

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 invention refers to use of a recombinant AAV FVIII virus in the manufacture of a medicament for treating a subject suffering from hemophilia A.

[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.

[0009] 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.

[0010] 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

sequences disclosed herein. The size of such single-stranded nucleic acids is provided in bases. A double-stranded AAV vector refers to nucleic acids that are present in the DNA of plasmids, e.g., pUC19, or genome of a double-stranded virus, e.g., baculovirus, used to express or transfer the AAV vector nucleic acids. The size of such double-stranded nucleic acids is provided in base pairs (bp).

[0011] The term “inverted terminal repeat (ITR)” as used herein refers to the art-recognized regions found at the 5’ and 3’ termini of the AAV genome which function in cis as origins of DNA replication and as packaging signals for the viral genome. AAV ITRs, together with the AAV rep coding region, provide for efficient excision and rescue from, and integration of a nucleotide sequence interposed between two flanking ITRs into a host cell genome. Sequences of certain AAV-associated ITRs are disclosed by Yan et al., *J. Virol.* 79(1):364-379 (2005) which is herein incorporated by reference in its entirety. ITR sequences that find use herein may be full length, wild-type AAV ITRs or fragments thereof that retain functional capability, or may be sequence variants of full-length, wild-type AAV ITRs that are capable of functioning in cis as origins of replication. AAV ITRs useful in the recombinant AAV FVIII vectors of the present disclosure may derive from any known AAV serotype and, in certain preferred examples, derive from the AAV2 or AAV5 serotype.

[0012] A “transcription regulatory element” refers to nucleotide sequences of a gene involved in regulation of genetic transcription including a promoter, plus response elements, activator and enhancer sequences for binding of transcription factors to aid RNA polymerase binding and promote expression, and operator or silencer sequences to which repressor proteins bind to block RNA polymerase attachment and prevent expression. The term “liver specific transcription regulatory element” refers to a regulatory element that modulates gene expression specifically in the liver tissue. Examples of liver specific regulatory elements include, but are not limited to, the mouse thyretin promoter (mTTR), the endogenous human factor VIII promoter (F8), human alpha-1-antitrypsin promoter (hAAT) and active fragments thereof, human albumin minimal promoter, and mouse albumin promoter. Enhancers derived from liver specific transcription factor binding sites are also contemplated, such as EBP, DBP, HNF1, HNF3, HNF4, HNF6, with Enh1.

[0013] In one example, the AAV vector of the disclosure comprises a nucleic acid encoding functionally active FVIII protein having the B domain replaced by the 14 amino acid SQ sequence. The SQ sequence is disclosed in Ward et al., *Blood*, 117:798-807, 2011, McIntosh et al., *Blood* 121:3335-3344, 2013, WO 2013/186563 and WO 2015/038625. The FVIII coding region sequence may be a codon-optimized FVIII-encoding sequence (see, e.g., WO 2011/005968, published January 13, 2011, WO 2015/038625, published March 19, 2015, and McIntosh et al., *Blood* 121:3335-3344, 2013, which are incorporated herein by reference in their entirety). In a preferred example, the nucleic acid encoding the functionally active human FVIII protein of the AAV vector or recombinant AAV virus particle consists of nucleotides 403 to 4776 of SEQ ID NO:1. This sequence is herein referred to as "FVIII - SQ".

[0014] In a first example, the recombinant AAV vector of the disclosure comprises Proto 1, which is depicted schematically in Figure 2A, and comprises the nucleic acid sequence set forth in SEQ ID NO:1.

[0015] In a second example, the recombinant AAV vector of the disclosure comprises Proto 1S, which is depicted schematically in Figure 2B, and comprises the nucleic acid sequence set forth in SEQ ID NO:2.

[0016] In a third example, the recombinant AAV vector of the disclosure comprises Proto 2S, which is depicted schematically in Figure 2C, and comprises the nucleic acid sequence set forth in SEQ ID NO:3.

[0017] In a fourth example, the recombinant AAV vector of the disclosure comprises Proto 3S, which is depicted schematically in Figure 2D, and comprises the nucleic acid sequence set forth in SEQ ID NO:4.

[0018] In another example, the recombinant AAV vector of the disclosure comprises a nucleic acid encoding functional FVIII lacking the entire B domain, including the SQ sequence, and the a3 domain, which is located just N-terminal to the light chain or C domain. The FVIII coding region sequence may be a codon-optimized sequence (see, e.g., WO 2011/005968, published January 13, 2011, WO 2015/038625, published March 19, 2015, and McIntosh et al., *Blood* 121:3335-3344, 2013).

[0019] In a first example, the recombinant AAV vector of the disclosure comprises Proto 4, which is depicted schematically in Figure 3A, and comprises the nucleic acid sequence set forth in SEQ ID NO:5.

[0020] In a second example, the recombinant AAV vector of the disclosure comprises Proto 5, which is depicted schematically in Figure 3B, and comprises the nucleic acid sequence set forth in SEQ ID NO:6.

[0021] In a third example, the recombinant AAV vector of the disclosure comprises Proto 6, which is depicted schematically in Figure 3C, and comprises the nucleic acid sequence set forth in SEQ ID NO:7.

[0022] In a fourth example, the recombinant AAV vector of the disclosure comprises Proto 7, which is depicted schematically in Figure 3D, and comprises the nucleic acid sequence set forth in SEQ ID NO:8.

[0023] In other examples, the recombinant AAV vector of the disclosure comprises a nucleic acid comprising an AAV2 5' inverted terminal repeat (ITR) (which may or may not be modified as known in the art), a liver-specific transcription regulatory region, a codon-optimized functionally active FVIII coding region, optionally one or more introns, a polyadenylation sequence, and an AAV2 3' ITR (which may or may not be modified as known in the art). In a preferred example, the liver-specific transcription regulatory region comprises a shortened ApoE enhancer sequence, a 186 base human alpha anti-trypsin (hAAT) proximal promoter, including 42 bases of the 5' untranslated region (UTR), and one or more enhancers selected from the group consisting of (i) a 34 base human ApoE/C1 enhancer, (ii) a 32 base human AAT promoter distal X region and (iii) 80 additional bases of distal element of the human AAT proximal promoter; and a codon-optimized functionally active FVIII coding region encoding the FVIII - SQ variant. In another preferred example, the liver specific transcription regulatory region comprises an α 1-microglobulin enhancer sequence and the 186 base human alpha anti-trypsin (AAT) proximal promoter.

[0024] In a first example, the recombinant AAV vector of the disclosure comprises Construct 100ATG comprising the nucleic acid sequence set forth in SEQ ID NO:9.

[0025] In a second example, the recombinant AAV vector of the disclosure comprises Construct 100ATG bGH poly A comprising the nucleic acid sequence set forth in SEQ ID NO:10.

[0026] In a third example, the recombinant AAV vector of the disclosure comprises Construct 100ATG short bGH polyA sequence set forth in SEQ ID NO:11.

[0027] In a fourth example, the recombinant AAV vector of the disclosure comprises Construct 103ATG comprising the nucleic acid sequence forth in SEQ ID NO:12.

[0028] In a fifth example, the recombinant AAV vector of the disclosure comprises Construct 103ATG short bGH poly A comprising the nucleic acid sequence set forth in SEQ ID NO:13.

[0029] In a sixth example, the recombinant AAV vector of the disclosure comprises Construct 105ATG bGH poly A comprising the nucleic acid sequence set forth in SEQ ID NO:14.

[0030] In a seventh example, the recombinant AAV vector of the disclosure comprises Construct DC172ATG FVIII comprising the nucleic acid sequence set forth in SEQ ID NO:15.

[0031] In an eighth example, the recombinant AAV vector of the disclosure comprises Construct DC172ATG FVIII hAAT comprising the nucleic acid sequence set forth in SEQ ID NO:16.

[0032] In a ninth example, the recombinant AAV vector of the i disclosure comprises Construct DC172 2xHCR ATG FVIII comprising the nucleic acid sequence set forth in SEQ ID NO:17.

[0033] In a tenth example, the recombinant AAV vector of the disclosure comprises Construct DC172 2xHCR ATG FVIII hAAT comprising the nucleic acid sequence set forth in SEQ ID NO:18.

[0034] In an eleventh example, the recombinant AAV vector of the disclosure comprises Construct 2x SerpinA hAAT ATG FVIII comprising the nucleic acid sequence set forth in SEQ ID NO:19.

[0035] In a twelfth example, the recombinant AAV vector of the disclosure comprises Construct 2x SerpinA hAAT ATG FVIII 2x μ -globulin enhancer comprising the nucleic acid sequence set forth in SEQ ID NO:20.

[0036] In a thirteenth example, the recombinant AAV vector of the disclosure Construct 100ATG short polyA 2x μ -globulin enhancer comprising the nucleic acid sequence set forth in SEQ ID NO:21.

[0037] In a fourteenth example, the recombinant AAV vector of the disclosure comprises Construct Factor VIII-BMN001 comprising the nucleic acid sequence set forth in SEQ ID NO:22.

[0038] In a fifteenth example, the recombinant AAV vector of the disclosure comprises Construct Factor VIII-BMN002 sequence set forth in SEQ ID NO:23.

[0039] In a sixteenth example, the recombinant AAV vector of the disclosure comprises Construct 99 comprising the nucleic acid sequence set forth in SEQ ID NO:24.

[0040] In a seventeenth example, the recombinant AAV vector of the disclosure comprises Construct 100 comprising the nucleic acid sequence set forth in SEQ ID NO:25.

[0041] In an eighteenth example, the recombinant AAV vector of the disclosure comprises Construct 100 reverse orientation comprising the nucleic acid sequence set forth in SEQ ID NO:26.

[0042] In a nineteenth example, the recombinant AAV vector of the disclosure Construct 100AT comprising the nucleic acid sequence set forth in SEQ ID NO:27.

[0043] In a twentieth example, the recombinant AAV vector of the disclosure Construct 100AT 2x MG comprising the nucleic acid sequence set forth in SEQ ID NO:28.

[0044] In a twenty-first example, the recombinant AAV vector of the disclosure comprises Construct 100AT 2x MG bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO:29.

[0045] In a twenty-second example, the recombinant AAV vector of the disclosure comprises Construct 100AT 2x MG (reverse) bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO:30.

[0046] In a twenty-third example, the recombinant AAV vector of the disclosure comprises Construct 100 bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO:31.

[0047] In a twenty-fourth example, the recombinant AAV vector of the disclosure comprises Construct 100-400 comprising the nucleic acid sequence set forth in SEQ ID NO:32.

[0048] In a twenty-fifth example, the recombinant AAV vector of the disclosure comprises Construct 101 comprising the nucleic acid sequence set forth in SEQ ID NO:33.

[0049] In a twenty-sixth example, the recombinant AAV vector of the disclosure comprises Construct 102 sequence comprising the nucleic acid sequence set forth in SEQ ID NO:34.

[0050] In a twenty-seventh example, the recombinant AAV vector of the disclosure comprises Construct 103 comprising the nucleic acid sequence set forth in SEQ ID NO:35.

[0051] In a twenty-ninth example, the recombinant AAV vector of the disclosure comprises Construct 103 reverse orientation comprising the nucleic acid sequence set forth in SEQ ID NO:36.

[0052] In a thirtieth example, the recombinant AAV vector of the disclosure comprises Construct 103AT comprising the nucleic acid sequence set forth in SEQ ID NO:37.

[0053] In a thirty-first example, the recombinant AAV vector of the disclosure comprises Construct 103AT 2xMG comprising the nucleic acid sequence set forth in SEQ ID NO:38.

[0054] In a thirty-second example, the recombinant AAV vector of the disclosure comprises Construct 103AT 2xMG bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO:39.

[0055] In a thirty-third example, the recombinant AAV vector of the disclosure comprises the Construct 103 bGH polyA comprising the nucleic acid sequence set forth in SEQ ID NO:40.

[0056] In a thirty-fourth example, the recombinant AAV vector of the disclosure comprises Construct 104 comprising the nucleic acid comprising the nucleic acid sequence set forth in SEQ ID NO:41.

[0057] In a thirty-fifth example, the recombinant AAV vector of the disclosure comprises Construct 105 comprising the nucleic acid sequence set forth in SEQ ID NO:42.

[0058] In a thirty-sixth example, the recombinant AAV vector of the disclosure comprises Construct 106 comprising the nucleic acid sequence set forth in SEQ ID NO:43.

[0059] In a thirty-seventh example, the recombinant AAV vector of the disclosure comprises Construct 106AT comprising the nucleic acid sequence set forth in SEQ ID NO:44.

[0060] In a thirty-eighth example, the recombinant AAV vector of the disclosure comprises p-100 ATGB, which comprises the nucleic acid sequence set forth in SEQ ID NO:45.

[0061] In yet other examples, the present disclosure is directed to vector constructs encoding a functional Factor VIII polypeptide, wherein said constructs comprise one or more of the individual elements of the above described constructs and combinations thereof, in one or more different orientation(s). The present disclosure is also directed to the above described constructs in an opposite orientation. The present disclosure is also directed to recombinant AAV virus particles comprising the herein described AAV FVIII vectors and their use for the treatment of hemophilia A.

[0062] The AAV vectors of the disclosure in single strand form are less than about 7.0 kb in length, or is less than 6.5kb in length, or is less than 6.4 kb in length, or is less than 6.3 kb in length, or is less than 6.2 kb in length, or is less than 6.0 kb in length, or is less than 5.8 kb in length, or is less than 5.6 kb in length, or is less than 5.5 kb in length, or is less than 5.4 kb in length, or is less than 5.4 kb in length, or is less than 5.2 kb in length or is less than 5.0 kb in length. The AAV vectors of the disclosure in single strand form range from about 5.0 kb to about 6.5 kb in length, or ranges from about 4.8 kb to about 5.2 k in length, or 4.8 kb to 5.3 kb in length, or ranges from about 4.9 kb to about 5.5 kb in length, or about 4.8 kb to about 6.0 kb in length, or about 5.0 kb to 6.2 kb in length or about 5.1 kb to about 6.3 kb in length ,or about 5.2 kb to about 6.4 kb in length, or about 5.5 kb to about 6.5 kb in length.

[0063] In another example, the disclosure provides for methods of producing a recombinant adeno-associated virus (AAV) particles comprising any of the AAV vectors of the disclosure. The methods comprise the steps of culturing a cell that has been transfected with any of the AAV vectors of the disclosure (in association with various AAV cap and rep genes) and recovering recombinant AAV FVIII virus particles from the supernatant of the transfected cell.

[0064] The cells of the disclosure useful for recombinant AAV production are any cell type susceptible to baculovirus infection, including insect cells such as High Five, Sf9, Se301, SeIZD2109, SeUCR1, Sf9, Sf900+, Sf21, BTI-TN-5B1-4, MG-1, Tn368, HzAm1, BM-N, Ha2302, Hz2E5 and Ao38. Preferred mammalian cells used can be HEK293, HeLa, CHO, NS0, SP2/0, PER.C6, Vero, RD, BHK, HT 1080, A549, Cos-7, ARPE-19 and MRC-5.

[0065] The disclosure also provides for a recombinant viral particle comprising any of the AAV vectors of the disclosure or any viral particle produced by the forgoing methods of the disclosure.

[0066] An "AAV virion" or "AAV viral particle" or "AAV vector particle" or "AAV virus" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide AAV vector as described herein. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "AAV vector particle" or simply an "AAV vector". Thus, production of AAV vector particles necessarily includes production of AAV vector, as such a vector is contained within an AAV vector particle.

[0067] The disclosure also provides for cells comprising any of the AAV vectors of the disclosure, and viral particles produced by these cells of the disclosure.

[0068] In another example, the disclosure provides for methods of treating a patient suffering from hemophilia A comprising administering to the patient a therapeutically effective amount of any of the AAV vectors of the disclosure, or a viral particle of the disclosure or a viral particle produced by a method of the disclosure.

[0069] In another example, the disclosure provides for methods of increasing circulating FVIII protein levels in a subject in need thereof comprising administering to the subject any of the AAV vectors of the disclosure, or a viral particle of the disclosure or a viral particle produced by a method of the disclosure

[0070] In another example, the disclosure provides for methods for inducing the expression of FVIII protein in a subject in need thereof comprising administering to the subject any of the AAV vectors of the disclosure, or viral particles of the disclosure or a viral particle produced by a method of the disclosure.

[0071] In another example, the disclosure provides for methods for increasing FVIII protein expression in a subject in need thereof comprising administering to the subject any of the AAV vectors of the disclosure, or viral particles of the disclosure or a viral particle produced by a method of the disclosure.

[0072] The disclosure also provides for any of the methods of the disclosure further comprising the step of determining the absence or presence of anti-AAV capsid antibodies in the serum of said subject after administration of said therapeutically effective amount of said recombinant AAV FVIII virus. In addition, the disclosure provides for any of the methods of the disclosure

further comprising the step of administering an effective amount of a corticosteroid to said subject after a determination of the presence of anti-AAV capsid antibodies in the serum of said subject is made.

[0073] In a further example, the disclosure provides for a use of any of the AAV vectors of the disclosure or recombinant AAV virus particles of the disclosure for preparation of a medicament for the treatment of hemophilia A. In one example, the medicament comprises an amount of AAV vector or recombinant AAV FVIII virus particle that expresses human FVIII in an amount effective to treat hemophilia A. The disclosure also provides for any of the uses of the disclosure wherein after administration of the medicament, the absence or presence of anti-AAV capsid antibodies in the serum of the subject is determined. If the subject is determined to have anti-AAV capsid antibodies in the serum, use of an effective amount of a corticosteroid for the preparation of a medicament for the administration to the subject having anti-AAV capsid antibodies in the serum.

[0074] In another example, the disclosure provides for a composition comprising any of the AAV vectors or recombinant AAV virus particles of the disclosure for the treatment of hemophilia A. In one example, the composition comprises an amount of AAV vector or recombinant AAV virus particles that expresses human FVIII in an amount effective to treat hemophilia A. In addition, any of the compositions of the disclosure are administered with an effective amount of a corticosteroid in a subject determined to have anti-AAV capsid antibodies in the serum after administration of the composition.

[0075] In another example, the AAV vectors of the disclosure are used to produce AAV viral particles that are useful for treating a patient suffering from hemophilia A.

[0076] In another example, the disclosure provides for pharmaceutical formulations comprising recombinant FVIII-encoding AAV virus particles as described herein. More specifically, in certain examples, the present disclosure is directed to pharmaceutical formulations that comprise a recombinant AAV FVIII-encoding virus, a buffering agent, an isotonicity agent, a bulking agent and a surfactant. In particularly preferred examples, the pharmaceutical formulations of the present disclosure comprise AAV5-FVIII-SQ, p-100 ATGB or any of the other herein described vectors and/or are stable during storage at $\leq 65^{\circ}\text{C}$ for at least 2 weeks. In yet other examples of the present disclosure, the pharmaceutical formulation comprises

sodium phosphate, dibasic at a concentration of from about 0.1 mg/ml to about 3 mg/ml, sodium phosphate monobasic monohydrate at a concentration of from about 0.1 mg/ml to about 3 mg/ml, sodium chloride at a concentration of from about 1 mg/ml to about 20 mg/ml, mannitol at a concentration of from about 5 mg/ml to about 40 mg/ml, and poloxamer 188 at a concentration of from about 0.1 mg/ml to about 4 mg/ml. In a particularly preferred example, the pharmaceutical formulation of the present disclosure comprises sodium phosphate, dibasic at a concentration of about 1.42 mg/ml, sodium phosphate monobasic monohydrate at a concentration of about 1.38 mg/ml, sodium chloride at a concentration of about 8.18 mg/ml, mannitol at a concentration of about 20 mg/ml, and poloxamer 188 at a concentration of about 2 mg/ml. The pharmaceutical formulations of the present disclosure may be in liquid form and may comprise the AAV FVIII virus particle at a concentration of from about 1E12 vg/ml to about 2E14 vg/ml, more preferably at a concentration of about 2E13 vg/ml. In one example, the pharmaceutical formulations of the disclosure are useful for intravenous administration to a human suffering from hemophilia A.

[0077] The present disclosure is also directed to methods for treating a subject suffering from hemophilia A which comprise the step of administering to the subject a therapeutically effective amount of a recombinant AAV FVIII virus, which optionally may be formulated as described above. In a preferred example, the subject suffering from hemophilia A is a human. In one example, the recombinant AAV FVIII virus is AAV5-FVIII-SQ. In one example, the step of administering is accomplished by intravenous (IV) administration. In certain examples of the present disclosure, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject, preferably at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject. In certain examples, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more weeks after administration. In certain examples, the therapeutically effective amount of AAV FVIII virus administered to the subject is least 2E13 vg/kg of body weight, sometimes at least 6E13 vg/kg of body weight. In certain examples, in addition to administration of a therapeutically effective amount of AAV FVIII virus, the subject is treated either prophylactically, therapeutically, or both with a corticosteroid to prevent and/or treat any hepatotoxicity associated with administration of the

AAV FVIII virus. In one example, associated hepatotoxicity is measured by comparing baseline (i.e., pre-dosing with FVIII AAV) alanine transaminase (ALT) levels to post-treatment ALT levels, wherein an increase in ALT levels post-dosing is evidence of associated hepatotoxicity. Prophylactic corticosteroid treatment refers to the administration of a corticosteroid to prevent hepatotoxicity and/or to prevent an increase in measured ALT levels in the subject. Therapeutic corticosteroid treatment refers to the administration of a corticosteroid to reduce hepatotoxicity caused by administration of an AAV FVIII virus and/or to reduce an elevated ALT concentration in the bloodstream of the subject caused by administration of an AAV FVIII virus. In certain examples, prophylactic or therapeutic corticosteroid treatment may comprise administration of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, or more mg/day of the corticosteroid to the subject. In certain examples, prophylactic or therapeutic corticosteroid treatment of a subject may occur over a continuous period of at least about 3, 4, 5, 6, 7, 8, 9, 10 weeks, or more.

[0078] The present disclosure is also directed to a composition comprising a therapeutically effective amount of a recombinant AAV FVIII virus for use in treating a subject suffering from hemophilia A. In one example, the AAV FVIII virus is AAV5-FVIII-SQ. In another example, the AAV FVIII virus comprises the p-100 ATGB vector. The composition optionally may be formulated as described above. In certain examples, compositions comprising a therapeutically effective amount of AAV FVIII virus are administered with a composition comprising a prophylactic and/or therapeutic corticosteroid for use in preventing and/or treating any hepatotoxicity associated with administration of the AAV FVIII virus. The composition comprising a prophylactic or therapeutic corticosteroid treatment may comprise at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, or more mg/day of the corticosteroid. In certain examples, compositions comprising a prophylactic or therapeutic corticosteroid may be administered over a continuous period of at least about 3, 4, 5, 6, 7, 8, 9, 10 weeks, or more.

[0079] The present disclosure is also directed to use of a therapeutically effective amount of recombinant AAV FVIII virus for the preparation of a medicament for the treatment of a subject suffering from hemophilia A. In certain examples, the AAV FVIII virus is AAV5-FVIII-SQ or a virus comprising the p-100 ATGB vector. The medicament optionally may be formulated as described above. In a preferred example, the subject suffering from hemophilia A is a human. In one example, the medicament is administered by intravenous (IV) administration.

In one example of the present disclosure, administration of the medicament results in expression of at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject, preferably at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject 16 weeks or more after administration. In certain examples, the medicament also comprises a prophylactic and/or therapeutic corticosteroid for the prevention and/or treatment of any hepatotoxicity associated with administration of the AAV FVIII virus. The medicament comprising a prophylactic or therapeutic corticosteroid treatment may comprise at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, or more mg/day of the corticosteroid. In certain examples, the medicament comprising a prophylactic or therapeutic corticosteroid may be administered over a continuous period of at least about 3, 4, 5, 6, 7, 8, 9, 10 weeks, or more.

[0080] The present disclosure is also directed to methods for reducing bleeding time during a bleeding episode in a subject suffering from hemophilia A which comprise the step of administering to the subject a therapeutically effective amount of a recombinant AAV FVIII virus as described herein, which optionally may be formulated as described above. In a preferred example, the subject suffering from hemophilia A is a human. In one example, the step of administering is accomplished by intravenous (IV) administration. In certain examples, the step of administering occurs at least about 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 weeks, or more, prior to the bleeding episode. In one example of the present disclosure, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject, preferably at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject. In certain examples, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more weeks after administration. In certain examples, in addition to administration of a therapeutically effective amount of AAV FVIII virus, the subject is treated either prophylactically, therapeutically, or both with a corticosteroid to prevent and/or treat any hepatotoxicity associated with administration of the AAV FVIII virus, as described above.

[0081] The present disclosure is also directed to a composition comprising a therapeutically effective amount of a recombinant AAV FVIII virus for use in reducing bleeding time of a bleeding episode in a subject suffering from hemophilia A. In one example, the AAVFVIII virus is AAV5-FVIII-SQ. The composition optionally may be formulated as described above.

In a preferred example, the subject suffering from hemophilia A is a human. The composition may be administered prior to the bleeding episode. In one example, the composition is administered by intravenous (IV) administration prior to the bleeding episode. In one example of the present disclosure, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject, preferably at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject. In certain examples, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more weeks after administration. In certain examples, compositions comprising a therapeutically effective amount of AAV FVIII virus for use in reducing bleeding time are administered with a composition comprising a prophylactic and/or therapeutic corticosteroid for use in preventing and/or treating any hepatotoxicity associated with administration of the AAV FVIII virus, as described above.

[0082] The disclosure also provides for any of the methods of reducing bleeding time further comprising the step of determining the absence or presence of anti-AAV capsid antibodies in the serum of said subject after administration of said therapeutically effective amount of said recombinant AAV FVIII virus. In addition, the disclosure provides for any of the methods of reducing bleeding time further comprising the step of administering an effective amount of a corticosteroid to said subject after a determination of the presence of anti-AAV capsid antibodies in the serum of said subject is made.

[0083] The present disclosure is also directed to use of a therapeutically effective amount of recombinant AAV FVIII virus for the preparation of a medicament for reducing bleeding time of a bleeding episode in a subject suffering from hemophilia A. In one example, the AAVFVIII virus is AAV5-FVIII-SQ. The medicament optionally may be formulated as described above. In a preferred example, the subject suffering from hemophilia A is a human. The medicament may be administered prior to the bleeding episode. In one example, the medicament is administered by intravenous (IV) administration prior to the bleeding episode. In one example of the present disclosure, administration of the medicament results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject, preferably at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject. In certain examples, administration of the medicament results in expression of at least about 1,

2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more weeks after administration. In certain examples, medicaments comprising a therapeutically effective amount of AAV FVIII virus for reducing bleeding time also comprise a prophylactic and/or therapeutic corticosteroid for preventing and/or treating any hepatotoxicity associated with administration of the AAV FVIII virus, as described above. In addition, any of the compositions of the disclosure for use in reducing bleeding time are administered with an effective amount of a corticosteroid in a subject determined to have anti-AAV capsid antibodies in the serum after administration of the composition.

[0084] The present disclosure is also directed to methods for inducing expression of a functional FVIII protein in a subject in need thereof which comprise the step of administering to the subject a recombinant AAV FVIII virus as described herein, which optionally may be formulated as described above, wherein such administration results in increased expression of functional FVIII protein or increased concentrations of functional FVIII protein in the bloodstream of the subject. In a preferred example, the subject in need is a human. In one example, the step of administering is accomplished by intravenous (IV) administration. In one example of the present disclosure, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject, preferably at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject. In certain examples, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more weeks after administration. In certain examples, in addition to administration of an AAV FVIII virus, the subject is treated either prophylactically, therapeutically, or both with a corticosteroid to prevent and/or treat any hepatotoxicity associated with administration of the AAV FVIII virus, as described above. In addition, in any of the uses of the disclosure after administration of the medicament to reduce bleeding time, the absence or presence of anti-AAV capsid antibodies in the serum of the subject is determined. If the subject is determined to have anti-AAV capsid antibodies in the serum, use of an effective amount of a corticosteroid for the preparation of a medicament for the administration to the subject having anti-AAV capsid antibodies in the serum is contemplated.

[0085] The present disclosure is also directed to methods for increasing expression of FVIII protein in a subject in need thereof which comprise the step of administering to the subject a recombinant AAV FVIII virus as described herein, which optionally may be formulated as described above, wherein such administration results in increased expression of functional FVIII protein or increased concentrations of functional FVIII protein in the bloodstream of the subject. In a preferred example, the subject in need is a human. In one example, the step of administering is accomplished by intravenous (IV) administration. In one example of the present disclosure, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject, preferably at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject. In certain examples, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more weeks after administration. In certain examples, in addition to administration of an AAV FVIII virus, the subject is treated either prophylactically, therapeutically, or both with a corticosteroid to prevent and/or treat any hepatotoxicity associated with administration of the AAV FVIII virus, as described above.

[0086] The present disclosure is also directed to a composition comprising a therapeutically effective amount of a recombinant AAV FVIII virus for use in increasing or inducing expression of FVIII protein in a subject in need thereof. In one example, the AAVFVIII virus is AAV5-FVIII-SQ. The composition optionally may be formulated as described above. In a preferred example, the subject in need is a human suffering from hemophilia A. The composition may be administered prior to the bleeding episode. In one example, the composition is administered by intravenous (IV) administration prior to the bleeding episode. In one example of the present disclosure, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject, preferably at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject. In certain examples, the step of administration results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more weeks after administration. In certain examples, compositions comprising a therapeutically effective amount of AAV FVIII virus for use in increasing or inducing expression of FVIII protein are administered with a composition

comprising a prophylactic and/or therapeutic corticosteroid for use in preventing and/or treating any hepatotoxicity associated with administration of the AAV FVIII virus, as described above.

[0087] The present disclosure is also directed to use of a therapeutically effective amount of recombinant AAV FVIII virus for the preparation of a medicament for increasing or inducing expression of FVIII protein in a subject in need. In one example, the subject in need is a human suffering from hemophilia A. In one example, the AAVFVIII virus is AAV5-FVIII-SQ. The medicament optionally may be formulated as described above. The medicament may be administered prior to the bleeding episode. In one example, the medicament is administered by intravenous (IV) administration prior to the bleeding episode. In one example of the present disclosure, administration of the medicament results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject, preferably at least about 5 IU/dl of Factor VIII protein in the bloodstream of the subject. In certain examples, administration of the medicament results in expression of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more IU/dl of Factor VIII protein in the bloodstream of the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more weeks after administration. In certain examples, medicaments comprising a therapeutically effective amount of AAV FVIII virus for increasing or inducing expression of FVIII protein also comprise a prophylactic and/or therapeutic corticosteroid for preventing and/or treating any hepatotoxicity associated with administration of the AAV FVIII virus, as described above.

[0088] The present disclosure is also directed to a method of treating a subject suffering from hemophilia A comprising the steps of (i) determining the absence of anti-AAV capsid antibodies in the serum of said subject, and (ii) administering to said subject a therapeutically effective amount of a recombinant AAV FVIII virus.

[0089] The present disclosure is also directed to use of a therapeutically effective amount of a recombinant AAV FVIII virus for the preparation of a medicament for the treatment of a subject suffering from hemophilia A, wherein anti-AAV capsid antibodies are absent from the serum of the subject.

[0090] The present disclosure is also directed to a composition comprising a therapeutically effective amount of a recombinant AAV FVIII virus for use in treating a subject suffering from hemophilia A, wherein anti-AAV capsid antibodies are absent from the subject's serum.

[0091] The present disclosure is also directed to a method of treating a subject suffering from hemophilia A comprising the steps of (i) administering to said subject a therapeutically effective amount of a recombinant AAV FVIII virus, and (ii) after administration of said therapeutically effective amount of said recombinant AAV FVIII virus, determining the absence or presence of anti-AAV capsid antibodies in the serum of said subject. In one example, the method further comprises the step of administering an effective amount of a corticosteroid to the subject after a determination of the presence of anti-AAV capsid antibodies in the serum of said subject is made.

[0092] The present disclosure is directed to use of a therapeutically effective amount of a recombinant AAV FVIII virus for the preparation of a medicament for the treatment of hemophilia A wherein after administration of the medicament, the absence or presence of anti-AAV capsid antibodies in the serum of the subject is determined. If the subject is determined to have anti-AAV capsid antibodies in the serum, use of an effective amount of a corticosteroid for the preparation of a medicament for administration to the subject having anti-AAV capsid antibodies in the serum. The present disclosure is also directed to a composition comprising an effective amount of recombinant AAV FVIII for treatment of hemophilia A, wherein this composition is administered with an effective amount of a corticosteroid in a subject determined to have anti-AAV capsid antibodies in the serum after administration of the composition.

[0093] Other examples of the present disclosure will be evident to one skilled in the art upon reading the present patent specification.

DESCRIPTION OF DRAWINGS

[0094] Figure 1 provides a schematic of an exemplary FVIII-encoding recombinant AAV vector. From left to right, the vector comprises an AAV2 5' ITR sequence, wild-type AAV2 viral sequence, a 34 base human ApoE/C1 enhancer sequence, a 32 base human AAT promoter distal X region sequence, a 186 base human AAT promoter sequence that includes 42 bases of 5' UTR sequence, a codon-optimized human FVIII SQ sequence, a 49 base synthetic Proudfoot polyadenylation sequence, wild-type AAV2 viral sequence, and an AAV2 3' ITR sequence (see WO 2011/005968, published January 13, 2011, which is incorporated herein by reference in its entirety, and McIntosh et al., *Blood* 121:3335-3344, 2013). This vector is 5081 bases in length.

What is claimed:

1. Use of a recombinant AAV FVIII virus in the manufacture of a medicament for treating a subject suffering from hemophilia A.
2. The use of Claim 2, wherein said recombinant AAV FVIII virus is AAV5-FVIII-SQ.
3. The use of Claim 1 or 2, wherein the medicament is to be administered intravenously.
4. The use of any one of Claims 1-3, wherein from 1E12 vg/kg to 1E14 vg/kg of the recombinant AAV FVIII virus is to be administered to said subject.
5. The use of any one of Claims 1-3, wherein from 6E12 vg/kg to 6E13 vg/kg of the recombinant AAV FVIII virus is to be administered to said subject.
6. The use of any one of Claims 1-5 which results in expression of at least about 5 IU/dl of functional Factor VIII protein in said subject.
7. The use of any one of Claims 1-6, wherein said subject expresses at least about 5 IU/dl of functional Factor VIII protein 3 weeks or more after administration.
8. The use of any one of Claims 1-6 which results in an increase in functional FVIII activity of at least about 1 IU/dl in said subject.
9. The use of any one of Claims 1-8, wherein the medicament further comprises a buffering agent, an isotonicity agent, a bulking agent and a surfactant.
10. The use of Claim 9, wherein the recombinant AAV FVIII virus is AAV5-FVIII-SQ.
11. The use of Claim 9 or 10, wherein the medicament is stable during storage at $\leq 65^{\circ}\text{C}$ for at least 2 weeks.
12. The use of any one of Claims 9-11, wherein the medicament comprises sodium phosphate, dibasic at a concentration of from 0.1 mg/ml to 3 mg/ml, sodium phosphate monobasic monohydrate 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.

13. The use of any one of Claims 9-12, wherein the medicament comprises sodium phosphate, dibasic at a concentration of about 1.42 mg/ml, sodium phosphate monobasic monohydrate at a concentration of about 1.38 mg/ml, sodium chloride at a concentration of about 8.18 mg/ml, mannitol at a concentration of about 20 mg/ml, and poloxamer 188 at a concentration of about 2 mg/ml.
14. The use of any one of Claims 9-13, wherein the medicament is liquid.
15. The use of any one of claims 9-14, wherein the medicament comprises said AAV FVIII virus at a concentration of from 1E12 vg/ml to 2E14 vg/ml.
16. The use of claim 15, wherein the medicament comprises said AAV FVIII virus at a concentration of about 2E13 vg/ml.
17. The use of any one of Claims 9-16, wherein the medicament is useful for IV administration to a patient suffering from hemophilia A
18. The use of any one of Claims 1-17, wherein said subject is to be treated prophylactically with a corticosteroid at a concentration ranging from 5 mg/day to 60 mg/day.
19. The use of any one of Claims 1-18, 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.
20. The use of any one of Claims 1-19, wherein said subject is to be treated therapeutically with a corticosteroid at a concentration ranging from 5 mg/day to 60 mg/day.
21. The use of any one of Claims 1-20, 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.
22. The use of any one of Claims 1-21 further comprising the step of determination of the absence or presence of anti-AAV capsid antibodies in the serum of said subject after administration of said therapeutically effective amount of said recombinant AAV FVIII virus.

23. The use of Claim 22 further comprising the step of administration of an effective amount of a corticosteroid to said subject after a determination of the presence of anti-AAV capsid antibodies in the serum of said subject is made.