fluoro modification, wherein the sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and, wherein the sense strand is conjugated to one or more GalNAc derivatives attached through a branched bivalent or trivalent linker at the 3'-terminus, thereby reducing the accumulation of misfolded Serpina1 in the liver of the subject having a Serpina1 deficiency variant.

In one embodiment, one of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the seed region of the antisense strand. In one embodiment, the antisense strand comprises a universal base at the mismatched nucleotide.

In one embodiment, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In one embodiment, the subject is a primate or rodent. In another embodiment, the subject is a human.

In one embodiment, the RNAi agent, *e.g.*, double stranded RNAi agent, is administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg. In another embodiment, the double stranded RNAi agent is administered at a dose of about 10 mg/kg to about 30 mg/kg.

In one embodiment, the RNAi agent, *e.g.*, double stranded RNAi agent, is administered subcutaneously or intravenously.

In another aspect, the present invention provides a double stranded RNAi agent for inhibiting expression of Serpina1 in a cell,

wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region,

wherein said antisense strand comprises at least 19 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5' – UUUUGUUCAAUCAUUAAGAAGAC – 3' (SEQ ID NO: 419),

wherein the sense strand and the antisense strand are each independently 19-25 nucleotides in length,

wherein substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, and

wherein at least one strand is conjugated to a ligand through a bivalent or trivalent branched linker.

In another aspect, the present invention provides a double stranded RNAi agent for inhibiting expression of Serpina1 in a cell,

wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5' – UUUUGUUCAAUCAUUAAGAAGAC -3' (SEQ ID NO: 419),

wherein the sense strand and the antisense strand are each independently 19-25 nucleotides in length,

wherein substantially all of the nucleotides of said sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification,

wherein said sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus,

wherein substantially all of the nucleotides of said antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification,

wherein said antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and

wherein said sense strand is conjugated to one or more GalNAc derivatives attached through a branched bivalent or trivalent linker at the 3'-terminus.

In another aspect, the present invention provides an isolated cell containing the double stranded RNAi agent as described herein.

In another aspect, the present invention provides an *in vitro* method of inhibiting Serpina1 expression in a cell, the method comprising:

(a) contacting the cell with the double stranded RNAi agent or the pharmaceutical composition as described herein; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a Serpina1 gene, thereby inhibiting expression of the Serpina1 gene in the cell.

In another aspect, the present invention provides the double stranded RNAi agent or the pharmaceutical composition as described herein for use in therapy.

In another aspect, the present invention provides a use of the double stranded RNAi agent or the pharmaceutical composition as described herein in the manufacture of a medicament for the treatment of a Serpina1-associated disorder in a subject, wherein the Serpina1-associated disorder is a liver disorder.

In another aspect, the present invention provides a use of the double stranded RNAi agent or the pharmaceutical composition as described herein in the manufacture of a medicament for reducing the accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant.

The present invention is further illustrated by the following detailed description and drawings.

Brief Description of the Drawings

Figure 1 is a graph depicting the *in vivo* efficacy and duration of response for the indicated siRNAs in transgenic mice expressing the Z-AAT form of human AAT.

Figures 2A-2B depict *in vivo* efficacy of five siRNAs with low IC50 values. Transgenic mice expressing the human Z-AAT allele were injected with 10 mg/kg siRNA duplex on day 0 and serum human AAT was followed for 21 days post dose (Figure 2A). Each point represents an average of three mice and the error bars reflect the standard deviation. Figure 2B depicts hAAT mRNA levels in liver normalized to GAPDH for each group. The bars reflect the average and the error bars reflect the standard deviation.

Figures 3A-3C depict durable AAT suppression in a dose responsive manner. Figure 3A specifically depicts the efficacy curve showing maximum knock-down of serum hAAT protein levels achieved at different doses of AD-59054 subcutaneously administered to transgenic mice. Each point is an average of three animals and the error bars represent the standard deviation. The duration of knock-down after a single dose of AAT siRNA at 0.3, 1, 3 or 10 mg/kg is shown in Figure 3B. The hAAT levels were normalized to the average of

Claims

1. A double stranded RNAi agent for inhibiting expression of Serpina1 in a cell,

wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double-stranded region,

wherein said antisense strand comprises at least 19 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5' – UUUUGUUCAAUCAUUAAGAAGAC – 3' (SEQ ID NO: 419),

wherein the sense strand and the antisense strand are each independently 19-25 nucleotides in length,

wherein substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, and

wherein at least one strand is conjugated to a ligand through a bivalent or trivalent branched linker.

2. The double stranded RNAi agent of claim 1, wherein one of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the seed region of the antisense strand.

3. The double stranded RNAi agent of claim 2, wherein the antisense strand comprises a universal base at the mismatched nucleotide.

4. The double stranded RNAi agent of claim 1, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand are modified nucleotides.

5. The double stranded RNAi agent of claim 1, wherein at least one of said modified nucleotides is selected from the group consisting of a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group.

6. The double stranded RNAi agent of any claim 1, wherein at least one strand comprises a 3’ overhang of at least 1 nucleotide.

7. The double stranded RNAi agent of claim 1, wherein at least one strand comprises a 3’ overhang of at least 2 nucleotides.

8. The double stranded RNAi agent of claim 1, wherein the double-stranded region is 19-25 nucleotide pairs in length.

9. The double stranded RNAi agent of claim 8, wherein the double-stranded region is 19-23 nucleotide pairs in length.

10. The double stranded RNAi agent of claim 8, wherein the double-stranded region is 19-21 nucleotide pairs in length.

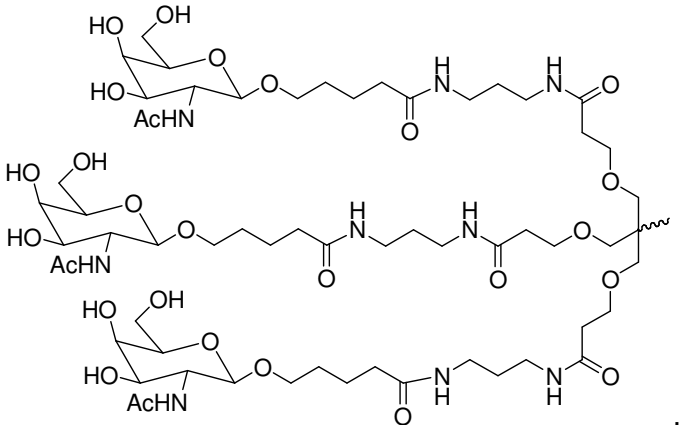
11. The double stranded RNAi agent of claim 8, wherein the double-stranded region is 21-23 nucleotide pairs in length.

12. The double stranded RNAi agent of claim 1, wherein each strand is independently 19-23 nucleotides in length.

13. The double stranded RNAi agent of claim 1, wherein each strand is independently 21-23 nucleotides in length.

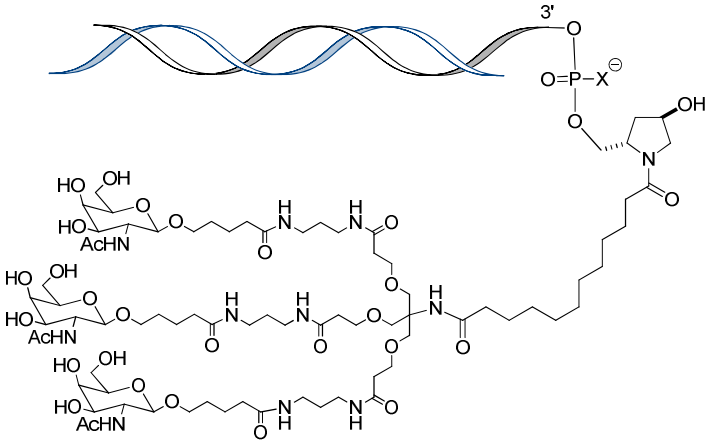
14. The double stranded RNAi agent of claim 1, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

15. The double stranded RNAi agent of claim 1, wherein the ligand is



16. The double stranded RNAi agent of claim 1, wherein the ligand is attached to the 3′ end of the sense strand.

17. The double stranded RNAi agent of claim 16, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

18. The double stranded RNAi agent of claim 1, wherein said agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

19. The double stranded RNAi agent of claim 18, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3’-terminus of one strand.

20. The double stranded RNAi agent of claim 19, wherein said strand is the antisense strand.

21. The double stranded RNAi agent of claim 19, wherein said strand is the sense strand.

22. The double stranded RNAi agent of claim 18, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

23. The double stranded RNAi agent of claim 22, wherein said strand is the antisense strand.

24. The double stranded RNAi agent of claim 22, wherein said strand is the sense strand.

25. The double stranded RNAi agent of claim 18, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand.

26. The double stranded RNAi agent of claim 25, wherein said strand is the antisense strand.

27. The double stranded RNAi agent of claim 18, wherein said RNAi agent comprises 6-8 phosphorothioate internucleotide linkages.

28. The double stranded RNAi of claim 27, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-terminus or the 3'-terminus.

29. The double stranded RNAi agent of claim 1, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

30. The double stranded RNAi agent of claim 1, wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

31. A double stranded RNAi agent for inhibiting expression of Serpina1 in a cell,

wherein said double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5' – UUUUGUUCAAUCAUUAAGAAGAC -3' (SEQ ID NO: 419),

wherein the sense strand and the antisense strand are each independently 19-25 nucleotides in length,

wherein substantially all of the nucleotides of said sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification,

wherein said sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus,

wherein substantially all of the nucleotides of said antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification,

wherein said antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and

wherein said sense strand is conjugated to one or more GalNAc derivatives attached through a branched bivalent or trivalent linker at the 3'-terminus.

32. The double stranded RNAi agent of claim 31, wherein one of the 3 nucleotide differences in the nucleotide sequence of the antisense strand is a nucleotide mismatch in the seed region of the antisense strand.

33. The double stranded RNAi agent of claim 32, wherein the antisense strand comprises a universal base at the mismatched nucleotide.

34. The double stranded RNAi agent of claim 31, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

35. An isolated cell containing the double stranded RNAi agent of any one of claims 1-34, and 56-67.

36. A pharmaceutical composition comprising the double stranded RNAi agent of any one of claims 1-34 and 56-67.

37. The pharmaceutical composition of claim 36, wherein RNAi agent is present in an unbuffered solution.

38. The pharmaceutical composition of claim 37, wherein said unbuffered solution is saline or water.

39. The pharmaceutical composition of claim 36, wherein said RNAi agent is present in a buffer solution.

40. The pharmaceutical composition of claim 39, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

41. The pharmaceutical composition of claim 40, wherein said buffer solution is phosphate buffered saline (PBS).

42. An *in vitro* method of inhibiting Serpina1 expression in a cell, the method comprising:

(a) contacting the cell with the double stranded RNAi agent of any one of claims 1-34 and 56-67, or the pharmaceutical composition of any one of claims 36-41; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a Serpina1 gene, thereby inhibiting expression of the Serpina1 gene in the cell.

43. The method of claim 42, wherein the Serpina1 expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, or about 100%.

44. The double stranded RNAi agent of any one of claims 1-34 and 56-67, or the pharmaceutical composition of any one of claims 36-41 for use in therapy.

45. Use of the double stranded RNAi agent of any one of claims 1-34 and 56-67, or the pharmaceutical composition of claim 36-41 in the manufacture of a medicament for the treatment of a Serpina1-associated disorder in a subject, wherein the Serpina1-associated disorder is a liver disorder

46. The use of claim 45, wherein the subject is a human.

47. The use of claim 45, wherein the liver disorder is selected from the group consisting of chronic liver disease, liver inflammation, cirrhosis, liver fibrosis, and/or hepatocellular carcinoma

48. The double stranded RNAi agent or pharmaceutical composition of claim 84 or use of claim 85, wherein the double stranded RNAi agent is for administration at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg.

49. The use of claim 48, wherein the double stranded RNAi agent is for subcutaneous administration.

50. The use of claim 48, wherein the double stranded RNAi agent is for intravenous administration.

51. Use of the double stranded RNAi agent of any one of claims 1-34 and 56-67, or the pharmaceutical composition of any one of claims 36-41 in the manufacture of a medicament for reducing the accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant.

52. The use of claim 51, wherein the subject is a human.

53. The use of claim 51, wherein the double stranded RNAi agent is to be administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg.

54. The use of claim 53, wherein the double stranded RNAi agent is for subcutaneous administration.

55. The use of claim 53, wherein the double stranded RNAi agent is for intravenous administration.

56. The double stranded RNAi agent of claim 1, wherein said sense strand comprises at least 19 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of CUUCUUAAUGAUUGAACAAAA (SEQ ID NO: 417).

57. The double stranded RNAi agent of claim 31, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

58. The double stranded RNAi agent of claim 31, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

59. The double stranded RNAi agent of claim 31, wherein the double-stranded region is 19-25 nucleotide pairs in length.

60. The double stranded RNAi agent of claim 59, wherein the double-stranded region is 19-23 nucleotide pairs in length.

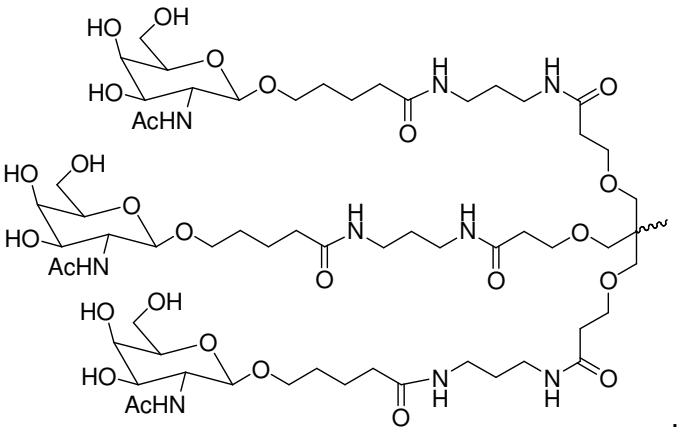
61. The double stranded RNAi agent of claim 59, wherein the double-stranded region is 19-21 nucleotide pairs in length.

62.The double stranded RNAi agent of claim 59, wherein the double-stranded region is 21-23 nucleotide pairs in length.

63. The double stranded RNAi agent of claim 31, wherein each strand is independently 19-23 nucleotides in length.

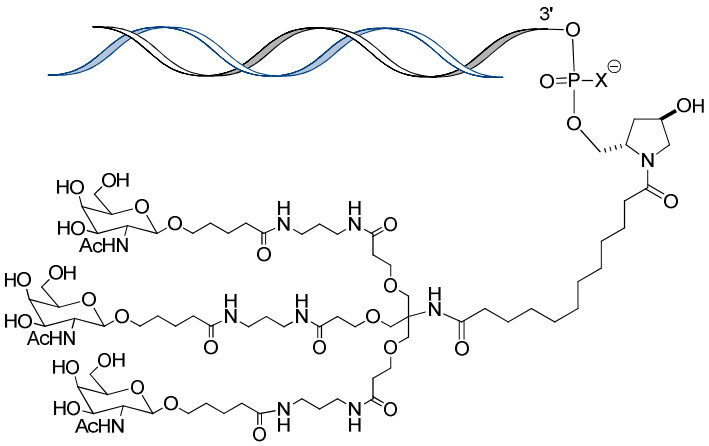
64. The double stranded RNAi agent of claim 31, wherein each strand is independently 21-23 nucleotides in length.

65. The double stranded RNAi agent of claim 31, wherein the one or more GalNac derivatives is



66. The double stranded RNAi agent of claim 31, wherein the one or more GalNAc derivatives is attached to the 3′ end of the sense strand.

67. The double stranded RNAi agent of claim 66, wherein the RNAi agent is conjugated to the one or more GalNAc derivatives as shown in the following schematic



wherein X is O or S.