There are currently very limited options for the treatment of patients with liver disease arising from alpha-1-antitrypsin deficiency, including hepatitis vaccination, supportive care, and avoidance of injurious agents (*e.g.*, alcohol and NSAIDs). Although replacement alpha-1-antitrypsin therapy is available, such treatment has no impact liver disease in these subjects and, although liver transplantation may be effective, it is a difficult, expensive and risky procedure and liver organs are not readily available.

Accordingly, there is a need in the art for effective treatments for Serpina1-associated diseases, such as a chronic liver disease, liver inflammation, cirrhosis, liver fibrosis, and/or hepatocellular carcinoma.

Summary ~~of the Invention~~

As described in more detail below, disclosed herein are compositions comprising agents, *e.g.,* single-stranded and double-stranded polynucleotides, *e.g.,* RNAi agents, *e.g.*, double-stranded iRNA agents, targeting Serpina1. Also disclosed are methods using the compositions of the ~~invention~~ disclosure for inhibiting Serpina1 expression and for treating Serpina1 associated diseases, *e.g.*, chronic liver disease, liver inflammation, cirrhosis, liver fibrosis, and/or hepatocellular carcinoma.

Accordingly, ~~in one aspect,~~ the present disclosure ~~invention~~ provides double stranded RNAi agents for inhibiting expression of Serpina1 in a cell. In one aspect, the present disclosure provides double stranded RNAi agents for inhibiting expression of Serpina1 in a cell, wherein the ~~The~~ double stranded RNAi agents comprise a sense strand and an antisense strand forming a double-stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25,

wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand is conjugated to a ligand attached at the 3'-terminus.

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 are modified nucleotides.

In one embodiment, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the sequences listed in any one of Tables 1, 2, 5, 7, 8, and 9.

In one embodiment, at least one of the 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.

In one embodiment, at least one strand comprises a 3' overhang of at least 1 nucleotide.

In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides.

In another aspect, the present disclosure ~~invention~~ provides RNAi agents, *e.g.*, double-stranded RNAi agents, capable of inhibiting the expression of Serpina1 in a cell, wherein the double stranded RNAi agent comprises a sense strand substantially complementary to an antisense strand, wherein the antisense strand comprises a region substantially complementary to part of an mRNA encoding Serpina1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p, n_p', n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. In one embodiment, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. In one embodiment, the strand is the antisense strand.

In one embodiment, the RNAi agent comprises 6-8 phosphorothioate internucleotide linkages. In one embodiment, 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.

In one embodiment, the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.

In one embodiment, the Y nucleotides contain a 2'-fluoro modification.

In one embodiment, the Y' nucleotides contain a 2'-O-methyl modification.

In one embodiment, p'>0. In another embodiment, p'=2.

In one embodiment, q'=0, p=0, q=0, and p' overhang nucleotides are complementary to the target mRNA. In another embodiment, q'=0, p=0, q=0, and p' overhang nucleotides are non-complementary to the target mRNA.

In one embodiment, the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

In one embodiment, at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage.

In one embodiment, all n_p' are linked to neighboring nucleotides via phosphorothioate linkages.

In one embodiment, the RNAi agent is selected from the group of RNAi agents listed in any one of Tables 1, 2, 5, 7, 8, and 9.

In one embodiment, the RNAi agent is selected from the group consisting of AD-58681, AD-59054, AD-61719, and AD-61444.

In another aspect, the present disclosure ~~invention~~ provides a double stranded RNAi agent for inhibiting expression of Serpina1 in a cell, wherein the. ~~The~~ double stranded RNAi agents comprise a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide

sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25 , wherein substantially all of the nucleotides of the sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-

fluoro modification, wherein the sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus, wherein substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-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.

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 said sense strand and all of the nucleotides of said antisense strand comprise a modification.

In another aspect, the present disclosure ~~invention~~ provides RNAi agents, *e.g.*, double stranded RNAi agents, capable of inhibiting the expression of Serpina1 in a cell, wherein the double stranded RNAi agent comprises a sense strand substantially complementary to an antisense strand, wherein the antisense strand comprises a region substantially complementary to part of an mRNA encoding Serpina1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p, n_p', n_q, and n_q', each of which may or may not be present independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand.

In yet another aspect, the present disclosure ~~invention~~ provides RNAi agents, *e.g.*, double stranded RNAi agents, capable of inhibiting the expression of Serpina1 in a cell, wherein the double stranded RNAi agent comprises a sense strand substantially complementary to an antisense strand, wherein the antisense strand comprises a region substantially complementary to part of an mRNA encoding Serpina1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

wherein:

i, j, k, and l are each independently 0 or 1;

each n_p, n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q′ are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X′X′X′, Y′Y′Y′, and Z′Z′Z′ each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In a further aspect, the present disclosure ~~invention~~ provides RNAi agents, *e.g.*, double stranded RNAi agents, capable of inhibiting the expression of Serpina1 in a cell, wherein the double stranded RNAi agent comprises a sense strand substantially complementary to an antisense strand, wherein the antisense strand comprises a region substantially complementary to part of an mRNA encoding Serpina1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

sense: $5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

wherein:

i, j, k, and l are each independently 0 or 1;

each n_p, n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In another aspect, the present disclosure ~~invention~~ provides RNAi agents, *e.g.*, double stranded RNAi agents capable of inhibiting the expression of Serpina1 in a cell, wherein the double stranded RNAi agent comprises a sense strand substantially complementary to an antisense strand, wherein the antisense strand comprises a region substantially complementary to part of an mRNA encoding Serpina1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

sense: $5' n_p\text{-}N_a\text{-}(X\,X\,X)_i\text{-}N_b\text{-}Y\,Y\,Y\text{-}N_b\text{-}(Z\,Z\,Z)_j\text{-}N_a\text{-}n_q\ 3'$

antisense: $3' n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

wherein:

i, j, k, and l are each independently 0 or 1;

each n_p, n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y';

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In yet another aspect, the present disclosure ~~invention~~ provides RNAi agents, *e.g.*, double stranded RNAi agents, capable of inhibiting the expression of Serpina1 in a cell, wherein the double stranded RNAi agent comprises a sense strand substantially complementary to an antisense strand, wherein the antisense strand comprises a region substantially complementary to part of an mRNA encoding Serpina1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

sense: $5' n_p - N_a - Y\ Y\ Y - N_a - n_q\ 3'$

antisense: $3' n_p'-N_a'- Y'Y'Y'- N_a'- n_q'\ 5'$ (IIIa)

wherein:

each n_p, n_q, and n_q', each of which may or may not be present, independently represents an overhang nucleotide;

p, q, and q' are each independently 0-6;

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

YYY and Y'Y'Y' each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2'-O-methyl or 2'-fluoro modifications;

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, Na' comprises 1-25 nucleotides, and wherein one of the 1-25 nucleotides at one of positions 2-9 from the 5'end is a nucleotide mismatch. In one embodiment, the mismatched base is a universal base.

The present disclosure ~~invention~~ also provides cells, vectors, host cells, and pharmaceutical compositions comprising the double stranded RNAi agents of the disclosure~~invention~~.

In one aspect, the present disclosure provides a vector containing the double stranded RNAi agent of the present disclosure. In another aspect, the present disclosure provides a pharmaceutical composition comprising the double stranded RNAi agent of the present disclosure.

In one ~~embodiment~~aspect, the present disclosure ~~invention~~ provides an RNAi agent selected from the group of RNAi agents listed in any one of Tables 1, 2, 5, 7, 8, and 9.

The present disclosure ~~invention~~ also provides a composition comprising a modified antisense polynucleotide agent. In one aspect, the present disclosure provides a composition comprising a modified antisense polynucleotide agent, wherein the ~~The~~ agent is capable of inhibiting the expression of Serpina1 in a cell, and comprises a sequence complementary to a sense sequence selected from the group of the sequences listed in any one of Tables 1, 2, 5, 7, 8, and 9, wherein the polynucleotide is about 14 to about 30 nucleotides in length.

In another aspect, the present ~~invention~~ disclosure provides a cell containing the double stranded RNAi agent of the disclosure~~invention~~.

In some embodiments, the RNAi agent is administered using a pharmaceutical composition.

In preferred embodiments, the RNAi agent is administered in a solution. In some such embodiments, the siRNA is administered in an unbuffered solution. In one embodiment, the siRNA is administered in water. In other embodiments, the siRNA is administered with a buffer solution, such as an acetate buffer, a citrate buffer, a prolamine buffer, a carbonate buffer, or a phosphate buffer or any combination thereof. In some embodiments, the buffer solution is phosphate buffered saline (PBS).

In one embodiment, the pharmaceutical compositions further comprise a lipid formulation. ~~In one aspect, t~~The present disclosure ~~invention~~ provides methods of inhibiting Serpina1 expression in a cell. The methods include contacting the cell with an RNAi agent, *e.g.*, a double stranded RNAi agent, composition, vector, or a pharmaceutical composition of the disclosure~~invention~~; and 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 one aspect, the present disclosure provides methods of inhibiting Serpina1 expression in a cell, the method comprising (a) contacting the cell *in vitro* with the double stranded RNAi agent of the present disclosure, the composition of the present disclosure, the vector of the present disclosure, or the pharmaceutical composition of the present disclosure; 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 one embodiment, the cell is within a subject.

In one embodiment, the subject is a human.

In one embodiment, the Serpina1 expression is inhibited by at least about 30% 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95%.

~~In another aspect, t~~The present ~~invention~~ disclosure provides methods of treating a subject having a Serpina1 associated disease. The methods include administering to the subject a therapeutically effective amount of an RNAi agent, *e.g.*, a double stranded RNAi agent, composition, vector, or a pharmaceutical composition of the disclosure~~invention~~, thereby treating the subject.

In one aspect, the present disclosure provides the double stranded RNAi agent of the present disclosure, the composition of the present disclosure, the vector of the present disclosure, or the pharmaceutical composition of the present disclosure, for use in therapy.

In another aspect, the present disclosure provides use of the double stranded RNAi agent of the present disclosure, the composition of the present disclosure, the vector of the present disclosure, or the pharmaceutical composition of the present disclosure, in the manufacture of a medicament for the treatment of a Serpina1-associated disorder in a subject.

~~In another aspect, t~~The present disclosure ~~invention~~ provides methods of treating a subject having a Serpina1-associated disorder. The methods include subcutaneously administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous

nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, wherein substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus,wherein substantially all of the nucleotides of the sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-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 treating the subject.

In another aspect, the present disclosure provides use of a double stranded RNAi agent in the manufacture of a medicament for the treatment of a Serpina1-associated disorder in a subject, wherein the medicament is via subcutaneous administration,

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

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25,

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'-fluoromodification,

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

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'-fluoromodification,

wherein said sense strand comprises two phosphorothioate internucleotide linkages at the 5'-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 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 Serpina1 associated disease is a liver disorder, *e.g.*, chronic liver disease, liver inflammation, cirrhosis, liver fibrosis, and/or hepatocellular carcinoma

In one embodiment, the administration of the RNAi agent to the subject results in a decrease in liver cirrhosis, fibrosis and/or Serpina1 protein accumulation in the liver. In another embodiment, the administration of the RNAi agent to the subject results, *e.g.*, further results, in a decrease in lung inflammation.

In one 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, about 0.5 mg/kg to about 50 mg/kg, about 10 mg/kg to about 30 mg/kg, about 10 mg/kg to about 20 mg/kg, about 15 mg/kg to about 20 mg/kg, about 15 mg/kg to about 25 mg/kg, about 15 mg/kg to about 30 mg/kg, or about 20 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 yet another aspect, t~~The present ~~invention~~ disclosure provides methods of inhibiting development of hepatocellular carcinoma in a subject having a Serpina1 deficiency variant.

The methods include administering to the subject a therapeutically effective amount of an RNAi agent, *e.g.*, a double stranded RNAi agent, composition, vector, or a pharmaceutical composition of the disclosure~~invention~~, thereby inihibiting the development of hepatocellular carcinoma in the subject.

In one aspect, the present disclosure provides use of the double stranded RNAi agent of the present disclosure, the composition of the present disclosure, the vector of the present disclosure, or the pharmaceutical composition of the present disclosure, in the manufacture of a medicament for the inhibition of development of hepatocellular carcinoma in a subject.

~~In another aspect, t~~The present ~~invention~~ disclosure provides methods of inhibiting development of hepatocellular carcinoma in a subject having a Serpina1 deficiency variant. The methods include subcutaneously administering to the subject a therapeutically effective amount of a double stranded RNAi agent, —wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, wherein substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, wherein substantially all of the nucleotides of the sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoromodification, 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 inhibiting development of hepatocellular carcinoma in the subject having a Serpina1 deficiency variant.

In another aspect, the present disclosure provides use of a double stranded RNAi agent in the manufacture of a medicament for the inhibition of development of hepatocellular carcinoma in a subject having a Serpina1 deficiency variant, wherein the medicament is via subcutaneous administration,

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

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25,

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'-fluoromodification,

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

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'-fluoromodification,

wherein said sense strand comprises two phosphorothioate internucleotide linkages at the 5'-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 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 at a dose of about 3 mg/kg. In another embodiment, the double stranded RNAi agent is administered at a dose of about 10 mg/kg. In yet another other embodiment, the double stranded RNAi agent is administered at a dose of about 0.5 mg/kg twice per week. In yet another embodiment, the double stranded RNAi agent is administered at a dose of about 10 mg/kg every other week. In yet another embodiment, the double stranded RNAi agent is administered at a dose of about 0.5 to about 1 mg/kg once per week.

In one embodiment, the RNAi agent, *e.g.*, double stranded RNAi agent, is administered twice per week. In another embodiment, the RNAi agent is administered every other week.

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

~~In another aspect, t~~The present ~~invention~~ disclosure provides methods for reducing the accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant. The methods include administering to the subject a therapeutically effective amount of an RNAi agent, *e.g.*, a double stranded RNAi agent, composition, vector, or a pharmaceutical composition of the disclosure~~invention~~, thereby reducing the accumulation of misfolded Serpina1 in the liver of the subject.

In one aspect, the present disclosure provides use of the double stranded RNAi agent of the present disclosure, the composition of the present disclosure, the vector of the present disclosure, or the pharmaceutical composition of the present disclosure, in the manufacture of a medicament for the reduction of accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant.

~~In another aspect, t~~The present disclosure ~~invention~~ provides methods of reducing the accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant. The methods include subcutaneously administering to the subject a therapeutically effective amount of a double stranded RNAi agent, wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25, wherein substantially all of the nucleotides of the antisense strand comprise a modification

selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, wherein substantially all of the nucleotides of the sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-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 another aspect, the present disclosure provides use of a double stranded RNAi agent in the manufacture of a medicament for the reduction of accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant, wherein the medicament is via subcutaneous administration,

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

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25,

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'-fluoromodification,

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

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'-fluoromodification,

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

The present disclosure ~~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

78. The pharmaceutical composition of claim 77, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

79. The pharmaceutical composition of claim 78, wherein said buffer solution is phosphate buffered saline (PBS).

80. A method of inhibiting Serpina1 expression in a cell, the method comprising:

(a) contacting the cell *in vitro* with the double stranded RNAi agent of any one of claims 1, 9, 59, and 63-70, the composition of claim 71, the vector of claim 72, or the pharmaceutical composition of any one of claims 74-79; 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.

~~81. The method of claim 80, wherein said cell is within a subject.~~

~~82. The method of claim 81, wherein the subject is a human.~~

~~83~~81. The method of ~~any one of~~ claims 80-~~82~~, 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%.

~~84~~82. ~~A method of treating a subject having a Serpina1-associated disease, comprising administering to the subject a therapeutically effective amount of t~~The double stranded RNAi agent of any one of claims 1, 9, 59, and 63-70, the composition of claim 71, ~~the~~ vector of claim 72, or the pharmaceutical composition of any one of claims 74-79~~-~~, for use in therapy ~~thereby treating said subject~~.

83. Use of the double stranded RNAi agent of any one of claims 1, 9, 59, and 63-70, the composition of claim 71, the vector of claim 72, or the pharmaceutical composition of any one of claims 74-79, in the manufacture of a medicament for the treatment of a Serpina1-associated disorder in a subject.

~~85~~84. Use of ~~A method of treating a subject having a Serpina1-associated disorder, comprising subcutaneously administering to the subject a therapeutically effective amount of~~ a double stranded RNAi agent in the manufacture of a medicament for the treatment of a Serpina1-associated disorder in a subject, wherein the medicament is via subcutaneous administration,

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

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25,

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'-fluoromodification,

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

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'-fluoromodification,

wherein said sense strand comprises two phosphorothioate internucleotide linkages at the 5'-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~~, thereby treating the subject~~.

~~86~~85. The ~~method~~ use of claim ~~85~~84, 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.

~~87~~86. The use ~~method~~ of claim ~~86~~85, wherein the antisense strand comprises a universal base at the mismatched nucleotide.

~~88~~87. The ~~method~~use of claim of claim ~~85~~84, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

~~89~~88. The use ~~method~~ of claim ~~84~~83 or ~~85~~84, wherein the subject is a human.

~~90~~89. The use ~~method~~ of claim ~~84~~ 83 or ~~85~~84, wherein the Serpina1 associated disease is a liver disorder.

~~91~~90. The use ~~method~~ of claim ~~90~~89, wherein the liver disorder is selected from the group consisting of chronic liver disease, liver inflammation, cirrhosis, liver fibrosis, and/or hepatocellular carcinoma

~~92~~91. The use ~~method~~ of claim ~~84~~ 83 or ~~85~~84, wherein the double stranded RNAi agent is for administration ~~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.

~~93~~92. The use ~~method~~ of claim ~~92~~91, wherein the double stranded RNAi agent is for administration ~~administered~~ at a dose of about 10 mg/kg to about 30 mg/kg.

~~94~~93. The use ~~method~~ of claim ~~92~~91, wherein the double stranded RNAi agent is for administration ~~administered~~ at a dose of about 3 mg/kg.

~~95~~94. The use ~~method~~ of claim ~~92~~91, wherein the double stranded RNAi agent is for administration ~~administered~~ at a dose of about 10 mg/kg.

~~96~~95. The use ~~method~~ of claim ~~92~~91, wherein the double stranded RNAi agent is for administration ~~administered~~ at a dose of about 0.5 mg/kg twice per week.

~~97~~96. The use ~~method~~ of claim ~~92~~91, wherein the double stranded RNAi agent is for administration ~~administered~~ at a dose of about 10 mg/kg every other week.

~~98~~97. The use ~~method~~ of claim ~~92~~91, wherein the double stranded RNAi agent is for administration ~~administered~~ at a dose of about 0.5-1 mg/kg once per week.

~~99~~98. The use ~~method~~ of claim ~~92~~91, wherein the double stranded RNAi agent is for subcutaneous administration~~administered subcutaneously~~.

~~10099~~. The use ~~method~~ of claim ~~9291~~, wherein the double stranded RNAi agent is for intravenous administration~~administered intravenously~~.

~~101~~100. The use ~~method~~ of claim ~~9291~~, wherein said RNAi agent is for administration ~~administered~~ in two or more doses.

~~102~~101. The use ~~method~~ of claim ~~101~~100, wherein said RNAi agent is for administration ~~administered~~ at intervals selected from the group consisting of once every about 12 hours, once every about 24 hours, once every about 48 hours, once every about 72 hours, and once every about 96 hours.

~~103~~102. The use ~~method~~ of claim ~~101~~100, wherein said RNAi agent is for administration ~~administered~~ twice per week.

~~104~~103. The use ~~method~~ of claim ~~101~~100, wherein said RNAi agent is for administration ~~administered~~ every other week.

~~105~~104. Use of ~~A method of inhibiting development of hepatocellular carcinoma in a subject having a Serpina1 deficiency variant, comprising administering to the subject a therapeutically effective amount of~~ the double stranded RNAi agent of any one of claims 1, 9, 59, and 63-70, the composition of claim 71, ~~the~~ vector of claim 72, or the pharmaceutical composition of any one of claims 74-79~~-~~, in the manufacture of a medicament for the inhibition of development of hepatocellular carcinoma in a subject~~thereby inhibiting development of hepatocellular carcinoma in the subject~~.

~~106~~105. Use of ~~A method of inhibiting development of hepatocellular carcinoma in a subject having a Serpina1 deficiency variant, comprising subcutaneously administering to the subject a therapeutically effective amount of~~ a double stranded RNAi agent in the manufacture of a medicament for the inhibition of development of hepatocellular carcinoma in a subject having a Serpina1 deficiency variant, wherein the medicament is via subcutaneous administration,

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

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID

NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25,

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'-fluoromodification,

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

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'-fluoromodification,

wherein said sense strand comprises two phosphorothioate internucleotide linkages at the 5'-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~~, thereby inhibiting development of hepatocellular carcinoma in the subject having a Serpina1 deficiency variant~~.

~~107~~106. The ~~method~~ use of claim ~~106~~105, 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.

~~108~~107. The use ~~method~~ of claim ~~107~~106, wherein the antisense strand comprises a universal base at the mismatched nucleotide.

~~109~~108. The ~~method~~ use of claim of claim ~~106~~105, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

~~110~~109. The use ~~method~~ of claim ~~105~~ 104 or ~~106~~105, wherein the subject is a primate or rodent.

~~111~~110. The use ~~method~~ of claim ~~105~~ 104 or ~~106~~105, wherein the subject is a human.

~~112~~111. The use ~~method~~ of claim ~~105~~ 104 or ~~106~~105, wherein the double stranded RNAi agent is for administration ~~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.

~~113~~112. The use ~~method~~ of claim ~~112~~111, wherein the double stranded RNAi agent is for administration ~~administered~~ at a dose of about 10 mg/kg to about 30 mg/kg.

~~114~~113. The use ~~method~~ of claim ~~112~~111, wherein said RNAi agent is for administration ~~administered~~ in two or more doses.

~~115~~114. The use ~~method~~ of claim ~~112~~111, wherein said RNAi agent is for administration ~~administered~~ at intervals selected from the group consisting of once every about 12 hours, once every about 24 hours, once every about 48 hours, once every about 72 hours, and once every about 96 hours.

~~116~~115. The use ~~method~~ of claim ~~112~~111, wherein said RNAi agent is for administration ~~administered~~ twice per week.

~~117~~116. The use ~~method~~ of claim ~~112~~111, wherein said RNAi agent is for administration ~~administered~~ every other week.

~~118~~117. The use ~~method~~ of claim ~~112~~111, wherein the double stranded RNAi agent is for subcutaneous administration~~administered subcutaneously~~.

~~119~~118. The use ~~method~~ of claim ~~112~~111, wherein the double stranded RNAi agent is for intravenous administration~~administered intravenously~~.

~~120~~119. Use of ~~A method of reducing the accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant, comprising administering to the subject a therapeutically effective amount of~~ the double stranded RNAi agent of any one of claims 1, 9, 59, and 63-70, the composition of claim 71, ~~the~~ vector of claim 72, or the pharmaceutical composition of any one of claims 74-79~~.~~, in the manufacture of a medicament for the reduction of accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant~~thereby reducing the accumulation of misfolded Serpina1 in the liver of the subject~~.

~~121~~120. Use of ~~A method of reducing the accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant, comprising subcutaneously administering to the subject a therapeutically effective amount of~~ a double stranded RNAi agent in the manufacture of a medicament for the reduction of accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant, wherein the medicament is via subcutaneous administration,

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

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, or SEQ ID NO:11, and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NO:15, SEQ ID NO:16, or SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, or SEQ ID NO:25,

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'-fluoromodification,

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

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'-fluoromodification,

wherein said sense strand comprises two phosphorothioate internucleotide linkages at the 5'-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~~, thereby reducing the accumulation of misfolded Serpina1 in the liver of the subject having a Serpina1 deficiency variant~~.

~~122~~121. The ~~method~~ use of claim ~~121~~120, 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.

~~123~~122. The use ~~method~~ of claim ~~122~~121, wherein the antisense strand comprises a universal base at the mismatched nucleotide.

~~124~~1213. The ~~method~~ use of claim of claim ~~121~~120, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

~~125~~124. The use ~~method~~ of claim ~~120~~ 119 or ~~121~~120, wherein the subject is a primate or rodent.

~~126~~125. The use ~~method~~ of claim ~~120~~ 119 or ~~121~~120, wherein the subject is a human.

~~127~~126. The use ~~method~~ of claim ~~120~~ 119 or ~~121~~120, wherein the double stranded RNAi agent is ~~administered~~ 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.

~~128~~127. The use ~~method~~ of claim ~~127~~126, wherein the double stranded RNAi agent is for administration ~~administered~~ at a dose of about 10 mg/kg to about 30 mg/kg.

~~129~~128. The use ~~method~~ of claim ~~127~~126, wherein said RNAi agent is for administration ~~administered~~ in two or more doses.

~~130~~129. The use ~~method~~ of claim ~~127~~126, wherein said RNAi agent is for administration ~~administered~~ twice per week.

~~131~~130. The use ~~method~~ of claim ~~127~~126, wherein said RNAi agent is for administration ~~administered~~ every other week.

~~132~~131. The use ~~method~~ of claim ~~127~~126, wherein said RNAi agent is for administration ~~administered~~ at intervals selected from the group consisting of once every about 12 hours, once every about 24 hours, once every about 48 hours, once every about 72 hours, and once every about 96 hours.

~~133~~132. The use ~~method~~ of claim ~~127~~126, wherein the double stranded RNAi agent is for subcutaneous administration~~administered subcutaneously~~.

~~134~~133. The use ~~method~~ of claim ~~127~~126, wherein the double stranded RNAi agent is for intravenous administration~~administered intravenously~~.