Claims

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.

6. The composition of claim 1, 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.

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

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

9. The composition of any one of claims 1, 6 or 8, or the use of any one of claims 3, 5, 7 or 8, wherein the guide RNA and the nucleic acid encoding the Cas9 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).

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

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

13. The composition of any one of claims 9, 11 or 12, or the use of any one of claims 9-12, wherein:

(i) the LNP comprises a lipid component and the lipid component comprises: 50-60 mol-% amine lipid such as Lipid A, 8-10 mol-% neutral lipid; and 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 6;

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

(iii) the LNP comprises a lipid component and the lipid component comprises: 50-60 mol-% amine lipid such as Lipid A; 5-15 mol-% neutral lipid; and 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 3-10;

(iv) the LNP comprises a lipid component and the lipid component comprises: 40-60 mol-% amine lipid such as Lipid A; 5-15 mol-% neutral lipid; and 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 6;

(v) the LNP comprises a lipid component and the lipid component comprises: 50-60 mol-% amine lipid such as Lipid A; 5-15 mol-% neutral lipid; and 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 6;

(vi) the LNP comprises a lipid component and the lipid component comprises: 40-60 mol-% amine lipid such as Lipid A; 0-10 mol-% neutral lipid; and 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 3-10;

(vii) the LNP comprises a lipid component and the lipid component comprises: 40-60 mol-% amine lipid such as Lipid A; less than 1 mol-% neutral lipid; and 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 3-10;

(viii) the LNP comprises a lipid component and the lipid component comprises: 40-60 mol-% amine lipid such as Lipid A; and 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 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: 50-60 mol-% amine lipid such as Lipid A; 8-10 mol-% neutral lipid; and 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 3-7;

(x) the LNP comprises a lipid component and the lipid component comprises: 50 mol-% amine lipid; 9 mol-% neutral lipid; 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 6; or

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

14. The composition of any one of claims 9 or 11-13, or the use of any one of claims 9-13, wherein the LNP has an N/P ratio of 6.

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

16. The use of any one of claims 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.

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

18. The use of any one of claims 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.

19. The use of any one of claims 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.

20. The use of any one of claims 2-5 or 7-19, wherein the composition is to be administered via a viral vector or via lipid nanoparticles.

21. The composition of any one of claims 1, 6, 8, 9, or 11-15, or the use of any of claims 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.