associated disorders, *e.g.*, iron overload associated disorders, such as thalassemia, *e.g.*, β-thalassemia, or hemochromatosis.

In one aspect, provided herein is a double stranded RNAi agent for inhibiting expression of TMPRSS6 in a cell, comprising a sense strand and an antisense strand forming a double-stranded region, wherein the sense strand comprises at least 17 contiguous nucleotides from the nucleotide sequence ACCUGCUUCUUCUGGUUCAUU (SEQ ID NO:139), and the antisense strand comprises at least 17 contiguous nucleotides from the nucleotide sequence AGAAUGAACCAGAAGAAGCAGGU (SEQ ID NO:187), wherein each strand is independently 17 to 30 nucleotides in length, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides selected from the group consisting of a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-deoxy-2'-fluoro (2'-F) modified nucleotide, and a 2'-deoxy modified nucleotide, wherein the double stranded RNAi agent comprises 6-8 phosphorothioate internucleotide linkages, and wherein at least one strand is conjugated to an N-acetylgalactosamine (GalNAc) ligand attached at the 3′-terminus, the 5′-terminus or both termini.

In another aspect, provided herein is a pharmaceutical composition for inhibiting expression of a TMPRSS6 gene comprising the double stranded RNAi agent as described herein.

In another aspect, provided herein is an *in vitro* method of inhibiting TMPRSS6 expression in a cell, the method comprising: (a) contacting the cell *in vitro* with the double stranded RNAi agent as described herein, or the pharmaceutical composition as described herein; and (b) maintaining the cell produced in step (a) *in vitro* 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 another aspect, provided herein is the use of a therapeutically effective amount of the double stranded RNAi agent as described herein, or the pharmaceutical composition as described herein, in the manufacture of a medicament for treating a subject having a TMPRSS6 associated disorder.

In another aspect, provided herein is an isolated cell containing the double stranded RNAi agent as described herein.

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

Accordingly, in one example, 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 example, all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand are modified nucleotides.

In one example, 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

CLAIMS

1. A double stranded RNAi agent for inhibiting expression of TMPRSS6 in a cell, comprising a sense strand and an antisense strand forming a double-stranded region, wherein the sense strand comprises at least 17 contiguous nucleotides from the nucleotide sequence ACCUGCUUCUUCUGGUUCAUU (SEQ ID NO:139), and the antisense strand comprises at least 17 contiguous nucleotides from the nucleotide sequence AGAAUGAACCAGAAGAAGCAGGU (SEQ ID NO:187),

wherein each strand is independently 17 to 30 nucleotides in length,

wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides selected from the group consisting of a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-deoxy-2'-fluoro (2'-F) modified nucleotide, and a 2'-deoxy modified nucleotide,

wherein the double stranded RNAi agent comprises 6-8 phosphorothioate internucleotide linkages, and

wherein at least one strand is conjugated to an N-acetylgalactosamine (GalNAc) ligand attached at the 3′-terminus, the 5′-terminus or both termini.

2. The double stranded RNAi agent of claim 1, wherein each strand is independently 17-25 nucleotides in length.

3. The double stranded RNAi agent of claim 1, wherein each strand is independently 19-25 nucleotides in length.

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

5. The double stranded RNAi agent of claim 1, wherein at least one strand comprises an overhang of at least 1 nucleotide.

6. The double stranded RNAi agent of claim 1, wherein the agent further comprises at least one methylphosphonate internucleotide linkage.

7. The double stranded RNAi agent of claim 1, wherein at least one of the phosphorothioate internucleotide linkages is at the 3'-terminus of one strand.

8. The double stranded RNAi agent of claim 7, wherein the strand is the antisense strand.

9. The double stranded RNAi agent of claim 7, wherein the strand is the sense strand.

10. The double stranded RNAi agent of claim 1, wherein at least one of the phosphorothioate internucleotide linkages is at the 5'-terminus of one strand.

11. The double stranded RNAi agent of claim 10, wherein the strand is the antisense strand.

12. The double stranded RNAi agent of claim 1, wherein at least one of the phosphorothioate internucleotide linkages is at both the 5'- terminus and 3'-terminus of one strand.

13. The double stranded RNAi agent of claim 12, wherein the strand is the antisense strand.

14. The double stranded RNAi agent of claim 6, wherein the methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

15. The double stranded RNAi agent of claim 14, wherein the strand is the antisense strand.

16. The double stranded RNAi agent of claim 14, wherein the strand is the sense strand.

17. The double stranded RNAi agent of claim 6, wherein the methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

18. The double stranded RNAi agent of claim 17, wherein the strand is the antisense strand.

19. The double stranded RNAi agent of claim 6, wherein the methylphosphonate internucleotide linkage is at both the 5'- terminus and 3'-terminus of one strand.

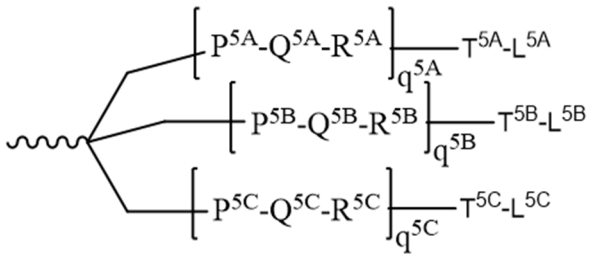
20. The double stranded RNAi agent of claim 19, wherein the strand is the antisense strand.

21. The double stranded RNAi agent of claim 1, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate

internucleotide linkages at the 3'-terminus, and the sense strand comprises two phosphorothioate internucleotide linkages at either the 5'-terminus or the 3'-terminus .

22. The double stranded RNAi agent of claim 1, wherein the N-acetylgalactosamine (GalNAc) ligand is attached through a trivalent branched linker.

23. The double stranded RNAi agent of claim 22, wherein the N-acetylgalactosamine (GalNAc) ligand is attached through a trivalent branched linker as shown in Formula (VII):



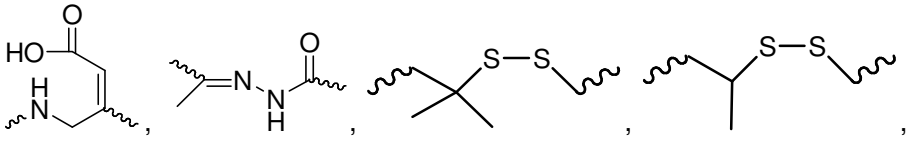
Formula (VII)

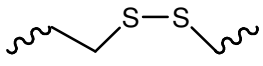
wherein: q^{5A}, q^{5B} and q^{5C} represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

P^{5A}, P^{5B}, P^{5C}, T^{5A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, or substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, C≡C or C(O);

R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, NHCH(R^a)C(O), -C(O)-CH(R^a)-NH-, CO, CH=N-O,





or heterocyclyl;

L^{5A}, L^{5B} and L^{5C} represent the N-acetylgalactosamine (GalNAc) ligand; and

R^a is H or amino acid side chain.

24. A pharmaceutical composition for inhibiting expression of a TMPRSS6 gene comprising the double stranded RNAi agent of any one of claims 1-23.

25. An *in vitro* 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 1-23, or the pharmaceutical composition of claim 24; and

(b) maintaining the cell produced in step (a) *in vitro* 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.

26. The method of claim 25, wherein the TMPRSS6 expression is inhibited by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98% or 100%; serum hepcidin concentration is increased by at least 10%, 25%, 50%, 100%, 150%, 200%, 250%, or 300%; serum iron concentration is decreased by at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98% or 100%; or percent transferrin saturation is decreased by at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98% or 100%.

27. Use of a therapeutically effective amount of the double stranded RNAi agent of any one of claims 1-23, or the pharmaceutical composition of claim 24, in the manufacture of a medicament for treating a subject having a TMPRSS6 associated disorder.

28. The use of claim 27, wherein the subject is a human.

29. The use of claim 27 or 28, wherein the TMPRSS6 associated disorder is hereditary hemochromatosis, β-thalassemia, erythropoietic porphyria, or a disorder associated with iron overload.

30. The use of any one of claims 27-29, wherein the double stranded RNAi agent is to be administered at a dose of 0.01 mg/kg to 10 mg/kg or 1 mg/kg to 10 mg/kg.

31. The use of any one of claims 27-30, wherein the double stranded RNAi agent is for subcutaneous administration.

32. The use of any one of claims 27-30, wherein the double stranded RNAi agent is for intravenous administration.

33. An isolated cell containing the double stranded RNAi agent of any one of claims 1-23.

34. The double stranded RNAi agent of any one of claims 1-23, or the pharmaceutical composition of claim 24 for use in therapy.