Claims

1. A composition for delivering an oligonucleotide to a liver cell *in vivo* comprising:
   a) an oligonucleotide covalently linked to a hydrophobic group having at least 20 carbon atoms; and,
   b) a reversibly masked and targeted amphipathic polymer comprising a terpolymer synthesized from amine-containing monomers, lower hydrophobic group-containing monomers, and higher hydrophobic group-containing monomers to which a plurality of galactose derivatives and PEG groups are individually linked to said polymer via pH labile disubstituted maleamic bonds and wherein cleavage of said pH labile disubstituted maleamic bonds yields amine groups thereby generating a membrane active polyamine.

2. A composition for delivering an oligonucleotide to a liver cell *in vivo* comprising:
   a) an oligonucleotide covalently linked to a galactose trimer; and,
   b) a reversibly masked and targeted amphipathic polymer comprising a terpolymer synthesized from amine-containing monomers, lower hydrophobic group-containing monomers, and higher hydrophobic group-containing monomers to which a plurality of galactose derivatives and PEG groups are individually linked to said polymer via pH labile disubstituted maleamic bonds and wherein cleavage of said pH labile disubstituted maleamic bonds yields amine groups thereby generating a membrane active polyamine.

3. A conjugate delivery system for delivering an RNA interference polynucleotide to a liver cell *in vivo* comprising:

$$N\text{–}A + P\begin{matrix}(L^2\text{–}M^1)_y \\ (L^2\text{–}M^2)_z,\end{matrix}$$

wherein,

P is a membrane active polyamine

L^2 is a pH labile linkage,

M^1 is a charge neutral masking agent containing a galactose derivative,

M^2 is a charge neutral masking agent containing a polyethylene glycol,

y and **z** are integers greater than 1 wherein the value of **y** and **z** together is greater than 50% on amines of **P**,

N is an RNA interference polynucleotide,

A is a galactose trimer or a hydrophobic group having at least 20 carbon atoms,

positive charge of **P** is neutralized and membrane activity of **P** is reversibly inhibited by modification of greater than 50% of **P** amines by attachment of $\mathbf{M^1}$ and $\mathbf{M^2}$ through the physiologically pH-labile linkages $\mathbf{L^2}$, and

cleavage of $\mathbf{L^2}$ in response to a decrease in pH restores amines and membrane activity of **P**.

4. A method of manufacturing an RNA oligonucleotide delivery composition comprising:
   a) forming a membrane active polyamine;
   b) forming a first masking agent comprising a charge neutral disubstituted maleic anhydride containing a galactose derivative;
   c) forming a second masking agent comprising a charge neutral disubstituted maleic anhydride containing a polyethylene glycol;
   d) reversibly inhibiting membrane activity of the membrane active polyamine wherein the inhibiting consists of modifying 50% or more of the amines on the polyamine by reacting the polyamine with the first and second masking agents thereby linking a plurality of galactose derivatives and a plurality of polyethylene glycols to the membrane active polymer via physiologically pH-labile disubstituted maleamate linkages; and,
   e) linking the RNA interference polynucleotide to galactose trimer or a hydrophobic group having at least 20 carbon atoms;
   f) providing the RNA interference polynucleotide and the reversibly inhibited membrane active polyamine in solution suitable for administration *in vivo*.

5. The composition of any of claims 1-4 wherein the RNA interference polynucleotide is selected from the groups consisting of: DNA, RNA, dsRNA, RNA interference polynucleotide, siRNA, and miRNA.

6. The composition of any of claims 1-4 wherein the liver cell consists of a hepatocyte.

7. The composition of any of claims 3 or 4 wherein the membrane active polymer contains two or more different monomers.

8. The composition of claim 7 wherein the membrane active polymer is composed of amine-containing monomers, lower hydrophobic group monomers, and higher hydrophobic group monomers.

9. The composition of any of claims 1-4 wherein the reversibly masked membrane active polyamine is soluble in water.

10. The composition of any of claims 1-4 wherein the membrane active polyamine is a random copolymer.

11. The composition of claim 10 wherein the random copolymer is selected from the group consisting of poly(vinyl ether) and poly(acrylate).

12. The composition of any of claims 1, 2, or 8 wherein the amine-containing monomers, lower alkyl monomers, and higher alkyl monomers are present in a ratio of 4-8 amine-containing monomers : 3-5 lower hydrophobic group monomers : 1 higher hydrophobic group monomers.

13. The composition of claim 12 wherein the lower hydrophobic group consists of a butyl group and the higher hydrophobic group consists of an octadecyl or dodecyl group.

14. The composition of any of claims 1-4 wherein the masking agents are reversibly linked to at least 70% of the amines on the polyamine.

15. The composition of claim 14 wherein the masking agents are reversibly linked to at least 80% of the amines on the polyamine.

16. The composition of any of claims 1-15 wherein the reversibly modified polymer has a zeta potential between +30 and −30 mV at pH 8.

17. The composition of claim 16 wherein the reversibly modified polymer has a zeta potential between +20 and −20 mV at pH 8.

18. The composition of claim 17 wherein the reversibly modified polymer has a zeta potential between +10 and −10 mV at pH 8.

19. The composition of any of claims 1-15 wherein the reversibly modified polymer has a zeta potential between 0 and −30 mV at pH 8.

20. The composition of claim 19 wherein the reversibly modified polymer has a zeta potential between 0 and −20 mV at pH 8.

21. The composition of claim 20 wherein the reversibly modified polymer has a zeta potential between 0 and −10 mV at pH 8.

22. The composition of any of claims 1-21 wherein the composition is provided in a pharmaceutically acceptable carrier or diluent.

23. The composition of 3 wherein $\mathbf{L}^2$ is a maleamate linkage.

24. The composition of 23 wherein $\mathbf{L}^2$ is a disubstituted maleamate linkage.

25. The composition of 3 wherein **N** is linked to **A** via a physiologically labile linkage $\mathbf{L}^1$.

26. The composition of 25 wherein $\mathbf{L}^1$ is a physiologically labile covalent linkage that is orthogonal to $\mathbf{L}^2$.

27. The composition of 3 wherein **A** contains a hydrophobic group having 20 or more carbon atoms.

28. The composition of 3 wherein **A** contains a galactose trimer.

29. The composition of any of claims 1-4 wherein the ratio of galactose derivative to PEG linked to the polymer is 1 to 05.-2.

30. The composition of any of claims 1-29 wherein the galactose derivative consists of an N-acetylgalactosamine.

31. The composition of any of claims 2-30 wherein the galactose trimer consists of an N-acetylgalactosamine trimer.

CLAIMS

1. A composition for delivering an oligonucleotide to a liver cell in vivo comprising:

$$N\text{–}A \quad \text{and} \quad P\begin{cases}(L^2\text{–}M^1)_y \\ (L^2\text{–}M^2)_z,\end{cases}$$

wherein,

P is an amphipathic membrane active polyamine,

L^2 is a disubstituted maleamate linkage,

M^1 is a charge neutral masking agent containing a galactose derivative having affinity for the asialoglycoprotein receptor equal to or greater than that of galactose,

M^2 is a charge neutral masking agent containing a polyethylene glycol,

y and **z** are integers greater than or equal to zero wherein the value of **y** and **z** together is greater than 50% of the number of amines of **P**,

N is an oligonucleotide,

A comprises a galactose trimer having affinity for the asialoglycoprotein receptor equal to or greater than that of galactose or a hydrophobic group having at least 20 carbon atoms,

positive charge of **P** is neutralized and membrane activity of **P** is reversibly inhibited by modification of greater than 50% of the number of primary amines of amphipathic membrane active polyamine **P** by attachment of M^1 and M^2 through the maleamate linkages L^2, and

cleavage of L^2 restores amines and membrane activity of **P**.

2. A method of manufacturing an oligonucleotide delivery composition comprising:
   a) forming an amphipathic membrane active polyamine;
   b) forming a first masking agent comprising a charge neutral disubstituted maleic anhydride containing a galactose derivative having affinity for the asialoglycoprotein receptor equal to or greater than that of galactose;
   c) forming a second masking agent comprising a charge neutral disubstituted maleic anhydride containing a polyethylene glycol;
   d) reversibly inhibiting membrane activity of the amphipathic membrane active polyamine wherein the inhibiting consists of modifying 50% or more of the number of amines on the

amphipathic membrane active polyamine by reacting the amphipathic membrane active polyamine with the first and second masking agents thereby linking a plurality of galactose derivatives and a plurality of polyethylene glycols to the amphipathic membrane active polyamine via physiologically pH-labile disubstituted maleamate linkages; and,

e) linking the oligonucleotide to a galactose trimer having affinity for the asialoglycoprotein receptor equal to or greater than that of galactose or a hydrophobic group having at least 20 carbon atoms;

f) providing the oligonucleotide and the reversibly inhibited amphipathic membrane active polyamine in solution suitable for administration in vivo.

3. The composition of claim 1 or claim 2 wherein the oligonucleotide is selected from the group consisting of: DNA, RNA, dsRNA, RNA interference polynucleotide, siRNA, and miRNA.
4. The composition claim 1 wherein the liver cell consists of a hepatocyte.
5. The composition of claim 1 or 2 wherein the amphipathic membrane active polyamine contains primary amine-containing monomers and hydrophobic group-containing monomers.
6. The composition of claim 5 wherein the amphipathic membrane active polyamine is composed of primary amine-containing monomers, lower hydrophobic group-containing monomers, and higher hydrophobic group-containing monomers.
7. The composition of claim 1 or 2 wherein the reversibly masked amphipathic membrane active polyamine is soluble in water.
8. The composition of any of claim 1 or 2 wherein the amphipathic membrane active polyamine is a random copolymer.
9. The composition of claim 8 wherein the random copolymer is selected from the group consisting of poly(vinyl ether) and poly(acrylate).
10. The composition of claim 6 wherein the primary amine-containing monomers, lower hydrophobic group-containing monomers, and higher hydrophobic group-containing monomers are present in a ratio of 4-8 primary amine-containing monomers : 3-5 lower hydrophobic group-containing monomers : 1 higher hydrophobic group-containing monomers.
11. The composition of claim 10 wherein the lower hydrophobic group consists of a butyl group and the higher hydrophobic group consists of an octadecyl or dodecyl group.

12. The composition of any of claims 1or 2 wherein the masking agents are reversibly linked to at least 70% of the number of primary amines on the amphipathic membrane active polyamine.
13. The composition of claim 12 wherein the masking agents are reversibly linked to at least 80% of the number of primary amines on the amphipathic membrane active polyamine.
14. The composition of any one of claims 1 and 3 to 13 wherein the reversibly modified amphipathic membrane active polyamine has a zeta potential between 0 and −20 mV at pH 8.
15. The composition of claim 14 wherein the reversibly modified amphipathic membrane active polyamine has a zeta potential between 0 and −10 mV at pH 8.
16. The composition of any of claims 1-15 wherein the composition is provided in a pharmaceutically acceptable carrier or diluent.
17. The composition of claim 1 wherein **N** is linked to **A** via a physiologically labile linkage $\mathbf{L^1}$.
18. The composition of claim 17 wherein $\mathbf{L^1}$ is a physiologically labile covalent linkage that is orthogonal to maleamate.
19. The composition of claim 1 wherein the ratio of galactose derivative to PEG linked to the amphipathic membrane active polyamine is 1 to 0.5-2.
20. The composition of any of claims 1-19, wherein the galactose derivative consists of an N-acetylgalactosamine.
21. The composition of any of claims 1 to 20, wherein the galactose trimer consists of an N-acetylgalactosamine trimer.
22. The composition of any of claims 1 to 20 wherein the hydrophobic group comprises a cholesterol.