Claims

1. A composition comprising:

(i) a messenger RNA (mRNA) encoding a Cas9 nuclease, wherein the mRNA comprises the sequence of SEQ ID NO: 377; and
(ii) a single guide RNA (sgRNA) comprising the sequence of SEQ ID NO: 87 (mA*mA*mA*GGCUGCUGAUGACACCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU, wherein * indicates a PS bond and "m" indicates that the nucleotide is a 2'-O-Me-modified nucleotide).

2. Use of a composition in the manufacture of a medicament for treating amyloidosis associated with deposition of transthyretin (ATTR) in a subject, wherein the composition comprises: (i) a messenger RNA (mRNA) encoding a Cas9 nuclease, wherein the mRNA comprises the sequence of SEQ ID NO: 377; and (ii) a single guide RNA (sgRNA) comprising the sequence of SEQ ID NO: 87 (mA*mA*mA*GGCUGCUGAUGACACCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU, wherein * indicates a PS bond and "m" indicates that the nucleotide is a 2'-O-Me-modified nucleotide).

3. Use of a composition in the manufacture of a medicament for treating amyloidosis associated with deposition of transthyretin (ATTR) in a human subject, wherein the composition comprises: (i) a messenger RNA (mRNA) comprising an open reading frame (ORF) encoding a Cas9 nuclease, wherein the mRNA comprises the sequence of SEQ ID NO: 311, wherein each thymidine in SEQ ID NO: 311 is replaced with a modified or unmodified uridine; and (ii) a single guide RNA (sgRNA) comprising the sequence of SEQ ID NO: 87 (mA*mA*mA*GGCUGCUGAUGACACCUGUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU, wherein * indicates a PS bond and "m" indicates that the nucleotide is a 2'-O-Me-modified nucleotide).

4. The composition of claim 1, for use in treating amyloidosis associated with deposition of transthyretin (ATTR) in a subject; or reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

5. The use of claim 2 or 3, wherein the composition is to be administered by infusion for more than 30 minutes.

6. The composition of claim 1, the use of any one of claims 2-3 and 5, or the composition for use of claim 4, wherein the composition results in editing of the *TTR* gene.

7. The composition of claim 1 or 6, the use of any one of claims 2-3, 5, and 6, or the composition for use of claim 4 or 6, wherein the sgRNA and the mRNA are associated with a lipid nanoparticle (LNP).

8. The composition of any one of claims 1, 6, and 7, the use of any one of 2, 3, and 5-7, or the composition for use of any one of claims 4 and 6-7, wherein the LNP comprises an amine lipid.

9. The composition of claim 8, the use of claim 8, or the composition for use of claim 8, wherein the LNP further comprises a helper lipid, a stealth lipid, and, optionally, a neutral lipid.

10. The composition of claim 9, the use of claim 9, or the composition for use of claim 9, wherein the amine lipid is (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate (lipid A); the helper lipid is cholesterol; the stealth lipid is 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DMG); or the neutral lipid is distearoylphosphatidylcholine (DSPC).

11. The composition of any one of claims 7-10, the use of any one of claims 7-10, or the composition for use of any one of claims 7-10, wherein the N/P ratio of the LNP composition is 6 ± 0.5.

12. The composition of any one of claims 7-11, the use of any one of claims 7-11, or the composition for use of any one of claims 7-11, wherein the LNP comprises a lipid component and the lipid component comprises:

(i) 50-60 mol-% amine lipid; 8-10 mol-% neutral lipid; 2.5-4 mol-% stealth lipid; and 25-40 mol% helper lipid or an amount of the helper lipid that brings the lipid component to 100 mol-%, wherein the N/P ratio of the LNP composition is 6;

(ii) 50-60 mol-% amine lipid; 8-10 mol-% neutral lipid; 2.5-4 mol-% stealth lipid; and 25-40 mol% helper lipid or an amount of the helper lipid that brings the lipid component to 100 mol-%, wherein the N/P ratio of the LNP composition is 5-7;

(iii) 50-60 mol-% amine lipid; 5-15 mol-% neutral lipid; 2.5-4 mol-% stealth lipid; and 25-40 mol% helper lipid or an amount of the helper lipid that brings the lipid component to 100 mol-%, wherein the N/P ratio of the LNP composition is 3-10;

(iv) 40-60 mol-% amine lipid; 5-15 mol-% neutral lipid; 2.5-4 mol-% stealth lipid; and 25-50 mol% helper lipid or an amount of the helper lipid that brings the lipid component to 100 mol-%, wherein the N/P ratio of the LNP composition is 6;

(v) 50-60 mol-% amine lipid; 5-15 mol-% neutral lipid; 1.5-10 mol-% stealth lipid; and 25-40 mol% helper lipid or an amount of the helper lipid that brings the lipid component to 100 mol-%, wherein the N/P ratio of the LNP composition is 6;

(vi) 40-60 mol-% amine lipid; 0-10 mol-% neutral lipid; 1.5-10 mol-% stealth lipid; and 25-55 mol-% helper lipid or an amount of the helper lipid that brings the lipid component to at least 99 mol-%, wherein the N/P ratio of the LNP composition is 3-10;

(vii) 40-60 mol-% amine lipid; less than 1 mol-% neutral lipid; 1.5-10 mol-% stealth lipid; and 30-50 mol% helper lipid or an amount of the helper lipid that brings the lipid component to 100 mol-%, wherein the N/P ratio of the LNP composition is 3-10;

(viii) 40-60 mol-% amine lipid; 1.5-10 mol-% stealth lipid; and 20-60 mol-% helper lipid or an amount of the helper lipid that brings the lipid component to at least 99 mol-%, wherein the N/P ratio of the LNP composition is 3-10 and the LNP composition is essentially free of or free of neutral phospholipid; or

(ix) 50-60 mol-% amine lipid; 8-10 mol-% neutral lipid; 2.5-4 mol-% stealth lipid; and 25-40 mol-% helper lipid or an amount of the helper lipid that brings the lipid component to 100 mol-%, wherein the N/P ratio of the LNP composition is 3-7.

13. The composition of any one of claims 7-11, the use of any one of claims 7-11, or the composition for use of any one of claims 7-11, wherein:

(i) the LNP comprises a lipid component and the lipid component comprises: 50-60 mol-% amine lipid, 8-10 mol-% neutral lipid; and 2.5-4 mol-% stealth 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 a lipid component and the lipid component comprises: 50-60 mol-% amine lipid ; 27-39.5 mol-% helper lipid; 8-10 mol-% neutral lipid; and 2.5-4 mol-% stealth lipid, wherein the N/P ratio of the LNP composition is 5-7 ;

(iii) the LNP comprises a lipid component and the lipid component comprises: 50-60 mol-% amine lipid ; 5-15 mol-% neutral lipid; and 2.5-4 mol-% stealth 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 ; 5-15 mol-% neutral lipid; and 2.5-4 mol-% stealth 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 ; 5-15 mol-% neutral lipid; and 1.5-10 mol-% stealth 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 ; 0-10 mol-% neutral lipid; and 1.5-10 mol-% stealth 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 , 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 ; and 1.5-10 mol-% stealth 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 ; 8-10 mol-% neutral lipid; and 2.5-4 mol-% stealth 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-% 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-% (9Z, 12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate; 9 mol-% DSPC; 3 mol-% 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 7-13, the use of any one of claims 7-13, or the composition for use of any one of claims 7-13, wherein the N/P ratio of the LNP composition is 6.

15. The use of any one of claims 3 and 5-14, wherein the mRNA further comprises (a) a 5' UTR with at least 90% identity to any one of SEQ ID NOs: 232, 234, 236, 238, 241, and 275-277, wherein each thymidine in the any one of SEQ ID NOs: 232, 234, 236, 238, 241, and 275-277 is replaced with a modified or unmodified uridine; or (b) a 3' UTR with at least 90% identity to any one of SEQ ID NOs: 233, 235, 237, 239, and 240, wherein each thymidine in any one of SEQ ID NO: 233, 235, 237, 239, and 240 is replaced with a modified or unmodified uridine.

16. The use of any one of claims 3 and 5-15, wherein the mRNA further comprises a 5' UTR and a 3' UTR from the same source, wherein the source is a constitutively expressed mRNA.

17. The composition of any one of claims 1 and 6-16, the use of any one of claims 2, 3, and 5-16, or the composition for use of any one of claims 4 and 6-16, wherein the mRNA further comprises a 5' cap selected from Cap0, Cap1, and Cap2.

18. The composition of any one of claims 1 and 6-17, or the use of any one of claims 2, 3, and 5-17, or the composition for use of any one of claims 4 and 6-17, wherein the mRNA further comprises a cap analog comprising 7-methylguanine 3'-methoxy-5'-triphosphate linked to the 5' position of a guanine ribonucleotide.

19. The composition of any one of claims 1 and 6-18, the use of any one of claims 2, 3, and 5-18, or the composition for use of any one of claims 4 and 6-18, wherein the composition is a pharmaceutical formulation.

20. The use of any one of claims 2, 3, and 5-19 or the composition for use of any one of claims 4 and 6-19, wherein the subject is human.

21. The use of any one of claims 2, 3, and 5-20 or the composition for use of any one of claims 4 and 6-20, wherein the subject has ATTRwt, hereditary ATTR, familial amyloid polyneuropathy, only or predominantly nerve symptoms of ATTR, familial amyloid cardiomyopathy, or only or predominantly cardiac symptoms of ATTR.

22. The use of any one of claims 2, 3, and 5-21 or the composition for use of any one of claims 4 and 6-21, 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.

23. The composition of any one of claims 1 and 6-22 the use of any of claims 2, 3, and 5-22, or the composition for use of any one of claims 4 and 6-22, wherein at least 10% of the uridine in the mRNA is a modified uridine.

24. The composition of any one of claims 1 and 6-23, the use of any of claims 2, 3, and 5-23, or the composition for use of any one of claims 4 and 6-23, wherein 100% of the uridine in the mRNA is modified uridine.

25. The composition of claim 23 or 24, the use of claim 23 or 24, or the composition for use of claim 23 or 24, wherein the modified uridine is one or more of N1-methyl-pseudouridine, pseudouridine, 5-methoxyuridine, and 5-iodouridine.

26. The composition of claim 24 or 25, the use of claim 24 or 25, or the composition for use of claim 24 or 25 wherein the modified uridine is N1-methyl-pseudouridine.