequence encoding a Factor VIII (FVIII) protein, and wherein the donor cassette is flanked on one or both sides by a gRNA target site.

53. The use of claim 52, wherein the donor cassette is flanked on both sides by a gRNA target site.

54. The use of claim 52 or 53, wherein the gRNA target site is a target site for the gRNA of (a).

55. The use of claim 54, wherein the gRNA target site of the donor template is the reverse complement of the gRNA target site in the cell genome for the gRNA of (a).

56. The use of any one of claims 41-55, wherein the donor template to be provided to the cell comprises that the donor template is to be administered to the subject.

57. The use of claim 56, wherein the administration is via intravenous route.

58. The use of any one of claims 41-57, wherein said DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

59. The use of claim 58, wherein said liposome or lipid nanoparticle also comprises the gRNA.

60. The use of claim 59, wherein the gRNA and the DNA endonuclease or nucleic acid encoding the DNA endonuclease to be provided to the cell comprises that the liposome or lipid nanoparticle is to be administered to the subject.

61. The use of claim 60, wherein the administration is via intravenous route.

62. The use of any one of claims 41-61, comprising that the DNA endonuclease pre-complexed with the gRNA is to be provided to the cell, forming a Ribonucleoprotein (RNP) complex.

63. The use of any one of claims 41-62, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are to be provided to the cell more than 4 days after the donor template of (c) is to be provided to the cell.

64. The use of any one of claims 41-63, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are to be provided to the cell at least 14 days after the donor template of (c) is to be provided to the cell.

65. The use of claim 63 or 64, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are to be provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b).

66. The use of claim 65, wherein one or more additional doses of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) are to be provided to the cell following the first dose of the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) until a target level of targeted integration of the nucleic acid sequence encoding a Factor VIII (FVIII) protein and/or a target level of expression of the nucleic acid sequence encoding a Factor VIII (FVIII) protein is achieved.

67. The use of any one of claims 63-66, wherein the gRNA of (a) and the DNA endonuclease or nucleic acid encoding the DNA endonuclease of (b) to be provided to the cell comprises that a lipid nanoparticle comprising nucleic acid encoding the DNA endonuclease and the gRNA is to be administered to the subject.

68. The use of any one of claims 63-67 wherein the donor template of (c) to be provided to the cell comprises that the donor template encoded in an AAV vector is to be administered to the subject.

69. The use of any one of claims 41-68, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) protein is expressed under the control of the endogenous albumin promoter.

70. The use of any one of claims 41-69, wherein said cell is a hepatocyte.

71. The use of any one of claims 41-70, wherein the nucleic acid sequence encoding a Factor VIII (FVIII) proteinis expressed in the liver of the subject.

72. Use of the genetically modified cell of any one of claims 37-40 in the manufacture of a medicament for treating Hemophilia A, wherein said medicament is to be administered to the subject.

73. The use of claim 72, wherein said genetically modified cell is autologous to the subject.

74. The use of claim 71 or 72 further comprising:

a biological sample obtained from the subject wherein the biological sample comprises a hepatocyte cell, wherein the genetically modified cell is prepared from the hepatocyte.

75. A kit comprising the elements of the system of any one of claims 1-15, and further comprising instructions for use.