TMPRSS6 iRNA COMPOSITIONS AND METHODS OF USE THEREOF

Related Applications

This application claims the benefit of priority to U.S. Provisional Patent Application No. 61/826,178, filed on May 22, 2013 and U.S. Provisional Patent Application No. 61/912,988, filed on December 6, 2013. This application is related to U.S. Provisional Application No. 61/561,710, filed on November 18, 2011, and PCT/US2012/065601, filed on November 16, 2012. The entire contents of each of the foregoing applications are hereby incorporated herein by reference.

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 21, 2014, is named 121301-00720_SL.txt and is 449,620 bytes in size.

Background of the Invention

TMPRSS6 (Transmembrane Protease, Serine 6) gene encodes TMPRSS6, also known as matriptase-2, a type II serine protease. It is primarily expressed in the liver, although high levels of TMPRSS6 mRNA are also found in the kidney, with lower levels in the uterus and much smaller amounts detected in many other tissues (Ramsay et al., *Haematologica* (2009), 94(6), 840-849). TMPRSS6 plays a role in iron homeostatis by binding and proteolytically degrading the hepcidin activator and BMP co-receptor HJV (hemojuvelin), which causes down-regulation of hepcidin levels.

TMPRSS6 consists of a short N-terminal intracytoplasmic tail, a type II transmembrane domain, a stem region composed of two extracellular CUB (complement factor Cls/Clr, urchin embryonic growth factor and BMP (bone morphogenetic protein)) domains, three LDLR (low-density-lipoprotein receptor class A) domains, and a C-terminal trypsin- like serine protease domain. There are also consensus sites for N-glycosylation in the extracellular domain, and a potential phosphorylation site in the intracytoplasmic tail region.

Numberous disorders can be associated with iron overload, a condition characterized by increased levels of iron. Iron overload can result in excess iron deposition in various tissues and can lead to tissue and organ damage. Accordingly, methods for effective treatment of disorders associated with iron overload are currently needed.

Summary ~~of the Invention~~

The present ~~invention~~ disclosure provides compositions comprising RNAi agents, *e.g.*, double-stranded iRNA agents, targeting TMPRSS6. The present disclosure ~~invention~~ also provides methods

using the compositions of the disclosure for inhibiting TMPRSS6 expression and for treating TMPRSS6 associated disorders, *e.g.*, iron overload associated disorders, such as thalassemia, *e.g.*, β-thalassemia, or hemochromatosis.

Accordingly, in one aspect, the present disclosure provides RNAi agents, *e.g.*, double-stranded RNAi agents, capable of inhibiting the expression of TMPRSS6 (matriptase-2) in a cell, 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, or SEQ ID NO:5, 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:6, SEQ ID NO:7, or SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10,

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, all of the nucleotides of said sense strand and all of the nucleotides of said 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 antisense sequences listed in any one of Tables 1, 2, 4, 5, 8, 10, and 12.

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, a nucleotide comprising a 5' phosphate or 5' phosphate mimic (see, *e.g.,* PCT Publication No. WO 2011/005860), 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 provides RNAi agents, *e.g.*, double-stranded RNAi agents, capable of inhibiting the expression of TMPRSS6 (matriptase-2) in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding TMPRSS6, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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, 4, 5, 8, 10, and 12.

In one embodiment, the RNAi agent is AD-59743. In another embodiment, the RNAi agent is AD-60940.

In one aspect, the present disclosure provides double stranded RNAi agents capable of ~~for~~ inhibiting expression of TMPRSS6 in a cell,

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, or SEQ ID NO:5, 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:6, SEQ ID NO:7, or SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10,

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, all of the nucleotides of the sense strand and all of the nucleotides of the 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 TMPRSS6 (matriptase-2) in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding TMPRSS6, 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 -(X X X) $_i$-N_b -Y Y Y -N_b -(Z Z Z)$_j$ -N_a - n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'- 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 TMPRSS6 (matriptase-2) in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding TMPRSS6, 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 -(X X X) $_i$-N_b -Y Y Y -N_b -(Z Z Z)$_j$ -N_a - n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'- 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 TMPRSS6 (matriptase-2) in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding TMPRSS6, 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 TMPRSS6 (matriptase-2) in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding TMPRSS6, 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 -(X X X) $_i$-N_b -Y Y Y -N_b -(Z Z Z)$_j$ -N_a - n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y'Y'Y'-N_b'-(Z'Z'Z')$_l$-N_a'- 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 TMPRSS6 (matriptase-2) in a cell, wherein the double stranded RNAi agent comprises a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding TMPRSS6, 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~~aspect, the present disclosure ~~invention~~ provides RNAi agent selected from the group of RNAi agents listed in any one of Tables 1, 2, 4, 5, 8, 19, and 12.

In one aspect, the present disclosure ~~invention~~ provides compositions comprising a modified antisense polynucleotide agent, wherein the agent is capable of inhibiting the expression of TMPRSS6 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, 4, 5, 8, 10, and 12, wherein the polynucleotide is about 14 to about 30 nucleotides in length.

The present disclosure ~~invention~~ also provides cells, vectors, host cells, and pharmaceutical compositions comprising, *e.g.,* the double stranded RNAi agents of the disclosure~~invention~~. In on aspect, the present disclosure provides a vector containing the double stranded RNAi agent of the disclosure. In another aspect, the present disclosure provides a cell containing the double stranded RNAi agent of the disclosure. In another aspect, the present disclosure provides a pharmaceutical composition comprising the double stranded RNAi agent of the disclosure, or the modified antisense polynucleotide of the disclosure, or the vector of the disclosure.

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 embodiment, the lipid formulation comprises a LNP, or XTC. In another embodiment, the lipid formulation comprises a MC3.

~~In one aspect, t~~The present ~~invention~~ disclosure provides methods of inhibiting TMPRSS6 expression in a cell. The methods include contacting the cell with an RNAi agent, *e.g.*, a double stranded RNAi agent, or a modified antisense polynucleotide agent of the disclosure ~~invention~~, or vector of the disclosure ~~invention~~, 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 TMPRSS6 gene, thereby inhibiting expression of the TMPRSS6 gene in the cell.

In one aspect, the present disclosure provides methods of inhibiting TMPRSS6 expression in a cell, the method comprising (a) contacting the cell *in vitro* with the double stranded RNAi agent of the present disclosure, or the modified antisense polynucleotide agent of the present disclosure, or the vector of the present disclosure, or a 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 TMPRSS6 gene, thereby inhibiting expression of the TMPRSS6 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 TMPRSS6 expression is inhibited by at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 100%.

In another embodiment, hepcidin gene expression is increased by at least about 1.5-fold, about 2-fold, about 3-fold, about 4-fold, or about 5-fold.

In yet another embodiment, serum hepcidin concentration is increased by at least about 10%, about 25%, about 50%, about 100%, about 150%, about 200%, about 250%, or about 300%.

In one embodiment, serum iron concentration is decreased by at least about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

In another embodiment,a percent transferrin saturation is decreased by at least about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

~~In another aspect, t~~The present ~~invention~~ disclosure provides methods of treating a subject having a disorder mediated by, or associated with, TMPRSS6 expression. The methods include administering to the subject a therapeutically effective amount of an RNAi agent, *e.g.*, a double stranded RNAi agent, of the disclosure~~invention~~, or a modified antisense polynucleotide agent of the disclosure~~invention~~, or a vector of the disclosure~~invention~~, 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, or the modified antisense polynucleotide agent of the present disclosure, or the vector of the present disclosure, or a pharmaceutical composition of the present disclosure, for use in therapy.

In one aspect, the present disclosure provides use of the double stranded RNAi agent of the present disclosure, or the modified antisense polynucleotide agent of the present disclosure, or the vector of the present disclosure, or a pharmaceutical composition of the present disclosure, in the manufacture of a medicament for the treatment of a TMPRSS6-associated disorder in a subject.

~~In one aspect, t~~The present disclosure ~~invention~~ provides methods of treating a subject having a TMPRSS6-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, or SEQ ID NO:5, 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:6, SEQ ID NO:7, or SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10,

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 one aspect, the present disclosure provides use of a double stranded RNAi agent in the manufacture of a medicament for the treatment of a TMPRSS6-associated disorder in a subject,

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, or SEQ ID NO:5, 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:6, SEQ ID NO:7, or SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10,

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, 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 human.

In one embodiment, the subject has a disorder associated with iron overload, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermiedia) erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia.

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 1 mg/kg to about 10 mg/kg, about 2 mg/kg to about 10 mg/kg, about 3 mg/kg to about 10 mg/kg, about 4 mg/kg to about 10 mg/kg, about 5 mg/kg to about 15 mg/kg, about 6 mg/kg to about 15 mg/kg, about 7 mg/kg to about 15 mg/kg,

about 8 mg/kg to about 15 mg/kg, about 9 mg/kg to about 15 mg/kg, about 10 mg/kg to about 20 mg/kg, about 12 mg/kg to about 20 mg/kg, about 13 mg/kg to about 20 mg/kg, about 14 mg/kg to about 20 mg/kg, about 15 mg/kg to about 20 mg/kg, about 16 mg/kg to about 20 mg/kg or about 18 mg/kg to about 20 mg/kg. In particular embodiments, the double stranded RNAi agent is administered at a dose of about 0.1 mg/kg, about 1.0 mg/kg, or about 3.0 mg/kg.

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

In one embodiment, the RNAi agent is administered in two or more doses. In a specific embodiment, the RNAi agent is 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, once every about 96 hours, once about every 7 days, or once about every 14 days. In particular embodiments, the RNAi agent is administered once a week for up to 2 weeks, up to 3 weeks, up to 4 weeks, up to 5 weeks, or longer.

~~In yet another aspect, t~~The present ~~invention~~ disclosure provides methods of treating an iron overload associated disorder in a subject. The methods include administering to the subject a therapeutically effective amount of an RNAi agent, *e.g.*, a double stranded RNAi agent, or the vector of the disclosure~~invention~~, thereby treating the subject.

In one embodiment, the iron overload associated disorder is hemochromatosis. In another embodiment, the iron overload associated disorder is a thalassemia, *e.g.*, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermiedia), or erythropoietic porphyria. In yet another embodiment, the iron overload associated disorder is a neurological disease, *e.g.*, Parkinson's Diasease, Alzheimer's Disease or Friedreich's Ataxia.

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, 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 one embodiment, the RNAi agent is administered in two or more doses. In a specific embodiment, the RNAi agent is 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, once every about 96 hours, once about every 7 days, or once about every 14 days.

In one embodiment, administering results in a decrease in iron levels, ferritin level and/or transferrin saturation level in the subject.

In one embodiment, the methods further comprise determining the iron level in the subject.

In one embodiment, the methods of the disclosure~~invention~~ which include administering an iRNA agent of the disclosure~~invention~~ (or pharmaceutical composition of the disclosure~~invention~~) to a subject are practiced in combination with administration of additional pharmaceuticals and/or other therapeutic methods. In one embodiment, the methods of the disclosure~~invention~~ further comprise administering an iron chelator, *e.g.*, deferiprone, deferoxamine, and deferasirox, to a subject.

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

Brief Description of the Drawings

Figure 1 is a graph showing relative levels of TMPRSS6 mRNA in the liver of wild-type mice following administration of a single dose of 1 mg/kg, 3 mg/kg or 10 mg/kg of the iRNA agent AD-59743.

Figure 2 is a graph showing relative levels of hepcidin mRNA in the liver of wild-type mice following administration of a single dose of 1 mg/kg, 3 mg/kg or 10 mg/kg of the iRNA agent AD-59743.

Figures 3A-3E show the levels of hepatic TMPRSS6 mRNA (Figure 3A), hepatic hepcidin mRNA (Figure 3B), serum hepcidin (Figure 3C), total serum iron (Figure 3D), and percent transferrin saturation (Figure 3E) in C57BL/6 mice at various time points following a

74. A method of inhibiting TMPRSS6 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, 7, 56, and 58-64 , or the modified antisense polynucleotide agent of claim 65, or the vector of claim 66, or a pharmaceutical composition of any one of claims 68-73; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TMPRSS6 gene, thereby inhibiting expression of the TMPRSS6 gene in the cell.

~~75. The method of claim 74, wherein said cell is within a subject.~~

~~76. The method of claim 75, wherein the subject is a human.~~

~~77~~75. The method of ~~any one of~~ claims 74~~-76~~, wherein the TMPRSS6 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%.

~~78~~76. The method of ~~any one of~~ claims 74~~-76~~, wherein hepcidin gene expression is increased by at least about 1.5-fold, about 2-fold, about 3-fold, about 4-fold or about 5-fold.

~~79~~77. The method of ~~any one of~~ claims 74~~-76~~, wherein serum hepcidin concentration is increased by at least about 10%, about 25%, about 50%, about 100%, about 150%, about 200%, about 250%, or about 300%.

~~80~~78. The method of ~~any one of~~ claims 74~~-76~~, wherein serum iron concentration is decreased by at least about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

~~81~~79. The method of ~~any one of~~ claims 74~~-76~~, wherein a percent transferrin saturation is decreased by at least about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

~~82~~80. The ~~A method of treating a subject having a TMPRSS6 associated disorder, comprising administering to the subject a therapeutically effective amount of the~~ double stranded RNAi agent of any one of claims 1, 7, 56, and 58-64~~-~~, or the modified antisense polynucleotide agent of claim 65, or the vector of claim 66, or a pharmaceutical composition of any one of claims 68-73~~-~~, for use in therapy. ~~thereby treating said subject.~~

81. Use of the double stranded RNAi agent of any one of claims 1, 7, 56, and 58-64, or the modified antisense polynucleotide agent of claim 65, or the vector of claim 66, or a pharmaceutical composition of any one of claims 68-73, in the manufacture of a medicament for the treatment of a TMPRSS6-associated disorder in a subject.

~~83~~82. Use of ~~A method of treating a subject having a TMPRSS6-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 TMPRSS6-associated disorder in a subject,

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, or SEQ ID NO:5, 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:6, SEQ ID NO:7, or SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10,

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~~.

~~84~~83. The ~~method~~ use of claim of claim ~~83~~82, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

~~85~~84. The use ~~method~~ of claim ~~82~~ 81 or ~~83~~82, wherein the subject is a human.

~~86~~85. The use ~~method~~ of claim ~~85~~84, wherein the human has hereditary hemochromatosis, β-thalassemia, or erythropoietic porphyria.

~~87~~86. The use ~~method~~ of claim ~~85~~84, wherein the human has β-thalassemia.

~~88~~87. The use ~~method~~ of claim ~~87~~86, wherein the β-thalassemia is thalassemia major.

~~89~~88. The use ~~method~~ of claim ~~87~~86, wherein the β-thalassemia is thalassemia intermedia.

~~90~~89. The use ~~method~~ of claim ~~85~~84, wherein the human has a disorder associated with iron overload.

~~91~~90. The use ~~method~~ of ~~90~~89, wherein the disorder associated with iron overload is Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia.

~~92~~91. The use ~~method~~ of claim ~~82~~81 or ~~83~~82, 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 1 mg/kg to about 10 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 0.1 mg/kg, about 1.0 mg/kg, or about 3.0 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 1 mg/kg to about 10 mg/kg.

~~95~~94. The use ~~method~~ of claim ~~92~~91, wherein the double stranded RNAi agent is for subcutaneous administration~~administered subcutaneously~~.

~~96~~95. The use ~~method~~ of claim ~~92~~91, wherein the double stranded RNAi agent is for intravenous administration~~administered intravenously~~.

~~97~~96. The use ~~method~~ of claim ~~92~~91, wherein said RNAi agent is for administration ~~administered~~ in two or more doses.

~~98~~97. The use ~~method~~ of claim ~~97~~96, 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.

~~99~~98. The use ~~method~~ of claim ~~97~~96, wherein said RNAi agent is for administration ~~administered~~ once a week for up to 2 weeks, up to 3 weeks, up to 4 weeks, up to 5 weeks, or longer.

~~100~~99. The use ~~method~~ of claim ~~82~~ 81 or ~~83~~82, further comprising ~~administering~~ an iron chelator ~~to said dusbject~~.

~~101~~100. The use ~~method~~ of claim ~~100~~99, wherein the iron chelator is selected from the group consisting of deferiprone, deferoxamine, and deferasirox.