wherein said antisense strand comprises at least 1915 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 a pharmaceutical composition comprising 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

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 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).

3~~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.

4~~3~~. The double stranded RNAi agent of claim 2, wherein the antisense strand comprises a universal base at the mismatched nucleotide.

5~~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.

6~~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.

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

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

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

109. The double stranded RNAi agent of claim 98, wherein the double-stranded region is 19-23 nucleotide pairs in length.

1110. The double stranded RNAi agent of claim 98, wherein the double-stranded region is 19-21 nucleotide pairs in length.

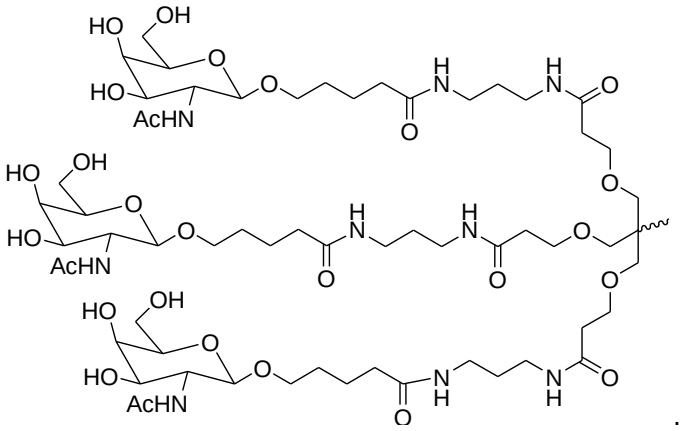
1211. The double stranded RNAi agent of claim 98, wherein the double-stranded region is 21-23 nucleotide pairs in length.

1312. The double stranded RNAi agent of claim 1, wherein each strand is independently 19-23 nucleotides in length.

1413. The double stranded RNAi agent of claim 1, wherein each strand is independently 21-23 nucleotides in length.

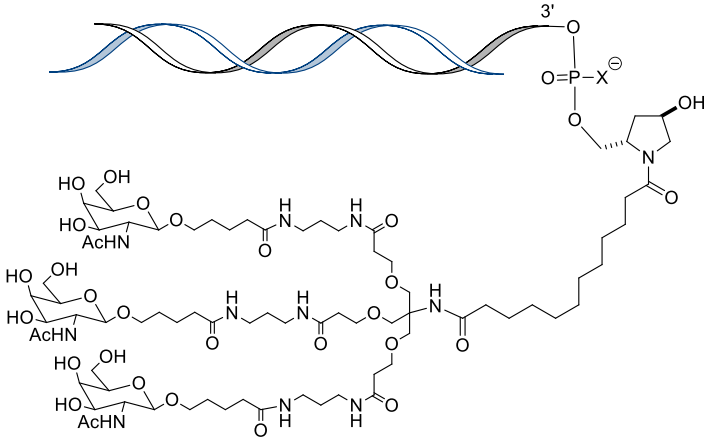
1514. The double stranded RNAi agent of claim 1, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

1615. The double stranded RNAi agent of claim 1, wherein the ligand is



17~~16~~. The double stranded RNAi agent of claim 1, wherein the ligand is attached to the 3′ end of the sense strand.

18~~17~~. The double stranded RNAi agent of claim 17~~16~~, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

19~~18~~. The double stranded RNAi agent of claim 1, wherein said agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

20~~19~~. The double stranded RNAi agent of claim 19~~18~~, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3’-terminus of one strand.

21~~20~~. The double stranded RNAi agent of claim 20~~19~~, wherein said strand is the antisense strand.

2221. The double stranded RNAi agent of claim 2019, wherein said strand is the sense strand.

2322. The double stranded RNAi agent of claim 1918, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

2423. The double stranded RNAi agent of claim 2322, wherein said strand is the antisense strand.

2524. The double stranded RNAi agent of claim 2322, wherein said strand is the sense strand.

2625. The double stranded RNAi agent of claim 1918, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand.

2726. The double stranded RNAi agent of claim 2625, wherein said strand is the antisense strand.

2827. The double stranded RNAi agent of claim 1918, wherein said RNAi agent comprises 6-8 phosphorothioate internucleotide linkages.

2928. The double stranded RNAi of claim 2827, 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.

3029. 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.

3130. 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.

3231. 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 1915 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.

33. The double stranded RNAi agent of claim 32, 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).

3432. The double stranded RNAi agent of claim 3231, 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.

3533. The double stranded RNAi agent of claim 3332, wherein the antisense strand comprises a universal base at the mismatched nucleotide.

3634. The double stranded RNAi agent of claim 3231, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

37. The double stranded RNAi agent of claim 32, wherein at least one strand comprises a 3’ overhang of at least 1 nucleotide.

38. The double stranded RNAi agent of claim 32, wherein at least one strand comprises a 3’ overhang of at least 2 nucleotides.

39. The double stranded RNAi agent of claim 32, wherein the double-stranded region is 19-25 nucleotide pairs in length.

40. The double stranded RNAi agent of claim 39, wherein the double-stranded region is 19-23 nucleotide pairs in length.

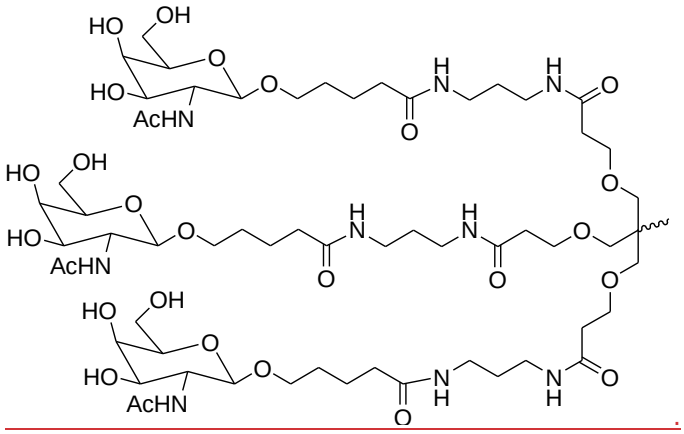
41. The double stranded RNAi agent of claim 39, wherein the double-stranded region is 19-21 nucleotide pairs in length.

42. The double stranded RNAi agent of claim 39, wherein the double-stranded region is 21-23 nucleotide pairs in length.

43. The double stranded RNAi agent of claim 32, wherein each strand is independently 19-23 nucleotides in length.

44. The double stranded RNAi agent of claim 32, wherein each strand is independently 21-23 nucleotides in length.

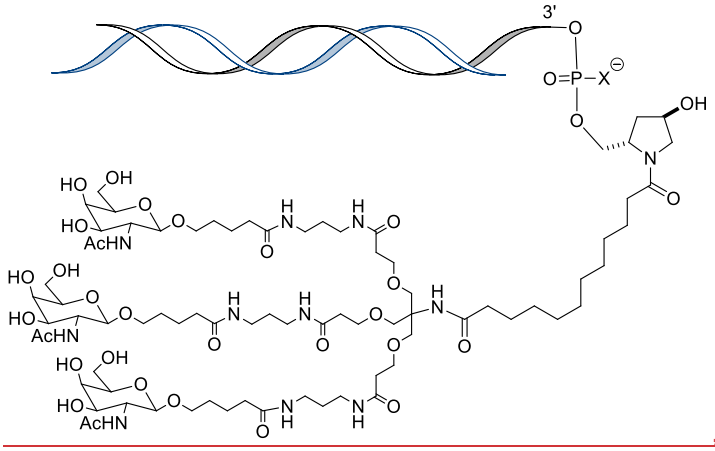
45. The double stranded RNAi agent of claim 32, wherein the one or more GalNac derivatives is



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46. The double stranded RNAi agent of claim 32, wherein the one or more GalNAc derivatives is attached to the 3′ end of the sense strand.

47. The double stranded RNAi agent of claim 46, 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.

4835. An isolated cell containing the double stranded RNAi agent of any one of claims 1-4734, and 56-67.

4936. A pharmaceutical composition comprising the double stranded RNAi agent of any one of claims 1-4734 and 56-67.

5037. The pharmaceutical composition of claim 4936, wherein RNAi agent is present in an unbuffered solution.

51~~38~~. The pharmaceutical composition of claim 50~~37~~, wherein said unbuffered solution is saline or water.

52~~39~~. The pharmaceutical composition of claim 49~~36~~, wherein said RNAi agent is present in a buffer solution.

53~~40~~. The pharmaceutical composition of claim 52~~39~~, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

54~~41~~. The pharmaceutical composition of claim 53~~40~~, wherein said buffer solution is phosphate buffered saline (PBS).

55~~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 1-47~~34 and 56-67~~, or the pharmaceutical composition of any one of claims 49-54~~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.

56~~43~~. The method of claim 55~~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%.

57~~44~~. The double stranded RNAi agent of any one of claims 1-47~~34 and 56-67~~, or the pharmaceutical composition of any one of claims 49-54~~36-41~~ for use in therapy.

58~~45~~. Use of the double stranded RNAi agent of any one of claims 1-47~~34 and 56-67~~, or the pharmaceutical composition of any one of claims 49-54~~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

59~~46~~. The use of claim 58~~45~~, wherein the subject is a human.

6047. The use of claim 5845, wherein the liver disorder is selected from the group consisting of chronic liver disease, liver inflammation, cirrhosis, liver fibrosis, and/or hepatocellular carcinoma.

6148. The ~~double stranded RNAi agent or pharmaceutical composition of claim 84 or~~ use of claim 5885, 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.

6249. The use of claim 6148, wherein the double stranded RNAi agent is for subcutaneous administration.

6350. The use of claim 6148, wherein the double stranded RNAi agent is for intravenous administration.

6451. Use of the double stranded RNAi agent of any one of claims 1-4734 ~~and 56-67~~, or the pharmaceutical composition of any one of claims 49-5436-41 in the manufacture of a medicament for reducing the accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant.

6552. The use of claim 6451, wherein the subject is a human.

6653. The use of claim 6451, 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.

6754. The use of claim 6653, wherein the double stranded RNAi agent is for subcutaneous administration.

6855. The use of claim 6653, 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.~~

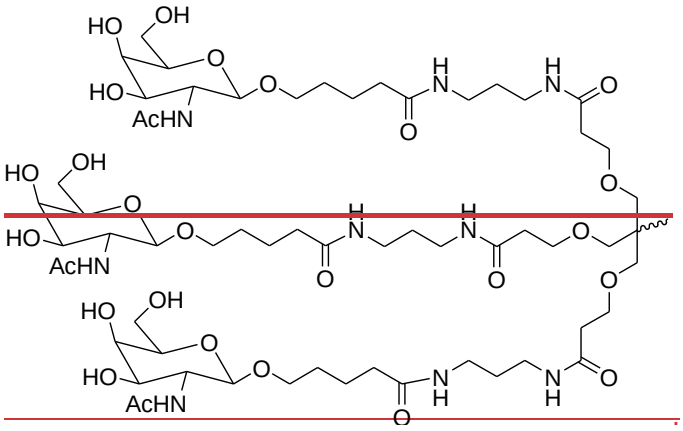
~~61. The double stranded RNAi agent of claim 59, wherein the double-stranded region is 19-21 nucleotide pairs in length.~~

~~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.~~

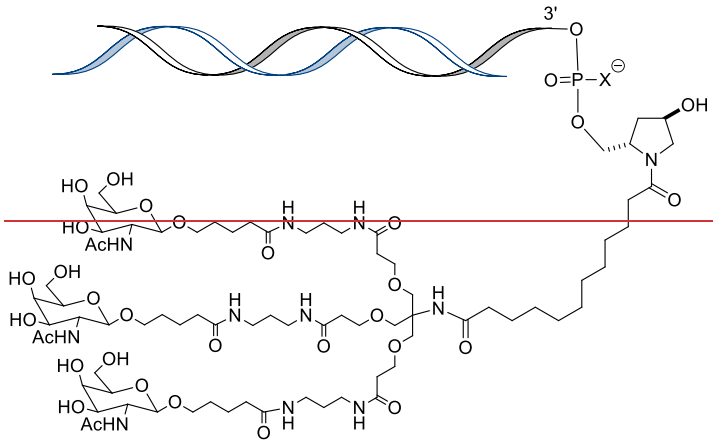
~~65. The double stranded RNAi agent of claim 31, wherein the one or more GalNac derivatives is~~



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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.