

vectors and/or recombinant Factor VIII AAV particles/viruses comprising any of the herein described AAV FVIII vectors, associated pharmaceutical formulations, and associated methods of administration for the treatment of hemophilia A in subjects suffering therefrom.

SUMMARY

[0007a] In one aspect, the present disclosure refers to use of a recombinant AAV5 FVIII-SQ virus in the manufacture of a medicament for treating a subject suffering from hemophilia A, wherein the medicament comprises (a) sodium phosphate, at a concentration of from 0.1 mg/ml to 3 mg/ml, sodium chloride at a concentration of from 1 mg/ml to 20 mg/ml, mannitol at a concentration of from 5 mg/ml to 40 mg/ml, and poloxamer 188 at a concentration of from 0.1 mg/ml to 4 mg/ml, or (b) sodium phosphate, dibasic at a concentration of 3.05 mg/ml, sodium phosphate monobasic at a concentration of 0.23 mg/ml, sodium chloride at a concentration of 8.18 mg/ml, mannitol at a concentration of 20 mg/ml, and poloxamer 188 at a concentration of 2 mg/ml, wherein AAV5-FVIII-SQ comprises an AAV2 5' inverted terminal repeat (ITR), a liver specific transcriptional regulatory region, a functionally active FVIII coding region, a polyadenylation sequence, an AAV2 3' ITR, and optionally one or more introns, wherein the functionally active FVIII coding region comprises nucleotides 403-4776 of SEQ ID NO: 1 and wherein the medicament is to be administered intravenously, and wherein the recombinant AAV5-FVIII-SQ comprises a liver specific regulatory element.

[0007b] In another aspect, the present disclosure refers to a pharmaceutical formulation comprising a recombinant AAV5-FVIII-SQ virus, a buffering agent, an isotonicity agent, a bulking agent and a surfactant, wherein the formulation comprises a) sodium phosphate, at a concentration of from 0.1 mg/ml to 3 mg/ml, sodium chloride at a concentration of from 1 mg/ml to 20 mg/ml, mannitol at a concentration of from 5 mg/ml to 40 mg/ml, and poloxamer 188 at a concentration of from 0.1 mg/ml to 4 mg/ml, or b) sodium phosphate, dibasic at a concentration of 3.05 mg/ml, sodium phosphate monobasic at a concentration of 0.23 mg/ml, sodium chloride at a concentration of 8.18 mg/ml, mannitol at a concentration of 20 mg/ml, and poloxamer 188 at a concentration of 2 mg/ml, wherein AAV5-FVIII-SQ comprises an AAV2 5' inverted terminal repeat (ITR), a liver specific transcriptional regulatory region, a functionally active FVIII coding region, a polyadenylation sequence, an AAV2 3' ITR, and optionally one or more introns, wherein the functionally active FVIII coding region comprises nucleotides 403-4776 of SEQ ID NO: 1 and wherein the medicament is to be administered intravenously, and wherein the recombinant AAV5-FVIII-SQ comprises a liver specific regulatory element.

[0008] The present disclosure provides AAV vectors encoding functionally active FVIII (referred to herein as “AAV FVIII vectors”). The recombinant AAV vectors of the present disclosure include non-naturally occurring derivatives of the AAV virus into which nucleic acid sequences encoding a functional FVIII protein have been introduced. The genomes encoding functionally active FVIII are preferably at most 7.0 kb in length, more preferably at most 6.5 kb in length, yet more preferably at most 6.0 kb in length, yet more preferably at most 5.5 kb in length, yet more preferably at most 5.0 kb in length, with enhanced promoter function.

As used herein, a “functionally active FVIII” is a FVIII protein that has the functionality of a wild-type FVIII protein *in vitro*, when expressed in cultured cells, or *in vivo*, when expressed in cells or body tissues. This includes, for example, functionally contributing in the blood coagulation cascade and/or reducing the time that it takes for blood to clot in a subject suffering from hemophilia A. Wild-type FVIII participates in blood coagulation via the coagulation cascade, acting as a co-factor for activated FIX (FIXa) which, in the presence of calcium ions and phospholipids forms a complex that converts Factor X (FX) into activated FX (FXa). Accordingly, a functionally active FVIII can form a complex with FIXa, which can convert FX to FXa. One example of a functionally active FVIII protein is a FVIII SQ protein as described in WO 2015/038625, herein incorporated by reference.

[0009] As used herein, an “AAV vector” refers to nucleic acids, either single-stranded or double-stranded, having an AAV 5’ inverted terminal repeat (ITR) sequence and an AAV 3’ ITR flanking a protein-coding sequence (preferably a functional Factor VIII-encoding sequence) operably linked to transcription regulatory elements that are heterologous to the AAV viral genome, i.e., one or more promoters and/or enhancers and, optionally, a polyadenylation sequence and/or one or more introns inserted between exons of the protein-coding sequence. A single-stranded AAV vector refers to nucleic acids that are present in the genome of an AAV virus particle, and can be either the sense strand or the anti-sense strand of the nucleic acid

CLAIMS

1. Use of a recombinant AAV5-FVIII-SQ virus in the manufacture of a medicament for treating a subject suffering from hemophilia A, wherein the medicament comprises
 - (a) sodium phosphate, at a concentration of from 0.1 mg/ml to 3 mg/ml, sodium chloride at a concentration of from 1 mg/ml to 20 mg/ml, mannitol at a concentration of from 5 mg/ml to 40 mg/ml, and poloxamer 188 at a concentration of from 0.1 mg/ml to 4 mg/ml, or
 - (b) sodium phosphate, dibasic at a concentration of 3.05 mg/ml, sodium phosphate monobasic at a concentration of 0.23mg/ml, sodium chloride at a concentration of 8.18 mg/ml, mannitol at a concentration of 20 mg/ml, and poloxamer 188 at a concentration of 2 mg/ml,wherein AAV5-FVIII-SQ comprises an AAV2 5' inverted terminal repeat (ITR), a liver specific transcriptional regulatory region, a functionally active FVIII coding region, a polyadenylation sequence, an AAV2 3' ITR, and optionally one or more introns, wherein the functionally active FVIII coding region comprises nucleotides 403-4776 of SEQ ID NO: 1

and wherein the medicament is to be administered intravenously, and wherein the recombinant AAV5-FVIII-SQ comprises a liver specific regulatory element.
2. The use of Claim 1, wherein from 1E12 vg/kg to 1E14 vg/kg recombinant AAV5-FVIII-SQ virus is to be administered to said subject.
3. The use of Claim 1, wherein from 6E12 vg/kg to 6E13 vg/kg recombinant AAV5-FVIII-SQ virus is to be administered to said subject.
4. The use of any one of Claims 1-3 which results in expression of at least 5 IU/dl of functional Factor VIII protein in said subject.
5. The use of any one of Claims 1-4, wherein said subject expresses at least 5 IU/dl of functional Factor VIII protein 3 weeks or more after said administration.
6. The use of any one of Claims 1-4 which results in an increase in functional FVIII activity of at least 1 IU/dl in said subject.

7. The use of any one of Claims 1-6, wherein the medicament is stable during storage at $\leq 65^{\circ}\text{C}$ for at least 2 weeks.
8. The use of any one of Claims 1-7, wherein the medicament is liquid.
9. The use of any one of Claims 1-8, wherein the medicament comprises said AAV5-FVIII-SQ virus at a concentration of from $1\text{E}12$ vg/ml to $2\text{E}14$ vg/ml.
10. The use of claim 9, wherein the medicament comprises said AAV5-FVIII-SQ virus at a concentration of $2\text{E}13$ vg/ml.
11. The use of any one of Claims 1-10, wherein the medicament is useful for IV administration to a patient suffering from hemophilia A.
12. The use of any one of Claims 1-11, wherein said subject is to be treated prophylactically with a corticosteroid at a concentration ranging from 5 mg/day to 60 mg/day.
13. The use of any one of Claims 1-12 wherein said subject is to be treated prophylactically with a corticosteroid at a concentration ranging from 5 mg/day to 60 mg/day over a continuous period of at least 3, 4, 5, 6, 7, 8, 9 or 10 weeks or greater.
14. The use of any one of Claims 1-13 wherein said subject is to be treated therapeutically with a corticosteroid at a concentration ranging from 5 mg/day to 60 mg/day.
15. The use of any one of Claims 1-14 wherein said subject is to be treated therapeutically with a corticosteroid at a concentration ranging from 5 mg/day to 60 mg/day over a continuous period of at least 3, 4, 5, 6, 7, 8, 9 or 10 weeks or greater.
16. The use of any one of Claims 1-15 wherein the absence or presence of anti-AAV capsid antibodies in the serum of said subject is to be determined before administration of said therapeutically effective amount of said recombinant AAV5-FVIII-SQ virus.
17. The use of any one of Claim 1-15 wherein the absence or presence of hepatotoxicity in the subject is to be determined after administration of said therapeutically effective amount of said recombinant AAV5-FVIII-SQ virus.

18. A pharmaceutical formulation comprising a recombinant AAV5-FVIII-SQ virus, a buffering agent, an isotonicity agent, a bulking agent and a surfactant, wherein the formulation comprises

a) sodium phosphate, at a concentration of from 0.1 mg/ml to 3 mg/ml, , sodium chloride at a concentration of from 1 mg/ml to 20 mg/ml, mannitol at a concentration of from 5 mg/ml to 40 mg/ml, and poloxamer 188 at a concentration of from 0.1 mg/ml to 4 mg/ml, or

b) sodium phosphate, dibasic at a concentration of 3.05 mg/ml, sodium phosphate monobasic at a concentration of 0.23 mg/ml, sodium chloride at a concentration of 8.18 mg/ml, mannitol at a concentration of 20 mg/ml, and poloxamer 188 at a concentration of 2 mg/ml,

wherein AAV5-FVIII-SQ comprises an AAV2 5' inverted terminal repeat (ITR), a liver specific transcriptional regulatory region, a functionally active FVIII coding region, a polyadenylation sequence, an AAV2 3' ITR, and optionally one or more introns, wherein the functionally active FVIII coding region comprises nucleotides 403-4776 of SEQ ID NO: 1

and wherein the medicament is to be administered intravenously, and wherein the recombinant AAV5-FVIII-SQ comprises a liver specific regulatory element.

19. The pharmaceutical formulation of Claim 18 which is liquid.

20. The pharmaceutical formulation of Claim 18 which comprises said AAV5-FVIII-SQ virus at a concentration of from 1E12 vg/ml to 2E14 vg/ml.

21. The pharmaceutical formulation of Claim 20 which comprises said AAV5-FVIII-SQ virus at a concentration of 2E13 vg/ml.