According to an aspect, the present invention provides an adeno-associated virus (AAV) vector comprising:

a first inverted terminal repeat (ITR) sequence;

a nucleic acid sequence encoding at least one apolipoprotein 4 (APOE4)-targeting miRNA comprising a nucleic acid sequence set forth in one or more of SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25;

an APOE4-targeting miRNA-resistant nucleic acid sequence encoding an apolipoprotein 2 (APOE2) polypeptide comprising a nucleic acid sequence having at least 95% sequence identity to any one of SEQ ID NO: 7 or SEQ ID NO: 26 – SEQ ID NO: 32;

and

a second ITR sequence.

In one example, the present disclosure provides a gene therapy vector for Alzheimer's disease. In one example, a gene therapy vector comprises an AAV expression vector encoding the human APOE2 gene and either in *cis* or in *trans* artificial microRNA(s) that target endogenous APOE4. This vector system silences the expression of detrimental endogenous APOE4 in combination with supplementation of the beneficial APOE2 gene from a gene therapy vector, e.g., an AAV vector. As disclosed herein, exemplary artificial microRNA sequences were designed that target the endogenous APOE4 mRNA for suppression. The microRNAs (miRNAs) may be incorporated in sequences that are 5' to the APOE2 coding sequence, e.g., in a n intron such as the CAG promoter intron, or sequences that are 3' to the APOE2 coding sequence, e.g., sequences that are 5' to the polyA tail of the vector transgene plasmid coding for the human APOE2 coding sequence. Alternatively, the microRNA(s) may be inserted between a PolIII promoter, e.g., a U6 promoter, and a terminator following the polyA site of the APOE2 expression cassette. The vector-derived human APOE2 DNA sequence optionally includes silent nucleotide changes to decrease or inhibit suppression by the microRNAs and in one example may include a tag such as a HA tag for detection, e.g., for pre-clinical detection studies. In one example, the expression construct is packaged into an AAV capsid of a serotype that targets astrocytes and glial cells (for example AAV9) the prominent sites of endogenous APOE expression in the CNS, but can be provided in other vectors, e.g., other viral vectors, plasmids, nanoparticle or liposomes.

In one aspect, the present disclosure refers to a gene therapy vector comprising a first promoter operably linked to a nucleic acid sequence comprising an open reading frame encoding APOE2 and a 3' untranslated region, and an isolated nucleotide sequence comprising one or more RNAi nucleic acid sequences for inhibition of APOE4 mRNA or a composition comprising the gene therapy vector which optionally comprises a pharmaceutically acceptable carrier.

In one example, a gene therapy vector is provided comprising a first promoter operably linked to a nucleic acid sequence comprising an open reading frame encoding APOE2 and a 3' untranslated region, and an isolated nucleotide sequence is provided comprising one or more RNAi nucleic acid sequences for inhibition of APOE4 mRNA. In one example, the vector comprises the nucleotide sequence. In one example, the nucleotide sequence is inserted 5' or 3' to the open reading frame. In one example, the nucleotide sequence is inserted 5' and 3' to the open reading frame. In one example, the nucleotide sequence is on a different vector. In one example, the isolated nucleotide sequence comprises a second promoter operably linked to the one or more RNAi nucleic acid sequences. In one example, the gene therapy vector is a viral

sequences and differs from the APOE4 RNAi sequences. In one example, the APOE4 that is inhibited has a sequence having at least 80%, 85%, 90%, 95% or more amino acid sequence identity to a polypeptide encoded by SEQ ID NO:22. In one example, the APOE2 has a sequence having at least 80%, 85%, 90%, 95% or more amino acid sequence identity to a polypeptide encoded by SEQ ID NO:9. In one example, the one or more RNAi nucleic acid sequences have at least 60%, 70%, 80%, 90% or more nucleotide sequence identity to one of SEQ ID Nos. 1-4 or 20-22 or the complement thereof. In one example, the vector has a first PolI promoter operably linked to a nucleic acid sequence comprising an open reading frame encoding human APOE2 and an isolated nucleotide sequence having one or more RNAi nucleic acid sequences for inhibition of human APOE4 mRNA. In one example, the nucleotide sequence is inserted 5' to the open reading frame. In one example, the nucleotide sequence is inserted 3' to the open reading frame. In one example, the nucleotide sequence is inserted 5' and 3' to the open reading frame. In one example, the isolated nucleotide sequence comprises a second promoter operably linked to the one or more RNAi nucleic acid sequences. In one example, the RNAi nucleic acid sequence is about 125 to 500, e.g., about 150 to 175, nucleotides in length. In one example the gene therapy vector may have 2, 3, 4 or more copies of the RNAi nucleic acid sequence which may include miRNA sequences, e.g., miRNA sequences which flank the APOE4 inhibitory sequences.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising the AAV vector of the invention.

In one aspect, the present disclosure refers to use of a pharmaceutical composition of the invention or the AAV vector of the invention in the manufacture of a medicament for preventing, inhibiting or treating Alzheimer's disease in a mammal.

In one example, a method to prevent, inhibit or treat Alzheimer's disease in a mammal is provided comprising: administering to the mammal an effective amount of a composition comprising the gene therapy vector. In one example, the composition comprises nanoparticles comprising the gene therapy vector or the different vector, or both. In one example, the gene therapy vector or the different vector, or both, comprise a viral vector. In one example, the mammal is a E2/E4 heterozygote. In one example, the mammal is a E4/E4 homozygote. In one example, the composition is systemically administered. In one example, the composition is orally administered. In one example, the composition is intravenously administered. In one example, the composition is locally administered. In one example, the composition is injected. In one example, the

WHAT IS CLAIMED IS:

1. An adeno-associated virus (AAV) vector comprising:

a first inverted terminal repeat (ITR) sequence;

a nucleic acid sequence encoding at least one apolipoprotein 4 (APOE4)-targeting miRNA comprising a nucleic acid sequence set forth in one or more of SEQ ID NO: 23, SEQ ID NO: 24, or SEQ ID NO: 25;

an APOE4-targeting miRNA-resistant nucleic acid sequence encoding an apolipoprotein 2 (APOE2) polypeptide comprising a nucleic acid sequence having at least 95% sequence identity to any one of SEQ ID NO: 7 or SEQ ID NO: 26 – SEQ ID NO: 32;

and

a second ITR sequence.

2. The AAV vector of claim 1, wherein the first and second ITR sequences are AAV2 ITR sequences.

3. The AAV vector of any one of claims 1 to 3, further comprising at least one promoter sequence.

4. The AAV vector of any one of claims 1 to 3, further comprising at least one enhancer sequence.

5. The AAV vector of any one of claims 1 to 4, wherein the promoter is a CAG promoter.

6. The AAV vector of any one of claims 1 to 5, wherein the enhancer sequence is a cytomegalovirus (CMV) enhancer sequence.

7. The AAV vector of any one of claims 1 to 6, further comprising a polyA sequence.

8. The AAV of any one of claims 1 to 7, wherein the at least one APOE4-targeting miRNA sequence is inserted into an intron or 3’ untranslated

region of the AAV vector.

9. The AAV vector of any one of claims 1 to 8, wherein the AAV vector is packaged as an AAV viral vector comprising an AAV capsid protein.

10. The AAV vector of any one of claims 1 to 9, wherein the AAV capsid protein is an AAV1 capsid protein, an AAV2 capsid protein, an AAV5 capsid protein, an AAV7 capsid protein, an AAV8 capsid protein, an AAV9 capsid protein, or an AAVrh10 capsid protein.

11. The AAV vector of any one of claims 1 to 10, wherein the AAV capsid protein is an AAVrh10 capsid protein.

12. The AAV vector of any one of claims 1 to 7, wherein the AAV vector comprises two, three, four, or more miRNA sequences.

13. A pharmaceutical composition comprising the AAV vector of any one of claims 1 to 12.

14. The pharmaceutical composition of claim 13, wherein the composition is formulated for systemic, oral, intravenous, or local administration.

15. The pharmaceutical composition of claim 13, wherein the composition is formulated for administration to the central nervous system.

16. Use of a pharmaceutical composition of any one of claims 13 to 15 or the AAV vector of any one of claims 1 to 12 in the manufacture of a medicament for preventing, inhibiting or treating Alzheimer's disease in a mammal.

17. The use of claim 16, wherein the composition comprises liposomes comprising the AAV vector.

18. The use of claim 16 or 17, wherein the composition comprises

nanoparticles comprising the AAV vector.

19. The use of any one of claims 16 to 18, wherein the mammal is a E3/E4 heterozygote or E2/E4 heterozygote.

20. The use of any one of claims 16 to 19, wherein the mammal is a E4/E4 homozygote.

21. The use of any one of claims 16 to 20, wherein the composition is to be systemically administered.

22. The use of any one of claims 16 to 21 wherein the composition is to be orally or intravenously administered.

23. The use of any one of claims 16 to 22 wherein the composition is to be locally administered.

24. The use of any one of claims 16 to 23 wherein the composition is to be injected.

25. The use of any one of claims 16 to 24 wherein the composition is to be administered to the central nervous system.

26. The use of any one of claims 16 to 25 wherein the composition is to be administered to the brain.

27. The use of any one of claims 16 to 26 wherein the composition is a sustained release composition.

28. The use of any one of claims 16 to 27 wherein the mammal is a human.