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.

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. 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. The use of claim 3 or 4, wherein the composition is to be administered by infusion for more than 30 minutes.

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

18. The composition, method, or use of claim 17, wherein the LNP comprises a CCD lipid, a helper lipid, a stealth lipid, or a neutral lipid.

19. The composition, method, or use of claim 18, wherein the CCD lipid is lipid A; the helper lipid is cholesterol; the stealth lipid is PEG2k-DMG; or the neutral lipid is DSPC.

20. The composition of any one of claims 17-19, method of any one of claims 17-19, or use of claim 17 or 18, 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. The composition of any one of claims 17-20, method of any one of claims 17-20, or use of any one of claims 17-18 or 20, 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. The composition of any one of claims 1, 4-5 or 8-24, method of any one of claims 2, 4 or 7-24, or use of any one of claims 3-4, 6-18 or 20-24, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.

26. 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, 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. The use of any one of claims 3-4 or 6-27, wherein the subject is human.

29. The use of any one of claims 3-4 or 6-28, 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. The use of any one of claims 3-4 or 6-29, 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. The method of any one of claims 2, 4 or 7-27, or use of any one of claims 3-4 or 6-30, 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. The composition of any one of claims 1, 4-5, 8-25, 27 or 32-35, method of any one of claims 2, 4, 7-27 or 31-35, or use of any of claims 3-4 or 6-35, wherein the nucleic acid is an mRNA in which at least 10% of the uridine is substituted with a modified uridine.