Claims

1. A composition comprising:

(i) a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent, wherein:

a. the open reading frame comprises a sequence with at least 93% identity to SEQ ID NO: 311;

b. the open reading frame has at least 93% identity to SEQ ID NO: 311 over at least its first 50, 200, 250, or 300 nucleotides, or at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides;

c. the open reading frame consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4, the low A set of Table 5, or the low A/U set of Table 5;

d. the open reading frame has an adenine content ranging from its minimum adenine content to 123% of the minimum adenine content; or

e. the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content; and

(ii) a guide RNA or a vector encoding a guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82.

2. A method of modifying the *TTR* gene or inducing a double-stranded break (DSB) within the *TTR* gene, comprising delivering a composition to a cell, wherein the composition comprises:

(i) a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent, wherein:

a. the open reading frame comprises a sequence with at least 93% identity to SEQ ID NO:311;

b. the open reading frame has at least 93% identity to SEQ ID NO: 311 over at least its first 50, 200, 250, or 300 nucleotides, or at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides;

c. the open reading frame consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4, the low A set of Table 5, or the low A/U set of Table 5;

d. the open reading frame has an adenine content ranging from its minimum adenine content to 123% of the minimum adenine content; or

e. the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content; and

(ii) a guide RNA or a vector encoding a guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82.

3. Use of a composition in the manufacture of a medicament for reducing TTR serum concentration, treating amyloidosis associated with TTR (ATTR), or reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in a subject, wherein the composition comprises:

(i) a nucleic acid comprising an open reading frame encoding an RNA-guided DNA binding agent, wherein:

a. the open reading frame comprises a sequence with at least 95% identity to SEQ ID NO:311;

b. the open reading frame has at least 95% identity to SEQ ID NO: 311 over at least its first 30, 50, 70, 100, 150, 200, 250, or 300 nucleotides;

c. the open reading frame consists of a set of codons of which at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons are codons listed in Table 4, the low A set of Table 5, or the low A/U set of Table 5;

d. the open reading frame has an adenine content ranging from its minimum adenine content to 150% of the minimum adenine content; or

e. the open reading frame has an adenine dinucleotide content ranging from its minimum adenine dinucleotide content to 150% of the minimum adenine dinucleotide content; and

(ii) a guide RNA or a vector encoding a guide RNA, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5-72, 74-78, and 80-82, thereby reducing TTR serum concentration, treating amyloidosis associated with TTR (ATTR), or reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in the subject.

1. A composition comprising:

(i) a nucleic acid encoding a Cas9, wherein the nucleic acid comprises the sequence of SEQ ID NO: 377; and

(ii) a single guide RNA (sgRNA) comprising the sequence of SEQ ID NO: 87.

2. Use of a composition in the manufacture of a medicament for treating amyloidosis associated with TTR (ATTR), reducing TTR serum concentration, or reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR, in a subject in need of, wherein the composition comprises: (i) a nucleic acid encoding a Cas9, wherein the nucleic acid comprises the sequence of SEQ ID NO: 377; and (ii) a single guide RNA (sgRNA) comprising the sequence of SEQ ID NO: 87.

3. Use of a composition in the manufacture of a medicament for treating amyloidosis associated with TTR (ATTR), reducing TTR serum concentration, or reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR, in a human subject in need of, wherein the composition comprises: (i) a nucleic acid comprising an open reading frame (ORF) encoding a Cas9, wherein the ORF comprises the sequence of SEQ ID NO: 311; and (ii) a single guide RNA (sgRNA) comprising the sequence of SEQ ID NO: 87.

4. Use of a composition in the manufacture of a medicament for modifying the *TTR* gene or inducing a double-stranded break (DSB) within the *TTR* gene, in a subject in need of, wherein the composition comprises: (i) a nucleic acid encoding a Cas9, wherein the nucleic acid comprises the sequence of SEQ ID NO: 377; and (ii) a single guide RNA (sgRNA) comprising the sequence of SEQ ID NO: 87.

5. Use of a composition in the manufacture of a medicament for modifying the *TTR* gene or inducing a double-stranded break (DSB) within the *TTR* gene, in a human subject in need of, wherein the composition comprises: (i) a nucleic acid comprising an open reading frame (ORF) encoding a Cas9, wherein the ORF comprises the sequence of SEQ ID NO: 311; and (ii) a single guide RNA (sgRNA) comprising the sequence of SEQ ID NO: 87.

~~4. The composition of claim 1, method of claim 2, or use of claim 3, wherein the guide RNA comprises a guide sequence selected from SEQ ID NOs: 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 22, 23, 27, 29, 30, 35, 36, 37, 38, 55, 61, 63, 65, 66, 68, or 69.~~

~~5.~~6. The composition of claim 1~~ or 4~~, for use in (1) inducing a double-stranded break (DSB) within the *TTR* gene in a cell or subject; (2) modifying the *TTR* gene in a cell or subject; (3) treating amyloidosis associated with TTR (ATTR) in a subject; (4) reducing TTR serum concentration in a subject; or (5) reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

~~6.~~7. The use of ~~claim 3 or 4~~ any one of claims 2-5, wherein the composition is to be administered by infusion for more than 30 minutes.

~~7.~~8. The ~~method of claim 2 or 4,~~ composition of claim 1 or 6 or the use of any one of claims ~~3-4 or 6~~ 2-5 or 7, wherein the composition results in editing of the TTR gene.

~~8. The composition of any one of claims 1 or 4-5, method of any one of claims 2, 4 or 7, or use of any one of claims 3-4 or 6-7, wherein the guide RNA comprises a crRNA that comprises the guide sequence and further comprises a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end.~~

~~9. The composition of any one of claims 1, 4-5 or 8, method of any one of claims 2, 4 or 7-8, or use of any one of claims 3-4 or 6-8, wherein the guide RNA is a single guide (sgRNA).~~

~~10. The composition, method, or use of claim 9, wherein the sgRNA comprises a guide sequence that has the pattern of SEQ ID NO: 3.~~

~~11. The composition, method, or use of claim 9 or 10, wherein the sgRNA comprises the sequence of SEQ ID NO: 3.~~

~~12. The composition, method, or use of any one of claims 9-11, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-72, 74-78, and 80-82 and the nucleotides of SEQ ID NO: 126.~~

~~13. The composition, method, or use of any one of claims 9-11, wherein the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID Nos: 87-113, 115-120, and 122-124 or a sequence selected from SEQ ID Nos: 87-113, 115-120, and 122-124.~~

~~14. The composition of any one of claims 1, 4-5 or 8-13, method of any one of claims 2, 4 or 7-13, or use of any one of claims 3-4 or 6-13, wherein the guide RNA comprises at least one modification.~~

~~15. The composition, method, or use of claim 14, wherein the at least one modification includes~~

~~(i) a 2'-O-methyl (2'-O-Me) modified nucleotide, a phosphorothioate (PS) bond between nucleotides, or a 2'-fluoro (2'-F) modified nucleotide;~~

~~(ii) a modification at one or more of the first five nucleotides at the 5' end or one or more of the last five nucleotides at the 3' end;~~

~~(iii) PS bonds between the first four nucleotides or between the last four nucleotides; or~~

~~(iv) 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end or 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end; or~~

~~combinations of one or more of (i)-(iv).~~

~~16. The composition, method, or use of claim 14 or 15, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.~~

~~17.~~9. The composition of any one of claims ~~1, 4-5 or 8-16~~ 1, 6 or 8, ~~method of any one of claims 2, 4 or 7-16,~~ or the use of any one of claims ~~3-4 or 6-16~~ 3, 5, 7 or 8, wherein the guide RNA and the nucleic acid encoding the Cas9~~comprising an open reading frame encoding an RNA-guided DNA binding agent~~ are associated with a lipid nanoparticle (LNP).

10. The use of any one of claims 2, 4, 7 or 8, wherein the sgRNA and the nucleic acid comprising an open reading frame encoding a Cas9 are associated with a lipid nanoparticle (LNP).

~~18.~~11. The composition of claim 9~~, method,~~ or the use of claim ~~17~~ 9 or 10, wherein the LNP comprises ~~a CCD~~ an amine lipid, a helper lipid, a stealth lipid, or a neutral lipid.

~~19.~~12. The composition of claim 9 or 11~~, method,~~ or the use of ~~claim 18~~ any one of claims 9-11, wherein the ~~CCD~~ amine lipid is lipid A; the helper lipid is cholesterol; the stealth lipid is ~~PEG2k-DMG~~ PEG-DMG; or the neutral lipid is DSPC.

~~20.~~13. The composition of any one of claims ~~17-19~~ 9, 11 or 12, ~~method of any one of claims 17-19,~~ or the use of ~~claim 17 or 18~~ any one of claims 9-12, wherein:

(i) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 50-60 mol-% amine lipid such as Lipid A, ~~about~~ 8-10 mol-% neutral lipid; and ~~about~~ 2.5-4 mol-% stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is ~~about~~ 6;

(ii) the LNP comprises ~~about~~ 50-60 mol-% amine lipid such as Lipid A; ~~about~~ 27-39.5 mol-% helper lipid; ~~about~~ 8-10 mol-% neutral lipid; and ~~about~~ 2.5-4 mol-% stealth lipid (e.g., a PEG lipid), wherein the N/P ratio of the LNP composition is ~~about~~ 5-7 (e.g., ~~about~~ 6);

(iii) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 50-60 mol-% amine lipid such as Lipid A; ~~about~~ 5-15 mol-% neutral lipid; and ~~about~~ 2.5-4 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is ~~about~~ 3-10;

(iv) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 40-60 mol-% amine lipid such as Lipid A; ~~about~~ 5-15 mol-% neutral lipid; and ~~about~~ 2.5-4 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is ~~about~~ 6;

(v) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 50-60 mol-% amine lipid such as Lipid A; ~~about~~ 5-15 mol-% neutral lipid; and ~~about~~ 1.5-10 mol-%

Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is ~~about~~ 6;

(vi) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 40-60 mol-% amine lipid such as Lipid A; ~~about~~ 0-10 mol-% neutral lipid; and ~~about~~ 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is ~~about~~ 3-10;

(vii) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 40-60 mol-% amine lipid such as Lipid A; less than ~~about~~ 1 mol-% neutral lipid; and ~~about~~ 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is ~~about~~ 3-10;

(viii) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 40-60 mol-% amine lipid such as Lipid A; and ~~about~~ 1.5-10 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, wherein the N/P ratio of the LNP composition is ~~about~~ 3-10, and wherein the LNP composition is essentially free of or free of neutral phospholipid;

(ix) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 50-60 mol-% amine lipid such as Lipid A; ~~about~~ 8-10 mol-% neutral lipid; and ~~about~~ 2.5-4 mol-% Stealth lipid (e.g., a PEG lipid), wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is ~~about~~ 3-7;

(x) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 50 mol-% amine lipid; ~~about~~ 9 mol-% neutral lipid; ~~about~~ 3 mol-% of stealth lipid, and the remainder of the lipid component is helper lipid wherein the N/P ratio of the LNP composition is ~~about~~ 6; or

(xi) the LNP comprises a lipid component and the lipid component comprises: ~~about~~ 50 mol-% Lipid A; ~~about~~ 9 mol-% DSPC; ~~about~~ 3 mol-% of PEG2k-DMG, and the remainder of the lipid component is cholesterol wherein the N/P ratio of the LNP composition is ~~about~~ 6.

~~21.~~14. The composition of any one of claims ~~17-20~~ 9 or 11-13, ~~method of any one of claims 17-20,~~ or the use of any one of claims ~~17-18 or 20~~ 9-13, wherein the LNP has an N/P ratio of ~~about~~ 6.

~~22. The composition of any one of claims 1, 4-5 or 8-21, method of any one of claims 2, 4 or 7-21, or use of any one of claims 3-4, 6-18 or 20-21, wherein the RNA-guided DNA binding agent is a Cas cleavase.~~

~~23. The composition, method, or use of claim 22, wherein the RNA-guided DNA binding agent is Cas9.~~

~~24. The composition of any one of claims 1, 4-5 or 8-23, method of any one of claims 2, 4 or 7-23, or use of any one of claims 3-4, 6-18 or 20-23, wherein the RNA-guided DNA binding agent is modified, optionally wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).~~

~~25.~~15. The composition of any one of claims ~~1, 4-5 or 8-24~~ 1, 6, 8, 9, or 11-14~~, method of any one of claims 2, 4 or 7-24,~~ or the use of any one of claims ~~3-4, 6-18 or 20-24~~ 2-5 or 7-14, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.

~~26.~~16. The ~~method of any one of claims 2, 4 or 7-25, or~~ use of any one of claims ~~3-4, 6-18 or 20-25~~ 2-5 or 7-15, wherein non-homologous end joining (NHEJ) leads to a mutation during repair of a DSB in the TTR gene that induces a frame shift or nonsense mutation in the *TTR* gene.

~~27. The composition of any one of claims 1, 4-5 or 8-25, method of any one of claims 2, 4 or 7-26, or use of any one of claims 3-4, 6-18 or 20-26, wherein the sequence of the guide RNA is:~~

~~a) SEQ ID NO: 92 or 104;~~

~~b) SEQ ID NO: 87, 89, 96, or 113;~~

~~c) SEQ ID NO: 100, 102, 106, 111, or 112; or~~

~~d) SEQ ID NO: 88, 90, 91, 93, 94, 95, 97, 101, 103, 108, or 109,~~

~~optionally wherein the guide RNA does not produce indels at off-target site(s) that occur in a protein coding region in the genome of primary human hepatocytes.~~

~~28.~~17. The use of any one of claims ~~3-4 or 6-27~~ 2, 4, or 7-16, wherein the subject is human.

~~29.~~18. The use of any one of claims ~~3-4 or 6-28~~ 2-5 or 7-17, wherein the subject has familial amyloid polyneuropathy, only or predominantly nerve symptoms of ATTR, familial amyloid cardiomyopathy, or only or predominantly cardiac symptoms of ATTR.

~~30.~~19. The use of any one of claims ~~3-4 or 6-29~~ 2-5 or 7-18, wherein the subject expresses TTR having a V30 mutation, such as V30A, V30G, V30L, or V30M; the subject expresses TTR having a T60 mutation, such as T60A; the subject expresses TTR having a V122 mutation, such as V122A, V122I, or V122(-); or the subject expresses wild-type TTR.

~~31.~~20. The ~~method of any one of claims 2, 4 or 7-27, or~~ use of any one of claims ~~3-4 or 6-30~~ 2-5 or 7-19, wherein the composition ~~or pharmaceutical formulation~~ is to be ~~delivered or~~ administered via a viral vector or via lipid nanoparticles.

~~32. The composition of any one of claims 1, 4-5, 8-25 or 27, method of any one of claims 2, 4, 7-27 or 31, or use of any one of claims 3-4 or 6-31, wherein the sequence selected from~~

~~SEQ ID NOs: 5-72, 74-78, and 80-82 is SEQ ID NO: 5, 6, 9, 13, 14, 15, 16, 17, 22, 23, 27, 30, 35, 36, 37, 38, 55, 63, 65, 66, 68, or 69.~~

~~33. The composition of any one of claims 1, 4-5, 8-25, 27 or 32, method of any one of claims 2, 4, 7-27 or 31-32, or use of any one of claims 3-4 or 6-32, wherein the open reading frame comprises a sequence with at least 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 311.~~

~~34. The composition of any one of claims 1, 4-5, 8-25, 27 or 32-33, method of any one of claims 2, 4, 7-27 or 31-33, or use of any one of claims 3-4 or 6-33, wherein at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% of the codons of the open reading frame are codons listed in Table 4, Table 5, or Table 7.~~

~~35. The composition of any one of claims 1, 4-5, 8-25, 27 or 32-34, method of any one of claims 2, 4, 7-27 or 31-34, or use of any one of claims 3-4 or 6-34, wherein the open reading frame comprises a sequence with at least 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 377.~~

~~36.~~21. The composition of any one of claims ~~1, 4-5, 8-25, 27 or 32-35~~ 1, 6, 8, 9, or 11-15, ~~method of any one of claims 2, 4, 7-27 or 31-35,~~ or the use of any of claims ~~3-4 or 6-35~~ 2-5 or 7-19, wherein the nucleic acid is an mRNA in which at least 10% of the uridine is substituted with a modified uridine.