Modified AAV Capsid Proteins for Treatment of Arthritic Disease

Field of the invention

The invention relates to the field of recombinant adeno-associated virus (rAAV) based gene therapy, in particular to the use of a mutant capsid rAAV in the treatment or prevention of an arthritic disease.

Background of the invention

Recombinant adeno-associated virus (rAAV) vectors have demonstrated excellent safety and efficacy profiles for the delivery of genes in humans *in vivo*. Therefore, rAAV vectors have been extensively used for *in vivo* gene therapy and have been shown safe and effective in pre-clinical models as well as in clinical trials. rAAV vectors have been successful in a number of gene therapy clinical trials for a range of diseases including haemophilia B, haemophilia A, cystic fibrosis, alpha-1 anti-trypsin deficiency, spinal muscular atrophy (SMA), Parkinson disease, Duchenne muscular dystrophy and Leber's congenital amaurosis (Selot *et al.*, Current Pharmaceutical Biotechnology, 2013, 14, 1072-1082). Alipogene tiparvovec (Glybera®, uniQure) has been granted marketing authorization in Europe as a gene therapy for the treatment of lipoprotein lipase deficiency (LPLD). Subsequently, gene therapy drug approval was granted for herpes-virus based Talimogene laherparepvec for the treatment of skin cancer (T-Vec, Imlygic®, Amgen) and for *ex vivo* stem cell retroviral-based gene therapy Strimvelis for the treatment of ADA-SCID (GSK).

rAAV vector-based gene therapy has also been applied in rheumatoid arthritis (RA), which is a chronic inflammatory disease that affects ~1% of the population. The pathology of RA extends throughout the synovial joint. The localized nature of the joint makes *in vivo* gene therapy very attractive. Therapies providing anti-inflammatory proteins aimed at shifting the balance in RA towards an anti-inflammatory state have been applied.

Much work has focused on the development of AAV capsid proteins with desired properties. Such properties can include higher transduction efficiency, tissue/organ tropism, de-targeting of non-desired tissues/organs, or avoidance of pre-existing neutralizing antibodies.

There is, however, still a need in the art to further improve rAAV gene therapy vectors. In particular, there is a need to improve the use of rAAV gene therapy vectors in arthritic disease and, more precisely, to improve the efficiency of delivering genetic material to the targeted tissue, such as the synovial joint or specific cell types within the synovial joint, preferably to fibroblast-like synoviocytes (FLS).

Summary

In one aspect, the present disclosure relates to a use of recombinant adeno-associated virus (rAAV) virion comprising (a) a modified VP1 capsid protein and (b) a promoter operably linked to a nucleotide sequence encoding a gene product of interest in the manufacture of a medicament for treating or preventing an arthritic disease or for treating or preventing a symptom associated

with an arthritic disease, wherein: the symptom is joint pain or the inflammation of one or more arthritic joints; the gene product of interest is an IL-6 inhibitor; and the modified VP1 capsid protein comprises EEEIxxxxPVATExxGxxxxNxQy – Z – $(x)_n$LPGMVWQxRDVYLQGPIWAKIPHTDG (SEQ ID NO: 27), wherein: (i) residues of sequence Z are exposed on the surface of the VP1 capsid protein; (ii) sequence Z comprises y – G – Q – x – G – $(x)_3$ – R – $(x)_3$ – y – A – Q – A – A (SEQ ID NO: 23); (iii) x represents a single amino acid residue; and (iv) y represents 0, 1 or 2 amino acid residues.

In another aspect, the present disclosure relates to a use of an rAAV composition in the manufacture of a medicament for treating or preventing an arthritic disease or for treating or preventing a symptom associated with an arthritic disease, wherein the symptom is joint pain or the inflammation of one or more arthritic joints and the rAAV composition comprises an rAAV virion as defined herein and a pharmaceutically acceptable carrier.

In yet another aspect, the present disclosure relates to a use of an rAAV composition and an immunosuppressant in the manufacture of a combination therapy for treating or preventing an arthritic disease or for treating or preventing symptoms associated with an arthritic disease, wherein the rAAV composition is as defined herein.

In some examples, the disclosure relates to the following numbered examples:

Example 1. A recombinant adeno-associated virus (rAAV) virion comprising a modified capsid protein and a promoter operably linked to a nucleotide sequence encoding a gene product of

Claims

1. Use of recombinant adeno-associated virus (rAAV) virion comprising (a) a modified VP1 capsid protein and (b) a promoter operably linked to a nucleotide sequence encoding a gene product of interest in the manufacture of a medicament for treating or preventing an arthritic disease or for treating or preventing a symptom associated with an arthritic disease, wherein:
the symptom is joint pain or the inflammation of one or more arthritic joints;
the gene product of interest is an IL-6 inhibitor; and
the modified VP1 capsid protein comprises EEEIxxxxPVATExxGxxxxNxQy – Z – $(x)_n$LPGMVWQxRDVYLQGPIWAKIPHTDG (SEQ ID NO: 27), wherein:
   (i) residues of sequence Z are exposed on the surface of the VP1 capsid protein;
   (ii) sequence Z comprises y – G – Q – x – G – $(x)_3$ – R – $(x)_3$ – y – A – Q – A – A (SEQ ID NO: 23);
   (iii) x represents a single amino acid residue; and
   (iv) y represents 0, 1 or 2 amino acid residues

2. The use according to claim 1, wherein the IL-6 inhibitor is selected from the group consisting of an IL-6 receptor antagonist, a humanised anti-IL-6 monoclonal antibody, a chimeric anti-IL-6 monoclonal antibody, a humanized rabbit anti-IL-6 monoclonal antibody and a soluble IL-6 receptor.

3. The use according to claim 1, wherein at least one of:
i) the IL-6 receptor antagonist is at least one of tocilizumab and sarilumab;
ii) the humanised anti-IL-6 monoclonal antibody is at least one of olokizumab and sirukumab;
iii) the chimeric anti-IL-6 monoclonal antibody is siltucimab; and
iv) the humanized rabbit anti-IL-6 monoclonal antibody is clazakizumab.

4. The use according to any one of the preceding claims, wherein the promoter is a constitutive promoter or an inducible promoter.

5. The use according to claim 4, wherein the promoter is an NFκB responsive promoter.

6. The use according to claim 5, wherein the NFκB responsive promoter is an NFκB responsive CMV promoter.

7. The use according to claim 6, wherein the NFκB responsive CMV promoter is an NFκB responsive minimal CMV promoter.

8. The use according to any one of claims 1 to 7, wherein the VP1 capsid protein comprises an amino acid sequence selected from the group consisting of:

i) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 1 and wherein amino acids at positions 588 – 602 of SEQ ID NO: 1 have at least 90% sequence identity with SEQ ID NO: 11,

ii) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 2 and wherein amino acids at positions 585 – 602 of SEQ ID NO: 2 have at least 90% sequence identity with SEQ ID NO: 10,

iii) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 3 and wherein amino acids at positions 587 – 601 of SEQ ID NO: 3 have at least 90% sequence identity with SEQ ID NO: 9,

iv) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 4 and wherein amino acids at positions 586 – 600 of SEQ ID NO: 4 have at least 90% sequence identity with SEQ ID NO: 8,

v) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 5 and wherein amino acids at positions 588 - 602 of SEQ ID NO: 5 have at least 90% sequence identity with SEQ ID NO: 9,

vi) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 6 and wherein amino acids at positions 588 - 602 of SEQ ID NO: 6 have at least 90% sequence identity with SEQ ID NO: 8, and

vii) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 7 and wherein amino acids at positions 587 – 604 of SEQ ID NO: 7 have at least 90% sequence identity with SEQ ID NO: 12,

wherein the modified VP1 capsid protein provides for an at least two-fold increase in expression in comparison to an unmodified VP1 capsid protein with an amino acid sequence selected from the group consisting of SEQ ID NO: 13 – 19, when tested under the same conditions.

9. The use according to claim 8, wherein the modified VP1 capsid protein provides for an at least two-fold increase in expression in human FLS cells, in comparison to an

unmodified VP1 capsid protein with an amino acid sequence selected from the group consisting of SEQ ID NO: 13 – 19, when tested under the same conditions.

10. The use according to claim 8 or 9, wherein the unmodified VP1 capsid protein has the amino acid sequence SEQ ID NO: 19 or has the same serotype as the modified VP1 capsid protein.

11. The use according to any one of claims 1 to 7, wherein the VP1 capsid protein comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO:1 – 7.

12. The use according to claim 11, wherein the VP1 capsid protein comprises or consists of SEQ ID NO: 4 or SEQ ID NO: 6.

13. Use of an rAAV composition in the manufacture of a medicament for treating or preventing an arthritic disease or for treating or preventing a symptom associated with an arthritic disease, wherein the symptom is joint pain or the inflammation of one or more arthritic joints and the rAAV composition comprises an rAAV virion as defined in any one of claims 1-12 and a pharmaceutically acceptable carrier.

14. The use according to any one of claims 1 to 13, wherein the arthritic disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile rheumatoid arthritis, osteoarthritis (OA), gout, pseudogout, spondyloarthritis (SpA), psoriatic arthritis, ankylosing spondylitis, septic arthritis, arthritis, juvenile idiopathic arthritis, joint replacement and Still's disease.

15. Use of an rAAV composition and an immunosuppressant in the manufacture of a combination therapy for treating or preventing an arthritic disease or for treating or preventing symptoms associated with an arthritic disease, wherein the rAAV composition is as defined in claim 13.

16. The use according to claim 15, wherein the arthritic disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile rheumatoid arthritis, osteoarthritis (OA), gout, pseudogout, spondyloarthritis (SpA), psoriatic arthritis, ankylosing spondylitis, septic arthritis, arthritis, juvenile idiopathic arthritis, joint replacement and Still's disease.

17. The use according to any one of claims 1 - 14, wherein the rAAV virion or the rAAV composition is to be administered systemically or locally.

18. The use according to claim 15 or 16, wherein at least one of the rAAV composition and the immunosuppressant is to be administered locally.

19. The use according to claim 18, wherein the local administration is intraarticular administration.