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 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 the nucleotide sequence of 5' – CUUCUUCUGGUUCAUUCUCCA -3' (SEQ ID NO:223), and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequences of 5' – UGGAGAAUGAACCAGAAGAAGCA – 3' (SEQ ID NO:292), wherein the sense strand and the antisense strand are each independently about 14 to about 30 nucleotides in length,

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.

Also disclosed are 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 a double stranded RNAi agents capable of 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 the nucleotide sequences of 5' – CUUCUUCUGGUUCAUUCUCCA -3' (SEQ ID NO:223), and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequences of 5' – UGGAGAAUGAACCAGAAGAAGCA – 3' (SEQ ID NO:292), wherein the sense strand and the antisense strand are each independently about 14 to about 30 nucleotides in length,

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.

Also disclosed are 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):

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 aspect, the present disclosure provides an RNAi agent comprising a sense strand comprising the nucleotide sequence of 5' – CUUCUUCUGGUUCAUUCUCCA -3' (SEQ ID NO:223), and an antisense strand comprising the nucleotide sequence of 5' – UGGAGAAUGAACCAGAAGAAGCA – 3' (SEQ ID NO:292), wherein the sense strand and the antisense strand are each independently about 14 to about 30 nucleotides in length.

In one aspect, the present disclosure 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 CUUCUUCUGGUUCAUUCUCCA, wherein the polynucleotide is about 14 to about 30 nucleotides in length.

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

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 a double stranded RNAi agent for use in 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 the nucleotide sequences of 5' – CUUCUUCUGGUUCAUUCUCCA -3' (SEQ ID NO:223), and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequences of 5' – UGGAGAAUGAACCAGAAGAAGCA – 3' (SEQ ID NO:292), wherein the sense strand and the antisense strand are each independently about 14 to about 30 nucleotides in length,

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,

We claim:

1. A double stranded RNAi agent capable of inhibiting expression of TMPRSS6 in a cell, 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 the nucleotide sequence of 5' – CUUCUUCUGGUUCAUUCUCCA -3' (SEQ ID NO:223), and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5' – UGGAGAAUGAACCAGAAGAAGCA – 3' (SEQ ID NO:292),

wherein the sense strand and the antisense strand are each independently about 14 to about 30 nucleotides in length,

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

wherein said sense strand is conjugated to a ligand attached at the 3'-terminus.

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

3. The double stranded RNAi agent of claim 1, wherein said sense strand and said antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides of the antisense sequence of 5' – UGGAGAAUGAACCAGAAGAAGCA – 3' (SEQ ID NO:292).

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

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

24. The double stranded RNAi agent of claim 21, wherein the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

25. The double stranded RNAi agent of claim 24, wherein at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage.

26. The double stranded RNAi agent of claim 25, wherein all n_p' are linked to neighboring nucleotides via phosphorothioate linkages.

27. The double stranded RNAi agent of claim 1, wherein said RNAi agent is selected from the group of RNAi agents listed in any one of Tables 1, 2, 4, 5, 8, 10, and 12.

28. A double stranded RNAi agent capable of inhibiting expression of TMPRSS6 in a cell,

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 the nucleotide sequences of sequence of 5' – CUUCUUCUGGUUCAUUCUCCA -3' (SEQ ID NO:223), and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5' – UGGAGAAUGAACCAGAAGAAGCA – 3' (SEQ ID NO:292),

wherein the sense strand and the antisense strand are each independently about 14 to about 30 nucleotides in length,

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

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

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

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

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

29. The double stranded RNAi agent of claim 28, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

30. An RNAi agent comprising a sense strand comprising the nucleotide sequence of 5' – CUUCUUCUGGUUCAUUCUCCA -3' (SEQ ID NO:223), and an antisense strand comprising the nucleotide sequence of 5' – UGGAGAAUGAACCAGAAGAAGCA – 3' (SEQ ID NO:292), wherein the sense strand and the antisense strand are each independently about 14 to about 30 nucleotides in length.

31. A composition comprising a modified antisense polynucleotide agent capable of inhibiting expression of TMPRSS6 in a cell, wherein said agent comprises a sequence complementary to a sense sequence CUUCUUCUGGUUCAUUCUCCA, wherein the polynucleotide is about 14 to about 30 nucleotides in length.

32. A cell containing the double stranded RNAi agent of any one of claims 1, 28, 30 and 66.

33. A pharmaceutical composition comprising the double stranded RNAi agent of any one of claims 1, 28, 30 and 66, or the modified antisense polynucleotide agent of claim 31.

34. The pharmaceutical composition of claim 33, wherein RNAi agent is to be administered in an unbuffered solution.

35. The pharmaceutical composition of claim 34, wherein said unbuffered solution is saline or water.

36. The pharmaceutical composition of claim 34, wherein said siRNA is to be administered with a buffer solution.

37. The pharmaceutical composition of claim 36, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

38. The pharmaceutical composition of claim 37, wherein said buffer solution is phosphate buffered saline (PBS).

39. 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, 28, 30, and 66, or the modified antisense polynucleotide agent of claim 31, or a pharmaceutical composition of any one of claims 33-38; 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.

40. The method of claim 39, 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%.

41. The method of claim 39, 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.

42. The method of claim 39, 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%.

43. The method of claim 39, 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%.

44. The method of claim 39, 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%.

45. The double stranded RNAi agent of any one of claims 1, 28, 30, and 66, or the modified antisense polynucleotide agent of claim 31, , or a pharmaceutical composition of any one of claims 33-38, for use in therapy.

46. The double stranded RNAi agent of any one of claims 1, 28, 30, and 66, or the modified antisense polynucleotide agent of claim 31, or a pharmaceutical composition of any one of claims 33-38 for use in the treatment of a TMPRSS6-associated disorder in a subject.

47. A double stranded RNAi agent for use in 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 the nucleotide sequence of 5' – CUUCUUCUGGUUCAUUCUCCA -3' (SEQ ID NO:223), and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5' – UGGAGAAUGAACCAGAAGAAGCA – 3' (SEQ ID NO:292),

wherein the sense strand and the antisense strand are each independently about 14 to about 30 nucleotides in length,

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.

48. The double stranded RNAi agent of claim 47, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

49. The double stranded RNAi agent of claim 46 or 47, wherein the subject is a human.

50. The double stranded RNAi agent of claim 49, wherein the human has hereditary hemochromatosis, β-thalassemia, or erythropoietic porphyria.

51. The double stranded RNAi agent of claim 49, wherein the human has β-thalassemia.