therapeutically effective amount of any of the dsRNAs described in the Summary, above. In related embodiments, the dsRNA is administered to the human at about 0.01, 0.1, 0.5, 1.0, 2.5, or 5.0 mg/kg. In a particular related embodiment, the dsRNA is administered to the human at about 1.0 mg/kg. In another related embodiment, the human has transthyretin amyloidosis, and/or a liver disorder. In yet another embodiment of the use provided by the invention, the treated human is further provided a liver transplant.

In yet another embodiment, the invention provides the use of a dsRNA in a method for inhibiting TTR expression in a cell, wherein the method comprises (a) contacting the cell with a dsRNA described in the Summary, above; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TTR gene, thereby inhibiting expression of the TTR gene in the cell.

The details of one or more embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

Description of the Drawings

FIG. 1 is a graph of TNFalpha and IFNalpha levels in cultured human PBMCs following transfection with TTR siRNAs.

FIG. 2A and 2B are dose response curves for AD-18324 and AD-18328, respectively, in HepG2 cells.

FIG. 3 is a dose response curve for AD-18246 in HepG2 cells.

FIG. 4A and FIG. 4B show inhibition of liver mRNA and plasma protein levels, respectively, in transgenic H129-mTTR-KO/iNOS-KO/hTTR mice by an intravenous bolus administration of TTR-dsRNA (AD-18324, AD-18328 and AD-18246) formulated in LNP01.

FIG. 5 is a graph summarizing the measurements of TTR mRNA levels in livers of non-human primates following 15-minute intravenous infusion of TTR-dsRNA (AD-18324 and AD-18328) formulated in SNALP.

FIG. 6A and FIG. 6B show inhibition of human V30M TTR liver mRNA and serum protein levels, respectively, in transgenic mice by an intravenous bolus administration of SNALP-18328. Group means were determined, normalized to the PBS control group, and then plotted. Error bars represent standard deviations. The percentage reduction of the group mean, relative to PBS, is indicated for the SNALP-1955 and SNALP-18328 groups. (*** $p < 0.001$, One-way ANOVA, with Dunn's post-hoc test).

CLAIMS

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of transthyretin (TTR), wherein

(a) said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region complementary to a part of a mRNA encoding transthyretin (TTR), wherein said region of complementarity is 19 nucleotides in length, the antisense strand comprises SEQ ID NO:170, and each strand of the dsRNA is 19, 20, 21, 22, 23, or 24 nucleotides in length; or

(b) said dsRNA comprises an antisense strand comprising a region complementary to 19 nucleotides of nucleotides 618-648 of SEQ ID NO: 1331 and wherein said antisense strand base pairs with the guanine at position 628 of SEQ ID NO:1331;

and wherein the dsRNA is formulated in a lipid formulation comprising a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle.

2. The dsRNA of claim 1, wherein said cationic lipid is (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino) butanoate (MC3).

3. The dsRNA of claim 1, wherein said non-cationic lipid is distearoylphosphatidylcholine (DSPC).

4. The dsRNA of claim 1, wherein said lipid that presents aggregation of the particle is a polyethyleneglycol (PEG)-lipid.

5. The dsRNA of any one of claims 1 to 4, wherein the lipid formulation further comprises cholesterol.

6. The dsRNA of claim 1, wherein the lipid formulation comprises MC3, DSPC, cholesterol and PEG_{2000}-C14.

7. The dsRNA of claim 1, wherein the lipid formulation comprises MC3/DSPC/cholesterol/PEG_{2000}-C14 in a ratio of about 50/10/38.5/1.5 mol%.

8. The dsRNA of any one of claims 1 to 7, wherein

(a) the sense strand consists of SEQ ID NO:449 and the antisense strand consists of SEQ ID NO:450;

(b) the sense strand consists of SEQ ID NO:729 and the antisense strand consists of SEQ ID NO:730; or

(c) the sense strand consists of SEQ ID NO:1009 and the antisense strand consists of SEQ ID NO:1010.

9. The dsRNA of any one of claims 1 to 7, wherein the sense strand comprises SEQ ID NO:169.

10. The dsRNA of any one of claims 1 to 7, wherein said dsRNA comprises at least one modified nucleotide, wherein preferably at least one of said modified nucleotides is chosen from the group of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

11. The dsRNA of any one of claims 1 to 7, wherein said dsRNA comprises at least one modified nucleotide, wherein said nucleotide is a 2'-O-methyl modified nucleotide.

12. The dsRNA of any one of claims 1 to 11, wherein the dsRNA is conjugated to a ligand.

13. A pharmaceutical composition for inhibiting expression of a TTR gene comprising the dsRNA of claims 1 to 12 and a pharmaceutically acceptable carrier.

14. A method of inhibiting TTR expression in a cell, the method comprising:

(a) contacting the cell with the dsRNA of claims 1 to 12; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TTR gene, thereby inhibiting expression of the TTR gene in the cell;

provided that any method of treatment of the human or animal body by therapy is excluded.

15. A dsRNA of claims 1 to 12 for use in treating in a human a disorder mediated by TTR expression, wherein

(a) the human has transthyretin amyloidosis; and/or

(b) the human has a liver disorder

wherein said transthyretin amyloidosis is preferably selected from familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/CNS amyloidosis, senile systemic amyloidosis (SSA) and senile cardiac amyloidosis (SCA).

16. The dsRNA for use of claim 15, wherein said dsRNA is to be administered to the human at about 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 0.543, 0.59, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 2.5, or 5.0 mg/kg.

17. The dsRNA for use of claim 15 or 16, wherein the human is additionally receiving a therapeutic method for treating a TTR amyloidosis, the therapeutic method selected from the group consisting of diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, dialysis, or a liver transplant.

18. The dsRNA for use of any one of claims 15 to 17, wherein the dsRNA is administered to the human at not more than 1, 2, 3, or 4 week intervals.

19. The dsRNA for use of any one of claims 15 to 18, wherein said dsRNA is to be administered intravenously or by intravenous infusion over 15 minutes time.

20. A use of a dsRNA of claims 1 to 12 in the manufacture of a medicament for the treatment of a human a disorder mediated by TTR expression, wherein

(a) the human has transthyretin amyloidosis; and/or

(b) the human has a liver disorder

wherein said transthyretin amyloidosis is preferably selected from familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/CNS amyloidosis, senile systemic amyloidosis (SSA) and senile cardiac amyloidosis (SCA).

21. The use of claim 20, wherein said dsRNA is to be administered to the human at about 0.01, 0.1, 0.2, 0.3, 0.4, 0.5, 0.543, 0.59, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 2.5, or 5.0 mg/kg.

22. The use of claim 20 or 21, wherein the human is additionally receiving a therapeutic method for treating a TTR amyloidosis, the therapeutic method selected from the group consisting of diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, dialysis, or a liver transplant.

23. The use of any one of claims 20 to 22, wherein the dsRNA is administered to the human at not more than 1, 2, 3, or 4 week intervals.

24. The use of any one of claims 20 to 22, wherein the dsRNA is administered every 3 weeks.

25. The use of any one of claims 20 to 23, wherein said dsRNA is to be administered intravenously or by intravenous infusion over 15 minutes time.

26. An in vitro method of inhibiting TTR expression in a cell, the method comprising:

(a) contacting the cell with a composition comprising a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of transthyretin (TTR) and a lipid formulation, wherein the dsRNA comprises a sense strand and an antisense strand comprising a region complementary to an mRNA encoding transthyretin (TTR), wherein the region

of complementarity comprises SEQ ID NO:170 and each strand is 19, 20, 21, 22, 23, or 24 nucleotides in length, the lipid formulation comprising the lipid (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3); and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TTR gene, thereby inhibiting expression of the TTR gene in the cell.

27 An in vitro method of inhibiting TTR expression in a cell, the method comprising:

(a) contacting the cell with a composition comprising a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of transthyretin (TTR) and a lipid formulation, wherein the dsRNA comprises a sense strand and an antisense strand comprising a region complementary to an mRNA encoding transthyretin (TTR), wherein the region of complementarity comprises SEQ ID NO:170 and each strand is 19, 20, 21, 22, 23, or 24 nucleotides in length, the lipid formulation comprising the lipid (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), wherein each strand is 21 nucleotides in length; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TTR gene, thereby inhibiting expression of the TTR gene in the cell.

28. An in vitro method of inhibiting TTR expression in a cell, the method comprising:

(a) contacting the cell with a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of transthyretin (TTR), wherein the dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region complementary to an mRNA encoding transthyretin (TTR), wherein the region of complementarity comprises SEQ ID NO:170 and each strand of the dsRNA is 19, 20, 21, 22, 23, or 24 nucleotides in length; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TTR gene, thereby inhibiting expression of the TTR gene in the cell.

29. The method of claim 28, wherein the region of complementarity consists of SEQ ID

NO:170.

30. The method of claim 28, wherein

(a) the sense strand consists of SEQ ID NO:449 and the antisense strand consists of SEQ ID NO:450;

(b) the sense strand consists of SEQ ID NO:729 and the antisense strand consists of SEQ ID NO:730; or

(c) the sense strand consists of SEQ ID NO:1009 and the antisense strand consists of SEQ ID NO:1010 and the dsRNA is formulated in a lipid formulation.

31. The method of claim 28, wherein each strand is 21 nucleotides in length.

32. The method of claim 28, wherein the dsRNA comprises at least one modified nucleotide.

33. The method of claim 28, wherein the dsRNA comprises at least one modified nucleotide chosen from the group of: a 2′-O-methyl modified nucleotide, a nucleotide comprising a 5′-phosphorothioate group, a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, a 2′-deoxy-2′-fluoro modified nucleotide, a 2′-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2′-amino-modified nucleotide, 2′-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

34. The method of claim 28, wherein the dsRNA comprises at least one 2′-O-methyl modified nucleotide.

35. The method of claim 28, wherein the dsRNA is conjugated to a ligand.

36. The method of claim 28 or 31, wherein the dsRNA is formulated in a lipid formulation.

37. The method of claim 28 or 31, wherein the dsRNA is formulated in a lipid formulation comprising MC3.

38. The method of claim 28, wherein contacting the cell with the dsRNA results in at least one of the following:

a) about 95% inhibition of TTR mRNA expression as measured by a real time PCR assay, wherein the cell is a HepG2 cell or a Hep3B cell, and the dsRNA concentration is 10 nM;

b) about 74% inhibition of TTR mRNA expression as measured by a branched DNA assay, wherein the cell is a HepG2 cell or a Hep3B cell and the dsRNA concentration is 10 nM;

c) the dsRNA has an IC50 of less than 10 pM in a HepG2 cell and the dsRNA concentration is 10 nM;

d) the dsRNA has an ED50 of about 1 mg/kg;

e) the dsRNA reduces TTR expression by 98.9% as measured by real-time PCR in a Hep3B cell and the dsRNA concentration is 0.1 nM;

f) the dsRNA reduces TTR expression by 99.4% as measured by real-time PCR in a Hep3B cell and the dsRNA concentration is 10 nM.

39. The method of claim 28, wherein the sense strand consists of SEQ ID NO:1009 and the antisense strand consists of SEQ ID NO:1010 and the dsRNA is lipid formulated in a formulation comprising MC3.