

CLAIMS:

1. A recombinant adeno-associated virus (rAAV) useful as a liver-directed therapeutic for hemophilia A, said rAAV comprising an AAV capsid, and a vector genome packaged therein, said vector genome comprising:

- (a) an AAV 5' inverted terminal repeat (ITR) sequence;
- (b) a transthyretin enhancer (enTTR);
- (c) a transthyretin (TTR) promoter;
- (d) a coding sequence encoding a human Factor VIII having coagulation

function; and

- (e) an AAV 3' ITR sequence;

wherein the coding sequence comprises a nucleotide sequence that is at least 95% identical to the nucleotide sequence as set forth in SEQ ID NO: 2.

~~2. The rAAV according to claim 1, wherein the human Factor VIII is a B-domain deleted factor VIII SQ which is about 1457 amino acid residues in length.~~

~~3. The rAAV according to claim 1 or claim 2, wherein the coding sequence of (d) is selected from SEQ ID NO: 1 and comprises the nucleotide sequence as set forth in SEQ ID NO: 2.~~

3. The rAAV according to claim 1 or claim 2, wherein the transthyretin (TTR) promoter comprises the nucleotide sequence as set forth in SEQ ID NO: 7.

4. The rAAV according to any one of claims 1 to 3, wherein the rAAV capsid is a hu37 capsid.

5. The rAAV according to any one of claims 1 to 4, wherein the AAV 5' ITR and/or the AAV 3' ITR is from AAV2.

6. The rAAV according to claim 1 or claim 2, wherein the enTTR comprises the nucleotide sequence as set forth in SEQ ID NO: 5.

~~67.~~ The rAAV according to any one of claims 1 to ~~56~~, wherein the vector genome further comprises a polyA ~~which is about 75 bp in size~~sequence.

8. The rAAV according to claim 7, wherein the polyA sequence comprises the nucleotide sequence as set forth in SEQ ID NO: 10.

~~79.~~ The rAAV according to any one of claims 1 to ~~68~~, wherein the vector genome is about 5 kilobases to about 5.5 kilobases in size.

10. The rAAV according to any one of claims 1 to 9, wherein the vector genome comprises nucleotides 1-5110 of the nucleotide sequence as set forth in SEQ ID NO: 13.

811. An aqueous suspension suitable for administration to a hemophilia A patient, said suspension comprising an aqueous suspending liquid and about 1×10^{12} GC/mL to about 1×10^{14} GC/mL of ~~a recombinant adeno-associated virus (rAAV) useful as a liver-directed therapeutic for hemophilia A, said~~the rAAV having an AAV capsid, and having packaged therein a vector genome comprising: according to any one of claims 1 to 10

- ~~— (a) an AAV 5' inverted terminal repeat (ITR) sequence;~~
- ~~— (b) a transthyretin enhancer (enTTR);~~
- ~~— (c) a transthyretin (TTR) promoter;~~
- ~~— (d) a coding sequence encoding a human Factor VIII having coagulation function;~~
- ~~— (e) an AAV 3' ITR.~~

912. The aqueous suspension according to claim 811, wherein the suspension is suitable for intravenous injection.

103. The aqueous suspension according to claim 811 or claim 912, wherein the suspension further comprises a surfactant, preservative, and/or buffer dissolved in the aqueous suspending liquid.

144. ~~A~~The rAAV according to any one claims 1 to 10 for use in ~~method of~~ treating a patient having hemophilia A ~~with an rAAV according to claim 1, wherein the rAAV is delivered about 1×10^{12} to about 1×10^{14} genome copies (GC)/kg in an aqueous suspension, wherein the GC are calculated as determined based on oqPCR.~~

15. Use of the rAAV virus according to any one of claims 1 to 10 in the preparation of a medicament for treating a patient having hemophilia A.

16. Use according to claim 15, wherein the rAAV is to be delivered at 1×10^{12} to 1×10^{14} genome copies (GC/kg) in an aqueous suspension

127. ~~The method~~Use according to claim 146, wherein the rAAV is to be readministered at first time point and a later time point.

13. ~~The rAAV according to claim 1, wherein the vector genome comprises nt 1-5110 of SEQ ID NO: 13.~~

14. ~~The rAAV according to claim 13, wherein the rAAV capsid is an hu37 capsid.~~

15. ~~The rAAV according to claim 1, wherein~~

~~the enTTR is SEQ ID NO:5;~~

~~the TTR promoter is SEQ ID NO:7;~~

~~the coding sequence is SED ID NO:2.~~

16. ~~The rAAV according to claim 15 wherein the AAV 5' ITR is SEQ ID NO:11 and the AAV 3' ITR is SEQ ID NO:12.~~

~~17. — The rAAV according to either of claims 15 or 16, further comprising a polyA sequence of SEQ ID NO:10.~~

~~18. — The rAAV according to any one of claims 15–17, wherein the rAAV capsid is an hu37 capsid.~~