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(54) **Title**: COMPOSITIONS AND METHODS FOR INHIBITING HEPCIDIN ANTIMICROBIAL PEPTIDE (HAMP) OR HAMP-RELATED GENE EXPRESSION

(57) **Abstract**: The invention relates to lipid formulated double-stranded ribonucleic acid (dsRNA) targeting a hepcidm antimicrobial peptide (HAMP) and/or HAMP-related gene, and methods of using the dsRNA to inhibit expression of HAMP and/or HAMP-related genes.

Compositions and methods for inhibiting hepcidin antimicrobial peptide (HAMP) or HAMP-related gene expression

Cross Reference to Related Applications

This application claims the benefit of U.S. Provisional Application Serial No. 61/499,516, filed June 21, 2011, and claims the benefit of U.S. Provisional Application Serial No. 61/569,054, filed December 9, 2011; each of which are incorporated herein by reference, in their entirety, for all purposes.

Reference to Sequence Listing

This application includes a Sequence Listing submitted electronically as a text file named _____.txt, created on _____, 2012, with a size of _____ bytes. The sequence listing is incorporated by reference.

Field

The disclosure relates to double-stranded ribonucleic acid (dsRNA) targeting HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1, and methods of using dsRNA to inhibit expression of HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1.

Background

The discovery of the hepcidin peptide and characterization of its gene, *HAMP,* has led to the revision of previous models for the regulation of iron homeostasis and the realization that the liver plays a key role in determining iron absorption from the gut and iron release from recycling and storage sites. In summary, the hepcidin model proposes that the rate of iron efflux into the plasma depends primarily on the plasma level of hepcidin; when iron levels are high the synthesis of hepcidin increases and the release of iron from enterocytes and macrophages is diminished. Conversely when iron stores drop, the synthesis of hepcidin is down-regulated and these cells release more iron. Hepcidin directly binds to ferroportin and decreases its functional activity by causing it to be internalized from the cell surface and degraded.

Hepcidin provides a unifying hypothesis to explain the behavior of iron in two diverse but common clinical conditions, the anemia of chronic disease and both *HFE* and non-*HFE* haemochromatosis. The pathophysiology of hepcidin has been sufficiently elucidated to offer promise of therapeutic intervention in both of these situations. Administering either hepcidin or an agonist could treat haemochromatosis, where the secretion of hepcidin is abnormally low.

The anemia of inflammation, commonly observed in patients with chronic infections, malignancy, trauma, and inflammatory disorders, is a well-known clinical entity. Until recently, little was understood about its pathogenesis. It now appears that the inflammatory cytokine IL-6 induces production of hepcidin, an iron-regulatory hormone that may be responsible for most or all of the features of this disorder. (Andrews NC. *J Clin Invest.* 2004 May 1; 113(9): 1251-1253). As such, down regulation of hepcidin in anemic patients will lead to a reduction in inflammation associated with such anemia.

Double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). WO 99/32619 (Fire et al.) discloses the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of genes in *C. elegans*. dsRNA has also been shown to degrade target RNA in other organisms, including plants (see, e.g., WO 99/53050, Waterhouse et al.; and WO 99/61631, Heifetz et al.), *Drosophila* (see, e.g., Yang, D., et al., *Curr. Biol.* (2000) 10:1191-1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer et al.). This natural mechanism has now become the focus for the development of a new class of pharmaceutical agents for treating disorders that are caused by the aberrant or unwanted regulation of a gene.

The following publications disclose dsRNA (siRNA) targeting the HAMP gene and are herein incorporated by reference for all purposes: WO 2008/036933 (International application no. PCT/US2007/079212, filed September 21, 2007); US 2009-0209478 (US patent application no. 11/859,288, filed September 21, 2007); US 2010-0204307 (US patent application no. 12/757,497, filed April 9, 2010); US 2011-0269823 (US patent application no. 13/184,087, filed July 15, 2011).

Description of the Drawings

Figure 1 shows the HAMP1 mRNA levels in mouse liver following various dosages of siRNA and the serum iron concentration (μg/dL) following various dosages of siRNA in mice.

Figure 2 shows the HAMP mRNA levels in liver following siRNA adminstration as well as the serum iron concentration (μg/dL) and the HAMP serum protein concentration (mg/mL) following siRNA administration to non-human primates.

Figure 3 shows the HAMP1 and TFR2 mRNA levels in mouse liver following various dosages of siRNA and the percent (%) transferrin saturation following various dosages of siRNA.

Figure 4 shows the HAMP1 and TFR2 mRNA levels in mouse liver following administration of siRNA and the percent (%) transferrin saturation over a 30 day time course.

Figure 5 shows the HAMP1 and TFR2 mRNA levels in rat liver following administration of siRNA. Figure 5 also shows the serum iron and Hb concentrations in rats at various time points.

Figure 6 shows the level of HAMP mRNA reduction in the liver of each animal following siRNA administration, compared to PBS controls.

Figure 7 shows the level of TFR2 mRNA reduction in the liver of each animal following siRNA administration, compared to PBS controls.

Figure 8 shows that serum iron concentration was increased in each animal after 1 mg/kg AD-52590 siRNA administration.

Figure 9 shows that the HAMP serum protein concentration was decreased in each animal following 1 mg/kg AD-52590 siRNA administration.

Figure 10 shows combinatorial use of dsRNAs targeting different HAMP-related mRNAs (HFE and TFR2) *in vivo*. Figure 10A shows relative mRNA levels for HFE (left bar), TFR2 (middle bar), and HAMP (right bar) for each group. Figure 10B shows UIBC (μg/dL) for each group. Figure 10C shows the percent transferring saturation for each group. Figure 10D shows serum iron concentration (μg/dL) for each group.

Summary

Disclosed herein is a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of hepcidin antimicrobial peptide (HAMP), wherein said dsRNA is selected from the dsRNAs listed in Table 2, 3, 4, or 5 with a start position of 379, 380, 382, or 385. In some aspects, the dsRNA consists of a dsRNA listed in Table 2, 3, 4, or 5 with a start position of 382.

Also described herein is a dsRNA for inhibiting expression of HAMP, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a HAMP mRNA transcript, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 2, 3, 4, or 5.

In some aspects, the region of complementarity is at least 17 nucleotides in length. In some aspects, the region of complementarity is between 19 and 21 nucleotides in length. In some aspects, the region of complementarity is 19 nucleotides in length. In some aspects, the region of complementarity consists of one of the antisense strand sequences of Table 2, 3, 4, or 5.

In some aspects, the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 2, 3, 4, or 5. In some aspects, the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 2, 3, 4, or 5. In some aspects, the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 2, 3, 4, or 5 and the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 2, 3, 4, or 5. In some aspects, the sense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the sense strand sequences of Table 2, 3, 4, or 5 and the antisense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the antisense strand sequences of Table 2, 3, 4, or 5. In some aspects, the sense strand comprises one of the sense strand sequences of Table 2, 3, 4, or 5. In some aspects, the antisense strand comprises one of the antisense strand sequences of Table 2, 3, 4, or 5. In some aspects, the sense strand comprises one of the sense strand sequences of Table 2, 3, 4, or 5 and the antisense strand comprises one of the antisense strand sequences of Table 2, 3, 4, or 5. In some aspects, the sense strand consists of one of the sense strand sequences of Table 2, 3, 4, or 5 and the antisense strand consists of one of the antisense strand sequences of Table 2, 3, 4, or 5. In some aspects, the dsRNA mediates degradation of HAMP mRNA.

In some aspects, said dsRNA further comprises at least one modified nucleotide. In some aspects, at least one of said modified nucleotides is chosen from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. In some aspects, said modified nucleotide is chosen from the group consisting of: a 2'-fluoro modified nucleotide, a 2'-fluoro modified nucleoside, a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

In some aspects, each strand is no more than 30 nucleotides in length. In some aspects, at least one strand comprises a 3' overhang of at least 1 nucleotide. In some aspects, at least one strand comprises a 3' overhang of at least 2 nucleotides. In some aspects, each strand comprises a 3' overhang of 2 nucleotides.

In some aspects, a dsRNA described above further comprises a ligand. In some aspects, the ligand is conjugated to the 3' end of the sense strand of the dsRNA. In some aspects, the dsRNA further comprises an N-Acetyl-Galactosamine (GalNac) conjugate.

In some aspects, a dsRNA described above is formulated in a nucleic acid lipid particle formulation. In some aspects, the nucleic acid lipid particle formulation is selected from Table A. In some aspects, the nucleic acid lipid particle formulation comprises MC3.

Also described herein is a cell comprising a dsRNA described above.

Also described herein is a vector encoding at least one strand of a dsRNA described above.

Also described herein is a cell comprising a vector described above.

Also described herein is a pharmaceutical composition for inhibiting expression of a HAMP gene comprising a dsRNA described above. In some aspects, the composition further comprises a lipid formulation. In some aspects, the lipid formulation is a nucleic acid lipid particle formulation.

Also described herein is a dsRNA for inhibiting expression of hemojuvelin (HFE2), wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a HFE2 mRNA transcript, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10A.

In some aspects, the region of complementarity is at least 17 nucleotides in length. In some aspects, the region of complementarity is between 19 and 21 nucleotides in length. In some aspects, the region of complementarity is 19 nucleotides in length. In some aspects, the region of complementarity consists of one of the antisense strand sequences of Table 10A.

In some aspects, the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 10A. In some aspects, the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 10A. In some aspects, the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 10A and the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 10A. In some aspects, the sense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the sense strand sequences of Table 10A and the antisense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the antisense strand sequences of Table 10A. In some aspects, the sense strand comprises one of the sense strand sequences of Table 10A. In some aspects, the antisense strand comprises one of the antisense strand sequences of Table 10A. In some aspects, the sense strand comprises one of the sense strand sequences of Table 10A and the antisense strand comprises one of the antisense strand sequences of Table 10A. In some aspects, the sense

strand consists of one of the sense strand sequences of Table 10A and the antisense strand consists of one of the antisense strand sequences of Table 10A. In some aspects, the dsRNA mediates degradation of HFE2 mRNA.

In some aspects, said dsRNA further comprises at least one modified nucleotide. In some aspects, at least one of said modified nucleotides is chosen from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. In some aspects, said modified nucleotide is chosen from the group consisting of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

In some aspects, each strand is no more than 30 nucleotides in length. In some aspects, at least one strand comprises a 3' overhang of at least 1 nucleotide. In some aspects, at least one strand comprises a 3' overhang of at least 2 nucleotides. In some aspects, each strand comprises a 3'overhang of 2 nucleotides.

In some aspects, a dsRNA described above further comprises a ligand. In some aspects, the ligand is conjugated to the 3' end of the sense strand of the dsRNA. In some aspects, a dsRNA described above further comprises a GalNac conjugate.

In some aspects, the dsRNA is formulated in a nucleic acid lipid particle formulation. In some aspects, the nucleic acid lipid particle formulation is selected from Table A. In some aspects, the nucleic acid lipid particle formulation comprises MC3.

Also described herein is a cell comprising a dsRNA described above.

Also described herein is a vector encoding at least one strand of a dsRNA described above.

Also described herein is a cell comprising a vector described above.

Also described herein is a pharmaceutical composition for inhibiting expression of a HFE2 gene comprising a dsRNA described above. In some aspects, the composition further comprises a lipid formulation. In some aspects, the lipid formulation is a nucleic acid lipid particle formulation.

Also described herein is a dsRNA for inhibiting expression of transferrin receptor 2 (TFR2), wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a TFR2 mRNA transcript, wherein the

antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10B or 13.

In some aspects, the region of complementarity is at least 17 nucleotides in length. In some aspects, the region of complementarity is between 19 and 21 nucleotides in length. In some aspects, the region of complementarity is 19 nucleotides in length. In some aspects, the region of complementarity consists of one of the antisense strand sequences of Table 10B or 13.

In some aspects, the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 10B or 13. In some aspects, the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 10B or 13. In some aspects, the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 10B or 13 and the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 10B or 13. In some aspects, the sense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the sense strand sequences of Table 10B or 13 and the antisense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the antisense strand sequences of Table 10B or 13. In some aspects, sense strand comprises one of the sense strand sequences of Table 10B or 13. In some aspects, the antisense strand comprises one of the antisense strand sequences of Table 10B or 13. In some aspects, the sense strand comprises one of the sense strand sequences of Table 10B or 13 and the antisense strand comprises one of the antisense strand sequences of Table 10B or 13. In some aspects, the sense strand consists of one of the sense strand sequences of Table 10B or 13 and the antisense strand consists of one of the antisense strand sequences of Table 10B or 13. In some aspects, the dsRNA mediates degradation of TFR2 mRNA.

In some aspects, said dsRNA further comprises at least one modified nucleotide. In some aspects, at least one of said modified nucleotides is chosen from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. In some aspects, said modified nucleotide is chosen from the group consisting of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

In some aspects, each strand is no more than 30 nucleotides in length. In some aspects, at least one strand comprises a 3' overhang of at least 1 nucleotide. In some aspects, at least one strand comprises a 3' overhang of at least 2 nucleotides. In some aspects, each strand comprises a 3 overhang of 2 nucleotides.

In some aspects, a dsRNA described above further comprises a ligand. In some aspects, the ligand is conjugated to the 3' end of the sense strand of the dsRNA. In some aspects, a dsRNA described above further comprises a GalNac conjugate.

In some aspects, the dsRNA is formulated in a nucleic acid lipid particle formulation. In some aspects, the nucleic acid lipid particle formulation is selected from Table A. In some aspects, the nucleic acid lipid particle formulation comprises MC3.

Also described herein is a cell comprising a dsRNA described above.

Also described herein is a vector encoding at least one strand of a dsRNA described above.

Also described herein is a cell comprising a vector described above.

Also described herein is a pharmaceutical composition for inhibiting expression of a TFR2 gene comprising a dsRNA described above. In some aspects, the composition further comprises a lipid formulation. In some aspects, the lipid formulation is a nucleic acid lipid particle formulation.

Also described herein is a composition comprising a first dsRNA for inhibiting expression of a HAMP gene and a second dsRNA for inhibiting expression of an HFE2 gene, wherein the first dsRNA comprises a first sense strand and an first antisense strand, the first antisense strand comprising a region of complementarity to a HAMP mRNA transcript, wherein the first antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 2, 3, 4, or 5; and wherein the second dsRNA comprises a second sense strand and a second antisense strand, the second antisense strand comprising a region of complementarity to a HFE2 mRNA transcript, wherein the second antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10A.

Also described herein is a composition comprising a first dsRNA for inhibiting expression of a HAMP gene and a second dsRNA for inhibiting expression of an TFR2 gene, wherein said first dsRNA comprises a first sense strand and a first antisense strand, the first antisense strand comprising a region of complementarity to a HAMP mRNA transcript, wherein the first antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 2, 3, 4, or 5; and wherein said second dsRNA comprises a second sense strand and a second antisense strand, the second antisense strand comprising a region of complementarity to a TFR2 mRNA transcript,

wherein the second antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10B or 13.

Also described herein is a composition comprising a first dsRNA for inhibiting expression of a TFR2 gene and a second dsRNA for inhibiting expression of a HFE2 gene, wherein said first dsRNA comprises a first sense strand and a first antisense strand, the first antisense strand comprising a region of complementarity to a TFR2 mRNA transcript, wherein the first antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10B or 13; and wherein said second dsRNA comprises a second sense strand and a second antisense strand, the second antisense strand comprising a region of complementarity to a HFE2 mRNA transcript, wherein the second antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10A.

Also described herein is a composition comprising a plurality of dsRNAs selected from the dsRNAs described above.

Also described herein is a method of inhibiting HAMP expression in a cell, the method comprising: (a) introducing into the cell a dsRNA described above; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a HAMP gene, thereby inhibiting expression of the HAMP gene in the cell. In some aspects, the HAMP expression is inhibited by at least 30%. In some aspects, the HAMP expression is inhibited by at least 80%.

Also described herein is a method of treating a disorder associated with HAMP expression comprising administering to a subject in need of such treatment a therapeutically effective amount of a dsRNA described above.

In some aspects, the subject has anemia. In some aspects, the subject has refractory anemia. In some aspects, the subject has anemia of chronic disease (ACD). In some aspects, the subject has iron-restricted erythropoiesis. In some aspects, the subject is a human.

In some aspects, the dsRNA is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

In some aspects, the dsRNA is lipid formulated. In some aspects, the dsRNA is lipid formulated in a formulation selected from Table A. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously. In some aspects, the dsRNA is conjugated to GalNac. In some aspects, the dsRNA is conjugated to

GalNac and administered subcutaneously. In some aspects, the dsRNA is administered subcutaneously.

Also described herein is a method for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of a HAMP dsRNA described above.

In some aspects, the dsRNA is lipid formulated. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously. In some aspects, the dsRNA is administered intravenously. In some aspects, the dsRNA is lipid formulated in a formulation selected from Table A. In some aspects, the dsRNA is conjugated to GalNac. In some aspects, the dsRNA is conjugated to GalNac and administered subcutaneously. In some aspects, the dsRNA is administered subcutaneously.

In some aspects, the subject is a primate or a rodent. In some aspects, the subject is a human.

In some aspects, the effective amount is a concentration of 0.01-5.0 mg/kg bodyweight of the subject.

In some aspects, the subject has fatigue, shortness of breath, headache, dizziness, or pale skin. In some aspects, the subject has reduced iron levels compared to a subject without anemia. In some aspects, the subject has haemoglobin (Hb) levels <9g/dL. In some aspects, the subject has chronic kidney disease (CKD), cancer, chronic inflammatory disease, rheumatoid arthritis (RA), or iron-resistant iron-deficient amemia (IRIDA). In some aspects, the subject has reduced renal erythropoietin (EPO) synthesis compared to a subject without CKD, a dietary deficiency, blood loss, or elevated hepcidin levels compared to a subject without CKD. In some aspects, the subject has decreased renal excretion of hepcidin compared to a subject without CKD or low grade inflammation characterized by increased interleukin-6 (IL-6) levels compared to a subject without CKD. In some aspects, the subject has a reticulocyte Hb of < 28 pg. In some aspects, the subject has >10% hypochromic red blood cells (RBCs). In some aspects, the method further comprises determining the complete blood count (CBC), serum iron concentration, Transferrin (Tf) saturation, or ferritin levels of the subject.

In some aspects, administering results in an increase in iron levels in the subject. In some aspects, administering results in a 2-fold increase in iron levels in the subject. In some aspects, administering results in an increase in Tf saturation in the subject.

In some aspects, the method further comprises determining the iron level in the subject. In some aspects, the method further comprises administering intravenous iron or ESAs to the subject.

Also described herein is a method of inhibiting HFE2 expression in a cell, the method comprising: (a) introducing into the cell a dsRNA described above; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a HFE2 gene, thereby inhibiting expression of the HFE2 gene in the cell. In some aspects, the HFE2 expression is inhibited by at least 30%. In some aspects, the HFE2 expression is inhibited by at least 80%.

Also described herein is a method of treating a disorder associated with HFE2 expression comprising administering to a subject in need of such treatment a therapeutically effective amount of a dsRNA described above.

In some aspects, the subject has anemia. In some aspects, the subject has refractory anemia. In some aspects, the subject has anemia of chronic disease (ACD). In some aspects, the subject has iron-restricted erythropoiesis. In some aspects, the subject is a human.

In some aspects, the dsRNA is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

In some aspects, the dsRNA is lipid formulated. In some aspects, the dsRNA is lipid formulated in a formulation selected from Table A. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously. In some aspects, the dsRNA is conjugated to GalNac. In some aspects, the dsRNA is conjugated to GalNac and administered subcutaneously. In some aspects, the dsRNA is administered subcutaneously.

Also described herein is a method for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of a HFE2 dsRNA described above.

In some aspects, the dsRNA is lipid formulated. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously. In some aspects, the dsRNA is administered intravenously. In some aspects, the dsRNA is lipid formulated in a formulation selected from Table A. In some aspects, the dsRNA is conjugated to GalNac. In some aspects, the dsRNA is conjugated to GalNac and administered subcutaneously. In some aspects, the dsRNA is administered subcutaneously.

In some aspects, the subject is a primate or a rodent. In some aspects, the subject is a human.

In some aspects, the effective amount is a concentration of 0.01-5.0 mg/kg bodyweight of the subject.

In some aspects, the subject has fatigue, shortness of breath, headache, dizziness, or pale skin. In some aspects, the subject has reduced iron levels compared to a subject without anemia. In some aspects, the subject has haemoglobin (Hb) levels <9g/dL. In some aspects, the subject has chronic kidney disease (CKD), cancer, chronic inflammatory disease, rheumatoid arthritis (RA), or iron-resistant iron-deficient amemia (IRIDA). In some aspects, the subject has reduced renal erythropoietin (EPO) synthesis compared to a subject without CKD, a dietary deficiency, blood loss, or elevated hepcidin levels compared to a subject without CKD. In some aspects, the subject has decreased renal excretion of hepcidin compared to a subject without CKD or low grade inflammation characterized by increased interleukin-6 (IL-6) levels compared to a subject without CKD. In some aspects, the subject has a reticulocyte Hb of < 28 pg. In some aspects, the subject has >10% hypochromic red blood cells (RBCs). In some aspects, the method further comprises determining the complete blood count (CBC), serum iron concentration, Transferrin (Tf) saturation, or ferritin levels of the subject.

In some aspects, administering results in an increase in iron levels in the subject. In some aspects, administering results in a 2-fold increase in iron levels in the subject. In some aspects, administering results in an increase in Tf saturation in the subject.

In some aspects, the method further comprises determining the iron level in the subject. In some aspects, the method further comprises administering intravenous iron or ESAs to the subject.

Also described herein is a method of inhibiting TFR2 expression in a cell, the method comprising: (a) introducing into the cell a dsRNA described above; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TFR2 gene, thereby inhibiting expression of the TFR2 gene in the cell. In some aspects, the TFR2 expression is inhibited by at least 30%. In some aspects, the TFR2 expression is inhibited by at least 80%.

Also described herein is a method of treating a disorder associated with TFR2 expression comprising administering to a subject in need of such treatment a therapeutically effective amount of a dsRNA described above.

In some aspects, the subject has anemia. In some aspects, the subject has refractory anemia. In some aspects, the subject has anemia of chronic disease (ACD). In some aspects, the subject has iron-restricted erythropoiesis. In some aspects, the subject is a human.

In some aspects, the dsRNA is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

In some aspects, the dsRNA is lipid formulated. In some aspects, the dsRNA is lipid formulated in a formulation selected from Table A. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously. In some aspects, the dsRNA is conjugated to GalNac. In some aspects, the dsRNA is conjugated to GalNac and administered subcutaneously. In some aspects, the dsRNA is administered subcutaneously.

Also described herein is a method for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of a TFR2 dsRNA described above.

In some aspects, the dsRNA is lipid formulated. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation. In some aspects, the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously. In some aspects, the dsRNA is administered intravenously. In some aspects, the dsRNA is lipid formulated in a formulation selected from Table A. In some aspects, the dsRNA is conjugated to GalNac. In some aspects, the dsRNA is conjugated to GalNac and administered subcutaneously. In some aspects, the dsRNA is administered subcutaneously.

In some aspects, the subject is a primate or a rodent. In some aspects, the subject is a human.

In some aspects, the effective amount is a concentration of 0.01-5.0 mg/kg bodyweight of the subject.

In some aspects, the subject has fatigue, shortness of breath, headache, dizziness, or pale skin. In some aspects, the subject has reduced iron levels compared to a subject without anemia. In some aspects, the subject has haemoglobin (Hb) levels <9g/dL. In some aspects, the subject has chronic kidney disease (CKD), cancer, chronic inflammatory disease, rheumatoid arthritis (RA), or iron-resistant iron-deficient amemia (IRIDA). In some aspects, the subject has reduced renal erythropoietin (EPO) synthesis compared to a subject without CKD, a dietary deficiency, blood loss, or elevated hepcidin levels compared to a subject without CKD. In some aspects, the subject has decreased renal excretion of hepcidin compared to a subject without CKD or low

grade inflammation characterized by increased interleukin-6 (IL-6) levels compared to a subject without CKD. In some aspects, the subject has a reticulocyte Hb of < 28 pg. In some aspects, the subject has >10% hypochromic red blood cells (RBCs). In some aspects, the method further comprises determining the complete blood count (CBC), serum iron concentration, Transferrin (Tf) saturation, or ferritin levels of the subject.

In some aspects, administering results in an increase in iron levels in the subject. In some aspects, administering results in a 2-fold increase in iron levels in the subject. In some aspects, administering results in an increase in Tf saturation in the subject.

In some aspects, the method further comprises determining the iron level in the subject. In some aspects, the method further comprises administering intravenous iron or ESAs to the subject.

Also described herein is a method of inhibiting HAMP, HFE2, and/or TFR2 expression in a cell, the method comprising: (a) introducing into the cell a plurality of dsRNAs selected from the dsRNAs described above; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a HAMP, HFE2, and/or TFR2 gene, thereby inhibiting expression of the HAMP, HFE2, and/or TFR2 gene in the cell.

In some aspects, the plurality of dsRNAs are introduced simultaneously. In some aspects, the plurality of dsRNAs are introduced concurrently. In some aspects, the plurality of dsRNAs are introduced individually. In some aspects, the plurality of dsRNAs are introduced together. In some aspects, the expression is inhibited by at least 30%. In some aspects, the expression is inhibited by at least 80%.

Also described herein is a method of treating a disorder associated with HAMP, HFE2, and/or TFR2 expression comprising administering to a subject in need of such treatment a therapeutically effective amount of a plurality of dsRNAs selected from the dsRNAs described above.

In some aspects, the plurality of dsRNAs are administered to the subject simultaneously. In some aspects, the plurality of dsRNAs are administered to the subject concurrently. In some aspects, the plurality of dsRNAs are administered to the subject individually. In some aspects, the plurality of dsRNAs are administered to the subject together.

In some aspects, the subject has anemia. In some aspects, the subject has refractory anemia. In some aspects, the subject has anemia of chronic disease (ACD). In some aspects, the subject has iron-restricted erythropoiesis. In some aspects, the subject is a human. In some

aspects, the plurality is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

Also described herein is a method for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of a plurality of dsRNAs selected from the dsRNAs described above.

In some aspects, the plurality of dsRNAs are administered to the subject simultaneously. In some aspects, the plurality of dsRNAs are administered to the subject concurrently. In some aspects, the plurality of dsRNAs are administered to the subject individually. In some aspects, the plurality of dsRNAs are administered to the subject together.

In some aspects, the plurality is lipid formulated. In some aspects, the plurality is lipid formulated in a nucleic acid lipid particle formulation. In some aspects, the plurality is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously. In some aspects, the plurality is administered intravenously. In some aspects, the plurality is lipid formulated in a formulation selected from Table A. In some aspects, the plurality is conjugated to GalNac. In some aspects, the plurality is conjugated to GalNac and administered subcutaneously. In some aspects, the plurality is administered subcutaneously.

In some aspects, the subject is a primate or a rodent. In some aspects, the subject is a human.

In some aspects, the effective amount is a concentration of 0.01-5.0 mg/kg bodyweight of the subject.

In some aspects, the subject has fatigue, shortness of breath, headache, dizziness, or pale skin. In some aspects, the subject has reduced iron levels compared to a subject without anemia. In some aspects, the subject has haemoglobin (Hb) levels <9g/dL. In some aspects, the subject has chronic kidney disease (CKD), cancer, chronic inflammatory disease, rheumatoid arthritis (RA), or iron-resistant iron-deficient amemia (IRIDA). In some aspects, the subject has reduced renal erythropoietin (EPO) synthesis compared to a subject without CKD, a dietary deficiency, blood loss, or elevated hepcidin levels compared to a subject without CKD. In some aspects, the subject has decreased renal excretion of hepcidin compared to a subject without CKD or low grade inflammation characterized by increased interleukin-6 (IL-6) levels compared to a subject without CKD. In some aspects, the subject has a reticulocyte Hb of < 28 pg. In some aspects, the subject has >10% hypochromic red blood cells (RBCs). In some aspects, the method further comprises determining the complete blood count (CBC), serum iron concentration, Transferrin (Tf) saturation, or ferritin levels of the subject.

In some aspects, administering results in an increase in iron levels in the subject. In some aspects, administering results in a 2-fold increase in iron levels in the subject. In some aspects, administering results in an increase in Tf saturation in the subject.

In some aspects, the method further comprises determining the iron level in the subject. In some aspects, the method further comprises administering intravenous iron or ESAs to the subject.

Detailed Description

The details of one or more embodiments are set forth in the description below. Other features, objects, and advantages will be apparent from the description and the drawings, and from the claims.

Provided herein are dsRNAs and methods of using the dsRNAs for inhibiting the expression of HAMP in a cell or a mammal where the dsRNA targets HAMP. Also provided are compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of HAMP. A HAMP dsRNA directs the sequence-specific degradation of HAMP mRNA.

Also provided herein are dsRNAs and methods of using the dsRNAs for inhibiting the expression of HFE2 in a cell or a mammal where the dsRNA targets HFE2. Also provided are compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of HFE2. A HFE2 dsRNA directs the sequence-specific degradation of HFE2 mRNA.

Also provided herein are dsRNAs and methods of using the dsRNAs for inhibiting the expression of HFE in a cell or a mammal where the dsRNA targets HFE. Also provided are compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of HFE. A HFE dsRNA directs the sequence-specific degradation of HFE mRNA.

Also provided herein are dsRNAs and methods of using the dsRNAs for inhibiting the expression of TFR2 in a cell or a mammal where the dsRNA targets TFR2. Also provided are compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of TFR2. A TFR2 dsRNA directs the sequence-specific degradation of TFR2 mRNA.

Also provided herein are dsRNAs and methods of using the dsRNAs for inhibiting the expression of BMPR1a in a cell or a mammal where the dsRNA targets BMPR1a. Also provided are compositions and methods for treating pathological conditions and diseases in a

mammal caused by the expression of BMPR1a. A BMPR1a dsRNA directs the sequence-specific degradation of BMPR1a mRNA.

Also provided herein are dsRNAs and methods of using the dsRNAs for inhibiting the expression of SMAD4 in a cell or a mammal where the dsRNA targets SMAD4. Also provided are compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of SMAD4. A SMAD4 dsRNA directs the sequence-specific degradation of SMAD4 mRNA.

Also provided herein are dsRNAs and methods of using the dsRNAs for inhibiting the expression of IL6R in a cell or a mammal where the dsRNA targets IL6R. Also provided are compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of IL6R. An IL6R dsRNA directs the sequence-specific degradation of IL6R mRNA.

Also provided herein are dsRNAs and methods of using the dsRNAs for inhibiting the expression of BMP6 in a cell or a mammal where the dsRNA targets BMP6. Also provided are compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of BMP6. A BMP6 dsRNA directs the sequence-specific degradation of BMP6 mRNA.

Also provided herein are dsRNAs and methods of using the dsRNAs for inhibiting the expression of NEO1 in a cell or a mammal where the dsRNA targets NEO1. Also provided are compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of NEO1. A NEO1 dsRNA directs the sequence-specific degradation of NEO1 mRNA.

Definitions

For convenience, the meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in other parts of this specification and its definition provided in this section, the definition in this section shall prevail.

"G," "C," "A" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, and uracil as a base, respectively. "T" and "dT" are used interchangeably herein and refer to a deoxyribonucleotide wherein the nucleobase is thymine, e.g., deoxyribothymine. However, it will be understood that the term "ribonucleotide" or "nucleotide" or "deoxyribonucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine,

cytosine, adenine, and uracil may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of the invention by a nucleotide containing, for example, inosine. Sequences comprising such replacement moieties are embodiments of the invention.

As used herein, "HAMP" refers to the hepcidin antimicrobial peptide gene, transcript, or protein (also known as LEAP). A human mRNA sequence for HAMP is Genbank accession NM_021175.2, included below as SEQ ID NO:1. Other examples of mammalian HAMP sequences are shown in Table B.

As used herein, "HFE2" refers to hemojuvelin gene, transcript, or protein. Examples of mammalian HFE2 sequences are shown in Table B.

As used herein, "TFR2" refers to transferrin receptor 2 gene, transcript, or protein. Examples of mammalian TFR2 sequences are shown in Table B.

As used herein, "HFE" refers to hemochromatosis gene, transcript, or protein. Examples of mammalian HFE sequences are shown in Table B.

As used herein, "BMPR1a" refers to bone morphogenetic protein receptor, type 1A gene, transcript, or protein. Examples of mammalian BMPR1a sequences are shown in Table B.

As used herein, "SMAD4" refers to SMAD family member 4 gene, transcript, or protein. Examples of mammalian SMAD4 sequences are shown in Table B.

As used herein, "IL6R" refers to interleukin 6 receptor gene, transcript, or protein. Examples of mammalian IL6R sequences are shown in Table B.

As used herein, "BMP6" refers to bone morphogenetic protein 6 gene, transcript, or protein. Examples of mammalian BMP6 sequences are shown in Table B.

As used herein, "NEO1" refers to neogenin homolog 1 gene, transcript, or protein. Examples of mammalian NEO1 sequences are shown in Table B.

As used herein, "HAMP-related" refers to a HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene, transcript, or protein.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene, including mRNA that is a product of RNA processing of a primary transcription product.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions may include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

This includes base-pairing of the oligonucleotide or polynucleotide comprising the first nucleotide sequence to the oligonucleotide or polynucleotide comprising the second nucleotide sequence over the entire length of the first and second nucleotide sequence. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as complementary with respect to a second sequence herein, the two sequences can be fully complementary, or they may be "substantially complementary," e.g., they may form one or more, but generally not more than 4, 3 or 2 mismatched base pairs upon hybridization, while retaining the ability to hybridize under the conditions most relevant to their ultimate application. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, may yet be referred to as "fully complementary" for the purposes described herein.

"Complementary" sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs includes, but not limited to, G:U Wobble or Hoogstein base pairing.

The terms "complementary," "fully complementary" and "substantially complementary" herein may be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1) including a 5' UTR, an open reading frame (ORF), or a 3' UTR. For example, a polynucleotide is complementary to at least a part of a HAMP mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding HAMP.

The term "double-stranded RNA" or "dsRNA," as used herein, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary, as defined above, nucleic acid strands. In general, the majority of nucleotides of each strand are ribonucleotides, but as described in detail herein, each or both strands can also include at least one non-ribonucleotide, e.g., a deoxyribonucleotide and/or a modified nucleotide. In addition, as used in this specification, "dsRNA" may include chemical modifications to ribonucleotides, including substantial modifications at multiple nucleotides and including all types of modifications disclosed herein or known in the art. Any such modifications, as used in an siRNA type molecule, are encompassed by "dsRNA" for the purposes of this specification and claims.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs. The term "siRNA" is also used herein to refer to a dsRNA as described above.

As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of a dsRNA when a 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand, or vice versa. "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the dsRNA, *i.e.*, no nucleotide overhang. A "blunt ended" dsRNA is a dsRNA that is double-stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule.

The term "antisense strand" refers to the strand of a dsRNA which includes a region that is complementary, e.g., fully complementary or substantially complementary to a target sequence. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is complementary to a sequence, for example a target sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated in the terminal regions and, if present, are generally in a terminal region or regions, *e.g.*, within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus.

The term "sense strand," as used herein, refers to the strand of a dsRNA that includes a region that is complementary, e.g., fully or substantially complementary to a region of the antisense strand.

The term "start position" refers to a nucleotide position on the target mRNA where the 5' most nucleotide of a dsRNA sense strand aligns with the nucleotide position on the target mRNA. For example, a dsRNA with a start position of 382 on NM_021175.2 (SEQ ID NO:1) would include AD-11459 because position 382 on NM_021175.2 (SEQ ID NO:1) is G and the sense sequence of AD-11459 is 5'-GAAcAuAGGucuuGGAAuAdTsdT-3' (SEQ ID NO:XX), where G is the 5' most nucleotide of the sense strand of AD-11459; thus G at position 382 on NM_021175.2 (SEQ ID NO:1) is the start position of AD-11459.

As used herein, the term "nucleic acid lipid particle" includes the term "SNALP" and refers to a vesicle of lipids coating a reduced aqueous interior comprising a nucleic acid such as a dsRNA or a plasmid from which a dsRNA is transcribed. Nucleic acid lipid particles, e.g., SNALP are described, *e.g.*, in U.S. Patent Application Publication Nos. 20060240093, 20070135372, and USSN 61/045,228 filed on April 15, 2008. These applications are hereby incorporated by reference.

"Introducing into a cell," when referring to a dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells *in vitro*; a dsRNA may also be

"introduced into a cell," wherein the cell is part of a living organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for *in vivo* delivery, dsRNA can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein or known in the art.

The terms "silence," "inhibit the expression of," "down-regulate the expression of," "suppress the expression of" and the like in as far as they refer to a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene, herein refer to the at least partial suppression of the expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene, as manifested by a reduction of the amount of mRNA which may be isolated from a first cell or group of cells in which a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is transcribed and which has or have been treated such that the expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition is usually expressed in terms of

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} * 100\%$$

Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene expression, *e.g.*, the amount of protein encoded by a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene which is secreted by a cell, or the number of cells displaying a certain phenotype, *e.g.*, apoptosis. In principle, HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene silencing may be determined in any cell expressing the target, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given dsRNA inhibits the expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene by a certain degree and therefore is encompassed by the instant invention, the assays provided in the Examples below shall serve as such reference.

For example, in certain instances, expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is suppressed by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of the double-stranded oligonucleotide featured in the invention. In some embodiments, a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is suppressed by at least about 60%, 70%, or 80% by

administration of the double-stranded oligonucleotide featured in the invention. In some embodiments, a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is suppressed by at least about 85%, 90%, or 95% by administration of the double-stranded oligonucleotide featured in the invention.

As used herein in the context of HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression, the terms "treat," "treatment," and the like, refer to relief from or alleviation of pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression. In the context of the present invention insofar as it relates to any of the other conditions recited herein below (other than pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression), the terms "treat," "treatment," and the like mean to relieve or alleviate at least one symptom associated with such condition, or to slow or reverse the progression of such condition.

As used herein, the phrases "effective amount" refers to an amount that provides a benefit in the treatment, prevention, or management of pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression or an overt symptom of pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression. The specific amount that is effective can be readily determined by an ordinary medical practitioner, and may vary depending on factors known in the art, such as, for example, the type of pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression, the patient's history and age, the stage of pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression, and the administration of other anti-pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression agents.

As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," or simply "effective amount" refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic or preventive result. For example, if a given clinical treatment is considered effective when there is at least a 25% reduction in a measurable parameter associated with a disease or disorder, a pharmacologically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 25% reduction in that parameter. For example, a pharmacologically effective amount of a dsRNA targeting HAMP can reduce HAMP serum levels by at least 25%.

The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

As used herein, a "transformed cell" is a cell into which a vector has been introduced from which a dsRNA molecule may be expressed.

Double-stranded ribonucleic acid (dsRNA)

As described in more detail herein, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene in a cell or mammal, where the dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene, and where the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and where said dsRNA, upon contact with a cell expressing said HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene, inhibits the expression of said HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene by at least 30% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by Western blot. Expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene can be reduced by at least 30% when measured by an assay as described in the Examples below. For example, expression of a HAMP gene in cell culture, such as in Hep3B cells, can be assayed by measuring HAMP mRNA levels, such as by bDNA or TaqMan assay, or by measuring protein levels, such as by ELISA assay. The dsRNA of the invention can further include one or more single-stranded nucleotide overhangs.

The dsRNA can be synthesized by standard methods known in the art as further discussed below, *e.g.*, by use of an automated DNA synthesizer, such as are commercially

available from, for example, Biosearch, Applied Biosystems, Inc. The dsRNA includes two RNA strands that are sufficiently complementary to hybridize to form a duplex structure. One strand of the dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence, derived from the sequence of an mRNA formed during the expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene, the other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. Generally, the duplex structure is between 15 and 30 or between 25 and 30, or between 18 and 25, or between 19 and 24, or between 19 and 21, or 19, 20, or 21 base pairs in length. In one embodiment the duplex is 19 base pairs in length. In another embodiment the duplex is 21 base pairs in length. When two different dsRNAs are used in combination, the duplex lengths can be identical or can differ. In one embodiment, the antisense strand of the dsRNA is sufficiently complementary to a target mRNA (e.g., a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 mRNA) so as to cause cleavage of the target mRNA.

Each strand of the dsRNA of invention is generally between 15 and 30, or between 18 and 25, or 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In other embodiments, each is strand is 25-30 nucleotides in length. Each strand of the duplex can be the same length or of different lengths. When two different siRNAs are used in combination, the lengths of each strand of each siRNA can be identical or can differ.

The dsRNA of the invention can include one or more single-stranded overhang(s) of one or more nucleotides. In one embodiment, at least one end of the dsRNA has a single-stranded nucleotide overhang of 1 to 4, generally 1 or 2 nucleotides. In another embodiment, the antisense strand of the dsRNA has 1-10 nucleotides overhangs each at the 3' end and the 5' end over the sense strand. In further embodiments, the sense strand of the dsRNA has 1-10 nucleotides overhangs each at the 3' end and the 5' end over the antisense strand. The dsRNA can include a 3' overhang of 2 nucleotides on both the sense and antisense strands.

A dsRNAs having at least one nucleotide overhang can have unexpectedly superior inhibitory properties than the blunt-ended counterpart. In some embodiments the presence of only one nucleotide overhang strengthens the interference activity of the dsRNA, without affecting its overall stability. A dsRNA having only one overhang has proven particularly stable and effective *in vivo*, as well as in a variety of cells, cell culture mediums, blood, and serum. Generally, the single-stranded overhang is located at the 3'-terminal end of the antisense strand or, alternatively, at the 3'-terminal end of the sense strand. The dsRNA can also have a blunt

end, generally located at the 5'-end of the antisense strand. Such dsRNAs can have improved stability and inhibitory activity, thus allowing administration at low dosages, *i.e.*, less than 5 mg/kg body weight of the recipient per day. Generally, the antisense strand of the dsRNA has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In one embodiment, a HAMP gene is a human HAMP gene. In specific embodiments, the sense strand of the dsRNA is one of the sense sequences from Tables 2, 3, 4, and 5, and the antisense strand is one of the antisense sequences of Tables 2, 3, 4, and 5. Alternative antisense agents that target elsewhere in the target sequence provided in Tables 2, 3, 4, and 5 can readily be determined using the target sequence and the flanking HAMP sequence.

The skilled person is well aware that dsRNAs having a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, EMBO 2001, 20:6877-6888). However, others have found that shorter or longer dsRNAs can be effective as well. In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Tables 2, 3, 4, and 5, the dsRNAs featured in the invention can include at least one strand of a length described herein. It can be reasonably expected that shorter dsRNAs having one of the sequences of Tables 2, 3, 4, and 5 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Tables 2, 3, 4, and 5, and differing in their ability to inhibit the expression of a HAMP gene in an assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further, dsRNAs that cleave within a desired HAMP target sequence can readily be made using the corresponding HAMP antisense sequence and a complementary sense sequence.

In addition, the dsRNAs provided in Tables 2, 3, 4, and 5 identify a site in a HAMP that is susceptible to RNAi based cleavage. As such, the present invention further features dsRNAs that target within the sequence targeted by one of the agents of the present invention. As used herein, a second dsRNA is said to target within the sequence of a first dsRNA if the second dsRNA cleaves the message anywhere within the mRNA that is complementary to the antisense strand of the first dsRNA. Such a second dsRNA will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Tables 2, 3, 4, and 5 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a HAMP gene.

In one embodiment, a HFE2 gene is a human HFE2 gene. In specific embodiments, the sense strand of the dsRNA is one of the sense sequences from Table 10A, and the antisense strand is one of the antisense sequences of Table 10A. Alternative antisense agents that target elsewhere in the target sequence provided in Table 10A can readily be determined using the target sequence and the flanking HFE2 sequence.

In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Table 10A, the dsRNAs featured in the invention can include at least one strand of a length described herein. It can be reasonably expected that shorter dsRNAs having one of the sequences of Table 10A minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Table 10A, and differing in their ability to inhibit the expression of a HFE2 gene in an assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further, dsRNAs that cleave within a desired HFE2 target sequence can readily be made using the corresponding HFE2 antisense sequence and a complementary sense sequence.

In addition, the dsRNAs provided in Table 10A identify a site in a HFE2 that is susceptible to RNAi based cleavage. As such, the present invention further features dsRNAs that target within the sequence targeted by one of the agents of the present invention. As used herein, a second dsRNA is said to target within the sequence of a first dsRNA if the second dsRNA cleaves the message anywhere within the mRNA that is complementary to the antisense strand of the first dsRNA. Such a second dsRNA will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Table 10A coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a HFE2 gene.

In one embodiment, a TFR2 gene is a human TFR2 gene. In specific embodiments, the sense strand of the dsRNA is one of the sense sequences from Table 10B or 13, and the antisense strand is one of the antisense sequences of Table 10B or 13. Alternative antisense agents that target elsewhere in the target sequence provided in Table 10B or 13 can readily be determined using the target sequence and the flanking TFR2 sequence.

In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Table 10B or 13, the dsRNAs featured in the invention can include at least one strand of a length described herein. It can be reasonably expected that shorter dsRNAs having one of the sequences of Table 10B or 13 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs

having a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Table 10B or 13, and differing in their ability to inhibit the expression of a TFR2 gene in an assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further, dsRNAs that cleave within a desired TFR2 target sequence can readily be made using the corresponding TFR2 antisense sequence and a complementary sense sequence.

In addition, the dsRNAs provided in Table 10B or 13 identify a site in a TFR2 that is susceptible to RNAi based cleavage. As such, the present invention further features dsRNAs that target within the sequence targeted by one of the agents of the present invention. As used herein, a second dsRNA is said to target within the sequence of a first dsRNA if the second dsRNA cleaves the message anywhere within the mRNA that is complementary to the antisense strand of the first dsRNA. Such a second dsRNA will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Table 10B or 13 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a TFR2 gene.

In one embodiment, a SMAD4 gene is a human SMAD4 gene. In specific embodiments, the sense strand of the dsRNA is one of the sense sequences from Table 15 or 16, and the antisense strand is one of the antisense sequences of Table 15 or 16. Alternative antisense agents that target elsewhere in the target sequence provided in Table 15 or 16 can readily be determined using the target sequence and the flanking SMAD4 sequence.

In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Table 15 or 16, the dsRNAs featured in the invention can include at least one strand of a length described herein. It can be reasonably expected that shorter dsRNAs having one of the sequences of Table 15 or 16 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Table 15 or 16, and differing in their ability to inhibit the expression of a SMAD4 gene in an assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further, dsRNAs that cleave within a desired SMAD4 target sequence can readily be made using the corresponding SMAD4 antisense sequence and a complementary sense sequence.

In addition, the dsRNAs provided in Table 15 or 16 identify a site in a SMAD4 that is susceptible to RNAi based cleavage. As such, the present invention further features dsRNAs that target within the sequence targeted by one of the agents of the present invention. As used herein, a second dsRNA is said to target within the sequence of a first dsRNA if the second

dsRNA cleaves the message anywhere within the mRNA that is complementary to the antisense strand of the first dsRNA. Such a second dsRNA will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Table 15 or 16 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a SMAD4 gene.

In one embodiment, a NEO1 gene is a human NEO1 gene. In specific embodiments, the sense strand of the dsRNA is one of the sense sequences from Table 17 or 18, and the antisense strand is one of the antisense sequences of Table 17 or 18. Alternative antisense agents that target elsewhere in the target sequence provided in Table 17 or 18 can readily be determined using the target sequence and the flanking NEO1 sequence.

In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Table 17 or 18, the dsRNAs featured in the invention can include at least one strand of a length described herein. It can be reasonably expected that shorter dsRNAs having one of the sequences of Table 17 or 18 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Table 17 or 18, and differing in their ability to inhibit the expression of a NEO1 gene in an assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further, dsRNAs that cleave within a desired NEO1 target sequence can readily be made using the corresponding NEO1 antisense sequence and a complementary sense sequence.

In addition, the dsRNAs provided in Table 17 or 18 identify a site in a NEO1 that is susceptible to RNAi based cleavage. As such, the present invention further features dsRNAs that target within the sequence targeted by one of the agents of the present invention. As used herein, a second dsRNA is said to target within the sequence of a first dsRNA if the second dsRNA cleaves the message anywhere within the mRNA that is complementary to the antisense strand of the first dsRNA. Such a second dsRNA will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Table 17 or 18 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a NEO1 gene.

In one embodiment, a BMP6 gene is a human BMP6 gene. In specific embodiments, the sense strand of the dsRNA is one of the sense sequences from Table 21, and the antisense strand is one of the antisense sequences of Table 21. Alternative antisense agents that target elsewhere in the target sequence provided in Table 21 can readily be determined using the target sequence and the flanking BMP6 sequence.

In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Table 21, the dsRNAs featured in the invention can include at least one strand of a length described herein. It can be reasonably expected that shorter dsRNAs having one of the sequences of Table 21 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Table 21, and differing in their ability to inhibit the expression of a BMP6 gene in an assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further, dsRNAs that cleave within a desired BMP6 target sequence can readily be made using the corresponding BMP6 antisense sequence and a complementary sense sequence.

In addition, the dsRNAs provided in Table 21 identify a site in a BMP6 that is susceptible to RNAi based cleavage. As such, the present invention further features dsRNAs that target within the sequence targeted by one of the agents of the present invention. As used herein, a second dsRNA is said to target within the sequence of a first dsRNA if the second dsRNA cleaves the message anywhere within the mRNA that is complementary to the antisense strand of the first dsRNA. Such a second dsRNA will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Table 21 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a BMP6 gene.

With regard to Tables 4, 10A, 10B, 13, 16, 18, and 21: It should be noted that unmodified versions of each of the modified sequences shown are included within the scope of the invention. "Unmodified version" refers to a sequence that does not include one or more chemical modifications, e.g., a 2'-O methyl group, a phosphorothioate, and/or a 2'-fluoro group. For example, included in the invention are unmodified versions of AD-47391, which targets HFE2. *See* Table 10A. Unmodified sense strand versions of AD-47391 include: AGAGUAGGGAAUCAUGGCUdTdT and AGAGUAGGGAAUCAUGGCU. Unmodified antisense strand versions of AD-47391 include: AGCCAUGAUUCCCUACUCUdTdT and AGCCAUGAUUCCCUACUCU. As another example, included in the invention are unmodified versions of AD-47826, which targets TFR2. *See* Table 10B. Unmodified sense strand versions of AD-47826 include: CAGGCAGCCAAACCUCAUUdTdT and CAGGCAGCCAAACCUCAUU. Unmodified antisense strand versions of AD-47826 include: AAUGAGGUUUGGCUGCCUG and AAUGAGGUUUGGCUGCCUGdTdT.

Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art. The cleavage site

on the target mRNA of a dsRNA can be determined using methods generally known to one of ordinary skill in the art, e.g., the 5'-RACE method described in Soutschek et al., *Nature*; 2004, Vol. 432, pp. 173-178 (which is herein incorporated by reference for all purposes). Included in the invention are dsRNA that cleave the RNA target at the same location as the dsRNA described in the tables herein.

The dsRNA featured in the invention can contain one or more mismatches to the target sequence. In one embodiment, the dsRNA featured in the invention contains no more than 3 mismatches. If the antisense strand of the dsRNA contains mismatches to a target sequence, it is preferable that the area of mismatch not be located in the center of the region of complementarity. If the antisense strand of the dsRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to 5 nucleotides from either end, for example 5, 4, 3, 2, or 1 nucleotide from either the 5' or 3' end of the region of complementarity. For example, for a 23 nucleotide dsRNA strand which is complementary to a region of a HAMP gene, the dsRNA generally does not contain any mismatch within the central 13 nucleotides. The methods described within the invention can be used to determine whether a dsRNA containing a mismatch to a target sequence is effective in inhibiting the expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene. Consideration of the efficacy of dsRNAs with mismatches in inhibiting expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is important, especially if the particular region of complementarity in a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is known to have polymorphic sequence variation within the population.

Modifications

In yet another embodiment, the dsRNA is chemically modified to enhance stability. The nucleic acids featured in the invention may be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Eds.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Specific examples of dsRNA compounds useful in this invention include dsRNAs containing modified backbones or no natural internucleoside linkages. As defined in this specification, dsRNAs having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified dsRNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Modified dsRNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those) having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference

Modified dsRNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or ore or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts. In some instances, dsRNAs can be made with "Light Fluoro" chemical modifications as follows: all pyrimidines (cytosine and uridine) in the sense strand can be replaced with corresponding 2′-Fluoro bases (2′ Fluoro C and 2′-Fluoro U). In the antisense strand, pyrimidines adjacent to (towards 5′ position) ribo A nucleoside can be replaced with their corresponding 2-Fluoro nucleosides.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704;

5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, each of which is herein incorporated by reference.

In other suitable dsRNA mimetics, both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, a dsRNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of a dsRNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen *et al.*, Science, 1991, 254, 1497-1500.

Other embodiments of the invention are dsRNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--CH_2--, --CH_2--$N(CH_3)$--O--CH_2--[known as a methylene (methylimino) or MMI backbone], --CH_2--O--$N(CH_3)$--CH_2--, --CH_2--$N(CH_3)$--$N(CH_3)$--CH_2-- and --$N(CH_3)$--CH_2--CH_2--[wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. Also preferred are dsRNAs having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

Modified dsRNAs may also contain one or more substituted sugar moieties. Preferred dsRNAs comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred dsRNAs comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an dsRNA, or a group for improving the pharmacodynamic properties of an dsRNA, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-

methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78, 486-504) *i.e.*, an alkoxy-alkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_2)_2$, also described in examples herein below.

Other preferred modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the dsRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. DsRNAs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

dsRNAs may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, DsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of

the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., DsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, also herein incorporated by reference.

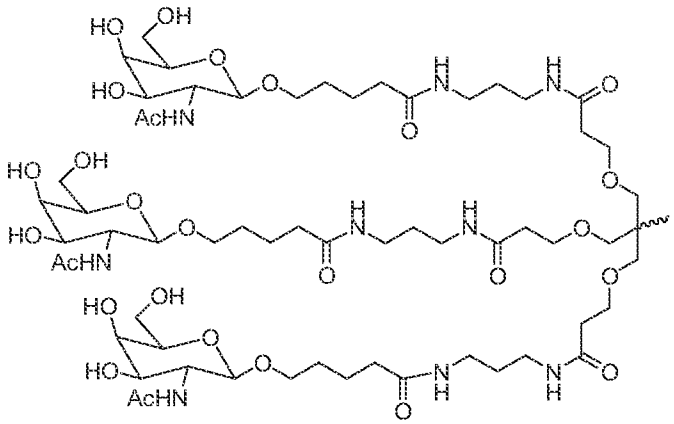
Conjugates

Another modification of the dsRNAs of the invention involves chemically linking to the dsRNA one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the dsRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, Proc. Natl. Acid. Sci. USA, 1989, 86: 6553-6556), cholic acid (Manoharan *et al.*, Biorg. Med. Chem. Let., 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, Ann. N.Y. Acad. Sci., 1992, 660:306-309; Manoharan *et al.*, Biorg. Med. Chem. Let., 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, Nucl. Acids Res., 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, EMBO J, 1991, 10:1111-1118; Kabanov *et al.*, FEBS Lett., 1990, 259:327-330; Svinarchuk *et al.*, Biochimie, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-Hphosphonate (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651-3654; Shea *et al.*, Nucl. Acids Res., 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, Nucleosides & Nucleotides, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, Biochim. Biophys. Acta, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, J. Pharmacol. Exp. Ther., 1996, 277:923-937).

In some embodiments of the compositions and methods of the invention, an dsRNA oligonucleotide further comprises a carbohydrate. The carbohydrate conjugated dsRNA are

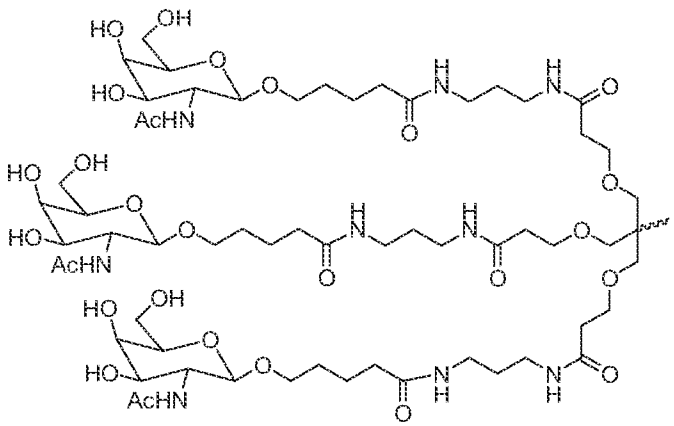
advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.*, C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.*, C5, C6, C7, or C8).

In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as

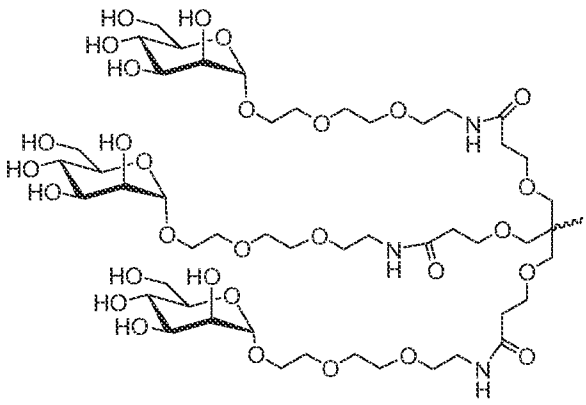


Formula II.

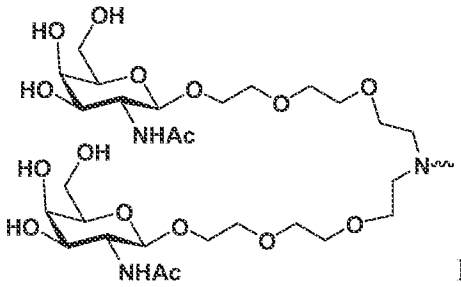
In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



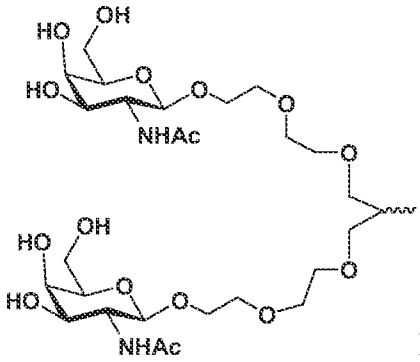
Formula II,



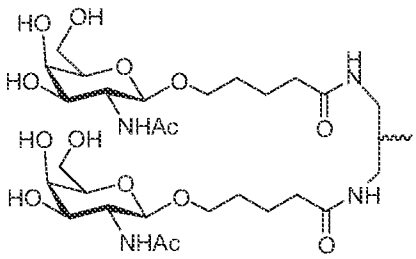
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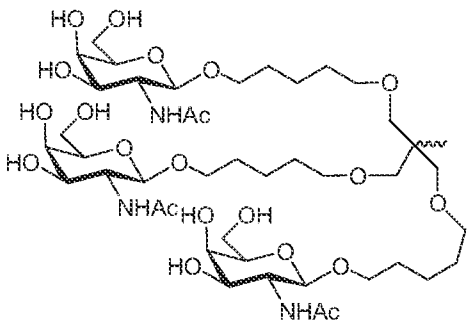
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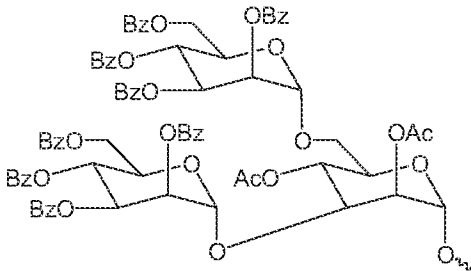
Formula V,



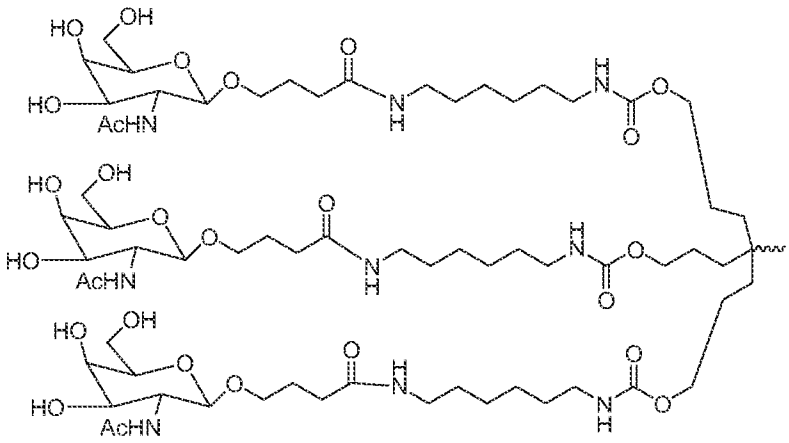
Formula VI,



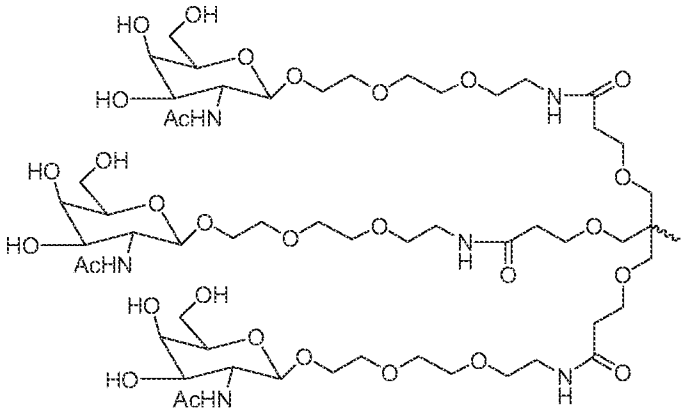
Formula VII,



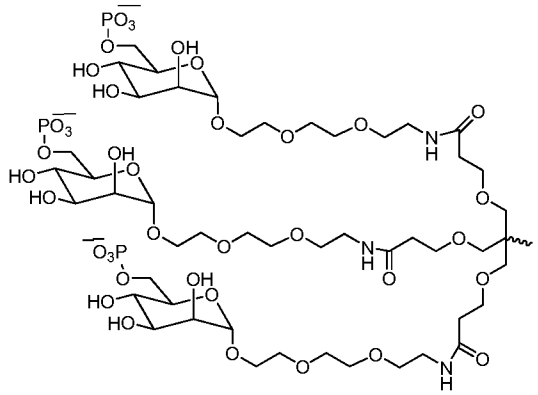
Formula VIII,



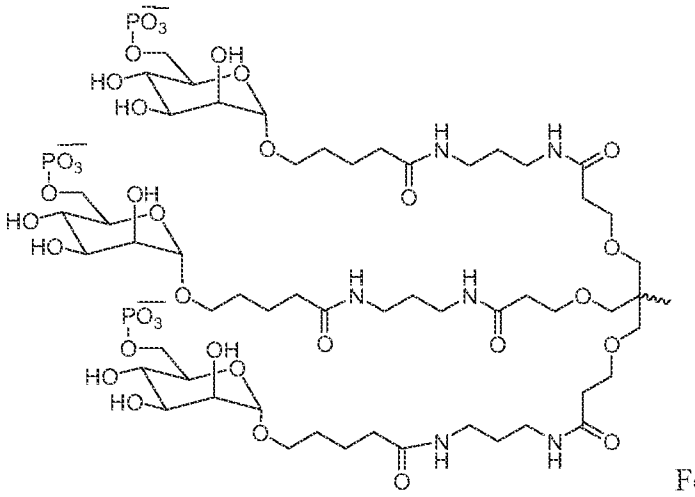
Formula IX,



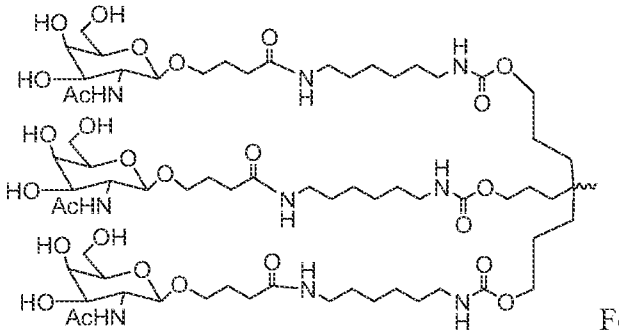
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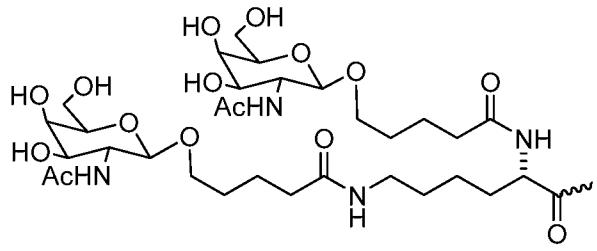
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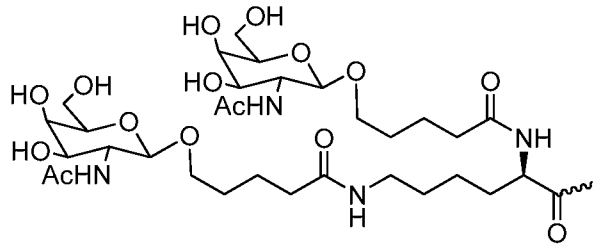
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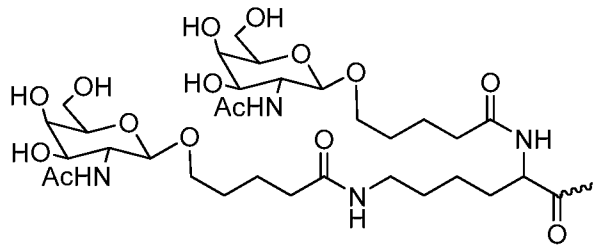
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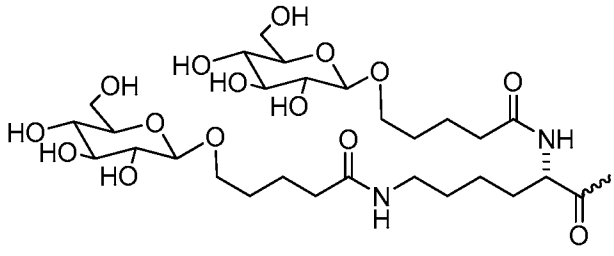
Formula XIV,



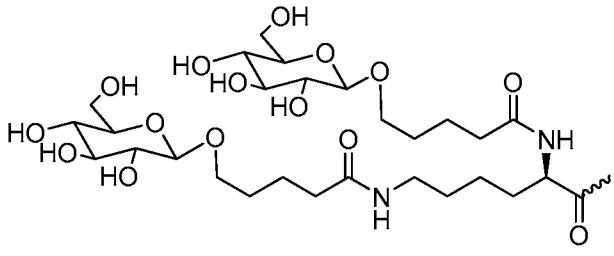
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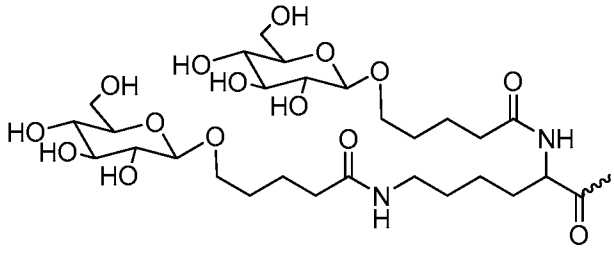
Formula XVI,



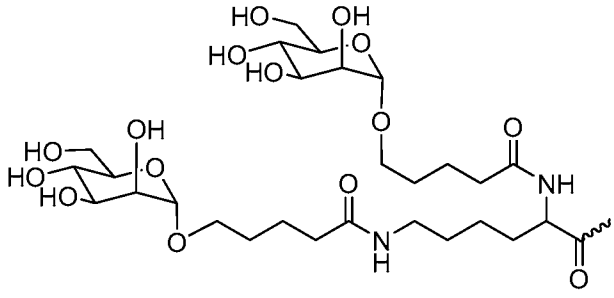
Formula XVII,



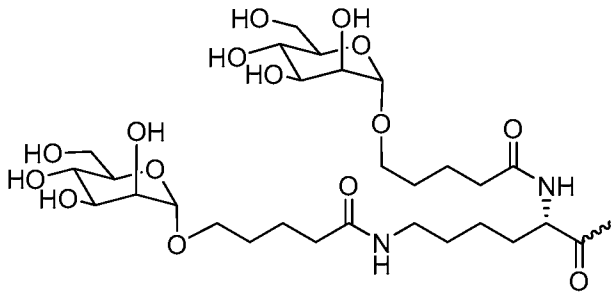
Formula XVIII,



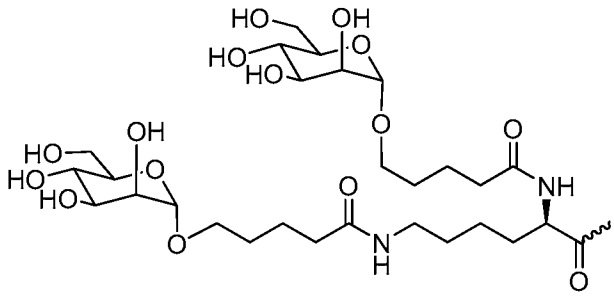
Formula XIX,



Formula XX,

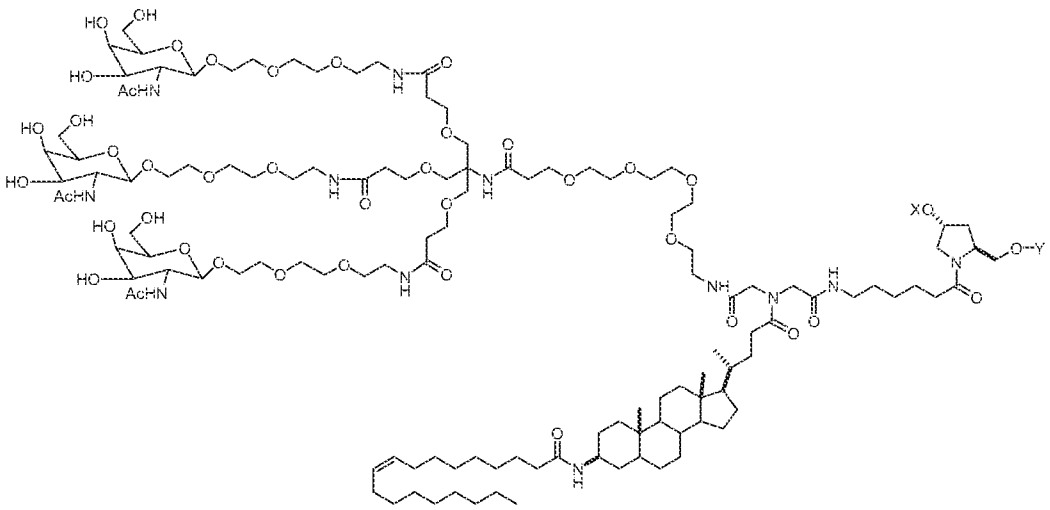


Formula XXI,



Formula XXII.

Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(Formula XXIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator and/or a cell permeation peptide.

In some embodiments, the conjugate or ligand described herein can be attached to an dsRNA oligonucleotide with various linkers that can be cleavable or non cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.*, covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be

interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times or more, or at least about 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

In one embodment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular dsRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

In another embodiment, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or

hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-. Preferred embodiments are -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O-, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-. A preferred embodiment is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

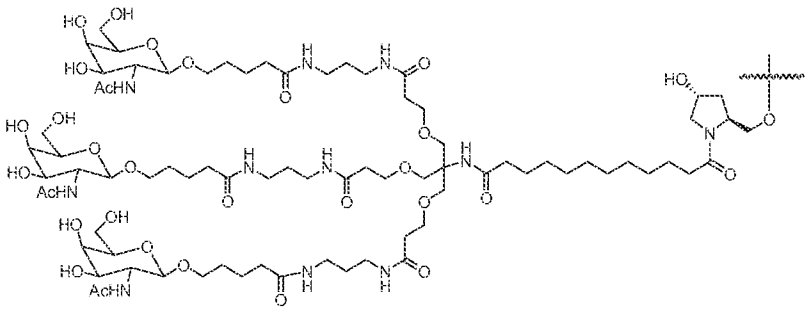
In another embodiment, a cleavable linker comprises an acid cleavable linking group. An *acid* cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.75, 5.5, 5.25, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula -C(O)O-, or -OC(O)-. These candidates can be evaluated using methods analogous to those described above.

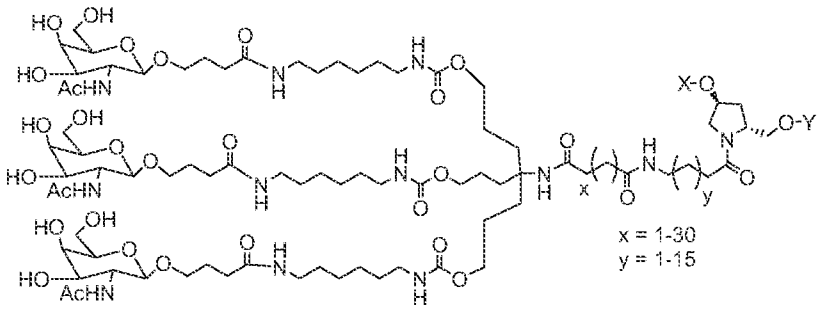
In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The

peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula – NHCHRAC(O)NHCHRBC(O)- (SEQ ID NO: 13), where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

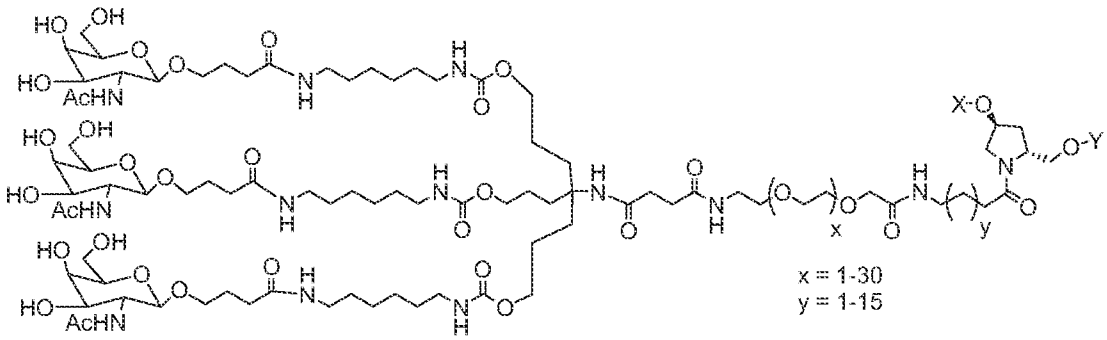
In one embodiment, an dsRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of dsRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



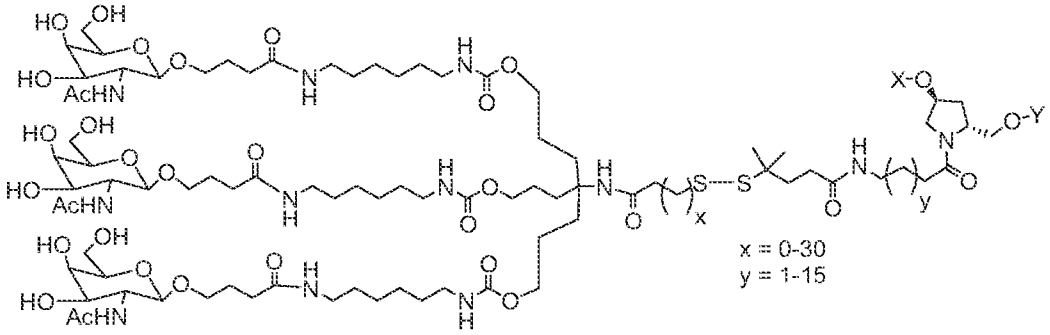
(Formula XXIV),



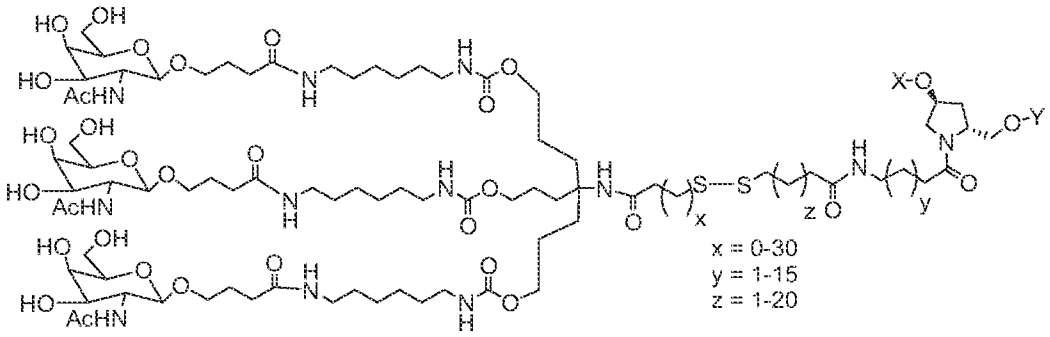
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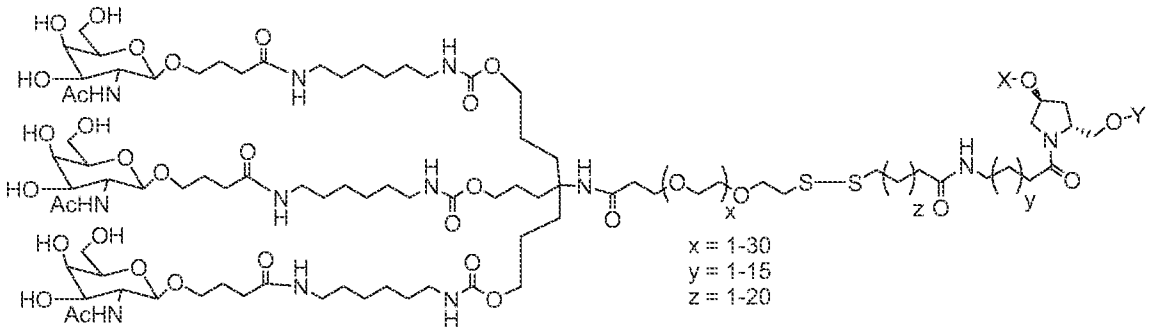
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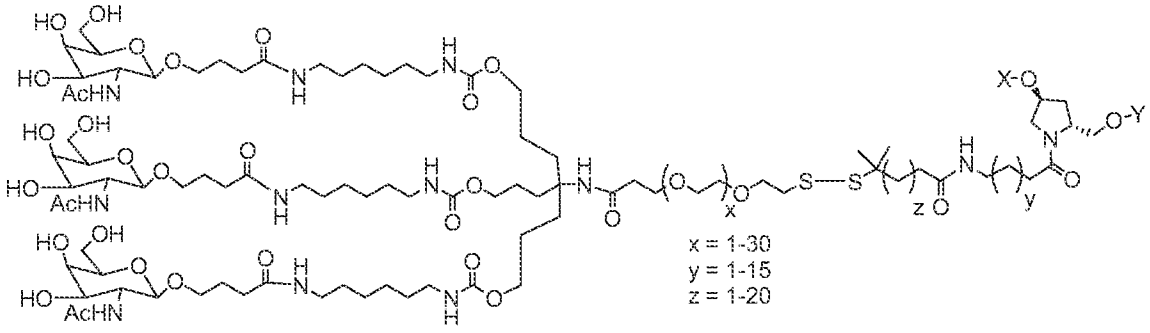
(Formula XXVII),



(Formula XXVIII),



(Formula XXIX), and



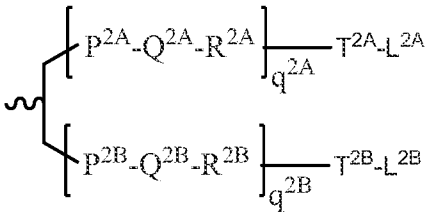
(Formula XXX), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the compositions and methods of the invention, a ligand is one or more GalNAc (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

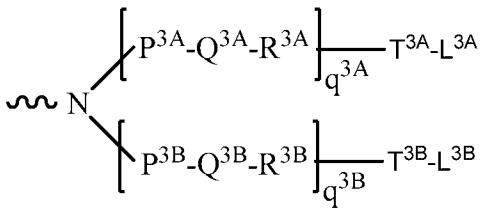
In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXI) – (XXXIV):

Formula XXXI

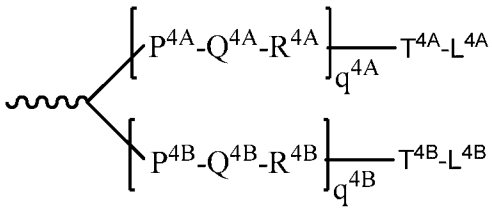
Formula XXXII

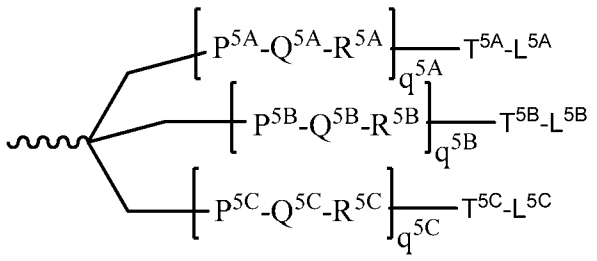


(IV)



(V)





Formula XXXIII

Formula XXXIV

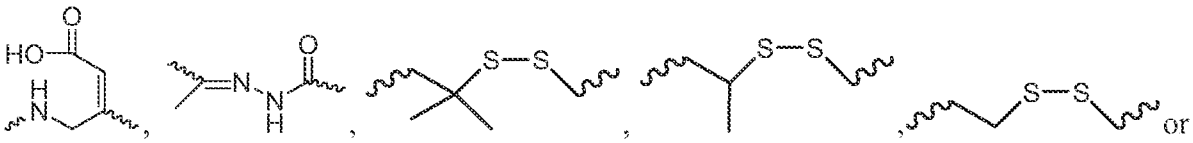
wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, 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^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherin one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R''), C≡C or C(O);

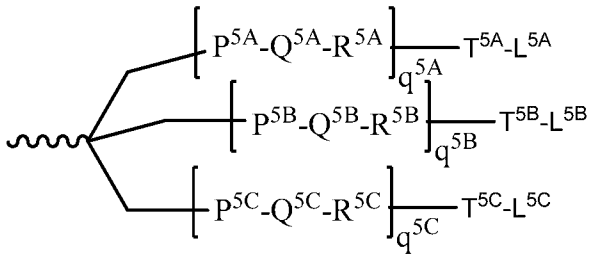
R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, $-C(O)-CH(R^a)-NH-$, CO, CH=N-O,



heterocyclyl;

L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; andR^a is H or amino acid side chain.Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XXXV):

Formula XXXV



Form ,

wherein L^{5A}, L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II_VII, XI, X, and XIII.

Representative U.S. patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single

compound or even at a single nucleoside within a dsRNA. The present invention also includes dsRNA compounds which are chimeric compounds. "Chimeric" dsRNA compounds or "chimeras," in the context of this invention, are dsRNA compounds, particularly dsRNAs, which contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of a dsRNA compound. These dsRNAs typically contain at least one region wherein the dsRNA is modified so as to confer upon the dsRNA increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the dsRNA may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of dsRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter dsRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxydsRNAs hybridizing to the same target region.

In certain instances, the dsRNA may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to dsRNAs in order to enhance the activity, cellular distribution or cellular uptake of the dsRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger *et al.*, Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan *et al.*, Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al.*, Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan *et al.*, Bioorg. Med. Chem. Let., 1993, 3:2765), a thiocholesterol (Oberhauser *et al.*, Nucl. Acids Res., 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, EMBO J., 1991, 10:111; Kabanov *et al.*, FEBS Lett., 1990, 259:327; Svinarchuk *et al.*, Biochimie, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651; Shea *et al.*, Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan *et al.*, Tetrahedron Lett., 1995, 36:3651), a palmityl moiety (Mishra *et al.*, Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al.*, J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such dsRNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of dsRNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated

using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the dsRNA still bound to the solid support or following cleavage of the dsRNA in solution phase. Purification of the dsRNA conjugate by HPLC typically affords the pure conjugate.

Vector encoded dsRNAs

In another aspect, HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 dsRNA molecules are expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), **12**:5-10; Skillern, A., *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a transgene integrated into the host genome. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) **92**:1292).

The individual strands of a dsRNA can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively each individual strand of the dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

The recombinant dsRNA expression vectors are generally DNA plasmids or viral vectors. dsRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka, *et al.*, *Curr. Topics Micro. Immunol.* (1992) 158:97-129)); adenovirus (see, for example, Berkner, *et al.*, BioTechniques (1998) 6:616), Rosenfeld *et al.* (1991, Science 252:431-434), and Rosenfeld *et al.* (1992), *Cell* 68:143-155)); or alphavirus as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, *in vitro* and/or *in vivo* (see, *e.g.*, Eglitis, *et al.*, *Science* (1985) 230:1395-1398; Danos and Mulligan, *Proc. Natl. Acad. Sci. USA* (1998) 85:6460-6464; Wilson *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:61416145; Huber *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury *et al.*, 1991, Science 254:1802-1805; van Beusechem. *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:7640-19 ; Kay *et al.*, 1992, Human Gene Therapy 3:641-647; Dai *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:10892-10895; Hwu *et al.*, 1993, J. Immunol. 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO

89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). Recombinant retroviral vectors capable of transducing and expressing genes inserted into the genome of a cell can be produced by transfecting the recombinant retroviral genome into suitable packaging cell lines such as PA317 and Psi-CRIP (Comette *et al.*, 1991, Human Gene Therapy 2:5-10; Cone *et al.*, 1984, Proc. Natl. Acad. Sci. USA 81:6349). Recombinant adenoviral vectors can be used to infect a wide variety of cells and tissues in susceptible hosts (*e.g.*, rat, hamster, dog, and chimpanzee) (Hsu *et al.*, 1992, J. Infectious Disease, 166:769), and also have the advantage of not requiring mitotically active cells for infection.

Any viral vector capable of accepting the coding sequences for the dsRNA molecule(s) to be expressed can be used, for example vectors derived from adenovirus (AV); adeno-associated virus (AAV); retroviruses (*e.g.*, lentiviruses (LV), Rhabdoviruses, murine leukemia virus); herpes virus, and the like. The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate.

For example, lentiviral vectors featured in the invention can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors featured in the invention can be made to target different cells by engineering the vectors to express different capsid protein serotypes. For example, an AAV vector expressing a serotype 2 capsid on a serotype 2 genome is called AAV 2/2. This serotype 2 capsid gene in the AAV 2/2 vector can be replaced by a serotype 5 capsid gene to produce an AAV 2/5 vector. Techniques for constructing AAV vectors which express different capsid protein serotypes are within the skill in the art; see, *e.g.*, Rabinowitz J E *et al.* (2002), J Virol 76:791-801, the entire disclosure of which is herein incorporated by reference.

Selection of recombinant viral vectors suitable for use in the invention, methods for inserting nucleic acid sequences for expressing the dsRNA into the vector, and methods of delivering the viral vector to the cells of interest are within the skill in the art. See, for example, Dornburg R (1995), Gene Therap. 2: 301-310; Eglitis M A (1988), Biotechniques 6: 608-614; Miller A D (1990), Hum Gene Therap. 1: 5-14; Anderson W F (1998), Nature 392: 25-30; and Rubinson D A *et al.*, Nat. Genet. 33: 401-406, the entire disclosures of which are herein incorporated by reference.

Viral vectors can be derived from AV and AAV. In one embodiment, the dsRNA featured in the invention is expressed as two separate, complementary single-stranded RNA

molecules from a recombinant AAV vector having, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter.

A suitable AV vector for expressing the dsRNA featured in the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H *et al.* (2002), *Nat. Biotech.* 20: 1006-1010.

Suitable AAV vectors for expressing the dsRNA featured in the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R *et al.* (1987), J. Virol. 61: 3096-3101; Fisher K J *et al.* (1996), J. Virol, 70: 520-532; Samulski R *et al.* (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

The promoter driving dsRNA expression in either a DNA plasmid or viral vector featured in the invention may be a eukaryotic RNA polymerase I (*e.g.*, ribosomal RNA promoter), RNA polymerase II (*e.g.*, CMV early promoter or actin promoter or U1 snRNA promoter) or generally RNA polymerase III promoter (*e.g.*, U6 snRNA or 7SK RNA promoter) or a prokaryotic promoter, for example the T7 promoter, provided the expression plasmid also encodes T7 RNA polymerase required for transcription from a T7 promoter. The promoter can also direct transgene expression to the pancreas (see, *e.g.*, the insulin regulatory sequence for pancreas (Bucchini *et al.*, 1986, Proc. Natl. Acad. Sci. USA 83:2511-2515)).

In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, *e.g.*, circulating glucose levels, or hormones (Docherty *et al.*, 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 -thiogalactopyranoside (EPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

Generally, recombinant vectors capable of expressing dsRNA molecules are delivered as described below, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of dsRNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the dsRNAs bind to target RNA and modulate its function or expression. Delivery of dsRNA expressing vectors can be systemic, such as by intravenous or

intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

dsRNA expression DNA plasmids are typically transfected into target cells as a complex with cationic lipid carriers (*e.g.*, Oligofectamine) or non-cationic lipid-based carriers (*e.g.*, Transit-TKO™). Multiple lipid transfections for dsRNA-mediated knockdowns targeting different regions of a single HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene or multiple HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 genes over a period of a week or more are also contemplated by the invention. Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of cells *ex vivo* can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (*e.g.*, antibiotics and drugs), such as hygromycin B resistance.

HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 specific dsRNA molecules can also be inserted into vectors and used as gene therapy vectors for human patients. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

Pharmaceutical compositions containing dsRNA

In one embodiment, the invention provides pharmaceutical compositions containing a dsRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition containing the dsRNA is useful for treating a disease or disorder associated with the expression or activity of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene, such as pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery, *e.g.*, by intravenous (IV) delivery. Another

example is compositions that are formulated for direct delivery into the brain parenchyma, *e.g.*, by infusion into the brain, such as by continuous pump infusion.

The pharmaceutical compositions featured herein are administered in dosages sufficient to inhibit expression of HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 genes.

In general, a suitable dose of dsRNA will be in the range of 0.01 to 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of 1 to 50 mg per kilogram body weight per day. For example, the dsRNA can be administered at 0.0059 mg/kg, 0.01 mg/kg, 0.0295 mg/kg, 0.05 mg/kg, 0.0590 mg/kg, 0.163 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.543 mg/kg, 0.5900 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.628 mg/kg, 2 mg/kg, 3 mg/kg, 5.0 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, or 50 mg/kg per single dose.

In one embodiment, the dosage is between 0.01 and 0.2 mg/kg. For example, the dsRNA can be administered at a dose of 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg 0.08 mg/kg 0.09 mg/kg , 0.10 mg/kg, 0.11 mg/kg, 0.12 mg/kg, 0.13 mg/kg, 0.14 mg/kg, 0.15 mg/kg, 0.16 mg/kg, 0.17 mg/kg, 0.18 mg/kg, 0.19 mg/kg, or 0.20 mg/kg.

In one embodiment, the dosage is between 0.005 mg/kg and 1.628 mg/kg. For example, the dsRNA can be administered at a dose of 0.0059 mg/kg, 0.0295 mg/kg, 0.0590 mg/kg, 0.163 mg/kg, 0.543 mg/kg, 0.5900 mg/kg, or 1.628 mg/kg.

In one embodiment, the dosage is between 0.2 mg/kg and 1.5 mg/kg. For example, the dsRNA can be administered at a dose of 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, or 1.5 mg/kg.

The dsRNA can be administered at a dose of 0.03 mg/kg, or 0.03, 0.1, 0.2, or 0.4 mg/kg.

The pharmaceutical composition may be administered once daily or the dsRNA may be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the dsRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, *e.g.*, using a conventional sustained release formulation which provides sustained release of the dsRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as could be used with the

agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

The effect of a single dose on HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 levels is long lasting, such that subsequent doses are administered at not more than 3, 4, or 5 day intervals, or at not more than 1, 2, 3, or 4 week intervals, or at not more than 5, 6, 7, 8, 9, or 10 week intervals.

The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and *in vivo* half-lives for the individual dsRNAs encompassed by the invention can be made using conventional methodologies or on the basis of *in vivo* testing using an appropriate animal model, as described elsewhere herein.

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression. Such models are used for *in vivo* testing of dsRNA, as well as for determining a therapeutically effective dose. A suitable mouse model is, for example, a mouse containing a plasmid expressing human HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1. Another suitable mouse model is a transgenic mouse carrying a transgene that expresses human HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful

doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The dsRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by target gene expression. In any event, the administering physician can adjust the amount and timing of dsRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

Administration

The present invention also includes pharmaceutical compositions and formulations which include the dsRNA compounds featured in the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, *e.g.*, intraparenchymal, intrathecal or intraventricular, administration.

The dsRNA can be delivered in a manner to target a particular tissue.

Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful. Suitable topical formulations include those in which the dsRNAs featured in the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.,* dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (*e.g.,* dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.,* dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). DsRNAs featured in the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, dsRNAs may be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine,

an acylcholine, or a C_{1-10} alkyl ester (*e.g.*, isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. Patent No. 6,747,014, which is incorporated herein by reference.

Liposomal formulations

There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages *in vivo*.

In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

Further advantages of liposomes include; liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes and as the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes

present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang *et al.*, Biochem. Biophys. Res. Commun., 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou *et al.*, Journal of Controlled Release, 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (*e.g.*, as a solution or as an emulsion) were ineffective (Weiner *et al.*, Journal of Drug Targeting, 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal

formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formulation was superior to aqueous administration (du Plessis *et al.*, Antiviral Research, 1992, 18, 259-265).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu *et al.* S.T.P.Pharma. Sci., 1994, 4, 6, 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, FEBS Letters, 1987, 223, 42; Wu *et al.*, Cancer Research, 1993, 53, 3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al.* (Ann. N.Y. Acad. Sci., 1987, 507, 64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon *et al.* (Proc. Natl. Acad. Sci. U.S.A., 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb *et al.*) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim *et al*).

Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto *et al.* (Bull. Chem. Soc. Jpn., 1980, 53, 2778) described liposomes comprising a nonionic detergent, $2C_{1215G}$, that

contains a PEG moiety. Illum *et al.* (FEBS Lett., 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (*e.g.*, PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534,899). Klibanov *et al.* (FEBS Lett., 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume *et al.* (Biochimica et Biophysica Acta, 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids, *e.g.*, DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle *et al.* (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin *et al.* (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Pat. No. 5,225,212 (both to Martin *et al.*) and in WO 94/20073 (Zalipsky *et al.*) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi *et al*). U.S. Pat. No. 5,540,935 (Miyazaki *et al.*) and U.S. Pat. No. 5,556,948 (Tagawa *et al.*) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

A number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry *et al.* discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa *et al.* discloses protein-bonded liposomes and asserts that the contents of such liposomes may include a dsRNA. U.S. Pat. No. 5,665,710 to Rahman *et al.* describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love *et al.* discloses liposomes comprising dsRNAs targeted to the raf gene.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, *e.g.*, they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to

the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

Nucleic acid lipid particles

In one embodiment, a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 dsRNA featured in the invention is fully encapsulated in the lipid formulation, e.g., a nucleic acid lipid particle, e.g., SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle. As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle, including SPLP. As used herein, the term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. Nucleic acid lipid particles typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate). Nucleic acid lipid particles are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site).

The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid- lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; and PCT Publication No. WO 96/40964.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. In some embodiments the lipid to dsRNA ratio can be about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, or 11:1.

In general, the lipid-nucleic acid particle is suspended in a buffer, e.g., PBS, for administration. In one embodiment, the pH of the lipid formulated siRNA is between 6.8 and 7.8, e.g., 7.3 or 7.4. The osmolality can be, e.g., between 250 and 350 mOsm/kg, e.g., around 300, e.g., 298, 299, 300, 301, 302, 303, 304, or 305.

The cationic lipid may be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3-

dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1 -(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (C12-200 or Tech G1), or a mixture thereof. The cationic lipid may comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

The non-cationic lipid may be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl- phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l- carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1 -trans PE, 1 -stearoyl-2-oleoyl- phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid may be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles may be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a

PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate may be, for example, a PEG-dilauryloxypropyl (C_{12}), a PEG-dimyristyloxypropyl (C_{14}), a PEG-dipalmityloxypropyl (C_{16}), or a PEG- distearyloxypropyl (C_{18}). Other examples of PEG conjugates include PEG-cDMA (N-[(methoxy poly(ethylene glycol)2000)carbamyl]-1,2-dimyristyloxlpropyl-3-amine), mPEG2000-DMG (mPEG-dimyrystylglycerol (with an average molecular weight of 2,000) and PEG-C-DOMG (R-3-[(ω-methoxy-poly(ethylene glycol)2000)carbamoyl)]-1,2-dimyristyloxlpropyl-3-amine). The conjugated lipid that prevents aggregation of particles may be from 0 mol % to about 20 mol % or about 1.0, 1.1., 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, or 55 mol % of the total lipid present in the particle.

LNP01

LNP01 formulations are described, *e.g.*, in International Application Publication No. WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary formulations are described in Table A.

Table A

	Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Mol % ratios Lipid:siRNA ratio
SNALP	DLinDMA	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
S-XTC	XTC	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA ~ 7:1
LNP05	XTC	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	XTC	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	XTC	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1
LNP08	XTC	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1

LNP09	XTC	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	ALN100	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	MC3	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	C12-200	C12-200/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1
LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

SNALP (l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009, which is hereby incorporated by reference.

XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane) comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/148,366, filed January 29, 2009; U.S. Provisional Serial No. 61/156,851, filed March 2, 2009; U.S. Provisional Serial No. filed June

10, 2009; U.S. Provisional Serial No. 61/228,373, filed July 24, 2009; U.S. Provisional Serial No. 61/239,686, filed September 3, 2009, and International Application No. PCT/US2010/022614, filed January 29, 2010, which are hereby incorporated by reference.

MC3 ((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate), (e.g., DLin-M-C3-DMA) comprising formulations are described, e.g., in U.S. Provisional Serial No. 61/244,834, filed September 22, 2009, U.S. Provisional Serial No. 61/185,800, filed June 10, 2009, and International Application No. PCT/US10/28224, filed June 10, 2010, which are hereby incorporated by reference.

ALNY-100 ((3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine) comprising formulations are described, e.g., International patent application number PCT/US09/63933, filed on November 10, 2009, which is hereby incorporated by reference.

C12-200, i.e., Tech G1, comprising formulations are described in U.S. Provisional Serial No. 61/175,770, filed May 5, 2009 and International Application No. PCT/US10/33777, filed May 5, 2010, which are hereby incorporated by reference.

Formulations prepared by either the standard or extrusion-free method can be characterized in similar manners. For example, formulations are typically characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles can be measured by light scattering using, for example, a Malvern Zetasizer Nano ZS (Malvern, USA). Particles should be about 20-300 nm, such as 40-100 nm in size. The particle size distribution should be unimodal. The total siRNA concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated siRNA can be incubated with an RNA-binding dye, such as Ribogreen (Molecular Probes) in the presence or absence of a formulation disrupting surfactant, *e.g.*, 0.5% Triton-X100. The total siRNA in the formulation can be determined by the signal from the sample containing the surfactant, relative to a standard curve. The entrapped fraction is determined by subtracting the "free" siRNA content (as measured by the signal in the absence of surfactant) from the total siRNA content. Percent entrapped siRNA is typically >85%. For a nucleic acid lipid formulation, the particle size is at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm, at least 100 nm, at least 110 nm, and at least 120 nm. The suitable range is typically about at least 50 nm to about at least 110 nm, about at least 60 nm to about at least 100 nm, or about at least 80 nm to about at least 90 nm.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.,* sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.,* p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, US Publn. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intraventricular or intrahepatic administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Emulsions

The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing

Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion may be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that may be incorporated into either phase of the emulsion. Emulsifiers may broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the

hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants may be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used may be free radical scavengers such as tocopherols, alkyl gallates, butylated

hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

In one embodiment of the present invention, the compositions of dsRNAs and nucleic acids are formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical

Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase may include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (Constantinides *et al.*, Pharmaceutical Research, 1994, 11, 1385-1390; Ritschel, Meth. Find. Exp. Clin. Pharmacol., 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (Constantinides *et al.*, Pharmaceutical Research, 1994, 11, 1385; Ho *et al.*, J. Pharm. Sci., 1996, 85, 138-143). Often microemulsions may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating thermolabile drugs, peptides or dsRNAs. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical

applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of dsRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of dsRNAs and nucleic acids.

Microemulsions of the present invention may also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the dsRNAs and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention may be classified as belonging to one of five broad categories--surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly dsRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers may be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants: In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of dsRNAs through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi *et al.*, J. Pharm. Pharmacol., 1988, 40, 252).

Fatty acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic

acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C.sub.1-10 alkyl esters thereof (*e.g.*, methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri *et al.*, J. Pharm. Pharmacol., 1992, 44, 651-654).

Bile salts: The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman *et al.* Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto *et al.*, J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita *et al.*, J. Pharm. Sci., 1990, 79, 579-583).

Chelating Agents: Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of dsRNAs through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Suitable chelating agents include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (*e.g.*, sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines)(Lee *et al.*, Critical Reviews in Therapeutic Drug

Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur *et al.*, J. Control Rel., 1990, 14, 43-51).

Non-chelating non-surfactants: As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of dsRNAs through the alimentary mucosa (Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita *et al.*, J. Pharm. Pharmacol., 1987, 39, 621-626).

Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (*i.e.*, does not possess biological activity per se) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao *et al.*, DsRNA Res. Dev., 1995, 5, 115-121; Takakura *et al.*, DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183.

Excipients

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, *etc.*, when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not

limited to, binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, *etc.*); fillers (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, *etc.*); lubricants (*e.g.*, magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, *etc.*); disintegrants (*e.g.*, starch, sodium starch glycolate, *etc.*); and wetting agents (*e.g.*, sodium lauryl sulphate, *etc*).

Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Formulations for topical administration of nucleic acids may include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Other Components

The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers,

wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more dsRNA compounds and (b) one or more anti-cytokine biologic agents which function by a non-RNAi mechanism. Examples of such biologics include, biologics that target IL1β (*e.g.*, anakinra), IL6 (tocilizumab), or TNF (etanercept, infliximab, adlimumab, or certolizumab).

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the dsRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R,

BMP6, and/or NEO1 expression. In any event, the administering physician can adjust the amount and timing of dsRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

Methods for inhibiting expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene

In yet another aspect, the invention provides a method for inhibiting the expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene in a mammal. The method includes administering a composition featured in the invention to the mammal such that expression of the target HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is silenced.

When the organism to be treated is a mammal such as a human, the composition may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection.

Methods for treating diseases caused by expression of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene

The invention relates in particular to the use of a dsRNA targeting HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 and compositions containing at least one such dsRNA for the treatment of a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1-mediated disorder or disease. For example, the compositions described herein can be used to treat anemia and other diseases associated with lowered iron levels.

Methods of using dsRNAs targeting HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1

In one aspect, the invention provides use of a siRNA for inhibiting the expression of HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 in a mammal. The method includes administering a composition of the invention to the mammal such that expression of the target HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is decreased. In some embodiments, HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 expression is decreased for an extended duration, *e.g.*, at least one week, two weeks, three weeks, or four weeks or longer. For example, in certain instances, expression of the HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6,

and/or NEO1 gene is suppressed by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% by administration of a siRNA described herein. In some embodiments, the HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is suppressed by at least about 60%, 70%, or 80% by administration of the siRNA. In some embodiments, the HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene is suppressed by at least about 85%, 90%, or 95% by administration of the double-stranded oligonucleotide.

The methods and compositions described herein can be used to treat diseases and conditions that can be modulated by down regulating HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene expression. For example, the compositions described herein can be used to treat anemia and other forms of iron imbalance such as refractory anemia, refractory anemia of chronic disease (ACD), iron-rstricted erythropoiesis, and the pathological conditions associated with these disorders. In some aspects, ACD subjects are those who are refractory to ESAs and i.v. iron administration. In some embodiments, the method includes administering an effective amount of a siRNA disclosd herein to a patient having lower iron levels relative to a control patient.

Therefore, the invention also relates to the use of a siRNA for the treatment of a disorder or disease mediated by or related to HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene expression. For example, a siRNA is used for treatment of anemia.

The effect of the decreased HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene expression preferably results in an enhancement of iron mobilization in the mammal. In some embodiments, iron mobilization is enhanced by at least 10%, 15%, 20%, 25%, 30%, 40%, 50%, or 60%, or more, as compared to pretreatment levels.

The effect of the decreased HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene expression preferably results in an Hb increase in the mammal. In some embodiments, Hb is increased by at least 10%, 15%, 20%, 25%, 30%, 40%, 50%, or 60%, or more, as compared to pretreatment levels.

The effect of the decreased HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene expression preferably results in a serum iron increase in the mammal. In some embodiments, serum iron is increased by at least 10%, 15%, 20%, 25%, 30%, 40%, 50%, or 60%, or more, as compared to pretreatment levels.

The effect of the decreased HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene expression preferably results in a transderrin (Tf) saturation increase

in the mammal. In some embodiments, Tf saturation is increased by at least 10%, 15%, 20%, 25%, 30%, 40%, 50%, or 60%, or more, as compared to pretreatment levels.

The effect of the decreased HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene expression preferably results in decreased levels of HAMP in the mammal. In some embodiments, HAMP is decreased by at least 10%, 15%, 20%, 25%, 30%, 40%, 50%, or 60%, or more, as compared to pretreatment levels.

The method includes administering a siRNA to the subject to be treated. The subject to be treated is generally a subject in need thereof. When the subject to be treated is a mammal, such as a human, the composition can be administered by any means known in the art including, but not limited to oral or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, and airway (aerosol) administration. In some embodiments, the compositions are administered by intravenous infusion or injection.

The method includes administering a siRNA, *e.g.*, a dose sufficient to depress levels of HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 mRNA for at least 5, more preferably 7, 10, 14, 21, 25, 30 or 40 days; and optionally, administering a second single dose of dsRNA, wherein the second single dose is administered at least 5, more preferably 7, 10, 14, 21, 25, 30 or 40 days after the first single dose is administered, thereby inhibiting the expression of the target gene in a subject.

In one embodiment, doses of siRNA are administered not more than once every four weeks, not more than once every three weeks, not more than once every two weeks, or not more than once every week. In another embodiment, the administrations can be maintained for one, two, three, or six months, or one year or longer.

In another embodiment, administration can be provided when Hb levels reach or drop lower than a predetermined minimal level, such as less than 8 g/dL, 9 g/dL, or 10 g/dL. In some aspects, administration is continued until Hb levels are >11 g/dL, e.g, 12 g/dL.

In another embodiment, administration can be provided when a patient presents with various known symptoms of disorders such as anemia. These can include fatigue, shortness of breath, headache, dizziness, or pale skin.

In another embodiment, administration can be provided when a patient is diagnosed with anemia via CBC.

In general, the siRNA does not activate the immune system, *e.g.*, it does not increase cytokine levels, such as TNF-alpha or IFN-alpha levels. For example, when measured by an assay, such as an *in vitro* PBMC assay, such as described herein, the increase in levels of TNF-

alpha or IFN-alpha, is less than 30%, 20%, or 10% of control cells treated with a control dsRNA, such as a dsRNA that does not target HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1.

In an aspect, a subject can be administered a therapeutic amount of siRNA, such as 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, or 2.5 mg/kg dsRNA. The siRNA can be administered by intravenous infusion over a period of time, such as over a 5 minute, 10 minute, 15 minute, 20 minute, or 25 minute period. The administration is repeated, for example, on a regular basis, such as biweekly (i.e., every two weeks) for one month, two months, three months, four months or longer. After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration biweekly for three months, administration can be repeated once per month, for six months or a year or longer. Administration of the siRNA can reduce HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 levels, e.g., in a cell, tissue, blood, urine or other compartment of the patient by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80 % or at least 90% or more.

Before administration of a full dose of the siRNA, patients can be administered a smaller dose, such as a 5% infusion reaction, and monitored for adverse effects, such as an allergic reaction, or for elevated lipid levels or blood pressure. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (*e.g.*, TNF-alpha or IFN-alpha) levels.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given siRNA drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Additional agents and co-administration

In further embodiments, administration of a siRNA is administered in combination an additional therapeutic agent. The siRNA and an additional therapeutic agent can be administered in combination in the same composition, *e.g.*, parenterally, or the additional

therapeutic agent can be administered as part of a separate composition or by another method described herein.

In one embodiment, the siRNA is administered to the patient, and then the additional therapeutic agent is administered to the patient (or vice versa). In another embodiment, the siRNA and the additional therapeutic agent are administered at the same time.

In some aspects, the additional agent can include one or more Erythropoiesis-stimulating agents (ESAs). ESAs are generally known in the art. ESAs can include Erythropoietin (EPO), Epoetin alfa (Procrit/Epogen), Epoetin beta (NeoRecormon), Darbepoetin alfa (Aranesp), and Methoxy polyethylene glycol-epoetin beta (Micera). ESAs can be administered in various doses, e.g., 7,000 U/week to 30,000 U/week.

In some aspects, the additional agent can include intravenous iron. Iron can be administered in various doses known in the art.

In some aspects, two or more dsRNAs are co-administered to a subject. In one embodiment, a first dsRNA is administered to the patient, and then a second dsRNA is administered to the patient (or vice versa). In another embodiment, the first dsRNA and the second dsRNA are administered at the same time.

In some aspects, a HAMP dsRNA is co-administered with one or more dsRNAs selected from HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1dsRNAs.

In some aspects, a HFE2 dsRNA is co-administered with one or more dsRNAs selected from HAMP, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1dsRNAs.

In some aspects, a HFE dsRNA is co-administered with one or more dsRNAs selected from HAMP, HFE2, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1dsRNAs.

In some aspects, a TFR2 dsRNA is co-administered with one or more dsRNAs selected from HAMP, HFE2, HFE, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1dsRNAs.

In some aspects, a BMPR1a dsRNA is co-administered with one or more dsRNAs selected from HAMP, HFE2, HFE, TFR2, SMAD4, IL6R, BMP6, and/or NEO1dsRNAs.

In some aspects, a SMAD4 dsRNA is co-administered with one or more dsRNAs selected from HAMP, HFE2, HFE, TFR2, BMPR1a, IL6R, BMP6, and/or NEO1dsRNAs.

In some aspects, an IL6R dsRNA is co-administered with one or more dsRNAs selected from HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, BMP6, and/or NEO1dsRNAs.

In some aspects, a BMP6 dsRNA is co-administered with one or more dsRNAs selected from HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, and/or NEO1dsRNAs.

In some aspects, a NEO1 dsRNA is co-administered with one or more dsRNAs selected from HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, and/or BMP6 dsRNAs.

In another aspect, the invention features, a method of instructing an end user, e.g., a caregiver or a subject, on how to administer a siRNA described herein. The method includes, optionally, providing the end user with one or more doses of the siRNA, and instructing the end user to administer the siRNA on a regimen described herein, thereby instructing the end user.

Identification of subjects in need of dsRNA administration

In one aspect, the invention provides a method of treating a patient by selecting a patient on the basis that the patient is in need of iron mobilization. The method includes administering to the patient a siRNA in an amount sufficient to increase the patient's iron mobilization.

In one aspect, the invention provides a method of treating a patient by selecting a patient on the basis that the patient is in need of increased Hb levels. Such a subject can have Hb levels of <9 g/dL. The method includes administering to the patient a siRNA in an amount sufficient to increase the patient's Hb levels. Typically target Hb levels are >11 g/dL, e.g., 11 g/dL or 12 g/dL.

In some aspects, a subject is identified as having anemia. In some aspects, a subject is identified as having a refractory form of anemia. In some aspects, a subject is identified as having ACD. Such subjects can be in need of administration of a dsRNA described herein. ACD can include a form of anemia wherein the subject is refractory to ESAs and/or i.v. iron administration. Typical clinical presentation of ACD includes fatigue, shortness of breadth, headache, dizziness, and/or pale skin. ACD can also be diagnosed via a CBC test, which is generally known in the art. ACD can also be diagnosed via serum iron levels, Tf saturation, and/or ferritin levels. ACD is typically diagnosed in certain settings such as subjects with CKD, cancer, chronic inflammatory diseases such as RA, or IRIDA. In some aspects, a subject with ACD has Hb levels of less than 9 g/dL. Such subjects typically become symptomatic for ACD.

CKD can result in reduced renal EPO synthesis, dietary hematinic deficiencies, blood loss, and/or elevated hepcidin levels. The elevation in hepcidin levels can be due to decreased renal excretion and/or low grade inflammation characterized by, e.g., interleukin (IL)-6.

In some aspects, a subject is identified as having iron-restricted erythropoiesis (IRE). Such subjects can be in need of administration of a dsRNA described herein. IRE can be assessed via reticulocyte Hb (CHr). Typically a result of <28 pg suggests IRE, where normal is in the range of 28-35 pg. IRE can also be assessed via percent (%) hypochromic RBCs. Typically a result of >10% suggests IRE, where 1-5% is generally considered normal.

A healthcare provider, such as a doctor, nurse, or family member, can take a family history before prescribing or administering a siRNA. In addition, a test may be performed to determine a genotype or phenotype. For example, a DNA test may be performed on a sample from the patient, e.g., a blood sample, to identify the relevant genotype and/or phenotype before a dsRNA is administered to the patient. In another embodiment, a test is performed to identify a related genotype and/or phenotype.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the dsRNAs and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

Example 1: dsRNA synthesis

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent may be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

siRNA synthesis

Single-stranded RNAs were produced by solid phase synthesis on a scale of 1 µmole using an Expedite 8909 synthesizer (Applied Biosystems, Applera Deutschland GmbH, Darmstadt, Germany) and controlled pore glass (CPG, 500Å, Proligo Biochemie GmbH, Hamburg, Germany) as solid support. RNA and RNA containing *2'-O*-methyl nucleotides were generated by solid phase synthesis employing the corresponding phosphoramidites and *2'-O*-methyl phosphoramidites, respectively (Proligo Biochemie GmbH, Hamburg, Germany). These building blocks were incorporated at selected sites within the sequence of the oligoribonucleotide chain using standard nucleoside phosphoramidite chemistry such as described in Current protocols in nucleic acid chemistry, Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA. Phosphorothioate linkages were introduced by replacement of the iodine oxidizer solution with a solution of the Beaucage reagent (Chruachem

Ltd, Glasgow, UK) in acetonitrile (1%). Further ancillary reagents were obtained from Mallinckrodt Baker (Griesheim, Germany).

Deprotection and purification of the crude oligoribonucleotides by anion exchange HPLC were carried out according to established procedures. Yields and concentrations were determined by UV absorption of a solution of the respective RNA at a wavelength of 260 nm using a spectral photometer (DU 640B, Beckman Coulter GmbH, Unterschleißheim, Germany). Double stranded RNA was generated by mixing an equimolar solution of complementary strands in annealing buffer (20 mM sodium phosphate, pH 6.8; 100 mM sodium chloride), heated in a water bath at 85 - 90°C for 3 minutes and cooled to room temperature over a period of 3 - 4 hours. The annealed RNA solution was stored at –20 °C until use.

Nucleic acid sequences are represented below using standard nomenclature, and specifically the abbreviations of Table 1.

Table 1: Abbreviations

Abbreviation	Nucleotide(s)
A	adenosine-3'-phosphate
C	cytidine-3'-phosphate
G	guanosine-3'-phosphate
U	uridine-3'-phosphate
N	any nucleotide (G, A, C, or T)
a	2'-O-methyladenosine-3'-phosphate
c	2'-O-methylcytidine-3'-phosphate
g	2'-O-methylguanosine-3'-phosphate
u	2'-O-methyluridine-3'-phosphate
T, dT	2'-deoxythymidine-3'-phosphate
sT; sdT	2'-deoxy-thymidine-5'phosphate-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Cf	2'-fluorocytidine-3'-phosphate
Gf	2'-fluoroguanosine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate

Example 2: HAMP siRNA Design

Transcripts

siRNA design was carried out to identify siRNAs targeting human, cynomolgus monkey (*Macaca fascicularis*; herein "cyno"), mouse, and rat HAMP transcripts annotated in the NCBI Gene database ncbi.nlm.nih.gov/gene website. In mouse, the HAMP gene is duplicated, yielding distinct HAMP1 and HAMP2 loci; duplex designs targeted only HAMP1. Design used the following transcripts from the NCBI RefSeq and GenBank collections: Human - NM_021175.2 (SEQ ID NO:1); Cyno -EU076443.1; Mouse - NM_032541.1; Rat -

NM_053469.1. Due to the short length of the HAMP transcripts and the high degree of primate/rodent HAMP sequence divergence, siRNA duplexes were designed in multiple separate batches. The separate batches are listed below and matched the various species as follows:

- human and cyno HAMP, exactly;
- only human HAMP, exactly;
- human and cyno HAMP, with mismatches to HAMP in both species allowed at sense-strand position 19 when a G or C HAMP targeting-nucleotide was replaced with a U or A, *i.e.* "UA-swap";
- human and cyno HAMP, with exact match to human HAMP and mismatches to cyno HAMP allowed at sense-strand positions 1, 2, and 19, *i.e.* "mismatch-to-cyno";
- mouse HAMP1, exactly;
- only rat HAMP, exactly.

All siRNA duplexes were designed that shared 100% identity with all listed human, cyno, mouse, or rat transcripts with the exception(s) of designated mismatched-to-target bases. Unless otherwise noted, duplexes themselves were 100% complementary and double-stranded.

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were selected that lacked repeats longer than 7 nucleotides. These siRNAs were used in comprehensive searches against the appropriate transcriptomes.

siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand.

Table 2 provides the sequences of the sense and antisense strands of 42 duplexes targeting the 3'UTR of the human HAMP gene.

Table 3 provides the sequences of the sense and antisense strands of 47 duplexes targeting the CDS of the human HAMP gene.

Table 4 provides the sequences of the sense and antisense strands of the modified duplexes targeting the HAMP gene.

Table 5 provides the sequences of the sense and antisense strands of the unmodified version of the duplexes shown in Table 4.

The antisense-derived human/cyno, mouse, rat, UA-swap, and mismatch-to-cyno oligonucleotides shown in Tables 3-4 were synthesized and formed into duplexes.

In some instances the duplexes contained no chemical modifications (unmodified).

In some instances the duplexes contained modifications (modified). For example, some duplexes were made with "Light Fluoro" chemical modifications as follows: all pyrimidines (cytosine and uridine) in the sense strand were replaced with corresponding 2′-Fluoro bases (2′ Fluoro C and 2′-Fluoro U). In the antisense strand, pyrimidines adjacent to (towards 5′ position) ribo A nucleoside was replaced with their corresponding 2-Fluoro nucleosides.

Example 3: HAMP siRNA screening

Cell culture and transfections:

Dual Luciferase system: COS7 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in DMEM (Gibco) supplemented with 10% FBS before being released from the plate by trypsinization. Cells were transfected with a psiCHECK2 vector (Promega) containing the human HAMP open reading frame (ORF). The ORF was introduced following the stop codon in the *renilla* luciferase sequence. Plasmid transfection was carried out by adding 19.8µl of Opti-MEM plus 0.2µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) and 2.5ng plasmid into a 96-well plate and incubated at room temperature for 15 minutes. 80µl of complete growth media containing ~2 x10^4 COS7 cells were then added. Cells were incubated for three hours, after which the media was removed from the wells and replaced with 80µl of complete growth media. Transfection of siRNA was accomplished out by preparing adding 14.8µl of Opti-MEM plus 0.2µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a new 96-well plate and incubated at room temperature for 15 minutes. The 20µl volumes containing the lipoplexes were then added over the culture plates and incubated for 48 hours. Single dose experiments were performed at final concentrations of 10nM and 0.1nM. An additional concentration of 0.01nM was performed for selected duplexes. Final duplex concentrations for dose response experiments were 10, 1.67, 0.278, 0.046, 0.0077, 0.0012, 0.0002, and 0.000035 nM.

Endogenous system (Human): For HAMP, HepG2 cells were used. HepG2 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in MEM (Gibco) supplemented with 10% FBS before being released from the plate by trypsinization. Transfection was carried out by adding 14.8µl of Opti-MEM plus 0.2µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 96-well plate and incubated at room temperature for 15 minutes. 80µl of complete growth media without antibiotic containing ~2 x10^4 HepG2 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments

were performed at final concentrations of 10nM and 0.1nM. An additional concentration of 0.01nM was performed for selected duplexes. Final duplex concentrations for dose response experiments were 10, 1.67, 0.278, 0.046, 0.0077, 0.0012, 0.0002, and 0.000035 nM.

Endogenous system (Cynomolgus): Transfection was carried out by adding 14.8µl of Opti-MEM plus 0.2µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 96-well plate and incubated at room temperature for 15 minutes. Primary cynomolgus hepatocytes (M003055-P, Celsis) were thawed and prepared in InVitroGRO CP plating medium (Z99029, Celsis). 80µl of complete growth media without antibiotic containing ~2 $x10^4$ cynomolgus hepatocytes were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at final concentrations of 10nM and 0.1nM. Final duplex concentrations for dose response experiments were 10, 1.67, 0.278, 0.046, 0.0077, 0.0012, 0.0002, and 0.000035 nM.

Dual Luciferase Assay (Promega part E2980):

For cells transfected with the psiCHECK2 vector containing the human HAMP ORF, the Dual Luciferase assay was performed to measure reduction in HAMP levels. Forty-eight hours after transfection, the media was removed over the cells, and cells received 150uL of a 1:1 mixture of complete growth medium and Dual-Glo Luciferase Reagent. As a control, these reagents were also added to empty wells; data derived from these samples were thus used as a blank measurement. Cells were then incubated for 30 minutes at room temperature on a shaker, protected from light. At this time, luminescence was determined using a SpectraMax M5 (Molecular Devices) with an integration time of 500ms, and resulting data defined as the firefly luciferase signal. Following measurement, 75µL of Dual-Glo Stop & Glo Reagent was added and the plates incubated in the dark at room temperature, without shaking. After an additional 10 minutes luminescence was again measured as above, and resulting data defined as the *renilla* luciferase signal. Data were background-subtracted, and the *renilla* values normalized to the firefly Luciferase values. Data were then expressed as percent mock-transfected or percent AD-1955.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen, part #: 610-12):

Cells were harvested and lysed in 150µl of Lysis/Binding Buffer then mixed for 5 minute at 850rpm using an Eppendorf Thermomixer (the mixing speed was the same throughout the process). Ten microliters of magnetic beads and 80µl Lysis/Binding Buffer mixture were added

to a round bottom plate and mixed for 1 minute. Magnetic beads were captured using magnetic stand and the supernatant was removed without disturbing the beads. After removing supernatant, the lysed cells were added to the remaining beads and mixed for 5 minutes. After removing supernatant, magnetic beads were washed 2 times with 150µl Wash Buffer A and mixed for 1 minute. Beads were capture again and supernatant removed. Beads were then washed with 150µl Wash Buffer B, captured and supernatant was removed. Beads were next washed with 150µl Elution Buffer, captured and supernatant removed. Beads were allowed to dry for 2 minutes. After drying, 50µl of Elution Buffer was added and mixed for 5 minutes at 70°C. Beads were captured on magnet for 5 minutes. 40µl of supernatant was removed and added to another 96 well plate.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):

A master mix of 2µl 10X Buffer, 0.8µl 25X dNTPs, 2µl Random primers, 1µl Reverse Transcriptase, 1µl RNase inhibitor and 3.2µl of H2O per reaction were added into 10µl total RNA. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25°C 10 min, 37°C 120 min, 85°C 5 sec, 4°C hold.

Real time PCR:

For human HAMP, 2µl of cDNA were added to a master mix containing 0.5µl GAPDH TaqMan Probe (Applied Biosystems Cat #4326317E), 0.5µl HAMP TaqMan probe (Applied Biosystems cat # Hs00221783_m1) and 5µl Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well 50 plates (Roche cat # 04887301001). For cynomolgus HAMP, 2µl of cDNA were added to a master mix containing 0.5µl 18s TaqMan Probe (Applied Biosystems Cat # 4319413E), 0.1µl 10x custom cynomolgus HAMP probe (Forward primer: CTCCGTTTTCCCACAACA (SEQ ID NO:XX); Reverse primer: CAGCACATCCCACACTTT (SEQ ID NO:XX); Probe: ACCCACTTCCCCATCTGCATT (SEQ ID NO:XX)), and 5µl Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well 50 plates (Roche cat # 04887301001). Real time PCR was done in an ABI 7900HT Real Time PCR system (Applied Biosystems) using the ΔΔCt(RQ) assay. Each duplex was tested in two independent transfections and each transfection was assayed in duplicate, unless otherwise noted in the summary tables.

To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC50s were calculated using a 4 parameter fit model using XLFit and

normalized to cells transfected with 10 nM AD-1955, mock transfected, or to the average lowest dose.

Table 6 shows the HAMP single dose screening data of the modified duplexes using the dual luciferase assay. Data are expressed as a percent of mock or AD-1955.

Table 7 shows the HAMP single dose screening data of the unmodified duplexes using the human endogenous assay. Data are expressed as a percent of mock.

Table 8 shows the HAMP single dose screening data of the modified duplexes using the human endogenous assay. Data are expressed as a percent of mock.

Table 9 shows the HAMP dose response data of modified and unmodified duplexes using the dual luciferase assay. Cells used included HepG2 and Cyno primary hepatocytes.

Example 4: HFE2 siRNA Design

siRNA design was carried out to identify siRNAs targeting human, rhesus (*Macaca mulatta*), mouse, and rat HFE2 transcripts annotated in the NCBI Gene database website noted above. There are at least 4 annotated human HFE2 transcripts and at least 3 annotated rhesus transcripts. Accordingly, we focused on the shortest annotated transcript for human, and the rhesus transcript which shared the greatest number of orthologous human exons, and designed on sequences held in common by the alternate transcripts. Design used the following transcripts from the NCBI RefSeq collection: Human - NM_213652.3; Rhesus - XM_001092987.1; Mouse - NM_027126.4; Rat - NM_001012080.1. Due to high primate/rodent sequence divergenge, siRNA duplexes were designed in two separate batches. The first batch matched human and rhesus; the second matched mouse and rat. All siRNA duplexes were designed that shared 100% identity with all listed human/rhesus or mouse/rat transcripts.

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were selected that lacked repeats longer than 7 nucleotides. These siRNAs were used in comprehensive searches against the appropriate transcriptomes.

siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand. We then selected duplexes whose antisense oligos lacked GC at the first position, lacked G at both positions 13 and 14, and had 4 or more Us or As in the seed region.

siRNA sequence selection

A total of 47 sense and 47 antisense derived human/rhesus, and 40 sense and 40 antisense derived mouse/rat siRNA oligos were synthesized and formed into duplexes.

Table 10A provides the sequences of the sense and antisense strands of the duplexes targeting the HFE2 gene.

Example 5: TFR2 siRNA Design

Transcripts

siRNA design was carried out to identify siRNAs targeting human, rhesus (*Macaca mulatta*), mouse, and rat TFR2 transcripts annotated in the NCBI Gene database website noted above. Design used the following transcripts from the NCBI RefSeq collection: Human - NM_003227.3, NM_001206855.1; Rhesus - XM_001113151.2; Mouse - NM_015799.3; Rat - NM_001105916.1. Due to high primate/rodent sequence divergenge, siRNA duplexes were designed in three separate batches. The first batch matched human and rhesus; the second matched human, rhesus, and mouse; the last batch matched mouse and rat. All siRNA duplexes were designed that shared 100% identity with all listed human/rhesus, human/rhesus/mouse, or mouse/rat transcripts.

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were selected that lacked repeats longer than 7 nucleotides. These siRNAs were used in comprehensive searches against the appropriate transcriptomes.

siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand. We then selected duplexes whose antisense oligos lacked GC at the first position, lacked G at both positions 13 and 14, and had 3 or more Us or As in the seed region.

siRNA sequence selection

A total of 40 sense and 40 antisense derived human/rhesus, 5 sense and 5 antisense derived human/rhesus/mouse, and 45 sense and 45 antisense derived mouse/rat siRNA oligos were synthesized and formed into duplexes.

Table 10B provides the sequences of the sense and antisense strands of the duplexes targeting the TFR2 gene.

Example 6: HFE2 and TFR2 siRNA Screening

Cell culture and transfections:

Endogenous system (Human): For TFR2, HepG2 cells were used; Hep3b cells were used for HFE2. HepG2 and Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in MEM (Gibco) supplemented with 10% FBS before being released from the plate by trypsinization. Transfection was carried out by adding 14.8µl of Opti-MEM plus 0.2µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 96-well plate and incubated at room temperature for 15 minutes. 80µl of complete growth media without antibiotic containing ~2 x10^4 HepG2 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at final concentrations of 10nM and 0.1nM. An additional concentration of 0.01nM was performed for selected duplexes. Final duplex concentrations for dose response experiments were 10, 1.67, 0.278, 0.046, 0.0077, 0.0012, 0.0002, and 0.000035 nM.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen, part #: 610-12):

Cells were harvested and lysed in 150µl of Lysis/Binding Buffer then mixed for 5 minute at 850rpm using an Eppendorf Thermomixer (the mixing speed was the same throughout the process). Ten microliters of magnetic beads and 80µl Lysis/Binding Buffer mixture were added to a round bottom plate and mixed for 1 minute. Magnetic beads were captured using magnetic stand and the supernatant was removed without disturbing the beads. After removing supernatant, the lysed cells were added to the remaining beads and mixed for 5 minutes. After removing supernatant, magnetic beads were washed 2 times with 150µl Wash Buffer A and mixed for 1 minute. Beads were capture again and supernatant removed. Beads were then washed with 150µl Wash Buffer B, captured and supernatant was removed. Beads were next washed with 150µl Elution Buffer, captured and supernatant removed. Beads were allowed to dry for 2 minutes. After drying, 50µl of Elution Buffer was added and mixed for 5 minutes at 70°C. Beads were captured on magnet for 5 minutes. 40µl of supernatant was removed and added to another 96 well plate.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):

A master mix of 2µl 10X Buffer, 0.8µl 25X dNTPs, 2µl Random primers, 1µl Reverse Transcriptase, 1µl RNase inhibitor and 3.2µl of H2O per reaction were added into 10µl total

RNA. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25°C 10 min, 37°C 120 min, 85°C 5 sec, 4°C hold.

Real time PCR:

2µl of cDNA were added to a master mix containing 0.5µl GAPDH TaqMan Probe (Applied Biosystems Cat #4326317E), 0.5µl HFE2 or TFR2 probes, and 5µl Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well 50 plates (Roche cat # 04887301001). HFE2 and TFR2 probes were Applied Biosystems cat # Hs02378779_s1 and Hs00162690_m1, respectively. Real time PCR was done in an ABI 7900HT Real Time PCR system (Applied Biosystems) using the ΔΔCt(RQ) assay. Each duplex was tested in two independent transfections and each transfection was assayed in duplicate, unless otherwise noted in the summary tables.

To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC50s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with 10 nM AD-1955, mock transfected, or to the average lowest dose.

Table 11 shows the HFE2 and TFR2 single dose screening data of the duplexes using the human endogenous assay.

Table 12 shows the HFE2 and TFR2 dose response data of the duplexes.

Example 7: HFE siRNA Design

Transcripts

siRNA design was carried out to identify siRNAs targeting human, rhesus (*Macaca mulatta*), mouse, and rat HFE transcripts annotated in the NCBI Gene database website noted above. There are at least 9 annotated human HFE transcripts, at least 5 annotated rhesus transcripts, and at least 4 annotated rat transcripts. Accordingly, we focused on the shortest annotated transcripts for human, rhesus, and rat HFE, and designed on sequences held in common by the alternate transcripts. Design used the following transcripts from the NCBI RefSeq collection: Human - NM_139006.2; Rhesus - XM_001085598.2; Mouse - NM_010424.4; Rat - NM_001173435.1. Due to high primate/rodent sequence divergenge, siRNA duplexes were designed in two separate batches. The first batch matched human and rhesus; the second matched mouse and rat. All siRNA duplexes were designed that shared 100% identity with all listed human/rhesus or mouse/rat transcripts.

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were selected that lacked repeats longer than 7 nucleotides. These siRNAs were used in comprehensive searches against the appropriate transcriptomes.

siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand. We then selected duplexes whose antisense oligos lacked GC at the first position, lacked G at both positions 13 and 14, and had 3 or more Us or As in the seed region.

siRNA sequence selection

A total of 46 sense and 46 antisense derived human/rhesus, and 24 sense and 24 antisense derived mouse/rat siRNA oligos are synthesized and formed into duplexes. The duplexes are screened using the methods described above. One or more duplexes are selected for further testing.

Example 8: BMPR1a siRNA Design

Transcripts

siRNA design was carried out to identify siRNAs targeting mouse and rat BMPR1A transcripts annotated in the NCBI Gene database website noted above. Design used the following transcripts from the NCBI RefSeq collection: Mouse - NM_009758.4; Rat - NM_030849.1. All siRNA duplexes were designed that shared 100% identity with all listed mouse/rat transcripts.

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were selected that lacked repeats longer than 7 nucleotides. These siRNAs were used in comprehensive searches against the appropriate transcriptomes.

siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand. We then selected duplexes whose antisense oligos lacked GC at the first position, lacked G at both positions 13 and 14, and had 4 or more Us or As in the seed region.

siRNA sequence selection

A total of 46 sense and 46 antisense derived mouse/rat siRNA oligos are synthesized and formed into duplexes. The duplexes are screened using the methods described above. One or more duplexes are selected for further testing.

Example 9: SMAD4 siRNA Design

Transcripts

siRNA design was carried out to identify siRNAs targeting human and mouse SMAD4 transcripts annotated in the NCBI Gene database website noted above. Design used the following transcripts from the NCBI RefSeq collection: Human - NM_005359.5; Mouse - NM_008540.2. All siRNA duplexes were designed that shared 100% identity with all listed human/mouse transcripts.

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were selected that lacked repeats longer than 7 nucleotides. These siRNAs were used in comprehensive searches against the appropriate transcriptomes.

siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand. We then selected duplexes whose antisense oligos lacked GC at the first position, lacked G at both positions 13 and 14, and had 4 or more Us or As in the seed region.

siRNA sequence selection

Tables 15-16 provide the sequences of the sense and antisense strands of the duplexes targeting SMAD4 mRNA at the indicated locations. Some duplexes were modified as indicated. These siRNA oligos were synthesized and formed into duplexes for further testing as described below.

Example 10: IL6R siRNA Design

Transcripts

siRNA design was carried out to identify siRNAs targeting mouse and rat IL6R transcripts annotated in the NCBI Gene database website noted above. Design used the following transcripts from the NCBI RefSeq collection: Mouse - NM_010559.2; Rat - NM_017020.3. All siRNA duplexes were designed that shared 100% identity with all listed mouse/rat transcripts.

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were selected that lacked repeats longer than 7 nucleotides. These siRNAs were used in comprehensive searches against the appropriate transcriptomes.

siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand. We then selected duplexes whose antisense oligos lacked GC at the first position and had 2 or more Us or As in the seed region.

siRNA sequence selection

A total of 44 sense and 44 antisense derived mouse/rat siRNA oligos are synthesized and formed into duplexes. The duplexes are screened using the methods described above. One or more duplexes are selected for further testing.

Example 11: BMP6 siRNA Design

Transcripts

siRNA design was carried out to identify siRNAs targeting human, rhesus (*Macaca mulatta*), mouse, and rat BMP6 transcripts annotated in the NCBI Gene database website noted above. Design used the following transcripts from the NCBI RefSeq collection: Human - NM_001718.4; Rhesus - XM_001085364.2; Mouse - NM_007556.2; Rat - NM_013107.1. Due to high primate/rodent sequence divergenge, siRNA duplexes were designed in three separate batches. The first batch matched human and rhesus; the second matched human, rhesus, and mouse; the last batch matched mouse and rat. All siRNA duplexes were designed that shared 100% identity with all listed human/rhesus, human/rhesus/mouse, or mouse/rat transcripts.

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were selected that lacked repeats longer than 7 nucleotides. These siRNAs were used in comprehensive searches against the appropriate transcriptomes.

siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand. We then selected duplexes whose antisense oligos lacked GC at the first position, lacked G at both positions 13 and 14, and had 3 or more Us or As in the seed region.

siRNA sequence selection

Table 21 provides the sequences of the sense and antisense strands of the duplexes targeting BMP6 mRNA. Some duplexes were modified as indicated. These siRNA oligos were synthesized and formed into duplexes for further testing using the methods described herein.

Example 12: Neo1 siRNA Design

Transcripts

siRNA design was carried out to identify siRNAs targeting human and mouse NEO1 transcripts annotated in the NCBI Gene database website noted above. There are 2 annotated mouse NEO1 transcripts. Accordingly, we focused on the shortest annotated transcripts for mouse NEO1, and designed on sequences held in common by the alternate transcripts. Design used the following transcripts from the NCBI RefSeq collection: Human - NM_002499.2; Mouse - NM_001042752.1. All siRNA duplexes were designed that shared 100% identity with all listed human/mouse transcripts.

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were selected that lacked repeats longer than 7 nucleotides. These siRNAs were used in comprehensive searches against the appropriate transcriptomes.

siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. We sorted by the specificity of the antisense strand. We then selected duplexes whose antisense oligos lacked GC at the first position, lacked G at both positions 13 and 14, and had 3 or more Us or As in the seed region.

siRNA sequence selection

Tables 17-18 provide the sequences of the sense and antisense strands of the duplexes targeting NEO1 mRNA at the indicated locations. Some duplexes were modified as indicated. These siRNA oligos were synthesized and formed into duplexes for further testing as described below.

Example 13: Activity of Murine siRNA in vivo

The efficacy of one or more siRNAs described above is determined in mice, e.g., normal 10 week old 129s6/svEvTac mice using AD-1955 targeting luciferase as a control. The siRNAs are formulated as described herein and administered, e.g., through i.v. bolus at a dose of, e.g., 10 mg/kg. Forty eight hours after injection, the liver and serum samples are harvested. The liver mRNA levels of the target mRNA are determined by qRT-PCR using gene specific primers and

serum iron levels are determined using Feroxcine (Randox Life Sciences) and Hitachi 717 instrument.

siRNAs that result in lowering of mRNA are selected for further evaluation.

Example 14: Activity of Murine Hepcidin siRNA in vivo

The efficacy of an HAMP siRNA AD-10812 was determined in mice using AF-011 formulated control siRNA and PBS as controls. Each siRNA was formulated with AF-011. AF-011 is also known as LNP11 (*See* Table A above; MC-3/DSPC/Cholesterol/PEG-DMG (50/10/38.5/1.5); Lipid:siRNA 10:1)).

position in mouse access. # NM_032541.1	sense strand sequence (5´-3´)	SEQ ID NO	antisense strand sequence (5´-3´)	SEQ ID NO	duplex name
245-263	uGcuGuAAcAAuucccAGuTsT		ACUGGGAAUUGUuAcAGcATsT		AD-10812

PBS and the siRNAs were administered at various dosages to the mice as shown in Figure 1: 1 mg/kg, 0.3 mg/kg, 0.1 mg/kg, 0.03 mg/kg, 0.01 mg/kg, and 0.003 mg/kg. A single siRNA dose was administered to each mouse. After injection, liver and serum samples were harvested from the mice. The liver Hamp1 mRNA levels were determined by qRT-PCR using Hamp1 specific primers and serum iron levels were determined. Figure 1 shows the HAMP1 mRNA levels in mouse liver following various dosages of siRNA and the serum iron concentration (μg/dL) following various dosages of siRNA.

Administration of AD-10812 HAMP siRNA to mice resulted in lowering of HAMP mRNA by >80% following a single dose. Administration of AD-10812 HAMP siRNA to mice resulted in an approximately 2-fold increase in serum iron following a single dose.

Example 15: Activity of Hepcidin siRNA in nonhuman primates (NHPs) in vivo

The efficacy of an HAMP siRNA AD-11459 was determined in male cynomolgus monkeys using AF-011 siRNA as a control.

Target	Duplex ID	Start Position	Sense Name	Sense Sequence	SEQ ID NO	Antisense Name	Antisense Sequence	SEQ ID NO
HAMP	AD-11459	382	A-18280.2	GAAcAuAGGucuuG GAAuAdTsdT		A-18304.1	uAuUCcAAGACCuAuGuUC dTsdT	

Each siRNA was formulated with AF-011. The siRNAs were administered intravenously at a dose of 1 mg/kg via a 15 minute infusion. A single siRNA dose was administered to each monkey. After injection, liver and serum samples were harvested. Liver samples were taken at 48 hours (h) post-injection. Serum samples were taken at Day -9, Day -6,

Day -3, 24h post-injection, and 48h post-injection. The liver Hamp mRNA levels were determined by qRT-PCR using Hamp specific primers and serum iron levels were determined. Serum HAMP protein levels were also determined. Figure 2 shows the HAMP mRNA levels in liver following siRNA adminstration as well as the serum iron concentration (μg/dL) and the HAMP serum protein concentration (mg/mL) following siRNA administration.

Single administration of LNP-siRNA AD-11459 resulted in rapid reduction of hepcidin mRNA and protein levels and elevation of serum iron levels in NHPs.

Example 16: Silencing of Murine TFR2 via siRNA in vivo

The efficacy of TFR2 siRNA AD-47882 (see table below for sequences) was determined in C57BL6 mice using AF-011 siRNA and PBS as controls. Each siRNA was formulated with AF-011. PBS and the siRNAs were administered at various dosages to the mice as shown in Figure 3: 1 mg/kg, 0.3 mg/kg, 0.1 mg/kg, and 0.03 mg/kg. After injection, liver and serum samples were harvested from the mice. The liver Hamp1 and TFR2 mRNA levels were determined by qRT-PCR using gene specific primers and transferrin saturation were determined at 48 hours post-injection. Figure 3 shows the HAMP1 and TFR2 mRNA levels in mouse liver following various dosages of siRNA and the percent (%) transferrin saturation following various dosages of siRNA.

Target	Duplex ID	Sense Sequence	SEQ ID NO	Antisense Sequence	SEQ ID NO
TFR2	AD-47882	ccAcGuGAuucuccu uucudTsdT		AGAAAGGAGAAUcACG UGGdTsdT	

Administration of AD-47882 siRNA to mice resulted in lowering of HAMP and TFR2 mRNA levels. Administration of AD-47882 siRNA to mice resulted in an increase in transferrin saturation.

Example 17: Silencing of Murine TFR2 via siRNA in vivo

The duration of TFR2 siRNA AD-47882 was determined in C57BL6 mice. The siRNAs were administered at in a single 0.3 mg/kg dose intravenously. Each siRNA was formulated with AF-011. After injection, liver and serum samples were harvested from the mice at various time points shown in Figure 4. The liver Hamp1 and TFR2 mRNA levels were determined by qRT-PCR using gene specific primers and transferrin saturation were determined. Figure 4 shows the HAMP1 and TFR2 mRNA levels in mouse liver following administration of siRNA and the percent (%) transferrin saturation over a 30 day time course.

Administration of AD-47882 siRNA to mice resulted in lowering of HAMP and TFR2 mRNA levels. Administration of AD-47882 siRNA to mice resulted in an increase in transferrin saturation.

Example 18: Silencing of Rat TFR2 via siRNA in vivo

The duration and efficacy of TFR2 siRNA AD-47882 was determined in male Lewis rats using the anemia of chronic disease (ACD) model described in Coccia et al., Exp. Hematology, 2001. Briefly, anemia was initiated in the rats with a single intraperaoneal (i.p.) injection of PG-APS (polymers from Group A Streptococci). The rats were then treated 3x per week with AD-47882 siRNA, AF-011 control siRNA, or saline control starting at day 21 post PG-APS. Each siRNA was formulated with AF-011. Serum and hematology parameters were measured biweekly and at 48 hours post final treatment. Serum samples were harvested from the rats at various time points as shown in Figure 5. Liver mRNA measurement was taken at 48 hours post final treatment. Figure 5 shows the HAMP1 and TFR2 mRNA levels in rat liver following administration of siRNA. Figure 5 also shows the serum iron and Hb concentrations at various time points.

Administration of AD-47882 siRNA resulted in lowering of HAMP and TFR2 mRNA levels. Administration of AD-47882 siRNA resulted in an approximate 2X increase in serum iron upon treatment. Administration of AD-47882 siRNA resulted in an increase in Hb levels to 11-12 g/dL with treatment.

Example 19: TFR2 siRNA Selection and Screening

siRNA sequence selection

Table 13 provides the sequences of the sense and antisense strands of the duplexes targeting the TFR2 gene at the indicated locations (64 or 239). These siRNA oligos were synthesized and formed into duplexes.

Cell culture and transfections:

Endogenous system (Human): For TFR2, HepG2 cells were used. HepG2 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in MEM (Gibco) supplemented with 10% FBS before being released from the plate by trypsinization. Transfection was carried out by adding 14.8µl of Opti-MEM plus 0.2µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 96-well plate and incubated at room temperature for 15 minutes. 80µl of complete growth media without antibiotic containing ~2 x10^4 HepG2 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments

were performed at final concentrations of 10nM and 0.1nM and 0.01 nM. An additional concentration of 0.01nM was performed for selected duplexes.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen, part #: 610-12):

Cells were harvested and lysed in 150µl of Lysis/Binding Buffer then mixed for 5 minute at 850rpm using an Eppendorf Thermomixer (the mixing speed was the same throughout the process). Ten microliters of magnetic beads and 80µl Lysis/Binding Buffer mixture were added to a round bottom plate and mixed for 1 minute. Magnetic beads were captured using magnetic stand and the supernatant was removed without disturbing the beads. After removing supernatant, the lysed cells were added to the remaining beads and mixed for 5 minutes. After removing supernatant, magnetic beads were washed 2 times with 150µl Wash Buffer A and mixed for 1 minute. Beads were capture again and supernatant removed. Beads were then washed with 150µl Wash Buffer B, captured and supernatant was removed. Beads were next washed with 150µl Elution Buffer, captured and supernatant removed. Beads were allowed to dry for 2 minutes. After drying, 50µl of Elution Buffer was added and mixed for 5 minutes at 70°C. Beads were captured on magnet for 5 minutes. 40µl of supernatant was removed and added to another 96 well plate.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):

A master mix of 2µl 10X Buffer, 0.8µl 25X dNTPs, 2µl Random primers, 1µl Reverse Transcriptase, 1µl RNase inhibitor and 3.2µl of H2O per reaction were added into 10µl total RNA. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25°C 10 min, 37°C 120 min, 85°C 5 sec, 4°C hold.

Real time PCR:

2µl of cDNA were added to a master mix containing 0.5µl GAPDH TaqMan Probe (Applied Biosystems Cat #4326317E), 0.5µl TFR2 probes, and 5µl Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well 50 plates (Roche cat # 04887301001). TFR2 probes were Applied Biosystems cat # Hs02378779_s1 and Hs00162690_m1, respectively. Real time PCR was done in an ABI 7900HT Real Time PCR system (Applied Biosystems) using the ΔΔCt(RQ) assay. Each duplex was tested in two independent transfections and each transfection was assayed in duplicate, unless otherwise noted in the summary tables.

To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC50s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with 10 nM AD-1955, mock transfected, or to the average lowest dose.

Table 14 shows the TFR2 dose response data of the duplexes.

Example 20: Activity of TFR2 and HAMP siRNA in non-human primates (NHPs) in vivo

The efficacy of AD-52590, AD-51707, and AD-48141 was determined in separate cynomolgus monkeys (3 each) using PBS as a control. The sequence of AD-52590, AD-51707, and AD-48141 are shown below and in Table 4, 10B, and 13.

Target	Duplex ID	Start Position	SEQ ID NO	Sense Sequence	SEQ ID NO	Antisense Sequence
TFR2	AD-52590	239		cAGGcAGcCAAAcCuCAuUdTsdT		AAUGAGGUuUGGCUGcCugdTsdT
TFR2	AD-51707	1051		ccuucAAucAAAcccAGuudTsdT		AACuGGGUuUGAuUGAAGGdTsdT
HAMP	AD-48141	382		GAAcAuAGGucuuGGAAuAdTdT		UAuUCcAAGACCuAuGuUCdTdT

Each siRNA was formulated with AF-011. The siRNAs were administered intravenously as indicated (0.1mg/kg, 0.03 mg/kg, or 1 mg/kg) via a 15 minute infusion. A single siRNA dose was administered to each monkey. After injection, liver and serum samples were harvested. Liver biopsy samples were taken at 48 hours (h) post-injection. Serum samples were taken at Day -9, Day -6, Day -3, 24h post-injection, and 48h post-injection. The liver Hamp mRNA levels were determined by qRT-PCR using Hamp specific primers. The liver TFR2 mRNA levels were determined by qRT-PCR using TFR2 specific primers. Serum iron levels were determined and are shown in μg/dL. Serum HAMP protein levels were also determined and are shown in ng/mL.

Figure 6 shows HAMP mRNA levels in the liver of each animal following siRNA administration, relative to PBS controls. Figure 7 shows TFR2 mRNA levels in the liver of each animal following siRNA administration, relative to PBS controls. Figure 8 shows that serum iron concentration was increased in each animal after 1 mg/kg AD-52590 siRNA administration. Figure 9 shows that the HAMP serum protein concentration was decreased in each animal following 1 mg/kg AD-52590 siRNA administration.

Single administration of AD-52590 resulted in rapid reduction of hepcidin mRNA and protein levels, TFR2 mRNA levels, and elevation of serum iron levels in NHPs.

Example 21: NEO1 and SMAD4 Duplex Screening

Human/mouse cross-reactive Neo1 and Smad4 siRNAs were screened in primary mouse hepatocytes. Duplexes are shown in Tables 15, 16, 17, and 18.

Cell culture and transfections:

Freshly isolated primary mouse hepatocytes (PMH) were transfected by adding 14.8µl of Opti-MEM plus 0.2µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 96-well plate and incubated at room temperature for 15 minutes. 80µl of primary hepatocyte media containing ~2 x10^4 PMH cells were then added to the siRNA mixture. Cells were incubated for either 24 prior to RNA purification. Single dose experiments were performed at 10nM and 0.1nM final duplex concentration.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen, part #: 610-12):

Cells were harvested and lysed in 150µl of Lysis/Binding Buffer then mixed for 5 minute at 850rpm using an Eppendorf Thermomixer (the mixing speed was the same throughout the process). Ten microliters of magnetic beads and 80µl Lysis/Binding Buffer mixture were added to a round bottom plate and mixed for 1 minute. Magnetic beads were captured using magnetic stand and the supernatant was removed without disturbing the beads. After removing supernatant, the lysed cells were added to the remaining beads and mixed for 5 minutes. After removing supernatant, magnetic beads were washed 2 times with 150µl Wash Buffer A and mixed for 1 minute. Beads were capture again and supernatant removed. Beads were then washed with 150µl Wash Buffer B, captured and supernatant was removed. Beads were next washed with 150µl Elution Buffer, captured and supernatant removed. Beads were allowed to dry for 2 minutes. After drying, 50µl of Elution Buffer was added and mixed for 5 minutes at 70°C. Beads were captured on magnet for 5 minutes. 40µl of supernatant was removed and added to another 96 well plate.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813):

A master mix of 2µl 10X Buffer, 0.8µl 25X dNTPs, 2µl Random primers, 1µl Reverse Transcriptase, 1µl RNase inhibitor and 3.2µl of H2O per reaction were added into 10µl total RNA. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25°C 10 min, 37°C 120 min, 85°C 5 sec, 4°C hold.

Real time PCR:

2µl of cDNA were added to a master mix containing 0.5µl of mouse GAPDH TaqMan Probe (Applied Biosystems Cat #4352932E), 0.5µl Neo1 or SMAD4 TaqMan probe (Applied Biosystems cat # Neo1-Mm00476326_m1 or SMAD4 Mm03023996_m1) and 5µl Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well 50 plates (Roche cat # 04887301001). Real time PCR was done in an ABI 7900HT Real Time PCR system (Applied Biosystems) using the ΔΔCt(RQ) assay. Each duplex was tested in two independent transfections and each transfection was assayed in duplicate, unless otherwise noted.

To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC50s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or naïve cells over the same dose range, or to its own lowest dose.

Table 19 shows the percent remaining mRNA remaining for each SMAD4 duplex tested at 0.1nM and 10nM. Controls were 10 nM AD-1955, mock transfected. Table 20 shows the percent remaining mRNA remaining for each NEO1 duplex tested at 0.1nM and 10nM. Controls were 10 nM AD-1955, mock transfected.

Example 22: *In vivo* combinatorial use of dsRNAs targeting HAMP-related mRNAs

The efficacy of TFR2 siRNA AD-47882 and HFE siRNA AD- 47320 (*see* table below for sequences) alone and in combination was determined in C57BL6 female mice using AF-011-Luc siRNA and PBS as controls. Each siRNA was formulated with AF-011. PBS and the siRNAs were administered at various (mg/kg) dosages to the mice as shown on the X-axis of each subfigure (A-D) of Figure 10. 48 hours after injection, liver and serum samples were harvested from the mice.

Target	Duplex ID	Accession Number	Sense Sequence	SEQ ID NO	Antisense Sequence	SEQ ID NO
HFE	AD-47320	NM_010424.4	uuuucuccAGuuAAGuucAdTsdT		UGAACUuAACUGGAGAAAAdTsdT	
HFE	Unmod AD-47320	NM_010424.4	UUUUCUCCAGUUAAGUUCA		UGAACUUAACUGGAGAAAA	

The liver Hamp1, HFE, and TFR2 mRNA levels were determined by qRT-PCR using gene specific primers. Blood was processed into serum to measure serum iron, transferrin saturation, and UIBC. Figure 10A shows the HAMP1, HFE, and TFR2 mRNA levels in mouse liver following various dosages of each siRNA group or PBS. Figures 10B-D shows serum iron concentration, transferrin saturation, and UIBC concentration in the serum of each group tested.

Example 23: Inhibition of HAMP in humans

A human subject is treated with a siRNA targeted to a HAMP gene to inhibit expression of the HAMP gene to treat a condition. In some instances, one or more additional siRNAs are co-administered, e.g., an siRNA targeted to a HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene.

A subject in need of treatment is selected or identified.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an siRNA is administered to the subject. The siRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated. This measurement can be accompanied by a measurement of target gene expression in said subject, and/or the products of the successful siRNA-targeting of mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

Example 24: Inhibition of HFE2 in humans

A human subject is treated with a siRNA targeted to a gene to inhibit expression of the HFE2 gene to treat a condition. In some instances, one or more additional siRNAs are co-administered, e.g., an siRNA targeted to a HAMP, HFE, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene.

A subject in need of treatment is selected or identified.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an siRNA is administered to the subject. The siRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated. This measurement can be accompanied by a measurement of target gene expression in said subject, and/or the products of the successful siRNA-targeting of mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

Example 25: Inhibition of HFE in humans

A human subject is treated with a siRNA targeted to a gene to inhibit expression of the HFE gene to treat a condition. In some instances, one or more additional siRNAs are co-administered, e.g., an siRNA targeted to a HAMP, HFE2, TFR2, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene.

A subject in need of treatment is selected or identified.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an siRNA is administered to the subject. The siRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated. This measurement can be accompanied by a measurement of target gene expression in said subject, and/or the products of the successful siRNA-targeting of mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

Example 26: Inhibition of TFR2 in humans

A human subject is treated with a siRNA targeted to a gene to inhibit expression of the TFR2 gene to treat a condition. In some instances, one or more additional siRNAs are co-administered, e.g., an siRNA targeted to a HAMP, HFE2, HFE, BMPR1a, SMAD4, IL6R, BMP6, and/or NEO1 gene.

A subject in need of treatment is selected or identified.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an siRNA is administered to the subject. The siRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated. This measurement can be accompanied by a measurement of target gene expression in said subject, and/or the products of the successful siRNA-targeting of mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

Example 27: Inhibition of BMPR1a in humans

A human subject is treated with a siRNA targeted to a gene to inhibit expression of the BMPR1a gene to treat a condition. In some instances, one or more additional siRNAs are co-administered, e.g., an siRNA targeted to a HAMP, HFE2, HFE, TFR2, SMAD4, IL6R, BMP6, and/or NEO1 gene.

A subject in need of treatment is selected or identified.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an siRNA is administered to the subject. The siRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated. This measurement can be accompanied by a measurement of target gene expression in said subject, and/or the products of the successful siRNA-targeting of mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

Example 28: Inhibition of SMAD4 in humans

A human subject is treated with a siRNA targeted to a gene to inhibit expression of the SMAD4 gene to treat a condition. In some instances, one or more additional siRNAs are co-administered, e.g., an siRNA targeted to a HAMP, HFE2, HFE, TFR2, BMPR1a, IL6R, BMP6, and/or NEO1 gene.

A subject in need of treatment is selected or identified.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an siRNA is administered to the subject. The siRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated. This measurement can be accompanied by a measurement of target gene expression in said subject, and/or the products of the successful siRNA-targeting of mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

Example 29: Inhibition of IL6R in humans

A human subject is treated with a siRNA targeted to a gene to inhibit expression of the IL6R gene to treat a condition. In some instances, one or more additional siRNAs are co-administered, e.g., an siRNA targeted to a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, BMP6, and/or NEO1 gene.

A subject in need of treatment is selected or identified.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an siRNA is administered to the subject. The siRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated. This measurement can be accompanied by a measurement of target gene expression in said subject, and/or the products of the successful

siRNA-targeting of mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

Example 30: Inhibition of BMP6 in humans

A human subject is treated with a siRNA targeted to a gene to inhibit expression of the BMP6 gene to treat a condition. In some instances, one or more additional siRNAs are co-administered, e.g., an siRNA targeted to a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, and/or NEO1 gene.

A subject in need of treatment is selected or identified.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an siRNA is administered to the subject. The siRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated. This measurement can be accompanied by a measurement of target gene expression in said subject, and/or the products of the successful siRNA-targeting of mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

Example 31: Inhibition of NEO1 in humans

A human subject is treated with a siRNA targeted to a gene to inhibit expression of the NEO1 gene to treat a condition. In some instances, one or more additional siRNAs are co-administered, e.g., an siRNA targeted to a HAMP, HFE2, HFE, TFR2, BMPR1a, SMAD4, IL6R, and/or BMP6 gene.

A subject in need of treatment is selected or identified.

The identification of the subject can occur in a clinical setting, or elsewhere, e.g., in the subject's home through the subject's own use of a self-testing kit.

At time zero, a suitable first dose of an siRNA is administered to the subject. The siRNA is formulated as described herein. After a period of time following the first dose, e.g., 7 days, 14 days, and 21 days, the subject's condition is evaluated. This measurement can be accompanied by a measurement of target gene expression in said subject, and/or the products of the successful siRNA-targeting of mRNA. Other relevant criteria can also be measured. The number and strength of doses are adjusted according to the subject's needs.

Tables

TABLE B

SEQ ID NO	DESCRIPTION	SEQUENCE
1	Human HAMP - NM_021175.2	gactgtcactcggtcccagacaccagagcaagctcaagacccagcagtgggacagcc agacagacggcacgatggcactgagctcccagatctgggccgcttgcctcctgctcc tcctcctcctcgccagcctgaccagtggctctgttttcccacaacagacgggacaac ttgcagagctgcaaccccaggacagagctggagccagggccagctggatgcccatgt tccagaggcgaaggaggcgagacacccacttcccccatctgcatttttctgctgcggct gctgtcatcgatcaaagtgtgggatgtgctgcaagacgtagaacctacctgccctgc ccccgtcccctccttccttatttattcctgctgccccagaacataggtcttggaat aaaatggctggttcttttgttttccaaaaaa
2	Cyno HAMP - EU076443.1	tcaagacctagcagtgggacagccagacagacggcacgatggcactgagctcccaga tctgggccacttgcctcctcctccttctcctcctcgccagcctgaccagtggctccg ttttcccacaacagacgggacaacttgcagagctgcaacctcaggacagagctggag ccagggccagctggacgcccatgctccagaggcgaaggaggcgagacacccacttcc ccatctgcattttctgctgcggctgctgtcatcgatcaaagtgtgggatgtgctgca ggacgtagaaccttcctgccctgcccccatcccctccttccttatttattcctgct gccccagaacacaggtcttggaataaaacggctgattcttttgttttcc
3	Mouse HAMP - NM_032541.1	agtccttagactgcacagcagaacagaaggcatgatggcactcagcactcggaccca ggctgcctgtctcctgcttctcctccttgccagcctgagcagcaccacctatctcca tcaacagatgagacagactacagagctgcagcctttgcacggggaagaaagcagggc agacattgcgataccaatgcagaagagaaggaagagagacaccaacttccccatctg catcttctgctgtaaatgctgtaacaattcccagtgtggtatctgttgcaaaacata gcctagagccacatcctgacctctctacacccctgcagcccctcaaccccattattt attcctgcctccccaccaatgaccttgaaataaagacgattttattttcaaaaaaa aaaaaaaaaa
4	Rat HAMP - NM_053469.1	cacgagggcaggacagaaggcaagatggcactaagcactcggatccaggctgcctgt ctcctgcttctcctcctggccagcctgagcagcggtgcctatctccggcaacagacg agacagactacggctctgcagccttggcatggggcagaaagcaagactgatgacagt gcgctgctgatgctgaagcgaaggaagcgagacaccaacttccccatatgcctcttc tgctgtaaatgctgtaagaattcctcctgtggtctctgttgcataacatagagagcc aagagccttgtcctgacctctcaacacactgcctcccctccgccccattatttattc ctgtcctaccccagcaatgaccttg
5	Human HFE2 - NM_213652.3	accgtcaactcagtagccacctccctcctgctcagctgtccagtactctggccagc catatactccccttcccccataccaaaccttctctggttccctgacctcagtgag acagcagccggcctggggacctgggggagacacggaggacccccctggctggagctga cccacagagtagggaatcatggctggagaattggatagcagagtaatgtttgacctc tggaaacactcaccatcatatttaagaacatgcaggaatgcattgatcagaaggtgt atcaggctgaggtggataatcttcctgtagcctttgaagatggttctatcaatggag gtgaccgacctgggggatccagtttgtcgattcaaactgctaaccctgggaaccatg tggagatccaagctgcctacattggcacaactataatcattcggcagacagctgggc agctctccttctccatcaaggtagcagaggatgtggccatggccttctcagctgaac aggacctgcagctctgtgttgggggtgccctccaagtcagcgactctctcgatcag agcgcaatcgtcggggagctataaccattgatactgccagacggctgtgcaaggaag ggcttccagtggaagatgcttacttccattcctgtgtctttgatgttttaatttctg gtgatcccaactttaccgtggcagctcaggcagcactggaggatgcccgagccttcc tgccagacttagagaagctgcatctcttcccctcagatgctggggttcctctttcct cagcaaccctcttagctccactcctttctgggctctttgttctgtggctttgcattc agtaaggggaccatcagtcccattactagtttggaaatgatttggagatacagattg gcatagaagaatgtaaagaatcattaaaggaagcagggcctaggagacacgtgaaac aatgacattatccagagtcagatgaggctgcagtccagggttgaaattatcacagaa taaggattctgggcaaggttactgcattccggatctctgtggggctcttcaccaatt tttccagcctcatttatagtaaacaaattgttctaatccatttactgcagatttcac ccttataagtttagaggtcatgaaggttttaatgatcagtaaagatttaagggttga gatttttaagaggcaagagctgaaagcagaagacatgatcattagccataagaaact caaaggaggaagacataattagggaaagaagtctatttgatgaatatgtgtgtgtaa ggtatgttctgctttcttgattcaaaaatgaagcaggcattgtctagctcttaggtg aagggagtctctgcttttgaagaatggcacaggtaggacagaagtatcatcctacc ccctaactaatctgttattaaagctacaaattcttcacaccatcaaaaaaaaaaaaa aaaaaa
6	Rhesus HFE2 -	cttctctggctccctgacctcagtgagacagcagccggcctggggacctgggggaga catggagaaagagacggaggacccccctggctggagctgacccacagagtagggaatc

	XM_001092987.1	atggctggagaattggatagcagagtaatgtttgacctctggaaacaccaaatttct tttttcagtcacttacagggcttccggtcaaaattcactaggtaggagggtcatcag ctgggaagaaccggcgcctggggaacctggctggataggtatgggggagcaaggcca gtcccctagtcccaggtcctcccatggcagtcccccaactctaagcactctcactct cctgctgctcctctgtggacatgctcattctcaatgcaagatcctccgctgcaatgc tgagtatgtatcgtccactctgagccttagaggtggcggttcatcaggagcacttcg aggaggaggaggaggaggaggccggggtggaggggtgggctctggcggcctctgtcg agccctccgctcctatgcgctctgcactcggcgcaccgcccgcacctgccgtgggga cctcgccttccattcggcggtacatggcatcgaagacctgatgatccagcacaactg ctcgcgccagggccctacagcccctcccccgccccggggcccccgccttccaggcgc aggctccggcctccctgcccccggaccttgtgactatgaaggccggttttcccggct gcatggtcgtcccccggggttcttgcattgcgcttccttcggggaccccccatgtgcg cagcttccaccaccattttcacacatgccgtgtccaaggagcttggcctctactgga taacgacttcctctttgtccaagccaccagctcccccatggcgttgggggccaacgc taccgctacccggaagctcaccatcatatttaagaacatgcaggaatgcattgatca gaaggtctatcaggctgaggtggataatcttcctgcagcctttgaagatggttctgt caatggaggtgaccgacctgggggatccagtttgtcgattcaaactgctaaccctgg gaaccacgtggagatccaagctgcctacattggcacaactataatcattcggcagac agctgggcagctctccttctccatcaaggtagcagaggatgtggccatggccttctc agctgaacaggacctgcagctctgtgttggggggtgccctccaagtcagcgactctc tcgatcagagcgcagtcgtccgggagctataaccattgatactgccagacggctgtg taaggaagggcttccagtggaagatgcttacttccattcctgtgtctttgatgtttt aatttctggtgatcccaactttactgtggcagctcaggcagcactggaggatgcccg agccttcctgccagacttagataagctgcatctcttcccttcagatgctggggtttc tctttcctcagcaaccttcctagccccactcctttctgggctctttgttctgtggct ttgcattcagtaaggaagccatcagtcctattactagtttggaaatgatttggggat agagattggcatagaagaatgtaaacaatcattaaaggaagcagggcccagaagaca catgaaacaatgacatcatccagagtcagatgaggctgcagtccagggttgaaatga tcacagaataaggattctgggcaaggtttctgcattccagacctcttcgccaaattt tccagccccatttacagtaaacaaattgttctttccatttactgcagatttcaccct ataagcttagaggtcatgaaggttttaacaatcagtaaagacttaagggttgagatt tttaagaggcaagagctgaaagcagaagacatgatcattagccataagaaactcaaa ggaagaagaaataattagggaaagaagtctatttgatgaatatgtgtgtgtaaggta tgttctgctttcttggttcaaaaatgaagcgggcgttgtctagctcttaggtgaagg gagtctctgctttggaagaacggcacaggtaggacagaagtatcatccctaccccta actgatctgttattaaagctacaaattcttcacaccgtc
7	Mouse HFE2 - NM_027126.4	ggctctctgacctgagtgagactgcagccattccggggcaatcatggagaaagagat gggggacccccctggctggagcagaccaacagaataggcaactatggctcgagaaccc agtatcagagtaatgcttgacctcgggaaacatcacagaagtacccagagaaattca ctaggtaggaggctcatcatctgggaagaaccggtgcctgggggacctggctggat aggtatgggccagtcccctagtccccggtcccccccacggcagccctccaactctaag caccctcactctcctgctgctcctctgtggacaggctcactcccagtgcaagatcct ccgctgcaatgccgagtatgtctcgtccactctgagtcttcggggaggtggctcacc ggacacgccgcgtggaggcggccgtggtgggctggcctcaggtggcttgtgtcgcgc cctgcgctcctacgctctctgcacgcggcgcacggcccgcacctgccgcggggacct tgctttccactctgcggtgcatggcatagaggacctgatgatccagcacaaactgctc acgccagggtcccacggccccgccccccggcccggggccccgccctgccccgggccgg gccagcgcccctgaccccagatccctgtgactatgaggcccggttttccaggctgca cggtcgagccccgggcttcttgcattgcgcatcctttggagatccccatgtgcgcag tttccacaaccaatttcacacatgccgtgtccaaggagcttggcccttgctagataa cgacttcctctttgtccaggccaccagctccccggtttcgtcgggagccaacgctac caccatccggaagatcactatcatatttaaaaacatgcaggaatgcattgaccagaa agtctaccaggctgaggtggacaatcttcctgcagcctttgaagatggttctatcaa tgggggcgaccgacctgggggctcgagtttgtccattcaaactgctaaccttgggag tcacgtggagattcgagctgcctacattggaacaactatcatcattcgacagacagc tgggcagctctccttctccatcagggtagcagaggatgtggcgcgggccttctccgc agagcaggacctacagctgtgtgttgggggatgccctccgagccagcgactctctcg ctcagagcgcaaccgccgtggggctatagccatagatactgccagaaggctgtgtaa ggaagggcttccggttgaagatgcctacttccaatcctgcgtctttgatgtttcagt ctccggtgaccccaactttactgtggcagctcagacagctctggacgatgcccgaat cttcttgacggatttagagaacttacatctctttccctcagatgcggggcctccct ctctcctgccatctgcctagtcccgcttctttcggccctctttgttctgtggctttg cttcagtaagtaggccagcaacccatgactggtttggaaacgatttgaggatagagg ttggtgtgagaaaccacaaagatgtgccaaaggaaacagcggggacaggagacaaca cttactcaatcagatgaggttgcagtccagggctgaaatgaccctagaataaagatt ctgggccagggttttgcactccagaccttggtgtgggctattcaccatggatttccc agttagtgatttcccacttgtaatgaaattccactctccatacacctgataccactc ctacaagcctagagattgtgagagtgctaatgaccagtgaaacattaaaggactgag atatcgtaaaggcaaaaacatgattctctttgagaaagtcaaaagaggagaagctaa

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8	Rat HFE2 - NM_001012080.1	cggggacagacatggagaaggagatggaggacccccctggctggagcagaccaacaga ataggcaactatggctggagaaccgggtatcagagtaatgcttgacctcgggaaaca ccaaatttcttcttccgatcgcagaagtagtactcggcgaaattcactaggtaggag gctcctcatctgggaagaaccggtgcctgggggacctggctggataggtatggggg atcgaggccggtcccctagtctccggtcccccccatggcagtcctccaactctaagca ccctcactctcctgctgctcctctgtggacaggctcactcccagtgcaagatcctcc gctgcaatgccgagtacgtctcgtccactctgagccttcggggaggggctcaccgg acacgccacatggaggcggccgtggtgggccggcctcaggtggcttgtgtcgcgccc tgcgctcctacgctctctgcacgcggcgcaccgcccgcacctgccgcggggacctcg ctttccactccgcggtgcatggcatagaggacctgatgatccagcacaactgctcac gccagggtcccacggcctcgcccccggcccgggtcctgccctgcccggggccggcc cagcgcccctgaccccagatccctgtgactatgaagcccggttttccaggctgcacg gtcgaaccccgggtttcttgcattgtgcttcctttggagacccccatgtgcgcagct tccacaatcactttcacacatgccgcgtccaaggagcttggcccctactagataacg acttcctctttgtccaagccaccagctccccggtagcatcgggagccaacgctacca ccatccggaagatcactatcatatttaaaaacatgcaggaatgcattgaccagaaag tctaccaggctgaggtagacaatcttcctgcagcctttgaagatggttctgtcaatg ggggcgaccgacctgggggctcgagtttgtccattcaaactgctaaccttgggagcc acgtggagattcgagctgcctacattggaacaactataatcgttcgtcagacagctg gacagctctccttctccatcagggtagcggaggatgtggcacgggccttctctgctg agcaggatctacagctgtgtgttgggggatgccctccgagccagcgactctctcgct cagagcgcaatcgccgtggggcgatagccatagatactgccagaaggttgtgtaagg aagggcttccggttgaagatgcctacttccaatcctgcgtctttgatgtttcagtct ccggtgaccccaactttactgtggcagctcagtcagctctggacgatgcccgagtct tcttgaccgatttggagaacttgcacctttttcccagtagatgcggggcctcccctct ctccagccacctgcctagtccggcttctttcggtcctctttgttctgtggttttgca ttcagtaagtaggccagcaacccgtgactagtttggaaacggtttgaggagagaggt tgatgtgagaaaacacaaagatgtgccaaaggaaacagtggggacaggagacaacga ccttactcaatcacacgaggttgcagtccagggctgaaatgaccctagaataaagat tctgagacagggttttgcactccagaccttggtatgggctccccatgaatttccccca ttagtgatttcccacttgtagtgaaattctactctctgtacacctgatatcactcct gcaaggctagagattgtgagagcgctaagggccagcaaaacattaaagggctgagat atcttaaaggcagaaactagaaaaggggaaaccatgattatctataagaaaatcaaa agaggggtttgggaatttagctcagtggtagagcacttgcctagcaagcgcaaggcc ctgggttcggtccccagctcctaaaaaagaaaaaaaaaatcaaaagagaaaaaacta attaaggcaagctttttggttcagaaatgaagtgggcattgtctggcagaggaagtc agcttttggagactggcaccaacatctccacccttcctactctgttattaaagtgac gaattccccaaaaaaaaaaaaaaaaaaaaaaaaaaaaaagg
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		gcggaaacagctgcccacccccccccatcccagcagctcagctaagccctgatgagaa tgaagccacaggagttgtctgaggtgaacccagccgctcagccacacatggaagcca ttgcctttgcacatagttcttgggttctttttcctaaaaaggtaaggagctgaggtg tgtggtttttttaatattaagaatatataatggaaaacacacgactgacgctcaggca tcttcccctactccccaacagatccccagaagacagcgtggaaggcagtgtagacag taaatcgggcttcagttctatagccaagaagagatcagctgctgaaaccaccagtgg gtaccccaggccacctgcctttgaacttggggatttgccatgtttgatcttgtcaca tacttgcttttttacaagatgaactctttgtatttatgatttgggggggcaatgaaag gtgcaatgcaggaactgctgctgccgagctcgctggtcacatgggggtgccaggcgg gattctggaaaaccagtgcacttaaactgatcctgaagagagctgtcccagcactct ggccaccaggagggccagattccccagaaactacctttgcccaaagaacatgctca gtatttggggcatttcctcccacaaaccctgactgcttctgttacctcagggccttg gtacctggatactgccacagaattggggcgggtgggggaggggcctatttttaaata aaataactgttcaaagttgggggttttttaaaaaattaagaaaaaggaaagctattc tgtattgcacctttcacaatttaatacattttcttacattttcctgtgattttcga aactaaaccattgtgtgtcctgtagtgtcctggttgagctgccgctcagcagcttcc tcgggggatttggaacacctgtgtctgtcgccgcactgcctgtgggaggggcccag agggctgctgggactggcgtctgtacacacttgtttggccttttctgtagttgatgc tgtaaactctatggctttttaaaaacgatttcatgtttttatttagtattggaaatc caatacacttttttaatccaatcaaaaaaaaaaaaaaaaaaaaaa
29	Mouse NEO1 - NM_001042752.1	gccccccctcgctctaccgtgaggagcccgagtcggcggcgggtggcggcgcctggaa cctggagagaccgagccacccccccggctctcggccggaatgtactgattctcctctg ctctcctccccgccccgctgcaggagggaggcgcccggagtctttcccccctgggcgc gcgagggggccgcgcgggccgggccgggccgggctggagccgagccctgcggcgcag agaccggctgaggcgcgctgagggaagggcgcgagcgctccgcggcgctatcgccgc cgccgccgccgccactcgtggggtagagatggcggcggagcgcgaagccgggcgact cctctgcacctcctcctccccggcgctgctgtccgccaccgccgctgctgctgttgct gccgctgctgctgctgctcggacgcccggcgtccggcgccgcggccacgaagagcgg ctccccgccgcagtccgcaggagccagtgttcgaacattcactccgttttatttttct ggtggagccagtagacaccctctcagttagaggctcttctgttatattaaattgctc ggcatattctgagccctctccaaacattgaatggaagaaagatgggacttttttaaa cttagaatcagatgatcgacgccagctactcccagatggatctttattcatcagcaa cgtggtgcattccaaacacaataagcctgacgaaggtttctatcagtgtgtagccac tgtggataatcttggaaccattgtcagcagaacagccaagctcacagtagcaggtct tccaagatttaccagccaaccagaaccttcttcagtctatgttggaaacagtgcaat tctgaattgtgaagttaatgcagatttggtcccatttgttaggtgggaacagaatcg acagccccttcttctagatgacaggattgtcaaacttccaagtggaacactggttat cagcaatgctactgaaggagatgggggactctaccgctgcattgttgaaagtggtgg gccaccaaagtttagtgacgaagctgaattgaaagttcttcaagatcctgaggaaat tgtagacttggtatttctgatgcgaccatcttctatgatgaaagtcactggtcagag tgcagtgttgccatgtgttgtctcagggcttcctgctccagttgttagatggatgaa aaacgaagaagtgcttgacacagaaagctctggcaggttggtcttgctagcaggagg ttgcttggagatcagtgatgtcactgaggatgatgctgggacttatttttgcatagc tgataatggaaataagacagttgaagctcaggcggagcttactgtgcaagtgccacc tggattcctgaaacaacctgctaacatatatgctcacgaatccatggacattgtatt tgaatgtgaagtcactgggaagccaactccaactgtgaagtgggtcaagaatgggga tgtggttatccccagtgattactttaaaattgtaaaggaacataatcttcaagtttt gggtctggtgaaatcagatgaagggttctatcaatgcattgctgagaatgatgttgg aaatgcacaagctggagcccagctgataatccttgagcatgatgttgccatcccaac attacctcccacttcactgaccagtgccactactgaccatctagcaccagccacaac gggaccattaccttcagctcctcgagacgtcgtggcctccctggtctctactcgctt cattaaattgacatggcgtacacctgcatcagaccctcatggagacaatctcaccta ctctgtgttctacaccaaggaaggggttgctagggagcgtgttgagaataccagcca gccaggagagatgcaggtgactattcaaaacttgatgccagcaactgtgtacatctt caaagttatggctcaaaataagcatggctctggagaaagttcagctcctcttcgagt agagacacagcctgaggttcagctccctggcccagcacctaatatccgtgcttatgc aacgtcacctacttctatcactgtcacctgggaaacaccgttatctggcaatgggga aattcaaaattacaaattgtactacatggaaaaaggaactgataaagaacaggatat tgatgtttcaagtcactcctacaccattaatggactgaagaaatacacagaatacag tttccgagtggtggcctacaataaacatggtcctggagtttctacacaagatgttgc tgttcgaacattatcagatgttcccagtgctgctcctcagaatctgtccttagaagt gagaaattcaaagagtatagtgatccactggcagccccttcctcaaccacacaaaa tgggcagataactggctacaagattcgatatcgaaaggcctcccgaaaaagtgatgt cactgagaccttggtaactgggacacagctgtctcagctgattgaaggtcttgatcg ggggacagaatataacttccgagtcgctgctctcacagtcaatggtacaggtccagc aactgattggctgtctgctgaaacttttgaaagcgacctagatgaaactcgtgttcc tgaagtgcccagctctcttcatgtccgtccgctcgtcactagcattgtagtgagctg gactcctccagagaaccagaacattgtggtccgaggttatgccatcggttacggcat tggcagccctcatgcccagaccatcaaagtggactataaacaacgttattacaccat

		cgaaaacttggatccaagctctcattacgtgattaccttgaaagcatttaacaatgt tggcgaaggcatccccctttatgagagtgctgtgaccagacctcacacagtgccaga tcccactcccatgatgccaccagtgggagttcaggcttccattctgagtcacgacac cataaggattacctgggcagacaactccctgcccaaacaccagaagattacagactc ccgctactacacagtccggtggaagaccaacatcccagcaaacacgaagtacaagaa tgcaaatgcaacgacgttaagctatttggttactggtttaaagccaaatacgctcta tgagttctctgtgatggtgaccaaaggcagaaggtcaagcacgtggagtatgacagc tcatggcgctacctttgaattagttcctacttctccacctaaggatgtgacagttgt gagtaaggaaggaaaacctagaaccatcatagtgaattggcagcctccctctgaagc taacggcaagattacaggttacatcatctattacagcacggatgtgaatgcagagat acatgactgggttattgaaccagttgtgggaaacagactgactcaccagattcaaga gttaacacttgatacgccatactacttcaaaatccaggcccggaactcaaagggcat ggggcccatgtctgaagctgtacagttcagaacacctaaagccttagggtcagcagg aaaaggaagccgactaccagacctgggatctgactacaaacctccaatgagtggcag caacagcccctcacgggagccccacctcccctctggacagcaacatgctgctggtcat cattgtctctgttggcgtcatcactatcgtggtggttgtggtcattgctgtcttttg tacccggcgcaccacctctcaccagaagaagaaacgagctgcgtgcaaatcagtgaa tggctcccataagtacaagggcaattgcaaagatgtgaagcctccagacctatggat ccatcacgagagactagagttgaagcctattgacaagtctccagatcctaaccctgt catgactgatactccaatccctcgaaactctcaagatatcacaccagtggacaattc catggatagcaatatccatcaaaggcggaattcatacagagggcatgagtcagagga cagcatgtctacactggctggaaggaggggaatgagaccaaaaatgatgatgcccct tgactctcagccacctcagcctgtgattagtgcccatcccatccattccctcgataa ccctcaccatcatttccactccagcagcctcgcttctccagcccgcagtcatctcta ccacccaagcagcccatggcccattggcacatccatgtccctttcagacagggccaa ttccacagaatctgttcgaaataccccccagcacggacaccatgccagcgtcctcgtc tcagacgtgctgcactgaccatcaggaccctgagggtgctactagctcctcttactt ggccagctcccaagaggaagactcaggccagagtcttcccacagcccatgtccgccc ttcccaccctctgaagagcttcgctgtgccagcaatcccaccccccaggacctcctct ctatgatcctgcactgccaagcacaccattactgtcccagcaagctctgaaccatca cattcactcagtgaaaacagcctccatcgggacgttaggaaggagccggcctcctat gccagtggttgttccgagtgcccctgaagtacaggagaccaccaggatgctggaaga ctccgagagtagctatgaaccagatgagctgaccaaagagatggcccacctggaagg actaatgaaggacctaaatgccatcacaacagcctgatgaccttcgcctggacatga ctccaagcctgagtctacaagtctcggaacttaaccttgaaaacaaggaattgtaca gagtacgagaggacagcacttgagagcaggagccagcaaaccagccagtgcctccat gtggggttggctccaggcacagccacctgccttctcctggtcagcctggattacact tgtgtggaggcagcttcccttgcctgctgagagcctgcaggactgggcactatggg ccaaaattttgtgtccagggaagaggcaagaagtacgacctgccttttgctttgtgg tcagtggcttgtgtctttgtgctgcaactgcatcacttttatggagtgtagacattg gcatttatgtacaattttgtgtcctatttttatttaccttaaaacactatcagaagc caagggagtctgtgatgttctctcaagcagttgacacttgactgtggttccagttac ttacggaaagtcatcaacagtgaggttgtttgacaccactgacaggcattggcttgt tgtgggtttcatttttattcttaattctgagacattgcatcctctgccagctgttaa tccatcactttgaggggaggacatgttgcattgctgtttgtaagcttttttattatt tttttattataattattaaaggcctgactttctcctctcatcactgtgagattacag atctatttgaatgaaatgtaacattgaaaagacttgtttgttgctttctgtgcagat tcagtattggggtgggattggggattgggaataggaaatggaggggctgctgaggcc ctgtgaatgtttctgtcaatgtactttgttccagaagcctgccgagaatgaaagcag catctttagtgtcctttcctggcatttccatcttcgtgtcactgcatagcaactgga gttttgtttggatctggttataaattcttgtacagtggatgactttggtgatttagc tgccctggtatcttggtcatttcctctttggagtgtccacactgaggtctctatcaa tgtatgtttaattgcttgagagatgccaagtagaaccagagcctgactgtgctctga gaagctacaaagcacagggtggagactccctttgtgttgctagtattggttctctct ggaaggttaaaatctaaggcaggatcttggtttcctattccaaataggatgcctgct tctctgggcaccagtcctcagccaggcagctctcgtggcattgcagaggctctcctg aaaaacatcaaccagggtgagagccaagatggggtggcacccatgacgcttccccac atgtttcttcaaggagcagaggacagagatagtggaaagagggtcagcagaagcagg tgccttcatctatcccagcagctcagccaaaccccagttagaatgaggcagcaggag attccaggtgtgctgagggttcagccacacgcagaagacgttgcagagtgttaaaga ggtaagctgaggtgtgtatttggttggctttgttgttgttgttaatgtataatgaaa agtataagactaaccctcaggcctcatgttctccaatagatccctggaagacagtat agaaagtcagtcgggcttgggctccttagccagtgagactactcagaccaccagtgg ctagcctagcctacctgtccttgaacatgggtgattttacccctttgaggtcttaac cctttttttacttttcaacaagatgagctctttgtatgattgcgggcgggggatatga aaatgcaatgatctaactcctgttgctcttctagctggtcacatgacggcaccaggc agggttctgggacacccggtgtgctttgactgttctacaaaaagctgtcagagcgtt ctggcctcctggaggctagattcctcagaaactgtctagcctttgcccacagagcat gctatgtaattagagcactccttcccatgaaccccagcacttgtgttacctcagggc cttggtacctggatactgccacagaatttccatggggcgggaagggatgtattttta

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Table 2: siRNA targeting HAMP 3' UTR

Duplex name	Start	SEQ ID NO	Sense (5'-3')	SEQ ID NO	Antisense (5'-3')
307-325_s	307		GGAUGUGCUGCAAGACGUA		UACGUCUUGCAGCACA UCC
309-327_s	309		AUGUGCUGCAAGACGUAGA		UCUACGUCUUGCAGCA CAU
310-328_s	310		UGUGCUGCAAGACGUAGAA		UUCUACGUCUUGCAGC ACA
313-331_s	313		GCUGCAAGACGUAGAACCU		AGGUUCUACGUCUUGC AGC
AD-11439.1_314-332_s	314		CUGCAAGACGUAGAACCUA		UAGGUUCUACGUCUUG CAG
322-340_s	322		CGUAGAACCUACCUGCCCU		AGGGCAGGUAGGUUCU ACG
347-365_s_G1A	347		GUCCCCUCCCUUCCUUAUU		AAUAAGGAAGGGAGGG GAC
348-366_s	348		UCCCCUCCCUUCCUUAUUU		AAAUAAGGAAGGGAGG GGA
349-367_s	349		CCCCUCCCUUCCUUAUUUA		UAAAUAAGGAAGGGAG GGG
350-368_s	350		CCCUCCCUUCCUUAUUUAU		AUAAAUAAGGAAGGGA GGG
351-369_s	351		CCUCCCUUCCUUAUUUAUU		AAUAAAUAAGGAAGGG AGG
352-370_s_C19A	352		CUCCCUUCCUUAUUUAUUA		UAAUAAAUAAGGAAGG GAG
352-370_s_C19U	352		CUCCCUUCCUUAUUUAUUU		AAAUAAAUAAGGAAGG GAG
354-372_s	354		CCCUUCCUUAUUUAUUCCU		AGGAAUAAAUAAGGAA GGG
355-373_s_G19A	355		CCUUCCUUAUUUAUUCCUA		UAGGAAUAAAUAAGGA AGG
355-373_s_G19U	355		CCUUCCUUAUUUAUUCCUU		AAGGAAUAAAUAAGGA AGG
356-374_s_C19A	356		CUUCCUUAUUUAUUCCUGA		UCAGGAAUAAAUAAGG AAG

356-374_s_C19U	356		CUUCCUUAUUUAUUCCUGU		ACAGGAAUAAAUAAGGAAG
357-375_s	357		UUCCUUAUUUAUUCCUGCU		AGCAGGAAUAAAUAAGGAA
358-376_s_G19A	358		UCCUUAUUUAUUCCUGCUA		UAGCAGGAAUAAAUAAGGA
358-376_s_G19U	358		UCCUUAUUUAUUCCUGCUU		AAGCAGGAAUAAAUAAGGA
359-377_s_C19A	359		CCUUAUUUAUUCCUGCUGA		UCAGCAGGAAUAAAUAAGG
359-377_s_C19U	359		CCUUAUUUAUUCCUGCUGU		ACAGCAGGAAUAAAUAAGG
363-381_s	363		AUUUAUUCCUGCUGCCCCA		UGGGGCAGCAGGAAUAAAU
365-383_s	365		UUAUUCCUGCUGCCCCAGA		UCUGGGGCAGCAGGAAUAA
366-384_s	366		UAUUCCUGCUGCCCCAGAA		UUCUGGGGCAGCAGGAAUA
369-387_s	369		UCCUGCUGCCCCAGAACAU		AUGUUCUGGGGCAGCAGGA
370-388_s	370		CCUGCUGCCCCAGAACAUA		UAUGUUCUGGGGCAGCAGG
373-391_s	373		GCUGCCCCAGAACAUAGGU		ACCUAUGUUCUGGGGCAGC
375-393_s	375		UGCCCCAGAACAUAGGUCU		AGACCUAUGUUCUGGGGCA
376-394_s	376		GCCCCAGAACAUAGGUCUU		AAGACCUAUGUUCUGGGGC
379-397_s	379		CCAGAACAUAGGUCUUGGA		UCCAAGACCUAUGUUCUGG
380-398_s	380		CAGAACAUAGGUCUUGGAA		UUCCAAGACCUAUGUUCUG
381-399_s	381		AGAACAUAGGUCUUGGAAU		AUUCCAAGACCUAUGUUCU
AD-11442.1_382-400_s	382		GAACAUAGGUCUUGGAAUA		UAUUCCAAGACCUAUGUUC
383-401_s	383		AACAUAGGUCUUGGAAUAA		UUAUUCCAAGACCUAUGUU
396-414_s	396		GAAUAAAAUGGCUGGUUCU		AGAACCAGCCAUUUUAUUC
398-416_s	398		AUAAAAUGGCUGGUUCUUU		AAAGAACCAGCCAUUUUAU
399-417_s	399		UAAAAUGGCUGGUUCUUUU		AAAAGAACCAGCCAUUUUA
402-420_s	402		AAUGGCUGGUUCUUUUGUU		AACAAAAGAACCAGCCAUU
403-421_s	403		AUGGCUGGUUCUUUUGUUU		AAACAAAAGAACCAGCCAU
407-425_s	407		CUGGUUCUUUUGUUUUCCA		UGGAAAACAAAAGAAC

					CAG
AD-11436.1_291-309 s	291		CAUCGAUCAAAGUGUGGGA		UCCCACACUUUGAUCGAUG
Note that an overhang (e.g. TT, dTsdT) can be added to the 3' end of any duplex.					

Table 3: siRNA targeting HAMP CDS

Duplex name	Start	SEQ ID NO	sense (5'-3')	SEQ ID NO	Antisense (5'-3')
62-80 s G19U	62		AGACGGCACGAUGGCACUU		AAGUGCCAUCGUGCCGUCU
67-85 s C19A	67		GCACGAUGGCACUGAGCUA		UAGCUCAGUGCCAUCGUGC
67-85 s C19U	67		GCACGAUGGCACUGAGCUU		AAGCUCAGUGCCAUCGUGC
74-92 s C19A	74		GGCACUGAGCUCCCAGAUA		UAUCUGGGAGCUCAGUGCC
74-92 s C19U	74		GGCACUGAGCUCCCAGAUU		AAUCUGGGAGCUCAGUGCC
76-94 s G19A	76		CACUGAGCUCCCAGAUCUA		UAGAUCUGGGAGCUCAGUG
76-94 s G19U	76		CACUGAGCUCCCAGAUCUU		AAGAUCUGGGAGCUCAGUG
132-150 s	132		CUGACCAGUGGCUCUGUUU		AAACAGAGCCACUGGUCAG
140-158 s	140		UGGCUCUGUUUUCCCACAA		UUGUGGGAAAACAGAGCCA
146-164_s_hcU1C_G19A	146		UGUUUUCCCACAACAGACA		UGUCUGUUGUGGGAAAACA
146-164_s_hcU1C_G19U	146		UGUUUUCCCACAACAGACU		AGUCUGUUGUGGGAAAACA
155-173 s	155		ACAACAGACGGGACAACUU		AAGUUGUCCCGUCUGUUGU
157-175 s C19A	157		AACAGACGGGACAACUUGA		UCAAGUUGUCCCGUCUGUU
157-175 s C19U	157		AACAGACGGGACAACUUGU		ACAAGUUGUCCCGUCUGUU
160-178 s	160		AGACGGGACAACUUGCAGA		UCUGCAAGUUGUCCCGUCU
161-179 s G19A	161		GACGGGACAACUUGCAGAA		UUCUGCAAGUUGUCCCGUC
161-179 s G19U	161		GACGGGACAACUUGCAGAU		AUCUGCAAGUUGUCCCGUC
162-180 s C19A	162		ACGGGACAACUUGCAGAGA		UCUCUGCAAGUUGUCCCGU

162-180_s_C19U	162		ACGGGACAACUUGCAGAGU		ACUCUGCAAGUUGUCCCGU
242-260_s_C19A	242		GAGGCGAGACACCCACUUA		UAAGUGGGUGUCUCGCCUC
242-260_s_C19U	242		GAGGCGAGACACCCACUUU		AAAGUGGGUGUCUCGCCUC
253-271_s	253		CCCACUUCCCCAUCUGCAU		AUGCAGAUGGGGAAGUGGG
258-276_s	258		UUCCCCAUCUGCAUUUUCU		AGAAAAUGCAGAUGGGGAA
261-279_s	261		CCCAUCUGCAUUUUCUGCU		AGCAGAAAAUGCAGAUGGG
275-293_s	275		CUGCUGCGGCUGCUGUCAU		AUGACAGCAGCCGCAGCAG
276-294_s_C19A	276		UGCUGCGGCUGCUGUCAUA		UAUGACAGCAGCCGCAGCA
276-294_s_C19U	276		UGCUGCGGCUGCUGUCAUU		AAUGACAGCAGCCGCAGCA
278-296_s	278		CUGCGGCUGCUGUCAUCGA		UCGAUGACAGCAGCCGCAG
279-297_s	279		UGCGGCUGCUGUCAUCGAU		AUCGAUGACAGCAGCCGCA
280-298_s_C19A	280		GCGGCUGCUGUCAUCGAUA		UAUCGAUGACAGCAGCCGC
280-298_s_C19U	280		GCGGCUGCUGUCAUCGAUU		AAUCGAUGACAGCAGCCGC
281-299_s	281		CGGCUGCUGUCAUCGAUCA		UGAUCGAUGACAGCAGCCG
AD-11443.1_282-300_s	282		GGCUGCUGUCAUCGAUCAA		UUGAUCGAUGACAGCAGCC
AD-11432.1_283-301_s	283		GCUGCUGUCAUCGAUCAAA		UUUGAUCGAUGACAGCAGC
284-302_s_G19A	284		CUGCUGUCAUCGAUCAAAA		UUUUGAUCGAUGACAGCAG
284-302_s_G19U	284		CUGCUGUCAUCGAUCAAAU		AUUUGAUCGAUGACAGCAG
AD-11441.1_285-303_s	285		UGCUGUCAUCGAUCAAAGU		ACUUUGAUCGAUGACAGCA
286-304_s_G19A	286		GCUGUCAUCGAUCAAAGUA		UACUUUGAUCGAUGACAGC
286-304_s_G19U	286		GCUGUCAUCGAUCAAAGUU		AACUUUGAUCGAUGACAGC
AD-11447.1_287-305_s	287		CUGUCAUCGAUCAAAGUGU		ACACUUUGAUCGAUGACAG
288-306_s_G19A	288		UGUCAUCGAUCAAAGUGUA		UACACUUUGAUCGAUGACA

288-306 s G19U	288		UGUCAUCGAUCAAAGUGUU		AACACUUUGAUCGAUGACA
290-308 s G19A	290		UCAUCGAUCAAAGUGUGGA		UCCACACUUUGAUCGAUGA
290-308 s G19U	290		UCAUCGAUCAAAGUGUGGU		ACCACACUUUGAUCGAUGA
295-313 s G19A	295		GAUCAAAGUGUGGGAUGUA		UACAUCCCACACUUUGAUC
295-313 s G19U	295		GAUCAAAGUGUGGGAUGUU		AACAUCCCACACUUUGAUC
299-317 s C19U	299		AAAGUGUGGGAUGUGCUGU		ACAGCACAUCCCACACUUU
Note that an overhang (e.g. TT, dTsdT) can be added to the 3' end of any duplex.					

Table 4: HAMP modified sequences

Table 4								
Target	Duplex ID	Start Position	Sense Name	Sense Sequence	SEQ ID NO	Antisense Name	Antisense Sequence	SEQ ID NO
HAMP	AD-45073	2	A-94166.1	AcuGucAcucGGucccAGAdTsdT		A-94167.1	UCUGGGACCGAGUGAcAGUdTsdT	
HAMP	AD-45079	7	A-94168.1	cAcucGGucccAGAcAccAdTsdT		A-94169.1	UGGUGUCUGGGACCGAGUGdTsdT	
HAMP	AD-45085	16	A-94170.1	ccAGAcAccAGAGcAAGcudTsdT		A-94171.1	AGCUUGCUCUGGUGUCUGGdTsdT	
HAMP	AD-29928	43	A-66808.1	AGcAGuGGGAcAGccAGAcdTsdT		A-66809.1	GUCUGGCUGUCCcACUGCUdTsdT	
HAMP	AD-45674	43	A-95618.1	AGcAGuGGGAcAGccAGAAdTsdT		A-95619.1	UUCUGGCUGUCCcACUGCUdTsdT	
HAMP	AD-45680	43	A-95620.1	AGcAGuGGGAcAGccAGAudTsdT		A-95621.1	AUCUGGCUGUCCcACUGCUdTsdT	
HAMP	AD-45686	48	A-95622.1	uGGGAcAGccAGAcAGAcGdTsdT		A-95623.1	CGUCUGUCUGGCUGUCCcAdTsdT	
HAMP	AD-45698	48	A-95626.1	uGGGAcAGccAGAcAGAcudTsdT		A-95627.1	AGUCUGUCUGGCUGUCCcAdTsdT	
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HAMP	AD-47028	363	A-98304.1	AUfUfUfAUfUfCfCfUfGCfUfGCfCfCfCfAdTsdT		A-98305.1	UGGGGCfAGCfAGGAACfAAAUdTsdT	
HAMP	AD-46991	365	A-98306.1	UfUfAUfUfCfCfUfGCfUfGCfCfCfCfAGAdTsdT		A-98307.1	UCUGGGGCfAGCfAGGAACfAAdTsdT	
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HAMP	AD-47003	369	A-98310.1	UfCfCfUfGCfUfGCfCfCfCfAG AACfAUfdTsdT		A-98311.1	AUGUUCUGGGGCfAG CfAGGAdTsdT	
HAMP	AD-45105	369	A-94208.1	uccuGcuGccccAGAAcAudTs dT		A-94209.1	AUGUUCUGGGGcAGc AGGAdTsdT	
HAMP	AD-47009	370	A-98312.1	CfCfUfGCfUfGCfCfCfCfAGAA CfAUfAdTsdT		A-98313.1	CfAUGUUCUGGGGCfA GCfAGGdTsdT	
HAMP	AD-45111	370	A-94210.1	ccuGcuGccccAGAAcAuAdTs dT		A-94211.1	uAUGUUCUGGGGcAG cAGGdTsdT	
HAMP	AD-47014	373	A-98314.1	GCfUfGCfCfCfCfAGAACfAUf AGGUfdTsdT		A-98315.1	ACCCfAUGUUCUGGGG CfAGCdTsdT	
HAMP	AD-45117	373	A-94212.1	GcuGccccAGAAcAuAGGudT sdT		A-94213.1	ACCuAUGUUCUGGGG cAGCdTsdT	
HAMP	AD-47019	375	A-98316.1	UfGCfCfCfCfAGAACfAUfAG GUfCfUfdTsdT		A-98317.1	AGACCCfAUGUUCUGG GGCfAdTsdT	
HAMP	AD-45076	375	A-94214.1	uGccccAGAAcAuAGGucudT sdT		A-94215.1	AGACCuAUGUUCUGG GGcAdTsdT	
HAMP	AD-48214	376	A-100244.1	GccccAGAAcAuAGGucuudT dT		A-100245.1	AAGACCuAUGUUCUG GGGCdTdT	
HAMP	AD-48219	376	A-100246.1	GcCCCAGAAcAuAGGucuudT dT		A-100247.1	AAGACCuaUGuuCUGG GGcdTdT	
HAMP	AD-47024	376	A-98318.1	GCfCfCfCfAGAACfAUfAGGU fCfUfUfdTsdT		A-98319.1	AAGACCCfAUGUUCUG GGGCdTsdT	
HAMP	AD-45082	376	A-94216.1	GccccAGAAcAuAGGucuudT sdT		A-94217.1	AAGACCuAUGUUCUG GGGCdTsdT	
HAMP	AD-48224	379	A-100248.1	ccAGAAcAuAGGucuuGGAd TdT		A-100249.1	UCcAAGACCuAUGUUC UCGdTdT	
HAMP	AD-48187	379	A-100248.2	ccAGAAcAuAGGucuuGGAd TdT		A-100250.1	uCCAAGACCuAUGuUC uggdTdT	
HAMP	AD-47029	379	A-98320.1	CfCfAGAACfAUfAGGUfCfUf UfGGAdTsdT		A-98321.1	UCCfAAGACCCfAUGU UCUGGdTsdT	
HAMP	AD-48192	379	A-100248.3	ccAGAAcAuAGGucuuGGAd TdT		A-100251.1	uCCAAGACCUaUgUuC uGgdTdT	
HAMP	AD-45088	379	A-94218.1	ccAGAAcAuAGGucuuGGAd TsdT		A-94219.1	UCcAAGACCuAUGUUC UGGdTsdT	
HAMP	AD-46992	380	A-98322.1	CfAGAACfAUfAGGUfCfUfUf GGAAdTsdT		A-98323.1	UUCCfAAGACCCfAUG UUCUGdTsdT	
HAMP	AD-45094	380	A-94220.1	cAGAAcAuAGGucuuGGAAd TsdT		A-94221.1	UUCcAAGACCuAUGUU CUGdTsdT	
HAMP	AD-46998	381	A-98324.1	AGAACfAUfAGGUfCfUfUfG GAAUfdTsdT		A-98325.1	AUUCCfAAGACCCfAU GUUCUdTsdT	
HAMP	AD-45100	381	A-94222.1	AGAAcAuAGGucuuGGAAud TsdT		A-94223.1	AUUCcAAGACCuAUGU UCUdTsdT	
HAMP	AD-48137	382	A-100195.1	GAAcAuAGGUCUUGGAAUA dTdT		A-98136.7	UAUUCCAAGACCUAU GUUCdTdT	
HAMP	AD-48196	382	A-100179.22	GAAcAuAGGucuuGGAAuAd TdT		A-100228.1	UAuUCCAAGaCCuAuG uucdTdT	
HAMP	AD-48195	382	A-98135.8	GAACAUAGGUCUUGGAAU AdTdT		A-100218.1	UAUUcCaAgAcCuAuGu UcdTdT	
HAMP	AD-48201	382	A-100179.23	GAAcAuAGGucuuGGAAuAd TdT		A-100229.1	UAUUCCAAGaCCuAuG uucdTdT	
HAMP	AD-48207	382	A-100179.24	GAAcAuAGGucuuGGAAuAd TdT		A-100230.1	UAUUCCAAgAcCuAuG uucdTdT	
HAMP	AD-48159	382	A-100179.5	GAAcAuAGGucuuGGAAuAd TdT		A-100184.1	UAUUCCAAGACCUAu GuUCdTdT	
HAMP	AD-48147	382	A-100179.3	GAAcAuAGGucuuGGAAuAd TdT		A-100182.1	UAUUCcAAGACCuAuG uUCdTdT	
HAMP	AD-48161	382	A-98135.4	GAACAUAGGUCUUGGAAU AdTdT		A-100188.2	UAUUCCAAGACCUAu GuUcdTdT	
HAMP	AD-48172	382	A-100193.1	GAAcAuAGGucUUGGAAUA dTdT		A-98136.5	UAUUCCAAGACCUAU GUUCdTdT	
HAMP	AD-48156	382	A-100194.4	GAAcAuAGGuCUUGGAAUA dTdT		A-100188.3	UAUUCCAAGACCUAu GuUcdTdT	
HAMP	AD-48195	382	A-98135.8	GAACAUAGGUCUUGGAAU AdTdT		A-100218.1	UAUUcCaAgAcCuAuGu UcdTdT	
HAMP	AD-48136	382	A-100179.9	GAAcAuAGGucuuGGAAuAd TdT		A-100187.1	UAUUCCAAGACCUAU GuUcdTdT	
HAMP	AD-48166	382	A-100192.1	GAAcAuAGGucuUGGAAUA dTdT		A-98136.4	UAUUCCAAGACCUAU GUUCdTdT	

HAMP	AD-48213	382	A-100179.25	GAAcAuAGGucuuGGAAuAdTdT		A-100231.1	UAuUCCAAgAcCuAuGuucdTdT	
HAMP	AD-48173	382	A-98135.6	GAACAUAGGUCUUGGAAUAdTdT		A-100190.2	UAUUCCAAGACcuAuGuUcdTdT	
HAMP	AD-48154	382	A-100179.12	GAAcAuAGGucuuGGAAuAdTdT		A-100190.1	UAUUCCAAGACcuAuGuUcdTdT	
HAMP	AD-48141	382	A-100179.2	GAAcAuAGGucuuGGAAuAdTdT		A-100181.1	UAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48216	382	A-100217.3	GAAcAuAGGuCUUGGAAUAdTsdT		A-100215.3	UAUUCCAAGACcuAuGuUcdTsdT	
HAMP	AD-48180	382	A-100194.8	GAAcAuAGGuCUUGGAAUAdTdT		A-100183.2	UAUUCCAAGACCuAuGuUCdTdT	
HAMP	AD-48143	382	A-100196.1	GAAcAUAGGUCUUGGAAUAdTdT		A-98136.8	UAUUCCAAGACCUAUGUUCdTdT	
HAMP	AD-48142	382	A-100179.10	GAAcAuAGGucuuGGAAuAdTdT		A-100188.1	UAUUCCAAGACCUAuGuUcdTdT	
HAMP	AD-48221	382	A-18280.13	GAAcAuAGGucuuGGAAuAdTsdT		A-15168.3	UAUUCCAAGACCUAUGUUCdTsdT	
HAMP	AD-48171	382	A-100179.7	GAAcAuAGGucuuGGAAuAdTdT		A-98136.2	UAUUCCAAGACCUAUGUUCdTdT	
HAMP	AD-48145	382	A-100195.4	GAAcAuAGGUCUUGGAAUAdTdT		A-100183.3	UAUUCCAAGACCuAuGuUCdTdT	
HAMP	AD-48160	382	A-100191.1	GAAcAuAGGucuuGGAAUAdTdT		A-98136.3	UAUUCCAAGACCUAUGUUCdTdT	
HAMP	AD-48144	382	A-100194.2	GAAcAuAGGuCUUGGAAUAdTdT		A-100190.5	UAUUCCAAGACcuAuGuUcdTdT	
HAMP	AD-48167	382	A-98135.5	GAACAUAGGUCUUGGAAUAdTdT		A-100189.2	UAUUCCAAGACCuAuGuUcdTdT	
HAMP	AD-48177	382	A-100179.8	GAAcAuAGGucuuGGAAuAdTdT		A-100186.1	UAUUCCAAGACCUAUGUUcdTdT	
HAMP	AD-48153	382	A-100179.4	GAAcAuAGGucuuGGAAuAdTdT		A-100183.1	UAUUCCAAGACCuAuGuUCdTdT	
HAMP	AD-48178	382	A-100194.1	GAAcAuAGGuCUUGGAAUAdTdT		A-98136.6	UAUUCCAAGACCUAUGUUCdTdT	
HAMP	AD-48155	382	A-98135.3	GAACAUAGGUCUUGGAAUAdTdT		A-100187.2	UAUUCCAAGACCUAUGuUcdTdT	
HAMP	AD-48174	382	A-100194.7	GAAcAuAGGuCUUGGAAUAdTdT		A-100197.1	UAUUCCAAGACcuAuGuUCdTdT	
HAMP	AD-48205	382	A-15167.2	GAACAUAGGUCUUGGAAUAdTsdT		A-100215.2	UAUUCCAAGACcuAuGuUcdTsdT	
HAMP	AD-48179	382	A-100196.2	GAAcAUAGGUCUUGGAAUAdTdT		A-100190.3	UAUUCCAAGACcuAuGuUcdTdT	
HAMP	AD-48168	382	A-100194.6	GAAcAuAGGuCUUGGAAUAdTdT		A-100186.3	UAUUCCAAGACCUAUGUUcdTdT	
HAMP	AD-48149	382	A-98135.2	GAACAUAGGUCUUGGAAUAdTdT		A-100186.2	UAUUCCAAGACCUAUGUUcdTdT	
HAMP	AD-48211	382	A-100217.2	GAAcAuAGGuCUUGGAAUAdTsdT		A-100214.2	UAUUCCAAGACCuAuGuUCdTsdT	
HAMP	AD-48200	382	A-100217.1	GAAcAuAGGuCUUGGAAUAdTsdT		A-15168.2	UAUUCCAAGACCUAUGUUCdTsdT	
HAMP	AD-48188	382	A-100179.20	GAAcAuAGGucuuGGAAuAdTdT		A-100205.1	UAuUCcAAGACCuAuGuUcdTdT	
HAMP	AD-48183	382	A-18280.10	GAAcAuAGGucuuGGAAuAdTsdT		A-100214.1	UAUUCCAAGACCuAuGuUCdTsdT	
HAMP	AD-48150	382	A-100194.3	GAAcAuAGGuCUUGGAAUAdTdT		A-100189.3	UAUUCCAAGACCuAuGuUcdTdT	
HAMP	AD-48162	382	A-100194.5	GAAcAuAGGuCUUGGAAUAdTdT		A-100187.3	UAUUCCAAGACCUAUGuUcdTdT	
HAMP	AD-48139	382	A-100195.3	GAAcAuAGGUCUUGGAAUAdTdT		A-100197.2	UAUUCCAAGACcuAuGuUCdTdT	
HAMP	AD-9942	382	A-15167.1	GAACAUAGGUCUUGGAAUAdTsdT		A-15168.1	UAUUCCAAGACCUAUGUUCdTsdT	
HAMP	AD-48138	382	A-100195.2	GAAcAuAGGUCUUGGAAUAdTdT		A-100190.4	UAUUCCAAGACcuAuGuUcdTdT	
HAMP	AD-11459	382	A-18280.2	GAAcAuAGGucuuGGAAuAdTsdT		A-18304.1	uAuUCcAAGACCuAuGuUCdTsdT	
HAMP	AD-48189	382	A-18280.11	GAAcAuAGGucuuGGAAuAdTsdT		A-100215.1	UAUUCCAAGACcuAuGuUcdTsdT	

HAMP	AD-48148	382	A-100179.11	GAAcAuAGGucuuGGAAuAdTdT		A-100189.1	UAUUCCAAGACCuAuGuUcdTdT	
HAMP	AD-48215	382	A-18280.8	GAAcAuAGGucuuGGAAuAdTsdT		A-100212.1	UAuUCcAAGACCuAuGuUCdTsdT	
HAMP	AD-48218	382	A-100179.26	GAAcAuAGGucuuGGAAuAdTdT		A-100232.1	uAuUCCAAgAcCuAuGuucdTdT	
HAMP	AD-48135	382	A-100179.1	GAAcAuAGGucuuGGAAuAdTdT		A-100180.1	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-47004	382	A-98326.1	GAACfAUfAGGUfCfUfUfGGAAUfAdTsdT		A-98327.1	CfAUUCCfAAGACCCfAUGUUCdTsdT	
HAMP	AD-48194	382	A-18280.12	GAAcAuAGGucuuGGAAuAdTsdT		A-100216.1	uAUUCCAAGACCuAuGuUCdTsdT	
HAMP	AD-48197	382	A-100239.1	GAAcAuAGGuCdTUdGGdAAdTAdTdT		A-100240.1	dTAdTUdCCdAAdGACCuAuGuucdTdT	
HAMP	AD-11459	382	A-18280.2	GAAcAuAGGucuuGGAAuAdTsdT		A-18304.1	uAuUCcAAGACCuAuGuUCdTsdT	
HAMP	AD-48164	382	A-100179.16	GAAcAuAGGucuuGGAAuAdTdT		A-100201.1	uAUUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48158	382	A-100179.15	GAAcAuAGGucuuGGAAuAdTdT		A-100200.1	uAUUCCAAGACCuAuGuUCdTdT	
HAMP	AD-48204	382	A-100208.1	GAAcAcAGGucuuGGAAuAdTdT		A-100209.1	uAuUCcAAGACCuGuGuUCdTdT	
HAMP	AD-48181	382	A-100192.2	GAAcAuAGGucuUGGAAUAdTdT		A-100180.7	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48223	382	A-100233.1	GAAcAuAGGucuuGGAAuAuu		A-100234.1	uAuUCcAAGACCuAuGuUCuu	
HAMP	AD-48190	382	A-100179.21	GAAcAuAGGucuuGGAAuAdTdT		A-100227.1	uAuUCCAAGaCCuAuGuucdTdT	
HAMP	AD-48163	382	A-100195.5	GAAcAuAGGUCUUGGAAUAdTdT		A-100180.4	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48140	382	A-100191.2	GAAcAuAGGucuuGGAAUAdTdT		A-100180.8	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48169	382	A-100194.9	GAAcAuAGGuCUUGGAAUAdTdT		A-100180.5	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48220	382	A-18280.9	GAAcAuAGGucuuGGAAuAdTsdT		A-100213.1	uAuUCcAAGACCuAuGuUcdTsdT	
HAMP	AD-48184	382	A-15167.3	GAACAUAGGUCUUGGAAUAdTsdT		A-18304.6	uAuUCcAAGACCuAuGuUCdTsdT	
HAMP	AD-48176	382	A-100179.18	GAAcAuAGGucuuGGAAuAdTdT		A-100203.1	uAUUCCAAGACCuAuGuUcdTdT	
HAMP	AD-48175	382	A-100193.2	GAAcAuAGGucUUGGAAUAdTdT		A-100180.6	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48146	382	A-100179.13	GAAcAuAGGucuuGGAAuAdTdT		A-100198.1	uAUUCCAAGACCUAUGUUcdTdT	
HAMP	AD-48182	382	A-100179.19	GAAcAuAGGucuuGGAAuAdTdT		A-100204.1	uAuUCcAAGACCuAuGuUcdTdT	
HAMP	AD-48199	382	A-100207.1	GAAcAuAGGUCUUGGAAuAdTdT		A-100180.10	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48157	382	A-100196.3	GAAcAUAGGUCUUGGAAUAdTdT		A-100180.3	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48206	382	A-100219.1	GAAcAcAGGucuuGGAAuAdTsdT		A-100220.1	uAuUCcAAGACCuGuGuUCdTsdT	
HAMP	AD-48193	382	A-100206.1	GAAcAuAGGuCuuGGAAuAdTdT		A-100180.9	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48152	382	A-100179.14	GAAcAuAGGucuuGGAAuAdTdT		A-100199.1	uAUUCCAAGACCUAUGUUCdTdT	
HAMP	AD-48151	382	A-98135.7	GAACAUAGGUCUUGGAAUAdTdT		A-100180.2	uAuUCcAAGACCuAuGuUCdTdT	
HAMP	AD-48170	382	A-100179.17	GAAcAuAGGucuuGGAAuAdTdT		A-100202.1	uAuUCUAAGACCuAuGuUCdTdT	
HAMP	AD-47010	383	A-98328.1	AACfAUfAGGUfCfUfUfGGAAUfAAdTsdT		A-98329.1	UCfAUUCCfAAGACCCfAUGUUdTsdT	
HAMP	AD-45106	383	A-94224.1	AAcAuAGGucuuGGAAuAAdTsdT		A-94225.1	UuAUUCcAAGACCuAUGUUdTsdT	
HAMP	AD-48222	385	A-100224.1	CAUAGGUCUUGGAAUAAAAdTdT		A-100225.1	UUUUAUUCCAAGACCUAUGdTdT	
HAMP	AD-48217	385	A-100221.2	cAuAGGucuuGGAAuAAAAdTdT		A-100223.1	UUUuAUUCcAAGACCuAUGdTdT	

HAMP	AD-48185	385	A-100221.3	cAuAGGucuuGGAAuAAAAdTdT		A-100226.1	uUUUAuuCCaaGACCUaugdTdT	
HAMP	AD-48212	385	A-100221.1	cAuAGGucuuGGAAuAAAAdTdT		A-100222.1	UuUuAuUCcAAGACCuAuGdTdT	
HAMP	AD-48198	396	A-100252.1	GAAuAAAAuGGcuGGuucudTdT		A-100253.1	AGAACcAGCcAUUUuAUUCdTdT	
HAMP	AD-48209	396	A-100252.3	GAAuAAAAuGGcuGGuucudTdT		A-100255.1	AGAACCAGcCaUuUuAuUcdTdT	
HAMP	AD-48203	396	A-100252.2	GAAuAAAAuGGcuGGuucudTdT		A-100254.1	AGAACcAGCCAuuUUAuucdTdT	
HAMP	AD-47015	396	A-98330.1	GAAUfAAAAUfGGCfUfGGUfUfCfUfdTsdT		A-98331.1	AGAACCfAGCCfAUUUCfAUUCdTsdT	
HAMP	AD-45112	396	A-94226.1	GAAuAAAAuGGcuGGuucudTsdT		A-94227.1	AGAACcAGCcAUUUuAUUCdTsdT	
HAMP	AD-47020	398	A-98332.1	AUfAAAAUfGGCfUfGGUfUfCfUfUfUfdTsdT		A-98333.1	AAAGAACCfAGCCfAUUUCfAUdTsdT	
HAMP	AD-45118	398	A-94228.1	AuAAAAuGGcuGGuucuuudTsdT		A-94229.1	AAAGAACcAGCcAUUUuAUdTsdT	
HAMP	AD-47025	399	A-98334.1	UfAAAAUfGGCfUfGGUfUfCfUfUfUfUfdTsdT		A-98335.1	AAAAGAACCfAGCCfAUUUCfAdTsdT	
HAMP	AD-45077	399	A-94230.1	uAAAAuGGcuGGuucuuuudTsdT		A-94231.1	AAAAGAACcAGCcAUUUuAdTsdT	
HAMP	AD-47030	402	A-98336.1	AAUfGGCfUfGGUfUfCfUfUfUfUfUfGUfUfUfdTsdT		A-98337.1	AACfAAAAGAACCfAGCCfAUUdTsdT	
HAMP	AD-45083	402	A-94232.1	AAuGGcuGGuucuuuuGuudTsdT		A-94233.1	AAcAAAAGAACcAGCcAUUdTsdT	
HAMP	AD-46993	403	A-98338.1	AUfGGCfUfGGUfUfCfUfUfUfUfGUfUfUfUfdTsdT		A-98339.1	AAACfAAAAGAACCfAGCCfAUdTsdT	
HAMP	AD-45089	403	A-94234.1	AuGGcuGGuucuuuuGuuudTsdT		A-94235.1	AAAcAAAAGAACcAGCcAUdTsdT	
HAMP	AD-46999	407	A-98340.1	CfUfGGUfUfCfUfUfUfUfGUfUfUfUfCfCfAdTsdT		A-98341.1	UGGAAAACfAAAAGAACCfAGdTsdT	

It should be noted that unmodified versions of each of the modified sequences shown are included within the scope of the invention.

Table 5: HAMP unmodified sequences

Table 5								
Target	Duplex ID	Start Position	Antisense Name	Antisense Sequence	SEQ ID NO	Sense Name	Sense Sequence	SEQ ID NO
HAMP	AD-47121	62	A-98153.1	AGACGGCACGAUGGCACUUdTdT		A-98154.1	AAGUGCCAUCGUGCCGUCUdTdT	
HAMP	AD-47133	67	A-98157.1	GCACGAUGGCACUGAGCUUdTdT		A-98158.1	AAGCUCAGUGCCAUCGUGCdTdT	
HAMP	AD-47127	67	A-98155.1	GCACGAUGGCACUGAGCUAdTdT		A-98156.1	UAGCUCAGUGCCAUCGUGCdTdT	
HAMP	AD-47145	74	A-98161.1	GGCACUGAGCUCCCAGAUUdTdT		A-98162.1	AAUCUGGGAGCUCAGUGCCdTdT	
HAMP	AD-47139	74	A-98159.1	GGCACUGAGCUCCCAGAUAdTdT		A-98160.1	UAUCUGGGAGCUCAGUGCCdTdT	
HAMP	AD-47157	76	A-98165.1	CACUGAGCUCCCAGAUCUUdTdT		A-98166.1	AAGAUCUGGGAGCUCAGUGdTdT	
HAMP	AD-47151	76	A-98163.1	CACUGAGCUCCCAGAUCUAdTdT		A-98164.1	UAGAUCUGGGAGCUCAGUGdTdT	
HAMP	AD-47163	132	A-98167.1	CUGACCAGUGGCUCUGUUUdTdT		A-98168.1	AAACAGAGCCACUGGUCAGdTdT	
HAMP	AD-47122	140	A-98169.1	UGGCUCUGUUUUCCCACAAdTdT		A-98170.1	UUGUGGGAAAACAGAGCCAdTdT	
HAMP	AD-47128	146	A-98171.1	UGUUUUCCCACAACAGACAdTdT		A-98172.1	UGUCUGUUGUGGGAAAACAdTdT	
HAMP	AD-47134	146	A-98173.1	UGUUUUCCCACAACAGACUdTdT		A-98174.1	AGUCUGUUGUGGGAAAACAdTdT	
HAMP	AD-47140	155	A-98175.1	ACAACAGACGGGACAACUUdTdT		A-98176.1	AAGUUGUCCCGUCUGUUGUdTdT	
HAMP	AD-47152	157	A-98179.1	AACAGACGGGACAACUUGUdTdT		A-98180.1	ACAAGUUGUCCCGUCUGUUdTdT	

HAMP	AD-47146	157	A-98177.1	AACAGACGGGACAAC UUGAdTdT		A-98178.1	UCAAGUUGUCCC GUCUGUUdTdT	
HAMP	AD-47158	160	A-98181.1	AGACGGGACAACUU GCAGAdTdT		A-98182.1	UCUGCAAGUUGU CCCGUCUdTdT	
HAMP	AD-47164	161	A-98183.1	GACGGGACAACUUG CAGAAdTdT		A-98184.1	UUCUGCAAGUUG UCCCGUCdTdT	
HAMP	AD-47123	161	A-98185.1	GACGGGACAACUUG CAGAUdTdT		A-98186.1	AUCUGCAAGUUG UCCCGUCdTdT	
HAMP	AD-47135	162	A-98189.1	ACGGGACAACUUGC AGAGUdTdT		A-98190.1	ACUCUGCAAGUU GUCCCGUdTdT	
HAMP	AD-47129	162	A-98187.1	ACGGGACAACUUGC AGAGAdTdT		A-98188.1	UCUCUGCAAGUU GUCCCGUdTdT	
HAMP	AD-47141	242	A-98191.1	GAGGCGAGACACCA CUUAdTdT		A-98192.1	UAAGUGGGUGUC UCGCCUCdTdT	
HAMP	AD-47147	242	A-98193.1	GAGGCGAGACACCA CUUUdTdT		A-98194.1	AAAGUGGGUGUC UCGCCUCdTdT	
HAMP	AD-47153	253	A-98195.1	CCCACUUCCCCAUCU GCAUdTdT		A-98196.1	AUGCAGAUGGGG AAGUGGGdTdT	
HAMP	AD-47159	258	A-98197.1	UUCCCCAUCUGCAU UUUCUdTdT		A-98198.1	AGAAAAUGCAGA UGGGGAAdTdT	
HAMP	AD-47165	261	A-98199.1	CCCAUCUGCAUUUU CUGCUdTdT		A-98200.1	AGCAGAAAAUGC AGAUGGGdTdT	
HAMP	AD-47124	275	A-98201.1	CUGCUGCGGCUGCU GUCAUdTdT		A-98202.1	AUGACAGCAGCCG CAGCAGdTdT	
HAMP	AD-47136	276	A-98205.1	UGCUGCGGCUGCUG UCAUUdTdT		A-98206.1	AAUGACAGCAGCC GCAGCAdTdT	
HAMP	AD-47130	276	A-98203.1	UGCUGCGGCUGCUG UCAUAdTdT		A-98204.1	UAUGACAGCAGCC GCAGCAdTdT	
HAMP	AD-47142	278	A-98207.1	CUGCGGCUGCUGUC AUCGAdTdT		A-98208.1	UCGAUGACAGCA GCCGCAGdTdT	
HAMP	AD-47148	279	A-98209.1	UGCGGCUGCUGUCA UCGAUdTdT		A-98210.1	AUCGAUGACAGC AGCCGCAdTdT	
HAMP	AD-47160	280	A-98213.1	GCGGCUGCUGUCAU CGAUUdTdT		A-98214.1	AAUCGAUGACAG CAGCCGCdTdT	
HAMP	AD-47154	280	A-98211.1	GCGGCUGCUGUCAU CGAUAdTdT		A-98212.1	UAUCGAUGACAG CAGCCGCdTdT	
HAMP	AD-47166	281	A-98215.1	CGGCUGCUGUCAUC GAUCAdTdT		A-98216.1	UGAUCGAUGACA GCAGCCGdTdT	
HAMP	AD-47125	282	A-98217.1	GGCUGCUGUCAUCG AUCAAdTdT		A-98218.1	UUGAUCGAUGAC AGCAGCCdTdT	
HAMP	AD-47131	283	A-98219.1	GCUGCUGUCAUCGA UCAAAdTdT		A-98220.1	UUUGAUCGAUGA CAGCAGCdTdT	
HAMP	AD-47137	284	A-98221.1	CUGCUGUCAUCGAU CAAAAdTdT		A-98222.1	UUUUGAUCGAUG ACAGCAGdTdT	
HAMP	AD-47143	284	A-98223.1	CUGCUGUCAUCGAU CAAAUdTdT		A-98224.1	AUUUGAUCGAUG ACAGCAGdTdT	
HAMP	AD-47149	285	A-98225.1	UGCUGUCAUCGAUC AAAGUdTdT		A-98226.1	ACUUUGAUCGAU GACAGCAdTdT	
HAMP	AD-47161	286	A-98229.1	GCUGUCAUCGAUCA AAGUUdTdT		A-98230.1	AACUUUGAUCGA UGACAGCdTdT	
HAMP	AD-47155	286	A-98227.1	GCUGUCAUCGAUCA AAGUAdTdT		A-98228.1	UACUUUGAUCGA UGACAGCdTdT	
HAMP	AD-47167	287	A-98231.1	CUGUCAUCGAUCAA AGUGUdTdT		A-98232.1	ACACUUUGAUCG AUGACAGdTdT	
HAMP	AD-47132	288	A-98235.1	UGUCAUCGAUCAAA GUGUUdTdT		A-98236.1	AACACUUUGAUC GAUGACAdTdT	
HAMP	AD-47126	288	A-98233.1	UGUCAUCGAUCAAA GUGUAdTdT		A-98234.1	UACACUUUGAUC GAUGACAdTdT	
HAMP	AD-47138	290	A-98237.1	UCAUCGAUCAAAGU GUGGAdTdT		A-98238.1	UCCACACUUUGA UCGAUGAdTdT	
HAMP	AD-47144	290	A-98239.1	UCAUCGAUCAAAGU GUGGUdTdT		A-98240.1	ACCACACUUUGAU CGAUGAdTdT	
HAMP	AD-47095	291	A-98151.1	CAUCGAUCAAAGUG UGGGAdTdT		A-98152.1	UCCCACACUUUGA UCGAUGdTdT	
HAMP	AD-47156	295	A-98243.1	GAUCAAAGUGUGGG AUGUUdTdT		A-98244.1	AACAUCCCACACU UUGAUCdTdT	
HAMP	AD-47150	295	A-98241.1	GAUCAAAGUGUGGG AUGUAdTdT		A-98242.1	UACAUCCCACACU UUGAUCdTdT	

HAMP	AD-47162	299	A-98245.1	AAAGUGUGGGAUGU GCUGUdTdT		A-98246.1	ACAGCACAUCCCA CACUUUdTdT	
HAMP	AD-47078	307	A-98067.1	GGAUGUGCUGCAAG ACGUAdTdT		A-98068.1	UACGUCUUGCAG CACAUCCdTdT	
HAMP	AD-47084	309	A-98069.1	AUGUGCUGCAAGAC GUAGAdTdT		A-98070.1	UCUACGUCUUGC AGCACAUdTdT	
HAMP	AD-47090	310	A-98071.1	UGUGCUGCAAGACG UAGAAdTdT		A-98072.1	UUCUACGUCUUG CAGCACAdTdT	
HAMP	AD-47096	313	A-98073.1	GCUGCAAGACGUAG AACCUdTdT		A-98074.1	AGGUUCUACGUC UUGCAGCdTdT	
HAMP	AD-47101	314	A-98075.1	CUGCAAGACGUAGA ACCUAdTdT		A-98076.1	UAGGUUCUACGU CUUGCAGdTdT	
HAMP	AD-47106	322	A-98077.1	CGUAGAACCUACCU GCCCUdTdT		A-98078.1	AGGGCAGGUAGG UUCUACGdTdT	
HAMP	AD-47111	347	A-98079.1	GUCCCCUCCCUUCCU UAUUdTdT		A-98080.1	AAUAAGGAAGGG AGGGGACdTdT	
HAMP	AD-47116	348	A-98081.1	UCCCCUCCCUUCCUU AUUUdTdT		A-98082.1	AAAUAAGGAAGG GAGGGGAdTdT	
HAMP	AD-47079	349	A-98083.1	CCCCUCCCUUCCUUA UUUAdTdT		A-98084.1	UAAAUAAGGAAG GGAGGGGdTdT	
HAMP	AD-47085	350	A-98085.1	CCCUCCCUUCCUUAU UUAUdTdT		A-98086.1	AUAAAUAAGGAA GGGAGGGdTdT	
HAMP	AD-47091	351	A-98087.1	CCUCCCUUCCUUAU UUAUUdTdT		A-98088.1	AAUAAAUAAGGA AGGGAGGdTdT	
HAMP	AD-47097	352	A-98089.1	CUCCCUUCCUUAUU UAUUAdTdT		A-98090.1	UAAUAAAUAAGG AAGGGAGdTdT	
HAMP	AD-47102	352	A-98091.1	CUCCCUUCCUUAUU UAUUUdTdT		A-98092.1	AAAUAAAUAAGG AAGGGAGdTdT	
HAMP	AD-47107	354	A-98093.1	CCCUUCCUUAUUUA UUCCUdTdT		A-98094.1	AGGAAUAAAUAA GGAAGGGdTdT	
HAMP	AD-47112	355	A-98095.1	CCUUCCUUAUUUAU UCCUAdTdT		A-98096.1	UAGGAAUAAAUA AGGAAGGdTdT	
HAMP	AD-47117	355	A-98097.1	CCUUCCUUAUUUAU UCCUUdTdT		A-98098.1	AAGGAAUAAAUA AGGAAGGdTdT	
HAMP	AD-47086	356	A-98101.1	CUUCCUUAUUUAUU CCUGUdTdT		A-98102.1	ACAGGAAUAAAU AAGGAAGdTdT	
HAMP	AD-47080	356	A-98099.1	CUUCCUUAUUUAUU CCUGAdTdT		A-98100.1	UCAGGAAUAAAU AAGGAAGdTdT	
HAMP	AD-47092	357	A-98103.1	UUCCUUAUUUAUUC CUGCUdTdT		A-98104.1	AGCAGGAAUAAA UAAGGAAdTdT	
HAMP	AD-47103	358	A-98107.1	UCCUUAUUUAUUCC UGCUUdTdT		A-98108.1	AAGCAGGAAUAA AUAAGGAdTdT	
HAMP	AD-47098	358	A-98105.1	UCCUUAUUUAUUCC UGCUAdTdT		A-98106.1	UAGCAGGAAUAA AUAAGGAdTdT	
HAMP	AD-47113	359	A-98111.1	CCUUAUUUAUUCCU GCUGUdTdT		A-98112.1	ACAGCAGGAAUA AAUAAGGdTdT	
HAMP	AD-47108	359	A-98109.1	CCUUAUUUAUUCCU GCUGAdTdT		A-98110.1	UCAGCAGGAAUA AAUAAGGdTdT	
HAMP	AD-47118	363	A-98113.1	AUUUAUUCCUGCUG CCCCAdTdT		A-98114.1	UGGGGCAGCAGG AAUAAAUdTdT	
HAMP	AD-47081	365	A-98115.1	UUAUUCCUGCUGCC CCAGAdTdT		A-98116.1	UCUGGGGCAGCA GGAAUAAdTdT	
HAMP	AD-47087	366	A-98117.1	UAUUCCUGCUGCCC CAGAAdTdT		A-98118.1	UUCUGGGGCAGC AGGAAUAdTdT	
HAMP	AD-47093	369	A-98119.1	UCCUGCUGCCCCAGA ACAUdTdT		A-98120.1	AUGUUCUGGGGC AGCAGGAdTdT	
HAMP	AD-47099	370	A-98121.1	CCUGCUGCCCCAGAA CAUAdTdT		A-98122.1	UAUGUUCUGGGG CAGCAGGdTdT	
HAMP	AD-47104	373	A-98123.1	GCUGCCCCAGAACAU AGGUdTdT		A-98124.1	ACCUAUGUUCUG GGGCAGCdTdT	
HAMP	AD-47109	375	A-98125.1	UGCCCCAGAACAUAG GUCUdTdT		A-98126.1	AGACCUAUGUUC UGGGGCAdTdT	
HAMP	AD-47114	376	A-98127.1	GCCCCAGAACAUAGG UCUUdTdT		A-98128.1	AAGACCUAUGUU CUGGGGCdTdT	
HAMP	AD-47119	379	A-98129.1	CCAGAACAUAGGUC UUGGAdTdT		A-98130.1	UCCAAGACCUAUG UUCUGGdTdT	
HAMP	AD-47082	380	A-98131.1	CAGAACAUAGGUCU UGGAAdTdT		A-98132.1	UUCCAAGACCUAU GUUCUGdTdT	

HAMP	AD-47088	381	A-98133.1	AGAACAUAGGUCUU GGAAUdTdT		A-98134.1	AUUCCAAGACCUA UGUUCUdTdT	
HAMP	AD-47094	382	A-98135.1	GAACAUAGGUCUUG GAAUAdTdT		A-98136.1	UAUUCCAAGACCU AUGUUCdTdT	
HAMP	AD-47094	382	A-98135.1	GAACAUAGGUCUUG GAAUAdTdT		A-98136.1	UAUUCCAAGACCU AUGUUCdTdT	
HAMP	AD-48210	382	A-100210.1	GAACACAGGUCUUG GAAUAdTdT		A-100211.1	UAUUCCAAGACCU GUGUUCdTdT	
HAMP	AD-47100	383	A-98137.1	AACAUAGGUCUUGG AAUAAdTdT		A-98138.1	UUAUUCCAAGACC UAUGUUdTdT	
HAMP	AD-47105	396	A-98139.1	GAAUAAAAUGGCUG GUUCUdTdT		A-98140.1	AGAACCAGCCAUU UUAUUCdTdT	
HAMP	AD-47110	398	A-98141.1	AUAAAAUGGCUGGU UCUUUdTdT		A-98142.1	AAAGAACCAGCCA UUUUAUdTdT	
HAMP	AD-47115	399	A-98143.1	UAAAAUGGCUGGUU CUUUUdTdT		A-98144.1	AAAAGAACCAGCC AUUUUAdTdT	
HAMP	AD-47120	402	A-98145.1	AAUGGCUGGUUCUU UUGUUdTdT		A-98146.1	AACAAAAGAACCA GCCAUUdTdT	
HAMP	AD-47083	403	A-98147.1	AUGGCUGGUUCUUU UGUUUdTdT		A-98148.1	AAACAAAAGAACC AGCCAUdTdT	
HAMP	AD-47089	407	A-98149.1	CUGGUUCUUUUGUU UUCCAdTdT		A-98150.1	UGGAAAACAAAA GAACCAGdTdT	

Table 6: HAMP single dose screen (Modified Duplexes, Dual Luciferase Assay)

Table 6								
			10 nM		0.1 nM		0.01 nM	
Target	Duplex ID	Start Position	Avg	SD	Avg	SD	Avg	SD
HAMP	AD-45073	2	107.73	0.50	92.94	7.41		
HAMP	AD-45079	7	110.26	7.14	101.78	5.79		
HAMP	AD-45085	16	90.81	0.48	96.06	2.19		
HAMP	AD-29928	43	102.01	15.80	96.01	1.70		
HAMP	AD-45674	43	94.81	4.68	108.44	7.69		
HAMP	AD-45680	43	109.80	2.04	111.06	5.64		
HAMP	AD-45686	48	89.78	15.04	110.29	0.29		
HAMP	AD-45698	48	103.33	8.83	112.53	1.57		
HAMP	AD-45692	48	110.03	6.99	115.05	0.14		
HAMP	AD-45354	51	111.45	7.56	105.64	4.49		
HAMP	AD-29929	54	99.33	11.26	104.08	6.92		
HAMP	AD-45091	55	116.71	3.20	102.27	0.81		
HAMP	AD-29930	59	88.47	0.38	102.18	7.79		
HAMP	AD-29931	60	104.54	3.36	104.80	3.55		
HAMP	AD-45704	60	142.74	0.80	122.02	1.37		
HAMP	AD-45710	60	135.87	3.55	129.05	1.72		
HAMP	AD-29932	61	103.48	6.29	108.36	1.31		
HAMP	AD-29933	62	110.13	1.03	104.36	6.68		
HAMP	AD-45675	62	113.15	1.01	107.56	0.54		
HAMP	AD-45716	62	111.06	12.39	113.09	8.16		
HAMP	AD-29934	63	101.68	3.60	96.37	6.01		
HAMP	AD-29935	64	100.63	8.13	93.98	8.75		
HAMP	AD-45687	64	103.09	8.83	105.61	3.09		
HAMP	AD-45681	64	117.87	2.59	111.72	1.69		
HAMP	AD-29936	66	98.38	12.53	98.56	13.20		
HAMP	AD-29937	67	93.41	2.34	97.50	10.28		
HAMP	AD-45699	67	47.01	9.59	98.55	3.80		
HAMP	AD-45693	67	84.68	3.15	113.79	5.11		
HAMP	AD-45711	68	113.03	9.72	108.10	3.83		
HAMP	AD-45717	68	99.40	12.84	110.38	0.04		
HAMP	AD-45705	68	110.22	3.84	117.90	9.96		
HAMP	AD-45682	69	96.60	3.60	103.41	1.06		
HAMP	AD-45688	69	100.44	9.14	104.93	5.18		
HAMP	AD-45676	69	106.83	9.15	106.73	1.89		
HAMP	AD-45360	70	92.88	0.12	93.73	2.85		
HAMP	AD-45366	71	92.46	2.58	99.04	0.39		
HAMP	AD-29938	72	62.08	21.83	75.55	6.85		
HAMP	AD-45372	73	59.85	2.76	96.31	5.86		

HAMP	AD-45700	74	12.85	5.11	63.97	14.79		
HAMP	AD-29939	74	36.40	19.57	67.18	9.10		
HAMP	AD-45694	74	17.85	4.97	90.43	0.13		
HAMP	AD-29940	75	49.83	6.31	76.05	7.08		
HAMP	AD-45712	76	32.07	2.85	63.27	3.48		
HAMP	AD-29941	76	81.10	0.03	97.49	9.32		
HAMP	AD-45706	76	43.48	6.67	97.60	1.61		
HAMP	AD-45097	88	50.62	0.50	71.18	1.94		
HAMP	AD-45103	91	53.20	9.02	96.52	7.45		
HAMP	AD-45378	116	95.96	1.21	103.17	3.99		
HAMP	AD-45383	117	99.99	2.44	104.79	5.38		
HAMP	AD-45388	118	98.52	4.10	105.96	3.21		
HAMP	AD-45393	120	103.62	5.17	102.44	6.38		
HAMP	AD-45355	121	73.28	0.51	96.56	1.06		
HAMP	AD-45361	122	98.67	0.23	99.82	4.47		
HAMP	AD-45367	123	90.48	1.28	102.75	4.07		
HAMP	AD-45373	126	106.01	8.36	99.38	4.05		
HAMP	AD-45109	132	85.86	5.55	95.06	3.75		
HAMP	AD-45115	140	100.97	1.25	90.90	9.28		
HAMP	AD-45074	142	95.53	2.12	95.37	2.74		
HAMP	AD-45677	146	58.20	5.06	80.37	5.46		
HAMP	AD-45683	146	67.80	1.12	88.08	7.96		
HAMP	AD-45718	146	76.16	3.48	100.37	3.35		
HAMP	AD-45080	149	52.89	1.28	84.16	0.02		
HAMP	AD-45379	150	48.97	3.64	103.43	1.19		
HAMP	AD-29942	151	88.17	4.85	97.49	7.95		
HAMP	AD-29943	152	43.37	9.93	73.15	23.90		
HAMP	AD-29944	153	80.38	9.90	92.54	7.85		
HAMP	AD-45695	153	65.57	2.52	92.72	5.87		
HAMP	AD-45689	153	72.67	0.78	93.00	2.67		
HAMP	AD-29945	154	69.81	13.13	76.49	17.11		
HAMP	AD-29946	155	75.80	1.18	80.39	14.15		
HAMP	AD-45713	157	70.69	1.76	94.45	0.39		
HAMP	AD-45707	157	71.62	6.17	94.94	2.22		
HAMP	AD-45701	157	79.39	1.97	101.46	0.70		
HAMP	AD-45384	159	89.86	1.67	102.53	2.37		
HAMP	AD-45389	160	41.14	0.44	90.29	3.94		
HAMP	AD-45678	161	55.04	0.96	76.03	3.63		
HAMP	AD-45719	161	55.02	5.94	84.45	2.01		
HAMP	AD-29947	161	81.45	6.55	89.78	7.92		
HAMP	AD-45690	162	105.99	3.29	97.21	2.61		
HAMP	AD-45696	162	105.48	0.08	99.13	0.28		
HAMP	AD-45684	162	96.14	6.48	104.09	2.52		
HAMP	AD-30016	163	57.89	8.28	90.60	14.06		
HAMP	AD-45394	164	87.68	5.01	108.27	2.32		
HAMP	AD-45702	165	70.60	2.02	93.12	5.12		
HAMP	AD-45708	165	74.75	3.73	98.51	2.26		
HAMP	AD-45714	165	73.26	2.24	102.34	12.47		
HAMP	AD-29949	166	102.90	8.09	91.62	0.16		
HAMP	AD-45086	167	120.81	3.27	106.79	7.19		
HAMP	AD-45356	168	81.17	4.40	93.13	0.76		
HAMP	AD-45685	169	114.45	9.16	98.53	0.41		
HAMP	AD-45679	169	105.22	9.07	101.56	5.80		
HAMP	AD-45720	169	121.03	5.25	110.57	1.75		
HAMP	AD-45703	170	44.33	1.60	79.12	4.45		
HAMP	AD-45697	170	46.91	2.65	87.12	1.48		
HAMP	AD-45691	170	54.15	1.94	92.73	6.86		
HAMP	AD-45362	189	40.88	0.51	88.62	2.54		
HAMP	AD-45368	190	31.23	1.19	95.59	2.85		
HAMP	AD-45374	199	101.82	3.03	101.10	0.65		
HAMP	AD-45092	222	87.17	5.48	98.58	1.36		
HAMP	AD-45721	228	46.67	6.31	81.09	9.13		
HAMP	AD-45715	228	49.86	3.40	88.14	4.98		
HAMP	AD-45709	228	77.17	5.09	98.27	3.31		
HAMP	AD-45380	230	62.83	3.09	103.76	1.31		
HAMP	AD-45385	231	98.28	0.86	102.61	0.12		
HAMP	AD-29950	232	55.13	8.89	67.22	10.51		

HAMP	AD-45390	233	43.22	3.42	94.61	0.86		
HAMP	AD-29951	234	37.28	9.48	53.43	13.93		
HAMP	AD-45395	235	60.56	0.93	96.88	1.63		
HAMP	AD-45727	239	41.79	5.36	73.07	5.68		
HAMP	AD-45732	239	40.15	8.90	73.60	14.88		
HAMP	AD-29952	239	97.66	18.17	104.87	4.70		
HAMP	AD-29953	240	86.68	10.48	88.35	7.38		
HAMP	AD-30017	241	33.76	16.25	60.73	30.76		
HAMP	AD-30018	242	41.44	14.83	70.97	23.75		
HAMP	AD-45737	242	17.97	4.49	71.13	9.76		
HAMP	AD-29956	246	89.56	4.13	97.82	5.97		
HAMP	AD-45357	247	82.69	2.17	93.22	4.53		
HAMP	AD-45363	248	93.32	5.91	91.24	0.20		
HAMP	AD-45747	251	70.65	8.85	97.73	2.26		
HAMP	AD-45752	251	89.51	3.39	98.35	2.19		
HAMP	AD-45757	251	82.94	6.75	102.34	3.76		
HAMP	AD-29957	252	81.99	11.58	93.00	9.35		
HAMP	AD-45399	253	64.38	0.64	97.91	2.54		
HAMP	AD-45098	255	82.48	2.01	76.84	2.07		
HAMP	AD-45400	256	41.85	0.69	73.87	3.09		
HAMP	AD-45381	257	33.48	1.75	76.90	0.24		
HAMP	AD-45401	258	20.19	1.67	47.65	3.20		
HAMP	AD-29958	261	56.65	14.92	84.66	28.04		
HAMP	AD-45391	262	24.94	0.82	89.45	2.12		
HAMP	AD-29959	267	31.48	11.63	63.85	30.99		
HAMP	AD-29960	268	79.91	12.47	93.49	6.68		
HAMP	AD-30019	270	63.27	10.61	74.99	18.30		
HAMP	AD-45396	271	119.24	2.30	111.41	3.10		
HAMP	AD-45358	272	94.71	8.18	101.07	2.21		
HAMP	AD-45364	273	84.76	0.19	96.53	5.62		
HAMP	AD-29962	274	76.15	9.81	86.71	13.14		
HAMP	AD-45370	275	72.03	3.07	96.93	2.90		
HAMP	AD-45728	276	16.69	2.26	33.05	13.53		
HAMP	AD-45722	276	14.19	2.15	36.38	8.98		
HAMP	AD-29963	276	33.66	10.79	68.80	33.38		
HAMP	AD-45104	278	68.72	2.74	87.22	0.49		
HAMP	AD-29964	279	71.02	18.87	76.03	27.29		
HAMP	AD-45738	280	50.02	8.64	70.44	7.26		
HAMP	AD-45733	280	57.29	5.28	84.47	5.76		
HAMP	AD-29965	281	55.85	8.35	72.34	23.30		
HAMP	AD-30020	283	68.86	8.88	66.02	23.24		
HAMP	AD-45748	284	21.85	1.77	35.95	6.98		
HAMP	AD-45743	284	29.01	1.73	42.99	5.69		
HAMP	AD-30021	284	42.30	7.75	66.28	27.47		
HAMP	AD-11441	285	15.04	8.59	34.60	10.87	63.42	16.67
HAMP	AD-45758	286	17.08	0.43	33.34	3.43		
HAMP	AD-45753	286	25.19	4.02	80.83	6.73		
HAMP	AD-29968	286	57.05	12.26	85.22	13.56		
HAMP	AD-29969	287	81.97	16.19	102.53	21.58		
HAMP	AD-45729	288	9.67	1.06	32.83	13.93		
HAMP	AD-45723	288	20.87	3.89	66.57	4.73		
HAMP	AD-29970	288	65.21	1.72	84.12	5.99		
HAMP	AD-45744	290	40.34	1.92	56.56	8.09		
HAMP	AD-45739	290	29.46	2.77	67.24	9.80		
HAMP	AD-45734	290	53.39	2.32	83.49	1.19		
HAMP	AD-11436	291	19.18	8.22	42.74	14.18	76.43	23.00
HAMP	AD-29971	291	29.02	9.90	52.08	14.09		
HAMP	AD-45376	292	47.54	0.51	87.50	1.26		
HAMP	AD-45382	293	37.05	0.44	93.25	4.03		
HAMP	AD-29972	294	32.08	7.08	53.51	17.12		
HAMP	AD-45754	295	30.78	4.04	62.74	0.50		
HAMP	AD-45749	295	48.49	11.81	92.97	1.08		
HAMP	AD-29973	295	101.69	8.46	97.65	8.24		
HAMP	AD-45730	296	78.23	2.00	86.13	5.69		
HAMP	AD-45724	296	82.07	4.46	86.67	2.23		
HAMP	AD-45759	296	92.10	6.68	97.71	2.40		
HAMP	AD-45110	297	69.77	2.74	90.01	2.11		

HAMP	AD-45387	298	98.34	5.75	108.20	1.85		
HAMP	AD-45740	299	124.12	4.66	101.03	6.78		
HAMP	AD-45745	299	131.69	10.22	103.23	1.76		
HAMP	AD-45735	299	111.96	3.19	103.86	6.46		
HAMP	AD-29974	300	34.04	6.78	53.98	24.23		
HAMP	AD-29975	301	53.80	12.05	67.73	22.08		
HAMP	AD-45116	306	25.93	2.21	55.49	5.82		
HAMP	AD-45075	307	19.84	2.98	63.83	1.45		
HAMP	AD-45081	309	14.66	0.55	38.67	3.25		
HAMP	AD-45087	310	11.95	0.17	29.23	1.61		
HAMP	AD-45093	313	14.07	0.68	45.44	2.30		
HAMP	AD-45099	322	108.37	1.23	107.51	2.82		
HAMP	AD-45105	369	102.93	6.40	101.31	4.27		
HAMP	AD-45111	370	117.00	3.72	104.04	2.33		
HAMP	AD-45117	373	99.33	0.61	102.95	0.52		
HAMP	AD-45076	375	62.84	4.39	90.32	1.71		
HAMP	AD-45082	376	75.58	1.59	95.17	0.21		
HAMP	AD-45088	379	83.27	12.84	101.84	1.83		
HAMP	AD-45094	380	99.51	2.23	102.51	2.51		
HAMP	AD-45100	381	112.68	6.11	107.46	7.75		
HAMP	AD-45106	383	138.19	1.98	112.49	0.89		
HAMP	AD-45112	396	128.11	6.65	106.21	3.64		
HAMP	AD-45118	398	116.86	12.51	103.78	4.53		
HAMP	AD-45077	399	98.64	1.22	103.30	2.55		
HAMP	AD-45083	402	114.82	2.26	104.50	0.17		
HAMP	AD-45089	403	107.59	7.70	103.43	1.69		

Data are expressed as percent of mock or AD-1955.

Table 7: HAMP single dose screen (Unmodified Duplexes, Human Endogenous)

Table 7								
			10 nM		0.1 nM		0.01 nM	
Target	Duplex ID	Start Position	Avg	SD	Avg	SD	Avg	SD
HAMP	AD-47121	62	22.18	1.49	60.31	16.26		
HAMP	AD-47133	67	27.53	1.12	53.58	2.92		
HAMP	AD-47127	67	20.45	4.63	54.16	7.57		
HAMP	AD-47145	74	19.51	6.65	54.67	10.88		
HAMP	AD-47139	74	20.72	0.17	58.12	2.90		
HAMP	AD-47157	76	10.07	0.35	28.24	6.49		
HAMP	AD-47151	76	12.08	1.83	33.95	2.14		
HAMP	AD-47163	132	8.58	0.51	44.65	17.97		
HAMP	AD-47122	140	25.66	0.45	72.63	2.29		
HAMP	AD-47128	146	30.88	3.04	64.36	2.04		
HAMP	AD-47134	146	48.07	0.91	72.33	12.48		
HAMP	AD-47140	155	15.20	1.25	34.69	0.30		
HAMP	AD-47152	157	13.21	6.55	28.17	1.17		
HAMP	AD-47146	157	14.77	0.68	32.02	1.58		
HAMP	AD-47158	160	9.73	1.66	32.92	1.87		
HAMP	AD-47164	161	5.71	0.44	32.90	2.89		
HAMP	AD-47123	161	7.88	3.02	39.31	19.09		
HAMP	AD-47135	162	26.84	0.87	74.06	15.78		
HAMP	AD-47129	162	27.18	1.57	83.62	13.59		
HAMP	AD-47141	242	110.80	16.98	127.17	42.39		
HAMP	AD-47147	242	116.01	9.55	132.52	28.61		
HAMP	AD-47153	253	34.69	7.47	66.88	2.08		
HAMP	AD-47159	258	33.41	1.23	57.26	7.97		
HAMP	AD-47165	261	25.12	0.71	85.70	6.86		
HAMP	AD-47124	275	36.35	7.66	87.65	11.44		
HAMP	AD-47136	276	6.06	0.70	40.72	13.94		
HAMP	AD-47130	276	8.76	0.58	46.31	13.29		
HAMP	AD-47142	278	24.10	2.89	56.75	18.44		
HAMP	AD-47148	279	19.36	1.09	57.95	18.21		
HAMP	AD-47160	280	8.75	0.73	35.24	4.98		
HAMP	AD-47154	280	15.01	3.91	36.32	0.45		
HAMP	AD-47166	281	11.98	0.47	51.40	12.88		

HAMP	AD-47125	282	14.62	1.15	54.37	11.47		
HAMP	AD-47131	283	8.74	0.45	42.66	12.21		
HAMP	AD-47137	284	9.97	0.73	36.35	5.96		
HAMP	AD-47143	284	9.66	0.84	39.72	8.37		
HAMP	AD-47149	285	13.85	0.47	47.21	9.13		
HAMP	AD-47161	286	7.28	0.89	31.75	8.03		
HAMP	AD-47155	286	8.27	0.74	36.03	14.37		
HAMP	AD-47167	287	8.98	0.14	41.61	6.60		
HAMP	AD-47132	288	9.08	0.17	38.01	4.01		
HAMP	AD-47126	288	8.59	3.66	40.28	10.49		
HAMP	AD-47138	290	41.75	1.27	81.70	14.64		
HAMP	AD-47144	290	60.81	13.34	107.58	15.10		
HAMP	AD-47095	291	34.79	5.48	58.98	7.36		
HAMP	AD-47156	295	39.09	4.26	90.08	4.38		
HAMP	AD-47150	295	53.01	9.58	99.42	9.61		
HAMP	AD-47162	299	122.90	8.44	123.74	15.71		
HAMP	AD-47078	307	26.81	9.00	59.79	15.73		
HAMP	AD-47084	309	31.16	5.91	59.33	19.95		
HAMP	AD-47090	310	15.07	5.19	49.74	11.70		
HAMP	AD-47096	313	49.34	9.86	68.64	0.71		
HAMP	AD-47101	314	13.36	5.68	38.13	10.64		
HAMP	AD-47106	322	29.91	1.99	61.30	1.25		
HAMP	AD-47111	347	21.57	4.45	46.65	10.06		
HAMP	AD-47116	348	32.95	7.34	65.00	10.06		
HAMP	AD-47079	349	10.10	2.16	24.36	4.46		
HAMP	AD-47085	350	8.08	5.13	20.39	9.47		
HAMP	AD-47091	351	20.73	6.86	42.28	5.15		
HAMP	AD-47097	352	10.57	2.97	24.58	3.18		
HAMP	AD-47102	352	15.48	7.81	25.60	8.37		
HAMP	AD-47107	354	50.89	12.39	61.80	6.52		
HAMP	AD-47112	355	42.93	6.42	53.00	3.93		
HAMP	AD-47117	355	33.82	2.18	60.78	7.57		
HAMP	AD-47086	356	16.50	3.69	34.88	9.79		
HAMP	AD-47080	356	13.76	3.39	38.95	7.09		
HAMP	AD-47092	357	35.01	5.39	48.61	6.81		
HAMP	AD-47103	358	45.09	9.10	66.18	7.81		
HAMP	AD-47098	358	63.20	11.74	70.69	1.23		
HAMP	AD-47113	359	27.42	9.95	49.88	7.22		
HAMP	AD-47108	359	30.30	9.89	52.33	12.30		
HAMP	AD-47118	363	7.45	0.35	19.20	1.31		
HAMP	AD-47081	365	4.25	1.97	22.94	6.70		
HAMP	AD-47087	366	9.49	2.18	37.51	12.04		
HAMP	AD-47093	369	4.75	1.38	23.36	3.30		
HAMP	AD-47099	370	5.05	0.01	17.71	7.66		
HAMP	AD-47104	373	32.32	8.82	37.72	8.15		
HAMP	AD-47109	375	25.45	4.46	35.56	1.25		
HAMP	AD-47114	376	10.65	4.55	17.30	6.01		
HAMP	AD-47119	379	7.99	0.50	17.44	6.45		
HAMP	AD-47082	380	13.13	1.08	27.19	8.88		
HAMP	AD-47088	381	5.80	2.75	12.26	7.13		
HAMP	AD-47094	382	5.59	2.35	11.95	7.39		
HAMP	AD-47094	382	8.63	3.05	14.02	2.30	22.83	0.56
HAMP	AD-48210	382	7.43	7.88	17.06	4.17	30.21	0.63
HAMP	AD-47100	383	3.80	2.75	8.41	3.86		
HAMP	AD-47105	396	6.56	2.25	12.11	4.90		
HAMP	AD-47110	398	10.42	5.14	21.44	0.24		
HAMP	AD-47115	399	4.86	0.27	9.25	1.57		
HAMP	AD-47120	402	5.78	0.12	15.68	0.67		
HAMP	AD-47083	403	4.36	1.88	14.49	5.26		
HAMP	AD-47089	407	17.68	1.22	21.61	6.91		

Data are expressed as percent of mock.

Table 8: HAMP single dose screen (Modified Duplexes, Human Endogenous)

Table 8						

			10 nM		0.1 nM		0.01 nM	
Target	Duplex ID	Start Position	Avg	SD	Avg	SD	Avg	SD
HAMP	AD-47031	62	15.53	6.23	42.68	4.24		
HAMP	AD-47043	67	21.87	1.62	50.05	4.67		
HAMP	AD-47037	67	23.85	4.92	53.84	0.43		
HAMP	AD-47055	74	31.38	2.06	59.08	4.48		
HAMP	AD-47049	74	30.11	3.10	64.35	3.66		
HAMP	AD-47067	76	8.71	1.38	28.60	2.87		
HAMP	AD-47061	76	11.78	3.07	29.53	0.18		
HAMP	AD-47032	140	37.89	8.04	60.90	3.74		
HAMP	AD-47038	146	33.92	2.79	53.24	7.14		
HAMP	AD-47044	146	39.99	7.45	60.74	5.99		
HAMP	AD-47050	155	14.55	1.46	35.51	0.33		
HAMP	AD-47062	157	13.42	1.10	45.34	3.82		
HAMP	AD-47056	157	23.31	0.44	46.02	0.80		
HAMP	AD-47068	160	24.68	0.67	56.12	4.66		
HAMP	AD-47033	161	11.56	4.54	36.94	0.19		
HAMP	AD-47074	161	9.99	1.07	44.47	0.95		
HAMP	AD-47039	162	63.29	2.38	80.39	15.70		
HAMP	AD-47045	162	86.89	5.22	96.60	14.33		
HAMP	AD-47057	242	66.74	2.06	82.90	10.89		
HAMP	AD-47051	242	72.68	0.12	86.34	3.07		
HAMP	AD-47063	253	26.21	0.40	58.94	7.25		
HAMP	AD-47069	258	30.01	3.26	41.02	3.03		
HAMP	AD-47075	261	30.80	2.74	75.66	1.48		
HAMP	AD-47034	275	54.15	10.01	75.48	16.26		
HAMP	AD-47046	276	13.55	0.80	30.18	6.37		
HAMP	AD-47040	276	18.09	3.87	40.15	14.54		
HAMP	AD-47058	279	36.00	4.98	64.23	1.93		
HAMP	AD-47070	280	12.74	1.13	34.84	9.02		
HAMP	AD-47064	280	17.08	0.13	49.50	0.21		
HAMP	AD-47076	281	12.07	1.81	36.35	5.58		
HAMP	AD-47035	282	31.01	7.32	61.60	1.15		
HAMP	AD-47041	283	16.92	0.64	39.03	10.75		
HAMP	AD-47053	284	10.31	0.77	23.40	7.24		
HAMP	AD-47047	284	12.12	0.18	30.96	7.74		
HAMP	AD-47059	285	20.79	0.79	45.23	6.52		
HAMP	AD-47071	286	15.36	1.48	36.67	8.67		
HAMP	AD-47065	286	19.45	0.16	53.77	19.91		
HAMP	AD-47077	287	9.85	0.40	45.43	2.39		
HAMP	AD-48208	288	9.71	4.88	14.16	3.25	40.26	4.14
HAMP	AD-47042	288	9.47	2.61	24.02	11.39		
HAMP	AD-48202	288	11.49	3.71	27.05	3.20	69.29	1.70
HAMP	AD-47036	288	10.22	1.87	38.40	8.79		
HAMP	AD-47048	290	38.00	2.44	80.14	9.40		
HAMP	AD-47054	290	46.82	5.24	87.19	6.81		
HAMP	AD-47005	291	34.54	2.08	63.87	11.34		
HAMP	AD-11436	291	43.37	7.53	74.23	14.15		
HAMP	AD-47066	295	37.84	6.67	66.36	3.03		
HAMP	AD-47060	295	52.68	4.93	83.68	16.47		
HAMP	AD-47072	299	74.58	22.86	117.51	7.68		
HAMP	AD-46988	307	39.46	7.63	78.38	1.82		
HAMP	AD-46994	309	91.00	6.12	100.96	9.30		
HAMP	AD-47000	310	42.88	7.35	65.34	6.09		
HAMP	AD-47006	313	27.81	0.36	71.03	9.01		
HAMP	AD-47011	314	24.50	4.38	63.98	14.14		
HAMP	AD-47016	322	65.73	3.26	90.84	9.34		
HAMP	AD-47021	347	80.76	0.51	86.40	9.24		
HAMP	AD-47026	348	71.64	5.09	81.58	5.61		
HAMP	AD-46989	349	90.45	10.46	99.05	11.53		
HAMP	AD-46995	350	20.68	3.39	75.89	9.25		
HAMP	AD-47001	351	74.47	1.50	80.49	21.50		
HAMP	AD-47012	352	71.82	13.01	84.03	5.63		
HAMP	AD-47007	352	82.28	15.46	89.63	9.85		
HAMP	AD-47017	354	66.26	8.83	100.80	21.89		
HAMP	AD-47022	355	63.73	4.49	87.39	9.12		
HAMP	AD-47027	355	68.87	6.64	108.08	32.59		

HAMP	AD-46996	356	37.91	1.83	48.04	6.32		
HAMP	AD-46990	356	41.87	4.92	54.43	6.70		
HAMP	AD-47002	357	16.19	0.33	42.98	3.19		
HAMP	AD-47013	358	22.95	0.97	44.27	7.76		
HAMP	AD-47008	358	20.38	2.71	50.46	16.50		
HAMP	AD-47023	359	77.40	10.39	95.51	9.29		
HAMP	AD-47018	359	95.24	14.37	97.19	8.72		
HAMP	AD-47028	363	28.25	2.86	62.93	4.48		
HAMP	AD-46991	365	15.53	2.49	29.41	0.30		
HAMP	AD-46997	366	31.51	3.85	48.07	6.21		
HAMP	AD-47003	369	9.85	2.64	34.31	6.01		
HAMP	AD-47009	370	6.69	1.11	24.11	3.57		
HAMP	AD-47014	373	55.85	3.19	60.89	10.51		
HAMP	AD-47019	375	28.54	1.87	49.45	14.83		
HAMP	AD-48214	376	12.63	3.25	16.69	1.38	29.21	0.40
HAMP	AD-48219	376	15.92	0.02	18.92	0.48	33.17	2.16
HAMP	AD-47024	376	19.61	1.81	42.20	5.93		
HAMP	AD-48224	379	22.20	5.60	33.45	1.62	52.72	1.81
HAMP	AD-48187	379	25.57	5.25	46.92	0.04	73.94	0.20
HAMP	AD-47029	379	24.31	0.26	47.37	6.54		
HAMP	AD-48192	379	19.69	0.78	55.32	4.62	88.04	0.76
HAMP	AD-46992	380	23.41	3.32	37.52	4.13		
HAMP	AD-46998	381	26.55	2.19	49.95	0.87		
HAMP	AD-48137	382	8.66	0.33	11.24	1.02	26.89	1.08
HAMP	AD-48196	382	6.92	3.59	11.81	1.33	22.33	0.98
HAMP	AD-48195	382	6.10	2.66	12.50	3.26	26.60	1.17
HAMP	AD-48201	382	12.95	1.61	13.01	2.07	25.66	6.95
HAMP	AD-48207	382	7.91	2.77	13.17	0.62	21.22	1.42
HAMP	AD-48159	382	15.26	0.13	13.59	1.45	28.89	4.67
HAMP	AD-48147	382	14.28	0.17	13.65	0.38	25.68	3.81
HAMP	AD-48161	382	9.61	3.69	13.77	1.67	22.95	0.59
HAMP	AD-48172	382	11.67	0.41	13.98	1.39	27.28	5.22
HAMP	AD-48156	382	12.14	0.96	14.06	1.75	28.85	2.18
HAMP	AD-48135	382	6.81	0.62	14.14	0.37	27.99	0.61
HAMP	AD-48136	382	10.81	4.42	14.16	1.41	29.89	1.13
HAMP	AD-48166	382	8.74	2.41	14.22	0.42	25.21	2.09
HAMP	AD-48213	382	8.35	1.61	14.49	3.71	22.38	0.33
HAMP	AD-48173	382	12.84	3.32	14.51	0.96	27.87	5.30
HAMP	AD-48154	382	9.93	2.07	14.80	0.75	23.47	0.94
HAMP	AD-48141	382	12.73	2.32	14.92	0.32	26.97	5.76
HAMP	AD-48216	382	10.39	0.95	15.18	2.11	22.38	1.02
HAMP	AD-48180	382	7.71	0.47	15.20	1.30	29.01	1.11
HAMP	AD-48143	382	7.00	2.73	15.44	1.88	24.35	2.09
HAMP	AD-48142	382	10.10	3.42	15.50	0.90	25.84	2.08
HAMP	AD-48221	382	9.54	1.43	15.56	0.24	23.59	1.06
HAMP	AD-48171	382	13.46	5.06	15.67	0.63	26.44	6.58
HAMP	AD-48145	382	10.87	0.68	15.70	2.69	28.65	2.93
HAMP	AD-48160	382	10.77	0.26	15.74	1.29	28.12	4.11
HAMP	AD-48144	382	9.88	0.46	15.75	0.94	33.60	0.84
HAMP	AD-48167	382	10.83	1.48	15.87	3.03	24.90	2.15
HAMP	AD-48177	382	15.05	2.15	15.87	0.86	28.68	8.05
HAMP	AD-48153	382	12.07	5.83	15.92	1.04	31.19	3.85
HAMP	AD-48178	382	11.02	0.05	16.06	0.45	27.12	2.27
HAMP	AD-48155	382	12.92	0.25	16.32	4.70	27.64	0.32
HAMP	AD-48174	382	11.50	2.09	16.39	0.74	27.90	4.46
HAMP	AD-48205	382	7.46	7.62	16.39	2.29	24.17	1.56
HAMP	AD-48179	382	10.80	3.42	16.50	0.71	27.82	3.57
HAMP	AD-48168	382	12.14	4.14	16.63	1.58	27.25	1.24
HAMP	AD-48149	382	10.42	0.41	16.71	3.88	28.30	1.91
HAMP	AD-48211	382	9.46	4.30	16.80	1.44	25.08	0.20
HAMP	AD-48200	382	9.05	1.30	16.97	2.20	28.99	0.38
HAMP	AD-48188	382	11.09	3.14	16.99	2.41	32.42	1.58
HAMP	AD-48183	382	9.79	11.87	17.20	0.54	42.02	0.63
HAMP	AD-48150	382	9.99	8.19	17.30	2.27	35.68	0.32
HAMP	AD-48162	382	8.48	2.96	17.38	1.26	29.63	0.85
HAMP	AD-48139	382	10.35	1.92	17.78	0.51	36.00	2.79
HAMP	AD-9942	382	8.96	1.21	18.03	1.85	30.09	0.13

HAMP	AD-48138	382	10.06	2.32	18.04	1.88	26.97	1.56
HAMP	AD-11459	382	7.07	0.09	18.93	3.13		
HAMP	AD-48189	382	8.39	0.33	19.16	0.02	26.30	0.33
HAMP	AD-48148	382	10.68	1.22	19.23	3.07	32.33	1.11
HAMP	AD-48215	382	12.87	3.87	19.50	0.69	35.59	3.70
HAMP	AD-48218	382	12.77	1.57	22.32	2.42	48.01	5.44
HAMP	AD-48135	382	13.43	1.45	26.06	4.80	57.92	1.12
HAMP	AD-47004	382	10.04	1.61	26.68	3.90		
HAMP	AD-48194	382	13.40	1.06	27.15	1.41	44.44	2.80
HAMP	AD-48197	382	13.40	6.22	27.28	1.85	47.77	5.73
HAMP	AD-11459	382	14.32	0.61	28.20	2.18	50.13	0.65
HAMP	AD-48164	382	16.52	2.52	28.92	3.09	55.94	0.41
HAMP	AD-48158	382	11.63	2.57	29.78	1.47	51.55	0.47
HAMP	AD-48204	382	11.53	13.16	29.90	1.81	68.66	2.16
HAMP	AD-48181	382	11.31	3.03	30.04	2.07	65.39	3.38
HAMP	AD-48223	382	12.35	5.46	30.39	3.74	60.92	1.91
HAMP	AD-48190	382	10.09	1.16	30.78	0.29	61.44	0.86
HAMP	AD-48163	382	13.43	2.71	31.64	4.03	60.73	1.22
HAMP	AD-48140	382	11.77	2.88	31.73	2.21	57.59	0.57
HAMP	AD-48169	382	12.50	0.13	32.00	4.43	60.20	3.22
HAMP	AD-48220	382	15.52	3.73	32.05	1.48	58.71	2.57
HAMP	AD-48184	382	13.23	0.16	33.25	3.63	56.78	2.43
HAMP	AD-48176	382	16.68	3.88	34.04	1.12	68.51	2.16
HAMP	AD-48175	382	13.60	9.49	34.34	2.07	63.89	2.17
HAMP	AD-48146	382	13.16	4.10	35.07	0.23	61.14	1.09
HAMP	AD-48182	382	13.71	8.96	36.24	0.98	71.73	0.19
HAMP	AD-48199	382	11.16	0.69	36.33	1.26	66.19	0.94
HAMP	AD-48157	382	11.27	0.12	36.54	2.61	71.05	0.88
HAMP	AD-48206	382	10.51	2.71	36.79	4.47	61.74	0.21
HAMP	AD-48193	382	13.00	3.25	37.58	2.12	73.07	0.26
HAMP	AD-48152	382	21.49	5.83	39.17	6.15	68.81	10.01
HAMP	AD-48151	382	13.62	10.83	39.31	6.55	66.68	1.47
HAMP	AD-48170	382	14.54	2.27	47.27	4.01	70.43	2.22
HAMP	AD-47010	383	41.47	1.29	64.00	4.87		
HAMP	AD-48222	385	9.49	0.13	10.82	1.20	18.78	2.86
HAMP	AD-48217	385	14.39	3.84	14.26	0.55	19.91	0.71
HAMP	AD-48185	385	14.22	4.44	18.35	1.29	37.50	0.03
HAMP	AD-48212	385	20.56	4.86	22.61	3.43	26.13	0.41
HAMP	AD-48198	396	8.31	23.06	13.10	3.95	50.72	1.39
HAMP	AD-48209	396	8.76	5.65	14.85	3.44	33.81	2.06
HAMP	AD-48203	396	9.38	2.78	15.08	2.34	35.67	2.38
HAMP	AD-47015	396	16.43	2.26	30.67	1.14		
HAMP	AD-47020	398	50.18	1.59	68.91	17.54		
HAMP	AD-47025	399	13.04	0.87	19.74	1.31		
HAMP	AD-47030	402	5.12	0.55	12.72	0.67		
HAMP	AD-46993	403	5.82	2.21	12.55	1.15		
HAMP	AD-46999	407	11.34	1.35	15.21	1.41		

Data are expressed as percent of mock.

Table 9: HAMP dose-response (Dual Luciferase, HepG2, Cyno primary hepatocytes; Unmodified & Modified duplexes)

Table 9						
				IC50 (nM)		
Target	Duplex ID	Start position	Modification status	Luc	HepG2	Cyno
HAMP	AD-29939	74	Modified	0.288		
HAMP	AD-45700	74	Modified		0.752	
HAMP	AD-29940	75	Modified	0.929		
HAMP	AD-29943	152	Modified	0.567		
HAMP	AD-29950	232	Modified	1.527		
HAMP	AD-29951	234	Modified	0.408		
HAMP	AD-30017	241	Modified	0.163		
HAMP	AD-30018	242	Modified	0.517		
HAMP	AD-29959	267	Modified	0.147		

HAMP	AD-29963	276	Modified	0.155		
HAMP	AD-45722	276	Modified		0.299	
HAMP	AD-29965	281	Modified	1.149		
HAMP	AD-30020	283	Modified	39.122		
HAMP	AD-30021	284	Modified	0.308		
HAMP	AD-11441	285	Modified	0.042	0.135	0.027
HAMP	AD-11458	285	Modified	0.358		
HAMP	AD-45729	288	Modified		0.068	0.016
HAMP	AD-48208	288	Modified		0.012	0.016
HAMP	AD-11436	291	Modified	0.054	> 10 nM	
HAMP	AD-11453	291	Modified	0.134		
HAMP	AD-29971	291	Modified	0.108		
HAMP	AD-29972	294	Modified	0.154		
HAMP	AD-29974	300	Modified	0.137		
HAMP	AD-29975	301	Modified	1.392		
HAMP	AD-45081	309	Modified		> 10 nM	
HAMP	AD-45087	310	Modified		> 10 nM	
HAMP	AD-45093	313	Modified		> 10 nM	
HAMP	AD-29979	352	Modified		> 10 nM	
HAMP	AD-45750	352	Modified		1.558	
HAMP	AD-45755	352	Modified		0.296	
HAMP	AD-45725	355	Modified		> 10 nM	
HAMP	AD-29981	357	Modified		> 10 nM	
HAMP	AD-45761	359	Modified		> 10 nM	
HAMP	AD-45377	364	Modified		> 10 nM	
HAMP	AD-29982	365	Modified		1.723	
HAMP	AD-29983	366	Modified		> 10 nM	
HAMP	AD-47099	370	Unmodified		0.017	0.081
HAMP	AD-47114	376	Unmodified		0.008	0.036
HAMP	AD-48214	376	Modified		0.008	1.575
HAMP	AD-47119	379	Unmodified		0.004	0.040
HAMP	AD-47088	381	Unmodified		0.007	>10nM
HAMP	AD-11442	382	Modified		0.028	0.010
HAMP	AD-11459	382	Unmodified		0.038	0.045
HAMP	AD-45062	382	Modified		0.088	0.030
HAMP	AD-47094	382	Unmodified		0.005	0.039
HAMP	AD-48141	382	Modified		0.004	0.023
HAMP	AD-48147	382	Modified		0.007	0.008
HAMP	AD-48154	382	Modified		0.006	0.019
HAMP	AD-48189	382	Modified		0.005	0.145
HAMP	AD-48195	382	Modified		0.011	0.009
HAMP	AD-48196	382	Modified		0.017	0.031
HAMP	AD-48201	382	Modified		0.007	0.009
HAMP	AD-48205	382	Modified		0.014	0.022
HAMP	AD-48207	382	Modified		0.007	0.017
HAMP	AD-48213	382	Modified		0.009	0.027
HAMP	AD-48216	382	Modified		0.014	0.035
HAMP	AD-47100	383	Unmodified		0.007	0.172
HAMP	AD-48217	385	Modified		0.028	0.021
HAMP	AD-47105	396	Unmodified		0.005	>10nM
HAMP	AD-48209	396	Modified		0.013	> 10nM
HAMP	AD-47115	399	Unmodified		0.007	>10nM
HAMP	AD-47030	402	Modified		0.015	>10nM
HAMP	AD-47120	402	Unmodified		0.006	>10nM
HAMP	AD-46993	403	Modified		0.015	>10nM
HAMP	AD-47083	403	Unmodified		0.007	>10nM
HAMP	AD-46999	407	Modified		0.011	>10nM

Tables 10A and 10B: Secondary Target sequences

Table 10A								
Target	Duplex ID	Start Position	Sense Name	Sense Sequence	SEQ ID NO	Antisense Name	Antisense Sequence	SEQ ID NO
HFE2	AD-47391	177	A-98855.1	AGAGuAGGGAAucAuGGcudTsdT		A-98856.1	AGCcAUGAUUCCCuACUCUdTsdT	

HFE2	AD-47397	193	A-98857.1	GcuGGAGAAuuGGAuAGcAdTsdT		A-98858.1	UGCuAUCcAAUUCUCcAGCdTsdT	
HFE2	AD-47403	195	A-98859.1	uGGAGAAuuGGAuAGcAGAdTsdT		A-98860.1	UCUGCuAUCcAAUUCUCcAdTsdT	
HFE2	AD-47409	199	A-98861.1	GAAuuGGAuAGcAGAGuAAdTsdT		A-98862.1	UuACUCUGCuAUCcAAUUCdTsdT	
HFE2	AD-47415	200	A-98863.1	AAuuGGAuAGcAGAGuAAudTsdT		A-98864.1	AUuACUCUGCuAUCcAAUUdTsdT	
HFE2	AD-47421	206	A-98865.1	AuAGcAGAGuAAuGuuuGAdTsdT		A-98866.1	UcAAAcAUuACUCUGCuAUdTsdT	
HFE2	AD-47427	211	A-98867.1	AGAGuAAuGuuuGAccucudTsdT		A-98868.1	AGAGGUcAAAcAUuACUCUdTsdT	
HFE2	AD-47433	244	A-98869.1	ucAuAuuuAAGAAcAuGcAdTsdT		A-98870.1	UGcAUGUUCUuAAAuAUGAdTsdT	
HFE2	AD-47392	257	A-98871.1	cAuGcAGGAAuGcAuuGAudTsdT		A-98872.1	AUcAAUGcAUUCCUGcAUGdTsdT	
HFE2	AD-47398	261	A-98873.1	cAGGAAuGcAuuGAucAGAdTsdT		A-98874.1	UCUGAUcAAUGcAUUCCUGdTsdT	
HFE2	AD-47404	290	A-98875.1	GGcuGAGGuGGAuAAucuudTsdT		A-98876.1	AAGAUuAUCcACCUcAGCCdTsdT	
HFE2	AD-47410	360	A-98877.1	uccAGuuuGucGAuucAAAdTsdT		A-98878.1	UUUGAAUCGAcAAACUGGAdTsdT	
HFE2	AD-47416	367	A-98879.1	uGucGAuucAAAcuGcuAAdTsdT		A-98880.1	UuAGcAGUUUGAAUCGAcAdTsdT	
HFE2	AD-47422	404	A-98881.1	GAuccAAGcuGccuAcAuudTsdT		A-98882.1	AAUGuAGGcAGCUUGGAUCdTsdT	
HFE2	AD-47428	415	A-98883.1	ccuAcAuuGGcAcAAcuAudTsdT		A-98884.1	AuAGUUGUGCcAAUGuAGGdTsdT	
HFE2	AD-47434	417	A-98885.1	uAcAuuGGcAcAAcuAuAAdTsdT		A-98886.1	UuAuAGUUGUGCcAAUGuAdTsdT	
HFE2	AD-47393	472	A-98887.1	ucAAGGuAGcAGAGGAuGudTsdT		A-98888.1	AcAUCCUCUGCuACCUUGAdTsdT	
HFE2	AD-47399	585	A-98889.1	GGAGcuAuAAccAuuGAuAdTsdT		A-98890.1	uAUcAAUGGUuAuAGCUCCdTsdT	
HFE2	AD-47405	587	A-98891.1	AGcuAuAAccAuuGAuAcudTsdT		A-98892.1	AGuAUcAAUGGUuAuAGCUdTsdT	
HFE2	AD-47417	638	A-98895.1	GGAAGAuGcuuAcuuccAudTsdT		A-98896.1	AUGGAAGuAAGcAUCUUCCdTsdT	
HFE2	AD-47423	642	A-98897.1	GAuGcuuAcuuccAuuccudTsdT		A-98898.1	AGGAAUGGAAGuAAGcAUCdTsdT	
HFE2	AD-47429	646	A-98899.1	cuuAcuuccAuuccuGuGudTsdT		A-98900.1	AcAcAGGAAUGGAAGuAAGdTsdT	
HFE2	AD-47435	656	A-98901.1	uuccuGuGucuuuGAuGuudTsdT		A-98902.1	AAcAUcAAAGAcAcAGGAAdTsdT	
HFE2	AD-47394	657	A-98903.1	uccuGuGucuuuGAuGuuudTsdT		A-98904.1	AAAcAUcAAAGAcAcAGGAdTsdT	
HFE2	AD-47400	678	A-98905.1	AuuucuGGuGAucccAAcudTsdT		A-98906.1	AGUUGGGAUcACcAGAAAUdTsdT	
HFE2	AD-47406	1121	A-98907.1	ccAuuuAcuGcAGAuuucAdTsdT		A-98908.1	UGAAAUCUGcAGuAAAUGGdTsdT	
HFE2	AD-47412	1151	A-98909.1	uuAGAGGucAuGAAGGuuudTsdT		A-98910.1	AAACCUUcAUGACCUCuAAdTsdT	
HFE2	AD-47418	1152	A-98911.1	uAGAGGucAuGAAGGuuuudTsdT		A-98912.1	AAAACCUUcAUGACCUCuAdTsdT	
HFE2	AD-47424	1203	A-98913.1	uuAAGAGGcAAGAGcuGAAdTsdT		A-98914.1	UUcAGCUCUUGCCUCUuAAdTsdT	
HFE2	AD-47430	1228	A-98915.1	AGAcAuGAucAuuAGccAudTsdT		A-98916.1	AUGGCuAAUGAUcAUGUCUdTsdT	
HFE2	AD-47436	1230	A-98917.1	AcAuGAucAuuAGccAuAAdTsdT		A-98918.1	UuAUGGCuAAUGAUcAUGUdTsdT	
HFE2	AD-47395	1233	A-98919.1	uGAucAuuAGccAuAAGAAdTsdT		A-98920.1	UUCUuAUGGCuAAUGAUcAdTsdT	
HFE2	AD-47401	1272	A-98921.1	AuuAGGGAAAGAAGucuAudTsdT		A-98922.1	AuAGACUUCUUUCCCuAAUdTsdT	
HFE2	AD-47407	1273	A-98923.1	uuAGGGAAAGAAGucuAuudTsdT		A-98924.1	AAuAGACUUCUUUCCCuAAdTsdT	
HFE2	AD-51740	1273	A-107281.4	uuAGGGAAAGAAGuCuAuUdTsdT		A-107275.3	AAuAGACUUCUUUCCCuAadTsdT	
HFE2	AD-51747	1273	A-107280.6	uuAGGGAAAGAAGucuAuUdTsdT		A-107277.2	AAuAGACUuCUuUCcCuAAdTsdT	

HFE2	AD-51744	1273	A-107281.5	uuAGGGAAAGAAGuCuAuUdTsdT		A-107276.3	AAuAGACUuCUuUCCCuAAdTsdT	
HFE2	AD-51731	1273	A-107280.2	uuAGGGAAAGAAGucuAuUdTsdT		A-107273.2	AAuAGACUUCUuUCCCuAAdTsdT	
HFE2	AD-51736	1273	A-107281.3	uuAGGGAAAGAAGuCuAuUdTsdT		A-107274.3	AAuAGACUUCUUUCcCuAAdTsdT	
HFE2	AD-51732	1273	A-107281.2	uuAGGGAAAGAAGuCuAuUdTsdT		A-107273.3	AAuAGACUUCUuUCCCuAAdTsdT	
HFE2	AD-51734	1273	A-98923.4	uuAGGGAAAGAAGucuAuudTsdT		A-107274.1	AAuAGACUUCUUUCcCuAAdTsdT	
HFE2	AD-51748	1273	A-107281.6	uuAGGGAAAGAAGuCuAuUdTsdT		A-107277.3	AAuAGACUuCUuUCcCuAAdTsdT	
HFE2	AD-51735	1273	A-107280.3	uuAGGGAAAGAAGucuAuUdTsdT		A-107274.2	AAuAGACUUCUUUCcCuAAdTsdT	
HFE2	AD-51749	1273	A-107282.6	uuAgGGAAAGAAGuCuAuUdTsdT		A-107277.4	AAuAGACUuCUuUCcCuAAdTsdT	
HFE2	AD-51752	1273	A-107281.7	uuAGGGAAAGAAGuCuAuUdTsdT		A-107278.3	AAuAGACUuCUuUCcCuAadTsdT	
HFE2	AD-51738	1273	A-98923.5	uuAGGGAAAGAAGucuAuudTsdT		A-107275.1	AAuAGACUUCUUUCCCuAadTsdT	
HFE2	AD-51730	1273	A-98923.3	uuAGGGAAAGAAGucuAuudTsdT		A-107273.1	AAuAGACUUCUuUCCCuAAdTsdT	
HFE2	AD-51745	1273	A-107282.5	uuAgGGAAAGAAGuCuAuUdTsdT		A-107276.4	AAuAGACUuCUuUCCCuAAdTsdT	
HFE2	AD-51737	1273	A-107282.3	uuAgGGAAAGAAGuCuAuUdTsdT		A-107274.4	AAuAGACUUCUUUCcCuAAdTsdT	
HFE2	AD-51743	1273	A-107280.5	uuAGGGAAAGAAGucuAuUdTsdT		A-107276.2	AAuAGACUuCUuUCCCuAAdTsdT	
HFE2	AD-51751	1273	A-107280.7	uuAGGGAAAGAAGucuAuUdTsdT		A-107278.2	AAuAGACUuCUuUCcCuAadTsdT	
HFE2	AD-51750	1273	A-98923.8	uuAGGGAAAGAAGucuAuudTsdT		A-107278.1	AAuAGACUuCUuUCcCuAadTsdT	
HFE2	AD-51741	1273	A-107282.4	uuAgGGAAAGAAGuCuAuUdTsdT		A-107275.4	AAuAGACUUCUUUCCCuAadTsdT	
HFE2	AD-51742	1273	A-98923.6	uuAGGGAAAGAAGucuAuudTsdT		A-107276.1	AAuAGACUuCUuUCCCuAAdTsdT	
HFE2	AD-51733	1273	A-107282.2	uuAgGGAAAGAAGuCuAuUdTsdT		A-107273.4	AAuAGACUUCUuUCCCuAAdTsdT	
HFE2	AD-51755	1273	A-107280.8	uuAGGGAAAGAAGucuAuUdTsdT		A-107279.2	AAUAGACUuCUuUCcCuAadTsdT	
HFE2	AD-51756	1273	A-107281.8	uuAGGGAAAGAAGuCuAuUdTsdT		A-107279.3	AAUAGACUuCUuUCcCuAadTsdT	
HFE2	AD-51728	1273	A-107281.1	uuAGGGAAAGAAGuCuAuUdTsdT		A-107272.3	AAuAGACUuCUUUCCCuAAdTsdT	
HFE2	AD-51729	1273	A-107282.1	uuAgGGAAAGAAGuCuAuUdTsdT		A-107272.4	AAuAGACUuCUUUCCCuAAdTsdT	
HFE2	AD-51726	1273	A-98923.2	uuAGGGAAAGAAGucuAuudTsdT		A-107272.1	AAuAGACUuCUUUCCCuAAdTsdT	
HFE2	AD-51746	1273	A-98923.7	uuAGGGAAAGAAGucuAuudTsdT		A-107277.1	AAuAGACUuCUuUCcCuAAdTsdT	

HFE2	AD-51757	1273	A-107282.8	uuAgGGAAAGAAGuCuAuUdTsdT		A-107279.4	AAUAGACUuCUuUCcCuAadTsdT	
HFE2	AD-51727	1273	A-107280.1	uuAGGGAAAGAAGucuAuUdTsdT		A-107272.2	AAuAGACUuCUUUCCCuAAdTsdT	
HFE2	AD-51753	1273	A-107282.7	uuAgGGAAAGAAGuCuAuUdTsdT		A-107278.4	AAuAGACUuCUuUCcCuAadTsdT	
HFE2	AD-51754	1273	A-98923.9	uuAGGGAAAGAAGucuAuudTsdT		A-107279.1	AAUAGACUuCUuUCcCuAadTsdT	
HFE2	AD-51739	1273	A-107280.4	uuAGGGAAAGAAGucuAuUdTsdT		A-107275.2	AAuAGACUUCUUUCCCuAadTsdT	
HFE2	AD-47413	1274	A-98925.1	uAGGGAAAGAAGucuAuuudTsdT		A-98926.1	AAAuAGACUUCUUUCCCuAdTsdT	
HFE2	AD-47419	1279	A-98927.1	AAAGAAGucuAuuuGAuGAdTsdT		A-98928.1	UcAUcAAAuAGACUUCUUUdTsdT	
HFE2	AD-47425	1280	A-98929.1	AAGAAGucuAuuuGAuGAAdTsdT		A-98930.1	UUcAUcAAAuAGACUUCUUdTsdT	
HFE2	AD-47431	1303	A-98931.1	uGuGuGuAAGGuAuGuucudTsdT		A-98932.1	AGAAcAuACCUuAcAcAcAdTsdT	
HFE2	AD-47437	1366	A-98933.1	GuGAAGGGAGucucuGcuudTsdT		A-98934.1	AAGcAGAGACUCCCUUcACdTsdT	
HFE2	AD-47396	1367	A-98935.1	uGAAGGGAGucucuGcuuudTsdT		A-98936.1	AAAGcAGAGACUCCCUUcAdTsdT	
HFE2	AD-47402	1396	A-98937.1	cAcAGGuAGGAcAGAAGuAdTsdT		A-98938.1	uACUUCUGUCCuACCUGUGdTsdT	
HFE2	AD-47408	1397	A-98939.1	AcAGGuAGGAcAGAAGuAudTsdT		A-98940.1	AuACUUCUGUCCuACCUGUdTsdT	
HFE2	AD-47414	1399	A-98941.1	AGGuAGGAcAGAAGuAucAdTsdT		A-98942.1	UGAuACUUCUGUCCuACCUdTsdT	
HFE2	AD-47420	1400	A-98943.1	GGuAGGAcAGAAGuAucAudTsdT		A-98944.1	AUGAuACUUCUGUCCuACCdTsdT	
HFE2	AD-47426	1404	A-98945.1	GGAcAGAAGuAucAuccuudTsdT		A-98946.1	AGGGAUGAuACUUCUGUCCdTsdT	
HFE2	AD-47432	1441	A-98947.1	uAuuAAAGcuAcAAAuucudTsdT		A-98948.1	AGAAUUUGuAGCUUuAAuAdTsdT	

It should be noted that unmodified versions of each of the modified sequences shown are included within the scope of the invention.

Table 10B						
Target	Duplex ID	Start Position	Sense Name	Sense Sequence	Antisense Name	Antisense Sequence
TFR2	AD-47814	64	A-99594.1	uccAGAGAGcGcAAcAAcudTsdT	A-99595.1	AGUUGUUGCGCUCUCUGGAdTsdT
TFR2	AD-47820	66	A-99596.1	cAGAGAGcGcAAcAAcuGudTsdT	A-99597.1	AcAGUUGUUGCGCUCUCUGdTsdT
TFR2	AD-47826	239	A-99598.1	cAGGcAGccAAAccucAuudTsdT	A-99599.1	AAUGAGGUUUGGCUGCCUGdTsdT
TFR2	AD-47819	772	A-99674.1	AGcuGGuGuAcGcccAcuAdTsdT	A-99675.1	uAGUGGGCGuAcACcAGCUdTsdT
TFR2	AD-47832	884	A-99600.1	ccAGAAGGuGAccAAuGcudTsdT	A-99601.1	AGcAUUGGUcACCUUCUGGdTsdT
TFR2	AD-47838	886	A-99602.1	AGAAGGuGAccAAuGcucAdTsdT	A-99603.1	UGAGcAUUGGUcACCUUCUdTsdT
TFR2	AD-47844	915	A-99604.1	GcucAAGGAGuGcucAuAudTsdT	A-99605.1	AuAUGAGcACUCCUUGAGCdTsdT
TFR2	AD-47849	916	A-99606.1	cucAAGGAGuGcucAuAuAdTsdT	A-99607.1	uAuAUGAGcACUCCUUGAGdTsdT
TFR2	AD-47854	920	A-99608.1	AGGAGuGcucAuAuAcccAdTsdT	A-99609.1	UGGGuAuAUGAGcACUCCUdTsdT
TFR2	AD-47815	922	A-99610.1	GAGuGcucAuAuAcccAGAdTsdT	A-99611.1	UCUGGGuAuAUGAGcACUCdTsdT
TFR2	AD-47825	1004	A-99676.1	AcAuGuGcAccuGGGAAcudTsdT	A-99677.1	AGUUCCcAGGUGcAcAUGUdTsdT
TFR2	AD-47821	1048	A-99612.1	cuuccuucAAucAAAcccAdTsdT	A-99613.1	UGGGUUUGAUUGAAGGAAGdTsdT
TFR2	AD-	1050	A-	uccuucAAucAAAcccAGudTsdT	A-	ACUGGGUUUGAUUGAAGGAdTsdT

	47827		99614.1		99615.1	
TFR2	AD-47833	1051	A-99616.1	ccuucAAucAAAcccAGuudTsdT	A-99617.1	AACUGGGUUUGAUUGAAGGdTsdT
TFR2	AD-51696	1051	A-107271.3	ccuucAAucAAAcccAGuUdTsdT	A-107257.2	AACUGGGUUUGAuUGAAGGdTsdT
TFR2	AD-51708	1051	A-107271.5	ccuucAAucAAAcccAGuUdTsdT	A-107259.2	AACUGGGUuUGAuUGAAGGdTsdT
TFR2	AD-51700	1051	A-99616.5	ccuucAAucAAAcccAGuudTsdT	A-107258.1	AACUGGGUuUGAUUGAAGGdTsdT
TFR2	AD-51701	1051	A-99616.13	ccuucAAucAAAcccAGuudTsdT	A-107266.1	AACuGGGUuUGAUUGAAGGdTsdT
TFR2	AD-51702	1051	A-107271.4	ccuucAAucAAAcccAGuUdTsdT	A-107258.2	AACUGGGUuUGAUUGAAGGdTsdT
TFR2	AD-51707	1051	A-99616.14	ccuucAAucAAAcccAGuudTsdT	A-107267.1	AACuGGGUuUGAuUGAAGGdTsdT
TFR2	AD-51694	1051	A-99616.4	ccuucAAucAAAcccAGuudTsdT	A-107257.1	AACUGGGUUUGAuUGAAGGdTsdT
TFR2	AD-51706	1051	A-99616.6	ccuucAAucAAAcccAGuudTsdT	A-107259.1	AACUGGGUuUGAuUGAAGGdTsdT
TFR2	AD-51695	1051	A-99616.12	ccuucAAucAAAcccAGuudTsdT	A-107265.1	AACuGGGUUUGAuUGAAGGdTsdT
TFR2	AD-51713	1051	A-99616.15	ccuucAAucAAAcccAGuudTsdT	A-107268.1	AACuGGGUuUGAuuGAAGGdTsdT
TFR2	AD-51714	1051	A-107271.6	ccuucAAucAAAcccAGuUdTsdT	A-107260.2	AACUGGGUuUGAuuGAAGGdTsdT
TFR2	AD-51683	1051	A-99616.10	ccuucAAucAAAcccAGuudTsdT	A-107263.1	AACuGGGUUUGAUUGAAGgdTsdT
TFR2	AD-51712	1051	A-99616.7	ccuucAAucAAAcccAGuudTsdT	A-107260.1	AACUGGGUuUGAuuGAAGGdTsdT
TFR2	AD-51720	1051	A-107271.7	ccuucAAucAAAcccAGuUdTsdT	A-107261.2	AACUGGGUuUGAuuGAAGgdTsdT
TFR2	AD-51719	1051	A-99616.16	ccuucAAucAAAcccAGuudTsdT	A-107269.1	AACuGGGUuUGAuuGAAGgdTsdT
TFR2	AD-51684	1051	A-107271.1	ccuucAAucAAAcccAGuUdTsdT	A-107255.2	AACUGGGUUUGAUUGAAGgdTsdT
TFR2	AD-51690	1051	A-107271.2	ccuucAAucAAAcccAGuUdTsdT	A-107256.2	AACUGGGUUUGAUuGAAGGdTsdT
TFR2	AD-51689	1051	A-99616.11	ccuucAAucAAAcccAGuudTsdT	A-107264.1	AACuGGGUUUGAUuGAAGGdTsdT
TFR2	AD-51682	1051	A-99616.2	ccuucAAucAAAcccAGuudTsdT	A-107255.1	AACUGGGUUUGAUUGAAGgdTsdT
TFR2	AD-51688	1051	A-99616.3	ccuucAAucAAAcccAGuudTsdT	A-107256.1	AACUGGGUUUGAUuGAAGGdTsdT
TFR2	AD-51718	1051	A-99616.8	ccuucAAucAAAcccAGuudTsdT	A-107261.1	AACUGGGUuUGAuuGAAGgdTsdT
TFR2	AD-51725	1051	A-99616.17	ccuucAAucAAAcccAGuudTsdT	A-107270.1	AACuGGGUuUGAUuGAAGgdTsdT
TFR2	AD-51724	1051	A-99616.9	ccuucAAucAAAcccAGuudTsdT	A-107262.1	AACUGGGUuUGAUuGAAGgdTsdT
TFR2	AD-47839	1067	A-99618.1	GuuccccuccAGuuGcAucAdTsdT	A-99619.1	UGAUGcAACUGGAGGGAACdTsdT
TFR2	AD-47845	1068	A-99620.1	uuccccuccAGuuGcAucAudTsdT	A-99621.1	AUGAUGcAACUGGAGGGAAdTsdT
TFR2	AD-47850	1299	A-99622.1	cGccucAGAGccAGAucAcudTsdT	A-99623.1	AGUGAUCUGGCUCUGAGCGdTsdT
TFR2	AD-47855	1355	A-99624.1	AGGAGcAGccuAAAuccGcudTsdT	A-99625.1	AGCGGAUUuAGCUGCUCCUdTsdT
TFR2	AD-47816	1441	A-99626.1	cccGcAGAAGucuccucuudTsdT	A-99627.1	AAGAGGAGACUUCUGCGGGdTsdT
TFR2	AD-47831	1548	A-99678.1	GuGuAcGuGAGccuGGAcAdTsdT	A-99679.1	UGUCcAGGCUcACGuAcACdTsdT
TFR2	AD-47822	1584	A-99628.1	GAcAAGuuucAuGccAAGAdTsdT	A-99629.1	UCUUGGcAUGAAACUUGUCdTsdT
TFR2	AD-47828	1612	A-99630.1	uucuGAcAAGucucAuuGAdTsdT	A-99631.1	UcAAUGAGACUUGUcAGAAdTsdT
TFR2	AD-47834	1614	A-99632.1	cuGAcAAGucucAuuGAGAdTsdT	A-99633.1	UCUcAAUGAGACUUGUcAGdTsdT
TFR2	AD-47840	1616	A-99634.1	GAcAAGucucAuuGAGAGudTsdT	A-99635.1	ACUCUcAAUGAGACUUGUCdTsdT
TFR2	AD-47846	1618	A-99636.1	cAAGucucAuuGAGAGuGudTsdT	A-99637.1	AcACUCUcAAUGAGACUUGdTsdT
TFR2	AD-47851	2140	A-99638.1	AGcGAcuGAcAcGcAuGuAdTsdT	A-99639.1	uAcAUGCGUGUcAGUCGCUdTsdT

TFR2	AD-47856	2142	A-99640.1	cGAcuGAcAcGcAuGuAcAdTsdT	A-99641.1	UGuAcAUGCGUGUcAGUCGdTsdT
TFR2	AD-47817	2143	A-99642.1	GAcuGAcAcGcAuGuAcAAdTsdT	A-99643.1	UUGuAcAUGCGUGUcAGUCdTsdT
TFR2	AD-47823	2146	A-99644.1	uGAcAcGcAuGuAcAAcGudTsdT	A-99645.1	ACGUUGuAcAUGCGUGUcAdTsdT
TFR2	AD-47837	2151	A-99680.1	cGcAuGuAcAAcGuGcGcAdTsdT	A-99681.1	UGCGcACGUUGuAcAUGCGdTsdT
TFR2	AD-47843	2152	A-99682.1	GcAuGuAcAAcGuGcGcAudTsdT	A-99683.1	AUGCGcACGUUGuAcAUGCdTsdT
TFR2	AD-47829	2154	A-99646.1	AuGuAcAAcGuGcGcAuAAdTsdT	A-99647.1	UuAUGCGcACGUUGuAcAUdTsdT
TFR2	AD-47835	2155	A-99648.1	uGuAcAAcGuGcGcAuAAudTsdT	A-99649.1	AUuAUGCGcACGUUGuAcAdTsdT
TFR2	AD-47841	2170	A-99650.1	uAAuGcGGGuGGAGuucuAdTsdT	A-99651.1	uAGAACUCcACCCGcAUuAdTsdT
TFR2	AD-51703	2170	A-99650.6	uAAuGcGGGuGGAGuucuAdTsdT	A-107249.1	UAGAACuCcACCCGcAUuAdTsdT
TFR2	AD-51710	2170	A-107254.2	uAAuGcGGGuGGAGuUCuAdTsdT	A-107246.3	UAGAACUCcACCCGcAuuAdTsdT
TFR2	AD-51697	2170	A-99650.5	uAAuGcGGGuGGAGuucuAdTsdT	A-107248.1	UAGAACUCcACCCGcAuuadTsdT
TFR2	AD-51692	2170	A-107253.3	uAAuGcGGGuGGAGuuCuAdTsdT	A-107247.2	UAGAACUCcACCCGcAUuadTsdT
TFR2	AD-51685	2170	A-99650.3	uAAuGcGGGuGGAGuucuAdTsdT	A-107246.1	UAGAACUCcACCCGcAuuAdTsdT
TFR2	AD-51691	2170	A-99650.4	uAAuGcGGGuGGAGuucuAdTsdT	A-107247.1	UAGAACUCcACCCGcAUuadTsdT
TFR2	AD-51698	2170	A-107253.4	uAAuGcGGGuGGAGuuCuAdTsdT	A-107248.2	UAGAACUCcACCCGcAuuadTsdT
TFR2	AD-51686	2170	A-107253.2	uAAuGcGGGuGGAGuuCuAdTsdT	A-107246.2	UAGAACUCcACCCGcAuuAdTsdT
TFR2	AD-51709	2170	A-99650.7	uAAuGcGGGuGGAGuucuAdTsdT	A-107250.1	UAGAACuCcACCCGcAuuAdTsdT
TFR2	AD-51679	2170	A-99650.2	uAAuGcGGGuGGAGuucuAdTsdT	A-107245.1	UAGAACUCcACCCGcAUuAdTsdT
TFR2	AD-51705	2170	A-107254.5	uAAuGcGGGuGGAGuUCuAdTsdT	A-107249.3	UAGAACuCcACCCGcAUuAdTsdT
TFR2	AD-51704	2170	A-107254.1	uAAuGcGGGuGGAGuUCuAdTsdT	A-107245.3	UAGAACUCcACCCGcAUuAdTsdT
TFR2	AD-51687	2170	A-107253.6	uAAuGcGGGuGGAGuuCuAdTsdT	A-107250.2	UAGAACuCcACCCGcAuuAdTsdT
TFR2	AD-51681	2170	A-107253.5	uAAuGcGGGuGGAGuuCuAdTsdT	A-107249.2	UAGAACuCcACCCGcAUuAdTsdT
TFR2	AD-51716	2170	A-107254.3	uAAuGcGGGuGGAGuUCuAdTsdT	A-107247.3	UAGAACUCcACCCGcAUuadTsdT
TFR2	AD-51693	2170	A-107253.7	uAAuGcGGGuGGAGuuCuAdTsdT	A-107251.2	UAGAACuCcACCCGcAUuadTsdT
TFR2	AD-51711	2170	A-107254.6	uAAuGcGGGuGGAGuUCuAdTsdT	A-107250.3	UAGAACuCcACCCGcAuuAdTsdT
TFR2	AD-51699	2170	A-107253.8	uAAuGcGGGuGGAGuuCuAdTsdT	A-107252.2	UAGAACuCcACCCGcAuuadTsdT
TFR2	AD-51722	2170	A-107254.4	uAAuGcGGGuGGAGuUCuAdTsdT	A-107248.3	UAGAACUCcACCCGcAuuadTsdT
TFR2	AD-51715	2170	A-99650.8	uAAuGcGGGuGGAGuucuAdTsdT	A-107251.1	UAGAACuCcACCCGcAUuadTsdT
TFR2	AD-51680	2170	A-107253.1	uAAuGcGGGuGGAGuuCuAdTsdT	A-107245.2	UAGAACUCcACCCGcAUuAdTsdT
TFR2	AD-51717	2170	A-107254.7	uAAuGcGGGuGGAGuUCuAdTsdT	A-107251.3	UAGAACuCcACCCGcAUuadTsdT
TFR2	AD-51723	2170	A-107254.8	uAAuGcGGGuGGAGuUCuAdTsdT	A-107252.3	UAGAACuCcACCCGcAuuadTsdT
TFR2	AD-51721	2170	A-99650.9	uAAuGcGGGuGGAGuucuAdTsdT	A-107252.1	UAGAACuCcACCCGcAuuadTsdT
TFR2	AD-47847	2178	A-99652.1	GuGGAGuucuAcuuccuuudTsdT	A-99653.1	AAAGGAAGuAGAACUCcACdTsdT
TFR2	AD-47852	2224	A-99654.1	cGuuccGccAcAucuucAudTsdT	A-99655.1	AUGAAGAUGUGGCGGAACGdTsdT
TFR2	AD-47857	2425	A-99656.1	GGAAcAuuGAuAAcAAcuudTsdT	A-99657.1	AAGUUGUuAUcAAUGUUCCdTsdT
TFR2	AD-47818	2602	A-99658.1	cAGcAcAGAuAuccAcAcAdTsdT	A-99659.1	UGUGUGGAuAUCUGUGCUGdTsdT
TFR2	AD-47824	2656	A-99660.1	GGucAuAcuGucGGuuAAudTsdT	A-99661.1	AUuAACCGAcAGuAUGACCdTsdT

TFR2	AD-47830	2658	A-99662.1	ucAuAcuGucGGuuAAucAdTsdT	A-99663.1	UGAUuAACCGAcAGuAUGAdTsdT
TFR2	AD-47836	2660	A-99664.1	AuAcuGucGGuuAAucAGAdTsdT	A-99665.1	UCUGAUuAACCGAcAGuAUdTsdT
TFR2	AD-47842	2662	A-99666.1	AcuGucGGuuAAucAGAGAdTsdT	A-99667.1	UCUCUGAUuAACCGAcAGUdTsdT
TFR2	AD-47848	2719	A-99668.1	GGuccuccAuAccuAGAGAdTsdT	A-99669.1	UCUCuAGGuAUGGAGGACCdTsdT
TFR2	AD-47853	2795	A-99670.1	ucGcuGGcAccAuAGccuudTsdT	A-99671.1	AAGGCuAUGGUGCcAGCGAdTsdT
TFR2	AD-47858	2802	A-99672.1	cAccAuAGccuuAuGGccAdTsdT	A-99673.1	UGGCcAuAAGGCuAUGGUGdTsdT
It should be noted that unmodified versions of each of the modified sequences shown are included within the scope of the invention.						

Table 11: Secondary Target single-dose

Table 11									
				10nM overall		0.1 nM overall		0.01 nM overall	
Target	Reactivity	Duplex Name	Start Position	Avg	SD	Avg	SD	Avg	SD
HFE2	Human	AD-47391	177	97.5	10.8	111.9	21.2		
HFE2	Human	AD-47397	193	27.3	4.2	36.9	3.3		
HFE2	Human	AD-47403	195	31.2	10.0	48.6	7.6		
HFE2	Human	AD-47409	199	82.3	15.8	89.5	11.4		
HFE2	Human	AD-47415	200	44.8	5.9	51.1	7.0		
HFE2	Human	AD-47421	206	27.8	8.1	28.8	0.9		
HFE2	Human	AD-47427	211	96.4	25.8	79.8	18.2		
HFE2	Human	AD-47433	244	7.5	1.3	21.2	4.0		
HFE2	Human	AD-47392	257	8.0	2.0	20.5	8.1		
HFE2	Human	AD-47398	261	30.0	6.5	45.9	6.4		
HFE2	Human	AD-47404	290	9.3	2.8	20.5	0.5		
HFE2	Human	AD-47410	360	28.7	9.8	36.7	1.8		
HFE2	Human	AD-47416	367	72.3	12.5	79.2	19.3		
HFE2	Human	AD-47422	404	20.4	2.5	35.4	2.5		
HFE2	Human	AD-47428	415	66.8	22.5	80.6	11.4		
HFE2	Human	AD-47434	417	34.7	5.9	28.6	3.4		
HFE2	Human	AD-47393	472	96.3	9.7	99.8	31.3		
HFE2	Human	AD-47399	585	10.0	6.6	16.3	0.6		
HFE2	Human	AD-47405	587	11.3	2.1	14.0	0.4		
HFE2	Human	AD-47417	638	39.3	2.0	62.6	7.6		
HFE2	Human	AD-47423	642	109.4	4.1	58.5	0.9		
HFE2	Human	AD-47429	646	56.0	13.0	76.3	21.8		
HFE2	Human	AD-47435	656	17.7	1.4	29.3	9.4		
HFE2	Human	AD-47394	657	8.8	7.3	9.8	6.3		
HFE2	Human	AD-47400	678	21.2	2.8	25.1	8.4		
HFE2	Human	AD-47406	1121	12.9	1.4	20.5	1.3		
HFE2	Human	AD-47412	1151	16.5	5.2	11.8	3.2		
HFE2	Human	AD-47418	1152	16.0	1.6	8.4	2.2		
HFE2	Human	AD-47424	1203	9.2	1.6	14.0	2.4		
HFE2	Human	AD-47430	1228	14.8	2.7	19.2	0.9		
HFE2	Human	AD-47436	1230	17.9	9.6	19.7	1.4		
HFE2	Human	AD-47395	1233	15.3	2.1	12.7	2.2		
HFE2	Human	AD-47401	1272	6.3	1.2	10.5	0.9		
HFE2	Human	AD-47407	1273	5.6	1.8	5.6	0.8		
HFE2	Human	AD-51740	1273	5.7	0.0	6.5	0.7	6.3	0.4
HFE2	Human	AD-51747	1273	7.1	1.6	6.0	0.1	7.0	0.1
HFE2	Human	AD-51744	1273	11.8	5.8	18.4	14.1	7.7	0.0
HFE2	Human	AD-51731	1273	6.2	0.7	7.1	0.2	8.1	3.2
HFE2	Human	AD-51736	1273	6.3	0.3	7.2	0.7	8.2	0.5
HFE2	Human	AD-51732	1273	6.0	1.0	8.2	0.5	8.3	0.6
HFE2	Human	AD-51734	1273	6.9	0.3	14.5	13.3	8.4	1.4
HFE2	Human	AD-51748	1273	6.6	0.2	7.7	0.9	8.5	1.3
HFE2	Human	AD-51735	1273	6.4	1.5	6.3	0.3	8.5	0.7
HFE2	Human	AD-51749	1273	6.8	1.0	8.3	0.4	8.7	2.2
HFE2	Human	AD-51752	1273	12.7	6.4	10.3	3.6	8.8	1.0
HFE2	Human	AD-51738	1273	5.8	0.6	9.2	3.0	8.9	1.4
HFE2	Human	AD-51730	1273	7.6	1.7	7.8	1.0	9.3	0.5
HFE2	Human	AD-51745	1273	5.8	0.4	6.5	1.6	9.5	1.2

HFE2	Human	AD-51737	1273	5.9	0.1	19.8	18.4	9.6	1.3
HFE2	Human	AD-51743	1273	6.5	1.6	7.0	1.5	9.9	2.0
HFE2	Human	AD-51751	1273	6.4	1.4	7.5	1.6	10.3	1.6
HFE2	Human	AD-51750	1273	6.9	0.2	8.8	0.3	10.7	1.0
HFE2	Human	AD-51741	1273	6.0	2.1	8.5	1.1	10.8	4.0
HFE2	Human	AD-51742	1273	7.0	1.0	6.1	0.2	11.0	0.9
HFE2	Human	AD-51733	1273	6.7	1.1	7.2	0.1	11.0	1.3
HFE2	Human	AD-51755	1273	6.1	0.8	13.4	6.9	11.2	2.8
HFE2	Human	AD-51756	1273	9.8	0.3	8.9	0.4	11.6	0.3
HFE2	Human	AD-51728	1273	7.1	0.8	8.2	0.2	11.6	0.6
HFE2	Human	AD-51729	1273	6.8	1.2	8.9	0.4	11.7	0.5
HFE2	Human	AD-51726	1273	7.1	0.6	9.0	1.0	12.6	2.4
HFE2	Human	AD-51746	1273	7.3	1.4	14.9	6.0	12.6	5.5
HFE2	Human	AD-51757	1273	9.1	2.0	10.4	0.6	13.1	1.9
HFE2	Human	AD-51727	1273	6.7	1.0	8.5	1.1	13.8	0.4
HFE2	Human	AD-51753	1273	7.2	0.3	13.6	8.4	14.2	7.8
HFE2	Human	AD-51754	1273	6.9	0.4	10.1	1.0	14.7	2.8
HFE2	Human	AD-51739	1273	6.1	0.1	8.2	0.1	14.8	8.9
HFE2	Human	AD-47413	1274	7.2	0.2	6.4	0.9		
HFE2	Human	AD-47419	1279	8.6	2.3	10.0	2.2		
HFE2	Human	AD-47425	1280	14.5	1.0	14.1	0.8		
HFE2	Human	AD-47431	1303	49.5	0.6	72.2	0.7		
HFE2	Human	AD-47437	1366	6.4	4.2	11.4	2.4		
HFE2	Human	AD-47396	1367	4.6	0.1	10.0	0.2		
HFE2	Human	AD-47402	1396	11.8	0.2	19.9	4.4		
HFE2	Human	AD-47408	1397	12.0	3.4	13.7	0.2		
HFE2	Human	AD-47414	1399	5.6	1.5	8.2	0.1		
HFE2	Human	AD-47420	1400	3.6	1.0	5.7	0.8		
HFE2	Human	AD-47426	1404	13.7	3.8	27.1	3.1		
HFE2	Human	AD-47432	1441	3.8	0.0	5.6	1.0		
TFR2	Human	AD-47814	64	7.8	0.4	16.3	0.1		
TFR2	Human	AD-47820	66	13.7	2.5	25.1	3.7		
TFR2	Human	AD-47826	239	13.5	1.8	25.4	4.3		
TFR2	Human	AD-47819	772	112.4	2.9	102.9	3.8		
TFR2	Human	AD-47832	884	24.2	1.8	52.4	2.7		
TFR2	Human	AD-47838	886	23.6	0.4	39.0	1.6		
TFR2	Human	AD-47844	915	19.5	3.9	40.9	4.5		
TFR2	Human	AD-47849	916	14.2	6.9	22.8	0.5		
TFR2	Human	AD-47854	920	69.4	4.2	88.3	0.8		
TFR2	Human	AD-47815	922	66.3	6.7	71.2	8.8		
TFR2	Human	AD-47825	1004	23.9	2.9	46.2	3.8		
TFR2	Human	AD-47821	1048	57.4	15.9	78.5	5.0		
TFR2	Human	AD-47827	1050	18.9	8.3	37.9	2.9		
TFR2	Human	AD-47833	1051	8.3	4.3	19.7	5.4		
TFR2	Human	AD-51696	1051	8.0	2.1	21.1	2.1	27.2	0.5
TFR2	Human	AD-51708	1051	8.8	1.2	17.7	0.8	28.5	3.7
TFR2	Human	AD-51700	1051	9.3	1.2	19.8	3.7	30.1	5.0
TFR2	Human	AD-51701	1051	9.4	0.6	22.3	8.1	30.8	2.7
TFR2	Human	AD-51702	1051	8.7	2.1	19.7	0.1	30.9	1.4
TFR2	Human	AD-51707	1051	8.1	2.5	19.1	2.6	32.2	8.2
TFR2	Human	AD-51694	1051	9.3	1.9	19.3	2.5	38.8	0.0
TFR2	Human	AD-51706	1051	8.4	0.3	19.5	1.5	39.9	6.9
TFR2	Human	AD-51695	1051	10.1	1.6	19.9	2.4	40.1	4.2
TFR2	Human	AD-51713	1051	14.6	1.8	45.3	2.5	59.0	1.6
TFR2	Human	AD-51714	1051	22.1	0.1	44.2	1.5	62.8	1.7
TFR2	Human	AD-51683	1051	9.7	0.6	36.5	2.4	66.0	2.4
TFR2	Human	AD-51712	1051	21.2	2.6	44.1	4.5	67.1	5.9
TFR2	Human	AD-51720	1051	34.5	6.1	58.3	10.4	67.4	0.0
TFR2	Human	AD-51719	1051	38.6	1.2	57.7	1.2	68.8	3.2
TFR2	Human	AD-51684	1051	14.7	3.5	48.3	1.9	69.3	3.5
TFR2	Human	AD-51690	1051	19.7	0.0	49.8	0.5	74.1	12.6
TFR2	Human	AD-51689	1051	40.5	2.4	53.1	9.7	75.0	6.4
TFR2	Human	AD-51682	1051	12.7	1.1	42.3	10.1	75.7	6.0
TFR2	Human	AD-51688	1051	34.9	2.9	62.2	6.9	78.1	3.2
TFR2	Human	AD-51718	1051	31.6	6.6	53.1	6.5	80.2	1.3
TFR2	Human	AD-51725	1051	47.9	5.0	76.1	1.8	83.7	3.2
TFR2	Human	AD-51724	1051	52.0	1.2	66.1	32.9	87.8	14.9
TFR2	Human	AD-47839	1067	54.0	3.1	71.5	8.4		
TFR2	Human	AD-47845	1068	105.7	20.1	98.0	3.0		
TFR2	Human	AD-47850	1299	16.7	4.8	21.3	3.2		
TFR2	Human	AD-47855	1355	64.6	0.5	66.1	8.0		

TFR2	Human	AD-47816	1441	10.6	2.6	30.6	6.9		
TFR2	Human	AD-47831	1548	22.8	0.2	36.6	9.5		
TFR2	Human	AD-47822	1584	57.2	7.0	72.6	1.6		
TFR2	Human	AD-47828	1612	38.2	5.9	61.2	9.9		
TFR2	Human	AD-47834	1614	9.2	3.6	20.1	3.0		
TFR2	Human	AD-47840	1616	50.1	3.7	55.6	3.8		
TFR2	Human	AD-47846	1618	75.0	7.9	94.6	4.3		
TFR2	Human	AD-47851	2140	94.1	0.4	101.3	10.6		
TFR2	Human	AD-47856	2142	63.3	4.1	60.7	3.1		
TFR2	Human	AD-47817	2143	50.2	2.7	50.3	6.5		
TFR2	Human	AD-47823	2146	26.1	2.3	40.9	3.3		
TFR2	Human	AD-47837	2151	119.5	21.7	89.5	6.9		
TFR2	Human	AD-47843	2152	20.6	1.7	34.9	7.8		
TFR2	Human	AD-47829	2154	53.4	4.1	60.3	0.5		
TFR2	Human	AD-47835	2155	15.5	1.8	18.3	2.4		
TFR2	Human	AD-47841	2170	26.6	1.5	24.7	2.0		
TFR2	Human	AD-51703	2170	25.2	2.8	27.9	3.5	23.2	1.1
TFR2	Human	AD-51710	2170	22.1	3.4	23.1	0.5	24.0	0.6
TFR2	Human	AD-51697	2170	30.9	3.6	25.3	0.9	24.5	0.8
TFR2	Human	AD-51692	2170	23.1	1.3	24.6	1.2	24.9	6.4
TFR2	Human	AD-51685	2170	24.6	2.2	23.9	0.6	25.6	1.7
TFR2	Human	AD-51691	2170	29.1	3.2	21.3	0.2	26.4	3.7
TFR2	Human	AD-51698	2170	23.1	2.3	25.8	3.0	26.8	2.8
TFR2	Human	AD-51686	2170	20.7	2.5	24.7	0.7	27.5	1.4
TFR2	Human	AD-51709	2170	23.1	1.3	25.1	2.7	27.7	2.1
TFR2	Human	AD-51679	2170	27.4	2.2	26.4	4.3	28.3	5.1
TFR2	Human	AD-51705	2170	27.8	5.3	24.6	2.0	28.8	2.4
TFR2	Human	AD-51704	2170	23.9	2.1	26.1	0.5	29.2	4.6
TFR2	Human	AD-51687	2170	20.8	3.9	27.7	2.8	29.4	1.0
TFR2	Human	AD-51681	2170	30.0	1.8	31.2	1.8	29.5	4.7
TFR2	Human	AD-51716	2170	20.0	1.7	25.9	2.2	30.2	1.1
TFR2	Human	AD-51693	2170	26.2	0.8	26.1	1.0	30.6	0.6
TFR2	Human	AD-51711	2170	20.8	0.5	24.8	3.2	31.3	3.0
TFR2	Human	AD-51699	2170	20.9	0.7	27.3	1.5	31.7	5.1
TFR2	Human	AD-51722	2170	28.3	3.7	30.0	0.5	32.1	1.2
TFR2	Human	AD-51715	2170	22.2	6.1	30.4	0.6	34.6	1.3
TFR2	Human	AD-51680	2170	26.4	2.5	26.7	5.4	36.6	2.6
TFR2	Human	AD-51717	2170	28.2	6.2	24.6	0.2	37.2	7.7
TFR2	Human	AD-51723	2170	25.9	4.0	30.7	4.0	40.7	3.1
TFR2	Human	AD-51721	2170	30.7	1.6	28.1	0.9	40.8	0.3
TFR2	Human	AD-47847	2178	21.7	2.1	25.1	3.5		
TFR2	Human	AD-47852	2224	71.4	2.2	66.7	7.1		
TFR2	Human	AD-47857	2425	37.4	4.8	29.5	5.4		
TFR2	Human	AD-47818	2602	48.3	4.8	50.8	4.3		
TFR2	Human	AD-47824	2656	19.9	3.3	25.7	0.1		
TFR2	Human	AD-47830	2658	25.8	7.7	25.8	6.4		
TFR2	Human	AD-47836	2660	34.6	0.1	37.4	6.1		
TFR2	Human	AD-47842	2662	19.2	6.8	26.3	1.1		
TFR2	Human	AD-47848	2719	76.8	2.2	90.1	9.7		
TFR2	Human	AD-47853	2795	28.1	6.3	43.7	3.8		
TFR2	Human	AD-47858	2802	66.9	8.2	73.6	3.4		

Data are expressed as percent of control (Mock transfected or 1955).

Table 12: Secondary Target dose-response

Table 12				
Target	Reactivity	Duplex Name	Start Position	IC50 (nM)
HFE2	HumaWn	AD-47394	657	0.004
HFE2	Human	AD-47395	1233	0.011
HFE2	Human	AD-47407	1273	0.002
HFE2	Human	AD-51747	1273	0.001
HFE2	Human	AD-51736	1273	0.001
HFE2	Human	AD-51734	1273	0.001
HFE2	Human	AD-51732	1273	0.002
HFE2	Human	AD-51731	1273	0.002
HFE2	Human	AD-51744	1273	0.002
HFE2	Human	AD-51748	1273	0.002
HFE2	Human	AD-51735	1273	0.002

HFE2	Human	AD-47407	1273	0.002
HFE2	Human	AD-51740	1273	0.003
HFE2	Human	AD-47413	1274	0.003
HFE2	Human	AD-47425	1280	0.021
HFE2	Human	AD-47437	1366	0.015
HFE2	Human	AD-47396	1367	0.013
HFE2	Human	AD-47414	1399	0.005
HFE2	Human	AD-47420	1400	0.010
HFE2	Human	AD-47432	1441	0.004
TFR2	Human	AD-47814	64	0.012
TFR2	Human	AD-47820	66	0.011
TFR2	Human	AD-47826	239	0.014
TFR2	Human	AD-47849	916	0.067
TFR2	Human	AD-47833	1051	0.013
TFR2	Human	AD-51701	1051	0.015
TFR2	Human	AD-51708	1051	0.017
TFR2	Human	AD-51700	1051	0.017
TFR2	Human	AD-47833	1051	0.023
TFR2	Human	AD-51696	1051	0.024
TFR2	Human	AD-47850	1299	0.011
TFR2	Human	AD-47834	1614	0.014
TFR2	Human	AD-47835	2155	0.023
TFR2	Human	AD-47841	2170	0.009
TFR2	Human	AD-51710	2170	0.003
TFR2	Human	AD-51703	2170	0.005
TFR2	Human	AD-51697	2170	0.006
TFR2	Human	AD-51692	2170	0.010
TFR2	Human	AD-47841	2170	0.024
TFR2	Human	AD-47847	2178	0.013

Table 13: TFR2 Duplex Sequences

Table 13						
Target	Duplex ID	Start Position	Sense Name	Sense Sequence	Antisense Name	Antisense Sequence
TFR2	AD-52549	64	A-108802.1	uccAGAGAGcGcAAcAAcUdTsdT	A-108798.2	AGUUGUUGCGCUCUCuGGAdTsdT
TFR2	AD-52550	64	A-108802.5	uccAGAGAGcGcAAcAAcUdTsdT	A-108803.2	AGUUGUUGCGCUCUCuGGadTsdT
TFR2	AD-52555	64	A-108802.2	uccAGAGAGcGcAAcAAcUdTsdT	A-108799.2	AGUUGUUGCGCUCuCuGGAdTsdT
TFR2	AD-52556	64	A-108802.6	uccAGAGAGcGcAAcAAcUdTsdT	A-108804.2	AGUUGUUGCGCUCuCuGGadTsdT
TFR2	AD-52561	64	A-108802.3	uccAGAGAGcGcAAcAAcUdTsdT	A-108800.2	AGUUGUUGCGCuCuCuGGAdTsdT
TFR2	AD-52562	64	A-108802.7	uccAGAGAGcGcAAcAAcUdTsdT	A-108805.2	AGUUGUUGCGCuCuCuGGadTsdT
TFR2	AD-52567	64	A-108802.4	uccAGAGAGcGcAAcAAcUdTsdT	A-108801.2	AGUUGuUGCGCuCuCuGGAdTsdT
TFR2	AD-52568	64	A-108802.8	uccAGAGAGcGcAAcAAcUdTsdT	A-108806.2	AGUUGuUGCGCuCuCuGGadTsdT
TFR2	AD-52572	64	A-99594.2	uccAGAGAGcGcAAcAAcudTsdT	A-108798.1	AGUUGUUGCGCUCUCuGGAdTsdT
TFR2	AD-52573	64	A-99594.6	uccAGAGAGcGcAAcAAcudTsdT	A-108803.1	AGUUGUUGCGCUCUCuGGadTsdT
TFR2	AD-52577	64	A-99594.3	uccAGAGAGcGcAAcAAcudTsdT	A-108799.1	AGUUGUUGCGCUCuCuGGAdTsdT
TFR2	AD-52578	64	A-99594.7	uccAGAGAGcGcAAcAAcudTsdT	A-108804.1	AGUUGUUGCGCUCuCuGGadTsdT
TFR2	AD-52582	64	A-99594.4	uccAGAGAGcGcAAcAAcudTsdT	A-108800.1	AGUUGUUGCGCuCuCuGGAdTsdT
TFR2	AD-52583	64	A-99594.8	uccAGAGAGcGcAAcAAcudTsdT	A-108805.1	AGUUGUUGCGCuCuCuGGadTsdT
TFR2	AD-52587	64	A-99594.5	uccAGAGAGcGcAAcAAcudTsdT	A-108801.1	AGUUGuUGCGCuCuCuGGAdTsdT
TFR2	AD-52588	64	A-99594.9	uccAGAGAGcGcAAcAAcudTsdT	A-108806.1	AGUUGuUGCGCuCuCuGGadTsdT
TFR2	AD-52551	239	A-108811.1	cAGGcAGccAAAccucAuUdTsdT	A-108810.2	AAUGAGGUuUGGCuGcCuGdTsdT
TFR2	AD-52552	239	A-108811.3	cAGGcAGccAAAccucAuUdTsdT	A-108816.1	AAuGAGGUuUGGCUGcCugdTsdT
TFR2	AD-52557	239	A-108812.1	cAGGcAGccAAAccuCAuUdTsdT	A-108810.3	AAUGAGGUuUGGCuGcCuGdTsdT
TFR2	AD-52558	239	A-108812.3	cAGGcAGccAAAccuCAuUdTsdT	A-108816.2	AAuGAGGUuUGGCUGcCugdTsdT

TFR2	AD-52563	239	A-108813.1	cAGGcAGccAAAcCuCAuUdTsdT	A-108810.4	AAUGAGGUuUGGCuGcCuGdTsdT
TFR2	AD-52564	239	A-108813.3	cAGGcAGccAAAcCuCAuUdTsdT	A-108816.3	AAuGAGGUuUGGCUGcCugdTsdT
TFR2	AD-52569	239	A-108814.1	cAGGcAGcCAAAcCuCAuUdTsdT	A-108810.5	AAUGAGGUuUGGCuGcCuGdTsdT
TFR2	AD-52570	239	A-108814.3	cAGGcAGcCAAAcCuCAuUdTsdT	A-108816.4	AAuGAGGUuUGGCUGcCugdTsdT
TFR2	AD-52574	239	A-99598.2	cAGGcAGccAAAccucAuudTsdT	A-108807.1	AAUGAGGUUUGGCUGCCuGdTsdT
TFR2	AD-52575	239	A-108811.2	cAGGcAGccAAAccucAuUdTsdT	A-108815.1	AAUGAGGUuUGGCUGcCugdTsdT
TFR2	AD-52579	239	A-99598.3	cAGGcAGccAAAccucAuudTsdT	A-108808.1	AAUGAGGUUUGGCUGcCuGdTsdT
TFR2	AD-52580	239	A-108812.2	cAGGcAGccAAAccuCAuUdTsdT	A-108815.2	AAUGAGGUuUGGCUGcCugdTsdT
TFR2	AD-52584	239	A-99598.4	cAGGcAGccAAAccucAuudTsdT	A-108809.1	AAUGAGGUUUGGCuGcCuGdTsdT
TFR2	AD-52585	239	A-108813.2	cAGGcAGccAAAcCuCAuUdTsdT	A-108815.3	AAUGAGGUuUGGCUGcCugdTsdT
TFR2	AD-52589	239	A-99598.5	cAGGcAGccAAAccucAuudTsdT	A-108810.1	AAUGAGGUuUGGCuGcCuGdTsdT
TFR2	AD-52590	239	A-108814.2	cAGGcAGcCAAAcCuCAuUdTsdT	A-108815.4	AAUGAGGUuUGGCUGcCugdTsdT
It should be noted that unmodified versions of each of the modified sequences shown are included within the scope of the invention.						

Table 14: TFR2 Dose Response

Table 14				
Target	Reactivity	Duplex Name	Start Position	IC50 (nM)
TFR2	Human	AD-47814	64	0.019
TFR2	Human	AD-52549	64	0.034
TFR2	Human	AD-52572	64	0.059
TFR2	Human	AD-52550	64	0.062
TFR2	Human	AD-52573	64	0.102
TFR2	Human	AD-52570	239	0.035
TFR2	Human	AD-47826	239	0.036
TFR2	Human	AD-52590	239	0.038
TFR2	Human	AD-52574	239	0.065
TFR2	Human	AD-52558	239	0.236

Table 15: SMAD4 Unmodified Duplexes

Table 15						
Duplex Name	Start	Target	Sense Oligo Name	Trans Seq	Antis Oligo Name	Trans Seq
AD-48090.1	481	SMAD4	A-100350.1	AUGCCUGUCUGAGCAUUGU	A-100351.1	ACAAUGCUCAGACAGGCAU
AD-48091.1	772	SMAD4	A-100366.1	AUGUUAAAUAUUGUCAGUA	A-100367.1	UACUGACAAUAUUUAACAU
AD-48092.1	817	SMAD4	A-100382.1	UCUGUGUGAAUCCAUAUCA	A-100383.1	UGAUAUGGAUUCACACAGA
AD-48093.1	1212	SMAD4	A-100398.1	ACUUACCAUCAUAACAGCA	A-100399.1	UGCUGUUAUGAUGGUAAGU
AD-48094.1	1351	SMAD4	A-100414.1	ACAAUGAGCUUGCAUUCCA	A-100415.1	UGGAAUGCAAGCUCAUUGU
AD-48095.1	1712	SMAD4	A-100430.1	UGUUCAUAAGAUCUACCCA	A-100431.1	UGGGUAGAUCUUAUGAACA
AD-48096.1	590	SMAD4	A-100352.1	AAAAGAUGAAUUGGAUUCU	A-100353.1	AGAAUCCAAUUCAUCUUUU
AD-48097.1	773	SMAD4	A-100368.1	UGUUAAAUAUUGUCAGUAU	A-100369.1	AUACUGACAAUAUUUAACA
AD-48098.1	819	SMAD4	A-100384.1	UGUGUGAAUCCAUAUCACU	A-100385.1	AGUGAUAUGGAUUCACACA
AD-48099.1	1232	SMAD4	A-100400.1	UACCACCUGGACUGGAAGU	A-100401.1	ACUUCCAGUCCAGGUGGUA
AD-48100.1	1362	SMAD4	A-100416.1	GCAUUCCAGCCUCCCAUUU	A-100417.1	AAAUGGGAGGCUGGAAUGC
AD-48101.1	1713	SMAD4	A-100432.1	GUUCAUAAGAUCUACCCAA	A-100433.1	UUGGGUAGAUCUUAUGAAC
AD-48102.1	602	SMAD4	A-100354.1	GGAUUCUUUAAUAACAGCU	A-100355.1	AGCUGUUAUUAAAGAAUCC
AD-48103.1	777	SMAD4	A-100370.1	AAAUAUUGUCAGUAUGCGU	A-100371.1	ACGCAUACUGACAAUAUUU
AD-48104.1	820	SMAD4	A-100386.1	GUGUGAAUCCAUAUCACUA	A-100387.1	UAGUGAUAUGGAUUCACAC
AD-48105.1	1238	SMAD4	A-100402.1	CUGGACUGGAAGUAGGACU	A-100403.1	AGUCCUACUUCCAGUCCAG

Table 15						
Duplex Name	Start	Target	Sense Oligo Name	Trans Seq	Antis Oligo Name	Trans Seq
AD-48106.1	1367	SMAD4	A-100418.1	CCAGCCUCCCAUUUCCAAU	A-100419.1	AUUGGAAAUGGGAGGCUGG
AD-48107.1	2816	SMAD4	A-100434.1	UAUUUCUAGGCACAAGGUU	A-100435.1	AACCUUGUGCCUAGAAAUA
AD-48108.1	608	SMAD4	A-100356.1	UUUAAUAACAGCUAUAACU	A-100357.1	AGUUAUAGCUGUUAUUAAA
AD-48109.1	778	SMAD4	A-100372.1	AAUAUUGUCAGUAUGCGUU	A-100373.1	AACGCAUACUGACAAUAUU
AD-48110.1	861	SMAD4	A-100388.1	AUUGAUCUCUCAGGAUUAA	A-100389.1	UUAAUCCUGAGAGAUCAAU
AD-48111.1	1250	SMAD4	A-100404.1	UAGGACUGCACCAUACACA	A-100405.1	UGUGUAUGGUGCAGUCCUA
AD-48112.1	1370	SMAD4	A-100420.1	GCCUCCCAUUUCCAAUCAU	A-100421.1	AUGAUUGGAAAUGGGAGGC
AD-48113.1	2984	SMAD4	A-100436.1	AAUAUUUUGGAAACUGCUA	A-100437.1	UAGCAGUUUCCAAAAUAUU
AD-48114.1	611	SMAD4	A-100358.1	AAUAACAGCUAUAACUACA	A-100359.1	UGUAGUUAUAGCUGUUAUU
AD-48115.1	781	SMAD4	A-100374.1	AUUGUCAGUAUGCGUUUGA	A-100375.1	UCAAACGCAUACUGACAAU
AD-48116.1	1090	SMAD4	A-100390.1	CUGUGGCUUCCACAAGUCA	A-100391.1	UGACUUGUGGAAGCCACAG
AD-48117.1	1257	SMAD4	A-100406.1	GCACCAUACACACCUAAUU	A-100407.1	AAUUAGGUGUGUAUGGUGC
AD-48118.1	1601	SMAD4	A-100422.1	GUUGGAAUGUAAAGGUGAA	A-100423.1	UUCACCUUUACAUUCCAAC
AD-48119.1	3013	SMAD4	A-100438.1	UAAAUACUGUGCAGAAUAA	A-100439.1	UUAUUCUGCACAGUAUUUA
AD-48120.1	659	SMAD4	A-100360.1	CAUACAGAGAACAUUGGAU	A-100361.1	AUCCAAUGUUCUCUGUAUG
AD-48121.1	783	SMAD4	A-100376.1	UGUCAGUAUGCGUUUGACU	A-100377.1	AGUCAAACGCAUACUGACA
AD-48122.1	1137	SMAD4	A-100392.1	AGUGAAGGACUGUUGCAGA	A-100393.1	UCUGCAACAGUCCUUCACU
AD-48123.1	1262	SMAD4	A-100408.1	AUACACACCUAAUUUGCCU	A-100409.1	AGGCAAAUUAGGUGUGUAU
AD-48124.1	1633	SMAD4	A-100424.1	UCAGGUGCCUUAGUGACCA	A-100425.1	UGGUCACUAAGGCACCUGA
AD-48125.1	698	SMAD4	A-100362.1	UCGGAAAGGAUUUCCUCAU	A-100363.1	AUGAGGAAAUCCUUUCCGA
AD-48126.1	784	SMAD4	A-100378.1	GUCAGUAUGCGUUUGACUU	A-100379.1	AAGUCAAACGCAUACUGAC
AD-48126.2	784	SMAD4	A-100378.2	GUCAGUAUGCGUUUGACUU	A-100379.2	AAGUCAAACGCAUACUGAC
AD-48127.1	1207	SMAD4	A-100394.1	CAGCUACUUACCAUCAUAA	A-100395.1	UUAUGAUGGUAAGUAGCUG
AD-48128.1	1272	SMAD4	A-100410.1	AAUUUGCCUCACCACCAAA	A-100411.1	UUUGGUGGUGAGGCAAAUU
AD-48129.1	1650	SMAD4	A-100426.1	CACGCGGUCUUUGUACAGA	A-100427.1	UCUGUACAAAGACCGCGUG
AD-48130.1	771	SMAD4	A-100364.1	CAUGUUAAAUAUUGUCAGU	A-100365.1	ACUGACAAUAUUUAACAUG
AD-48131.1	791	SMAD4	A-100380.1	UGCGUUUGACUUAAAAUGU	A-100381.1	ACAUUUUAAGUCAAACGCA
AD-48132.1	1209	SMAD4	A-100396.1	GCUACUUACCAUCAUAACA	A-100397.1	UGUUAUGAUGGUAAGUAGC
AD-48133.1	1273	SMAD4	A-100412.1	AUUUGCCUCACCACCAAAA	A-100413.1	UUUUGGUGGUGAGGCAAAU
AD-48134.1	1652	SMAD4	A-100428.1	CGCGGUCUUUGUACAGAGU	A-100429.1	ACUCUGUACAAAGACCGCG
Note that an overhang (e.g. TT, dTsdT) can be added to the 3' end of any duplex.						

Table 16: SMAD4 Modified Duplexes

Table 16						
Target	Duplex Name	Start	Sense Oligo Name	Oligo Seq	Antis Oligo Name	Oligo Seq
SMAD4	AD-48090.1	481	A-100350.1	AuGccuGucuGAGcAuuGudTsdT	A-100351.1	AcAAUGCUcAGAcAGGcAUdTsdT
SMAD4	AD-48091.1	772	A-100366.1	AuGuuAAAuAuuGucAGuAdTsdT	A-100367.1	uACUGAcAAuAUUuAAcAUdTsdT
SMAD4	AD-48092.1	817	A-100382.1	ucuGuGuGAAuccAuAucAdTsdT	A-100383.1	UGAuAUGGAUUcAcAcAGAdTsdT
SMAD4	AD-48093.1	1212	A-100398.1	AcuuAccAucAuAAcAGcAdTsdT	A-100399.1	UGCUGUuAUGAUGGuAAGUdTsdT
SMAD4	AD-48094.1	1351	A-100414.1	AcAAuGAGcuuGcAuuccAdTsdT	A-100415.1	UGGAAUGcAAGCUcAUUGUdTsdT
SMAD4	AD-48095.1	1712	A-100430.1	uGuucAuAAGAucuAcccAdTsdT	A-100431.1	UGGGuAGAUCUuAUGAAcAdTsdT
SMAD4	AD-48096.1	590	A-100352.1	AAAAGAuGAAuuGGAuucudTsdT	A-100353.1	AGAAUCcAAUUcAUCUUUUdTsdT
SMAD4	AD-48097.1	773	A-100368.1	uGuuAAAuAuuGucAGuAudTsdT	A-100369.1	AuACUGAcAAuAUUuAAcAdTsdT
SMAD4	AD-48098.1	819	A-100384.1	uGuGuGAAuccAuAucAcudTsdT	A-100385.1	AGUGAuAUGGAUUcAcAcAdTsdT
SMAD4	AD-48099.1	1232	A-100400.1	uAccAccuGGAcuGGAAGudTsdT	A-100401.1	ACUUCcAGUCcAGGUGGuAdTsdT
SMAD4	AD-48100.1	1362	A-100416.1	GcAuuccAGccucccAuuudTsdT	A-100417.1	AAAUGGGAGGCUGGAAUGCdTsdT
SMAD4	AD-48101.1	1713	A-100432.1	GuucAuAAGAucuAcccAAdTsdT	A-100433.1	UUGGGuAGAUCUuAUGAACdTsdT
SMAD4	AD-48102.1	602	A-100354.1	GGAuucuuuAAuAAcAGcudTsdT	A-100355.1	AGCUGUuAUuuAAAGAAUCCdTsdT
SMAD4	AD-48103.1	777	A-100370.1	AAAuAuuGucAGuAuGcGudTsdT	A-100371.1	ACGcAuACUGAcAAuAUUUdTsdT
SMAD4	AD-48104.1	820	A-100386.1	GuGuGAAuccAuAucAcuAdTsdT	A-100387.1	uAGUGAuAUGGAUUcAcACdTsdT
SMAD4	AD-48105.1	1238	A-100402.1	cuGGAcuGGAAGuAGGAcudTsdT	A-100403.1	AGUCCuACUUCcAGUCcAGdTsdT
SMAD4	AD-48106.1	1367	A-100418.1	ccAGccucccAuuuccAAudTsdT	A-100419.1	AUUGGAAAUGGGAGGCUGGdTsdT
SMAD4	AD-48107.1	2816	A-100434.1	uAuuucuAGGcAcAAGGuudTsdT	A-100435.1	AACCUUGUGCCuAGAAAuAdTsdT
SMAD4	AD-48108.1	608	A-100356.1	uuuAAuAAcAGcuAuAAcudTsdT	A-100357.1	AGUuAuAGCUGUuAUuAAAdTsdT
SMAD4	AD-48109.1	778	A-100372.1	AAuAuuGucAGuAuGcGuudTsdT	A-100373.1	AACGcAuACUGAcAAuAUUdTsdT
SMAD4	AD-48110.1	861	A-100388.1	AuuGAucucucAGGAuuAAdTsdT	A-100389.1	UuAAUCCUGAGAGAUcAAUdTsdT
SMAD4	AD-48111.1	1250	A-100404.1	uAGGAcuGcAccAuAcAcAdTsdT	A-100405.1	UGUGuAUGGUGcAGUCCuAdTsdT
SMAD4	AD-48112.1	1370	A-100420.1	GccucccAuuuccAAucAudTsdT	A-100421.1	AUGAUUGGAAAUGGGAGGCdTsdT
SMAD4	AD-48113.1	2984	A-100436.1	AAuAuuuuGGAAAcuGcuAdTsdT	A-100437.1	uAGcAGUUUCcAAAAuAUUdTsdT
SMAD4	AD-48114.1	611	A-100358.1	AAuAAcAGcuAuAAcuAcAdTsdT	A-100359.1	UGuAGUuAuAGCUGUuAUUdTsdT

Table 16						
Target	Duplex Name	Start	Sense Oligo Name	Oligo Seq	Antis Oligo Name	Oligo Seq
SMAD4	AD-48115.1	781	A-100374.1	AuuGucAGuAuGcGuuuGAdTsdT	A-100375.1	UcAAACGcAuACUGAcAAUdTsdT
SMAD4	AD-48116.1	1090	A-100390.1	cuGuGGcuuccAcAAGucAdTsdT	A-100391.1	UGACUUGUGGAAGCcAcAGdTsdT
SMAD4	AD-48117.1	1257	A-100406.1	GcAccAuAcAcAccuAAuudTsdT	A-100407.1	AAUuAGGUGUGuAUGGUGCdTsdT
SMAD4	AD-48118.1	1601	A-100422.1	GuuGGAAuGuAAAGGuGAAdTsdT	A-100423.1	UUcACCUUuAcAUUCcAACdTsdT
SMAD4	AD-48119.1	3013	A-100438.1	uAAAuAcuGuGcAGAAuAAdTsdT	A-100439.1	UuAUUCUGcAcAGuAUUuAdTsdT
SMAD4	AD-48120.1	659	A-100360.1	cAuAcAGAGAAcAuuGGAudTsdT	A-100361.1	AUCcAAUGUUCUCUGuAUGdTsdT
SMAD4	AD-48121.1	783	A-100376.1	uGucAGuAuGcGuuuGAcudTsdT	A-100377.1	AGUcAAACGcAuACUGAcAdTsdT
SMAD4	AD-48122.1	1137	A-100392.1	AGuGAAGGAcuGuuGcAGAdTsdT	A-100393.1	UCUGcAAcAGUCCUUcACUdTsdT
SMAD4	AD-48123.1	1262	A-100408.1	AuAcAcAccuAAuuuGccudTsdT	A-100409.1	AGGcAAAUuAGGUGUGuAUdTsdT
SMAD4	AD-48124.1	1633	A-100424.1	ucAGGuGccuuAGuGAccAdTsdT	A-100425.1	UGGUcACuAAGGcACCUGAdTsdT
SMAD4	AD-48125.1	698	A-100362.1	ucGGAAAGGAuuuccucAudTsdT	A-100363.1	AUGAGGAAAUCCUUUCCGAdTsdT
SMAD4	AD-48126.1	784	A-100378.1	GucAGuAuGcGuuuGAcuudTsdT	A-100379.1	AAGUcAAACGcAuACUGACdTsdT
SMAD4	AD-48126.2	784	A-100378.2	GucAGuAuGcGuuuGAcuudTsdT	A-100379.2	AAGUcAAACGcAuACUGACdTsdT
SMAD4	AD-48127.1	1207	A-100394.1	cAGcuAcuuAccAucAuAAdTsdT	A-100395.1	UuAUGAUGGuAAGuAGCUGdTsdT
SMAD4	AD-48128.1	1272	A-100410.1	AAuuuGccucAccAccAAAdTsdT	A-100411.1	UUUGGUGGUGAGGcAAAUUdTsdT
SMAD4	AD-48129.1	1650	A-100426.1	cAcGcGGucuuuGuAcAGAdTsdT	A-100427.1	UCUGuACAAAGACCGCGUGdTsdT
SMAD4	AD-48130.1	771	A-100364.1	cAuGuuAAAuAuuGucAGudTsdT	A-100365.1	ACUGAcAAuAUUuAAcAUGdTsdT
SMAD4	AD-48131.1	791	A-100380.1	uGcGuuuGAcuuAAAAuGudTsdT	A-100381.1	AcAUUUuAAGUcAAACGcAdTsdT
SMAD4	AD-48132.1	1209	A-100396.1	GcuAcuuAccAucAuAAcAdTsdT	A-100397.1	UGUuAUGAUGGuAAGuAGCdTsdT
SMAD4	AD-48133.1	1273	A-100412.1	AuuuGccucAccAccAAAAdTsdT	A-100413.1	UUUUGGUGGUGAGGcAAAUdTsdT
SMAD4	AD-48134.1	1652	A-100428.1	cGcGGucuuuGuAcAGAGudTsdT	A-100429.1	ACUCUGuAcAAAGACCGCGdTsdT

It should be noted that unmodified versions of each of the modified sequences shown are included within the scope of the invention.

Table 17: NEO1 Unmodified Duplexes

Table 17						
Target	Duplex Name	Start	Sense OligoName	Trans Seq	Antis Oligo Name	Trans Seq
NEO1	AD-48273.1	4618	A-100622.1	CUCCGAGAGUAGCUAUGAA	A-100623.1	UUCAUAGCUACUCUCGGAG
NEO1	AD-48287.1	546	A-100564.1	GCUCUUCUGUUAUAUUAAA	A-100565.1	UUUAAUAUAACAGAAGAGC
NEO1	AD-48274.1	5060	A-100638.1	GAGUGUAGACAUUGGCAUU	A-100639.1	AAUGCCAAUGUCUACACUC
NEO1	AD-48309.1	4778	A-100634.1	GGAAUUGUACAGAGUACGA	A-100635.1	UCGUACUCUGUACAAUUCC
NEO1	AD-48309.2	4778	A-100634.2	GGAAUUGUACAGAGUACGA	A-100635.2	UCGUACUCUGUACAAUUCC
NEO1	AD-48297.1	4674	A-100630.1	GACUAAUGAAGGACCUAAA	A-100631.1	UUUAGGUCCUUCAUUAGUC
NEO1	AD-48296.1	4495	A-100614.1	GAACCAUCACAUUCACUCA	A-100615.1	UGAGUGAAUGUGAUGGUUC
NEO1	AD-48280.1	5062	A-100640.1	GUGUAGACAUUGGCAUUUA	A-100641.1	UAAAUGCCAAUGUCUACAC
NEO1	AD-48275.1	535	A-100560.1	CUCAGUUAGAGGCUCUUCU	A-100561.1	AGAAGAGCCUCUAACUGAG
NEO1	AD-48276.1	1283	A-100576.1	GAUGAUGCUGGGACUUAUU	A-100577.1	AAUAAGUCCCAGCAUCAUC
NEO1	AD-48269.1	533	A-100558.1	CUCUCAGUUAGAGGCUCUU	A-100559.1	AAGAGCCUCUAACUGAGAG
NEO1	AD-48286.1	5069	A-100642.1	CAUUGGCAUUUAUGUACAA	A-100643.1	UUGUACAUAAAUGCCAAUG
NEO1	AD-48299.1	791	A-100568.1	GCAGGUCUUCCAAGAUUUA	A-100569.1	UAAAUCUUGGAAGACCUGC
NEO1	AD-48295.1	2602	A-100598.1	CCUAGAUGAAACUCGUGUU	A-100599.1	AACACGAGUUUCAUCUAGG
NEO1	AD-48292.1	5329	A-100644.1	GCAUUGCUGUUUGUAAGCU	A-100645.1	AGCUUACAAACAGCAAUGC
NEO1	AD-48293.1	686	A-100566.1	GUGGUGCAUUCCAAACACA	A-100567.1	UGUGUUUGGAAUGCACCAC
NEO1	AD-48288.1	1535	A-100580.1	GUUUUGGGUCUGGUGAAAU	A-100581.1	AUUUCACCAGACCCAAAAC
NEO1	AD-48307.1	4066	A-100602.1	GCCUGUGAUUAGUGCCCAU	A-100603.1	AUGGGCACUAAUCACAGGC
NEO1	AD-48270.1	1282	A-100574.1	GGAUGAUGCUGGGACUUAU	A-100575.1	AUAAGUCCCAGCAUCAUCC
NEO1	AD-48300.1	1949	A-100584.1	GCUCAAAAUAAGCAUGGCU	A-100585.1	AGCCAUGCUUAUUUUGAGC
NEO1	AD-48306.1	2227	A-100586.1	CCGAGUGGUGGCCUACAAU	A-100587.1	AUUGUAGGCCACCACUCGG

Table 17						
Target	Duplex Name	Start	Sense OligoName	Trans Seq	Antis Oligo Name	Trans Seq
NEO1	AD-48315.1	5059	A-100636.1	GGAGUGUAGACAUUGGCAU	A-100637.1	AUGCCAAUGUCUACACUCC
NEO1	AD-48291.1	4673	A-100628.1	GGACUAAUGAAGGACCUAA	A-100629.1	UUAGGUCCUUCAUUAGUCC
NEO1	AD-48272.1	4096	A-100606.1	CCUCGAUAACCCUCACCAU	A-100607.1	AUGGUGAGGGUUAUCGAGG
NEO1	AD-48271.1	2273	A-100590.1	GAUGUUGCUGUUCGAACAU	A-100591.1	AUGUUCGAACAGCAACAUC
NEO1	AD-48294.1	1540	A-100582.1	GGGUCUGGUGAAAUCAGAU	A-100583.1	AUCUGAUUUCACCAGACCC
NEO1	AD-48278.1	4123	A-100608.1	CUCCAGCAGCCUCGCUUCU	A-100609.1	AGAAGCGAGGCUGCUGGAG
NEO1	AD-48277.1	2312	A-100592.1	GCUCCUCAGAAUCUGUCCU	A-100593.1	AGGACAGAUUCUGAGGAGC
NEO1	AD-48313.1	4086	A-100604.1	CCAUCCAUUCCCUCGAUAA	A-100605.1	UUAUCGAGGGAAUGGAUGG
NEO1	AD-48289.1	2484	A-100596.1	CUCAGCUGAUUGAAGGUCU	A-100597.1	AGACCUUCAAUCAGCUGAG
NEO1	AD-48290.1	4179	A-100612.1	GGCCCAUUGGCACAUCCAU	A-100613.1	AUGGAUGUGCCAAUGGGCC
NEO1	AD-48284.1	4174	A-100610.1	CCCAUGGCCCAUUGGCACA	A-100611.1	UGUGCCAAUGGGCCAUGGG
NEO1	AD-48298.1	6731	A-100646.1	GUACCUGGAUACUGCCACA	A-100647.1	UGUGGCAGUAUCCAGGUAC
NEO1	AD-48311.1	852	A-100572.1	CAAUUCUGAAUUGUGAAGU	A-100573.1	ACUUCACAAUUCAGAAUUG
NEO1	AD-48285.1	4664	A-100626.1	CACCUGGAAGGACUAAUGA	A-100627.1	UCAUUAGUCCUUCCAGGUG
NEO1	AD-48282.1	1448	A-100578.1	CCAACUCCAACUGUGAAGU	A-100579.1	ACUUCACAGUUGGAGUUGG
NEO1	AD-48302.1	4542	A-100616.1	GAAGGAGCCGGCCUCCUAU	A-100617.1	AUAGGAGGCCGGCUCCUUC
NEO1	AD-48303.1	4767	A-100632.1	CUUGAAAACAAGGAAUUGU	A-100633.1	ACAAUUCCUUGUUUUCAAG
NEO1	AD-48279.1	4629	A-100624.1	GCUAUGAACCAGAUGAGCU	A-100625.1	AGCUCAUCUGGUUCAUAGC
NEO1	AD-48301.1	3361	A-100600.1	GAUACAUGACUGGGUUAUU	A-100601.1	AAUAACCCAGUCAUGUAUC
NEO1	AD-48314.1	4613	A-100620.1	GAAGACUCCGAGAGUAGCU	A-100621.1	AGCUACUCUCGGAGUCUUC
NEO1	AD-48312.1	2236	A-100588.1	GGCCUACAAUAAACAUGGU	A-100589.1	ACCAUGUUUAUUGUAGGCC
NEO1	AD-48304.1	7033	A-100648.1	GUACACACUUGUUUGGCCU	A-100649.1	AGGCCAAACAAGUGUGUAC
NEO1	AD-48310.1	7043	A-100650.1	GUUUGGCCUUUUCUGUAGU	A-100651.1	ACUACAGAAAAGGCCAAAC
Note that an overhang (e.g. TT, dTsdT) can be added to the 3′ end of any duplex.						

Table 18: NEO1 Modified Duplexes

Table 18						
Duplex Name	Target	Start	Sense Oligo Name	Oligo Seq	Antis Oligo Name	Oligo Seq
AD-48273.1	NEO1	4618	A-100622.1	cuccGAGAGuAGcuAuGAAdTsdT	A-100623.1	UUcAuAGCuACUCUCGGAGdTsdT
AD-48287.1	NEO1	546	A-100564.1	GcucuucuGuuAuAuuAAAdTsdT	A-100565.1	UUuAAuAuAAcAGAAGAGCdTsdT
AD-48274.1	NEO1	5060	A-100638.1	GAGuGuAGAcAuuGGcAuudTsdT	A-100639.1	AAUGCcAAUGUCuAcACUCdTsdT
AD-48309.1	NEO1	4778	A-100634.1	GGAAuuGuAcAGAGuAcGAdTsdT	A-100635.1	UCGuACUCUGuAcAAUUCCdTsdT
AD-48309.2	NEO1	4778	A-100634.2	GGAAuuGuAcAGAGuAcGAdTsdT	A-100635.2	UCGuACUCUGuAcAAUUCCdTsdT
AD-48297.1	NEO1	4674	A-100630.1	GAcuAAuGAAGGAccuAAAdTsdT	A-100631.1	UUuAGGUCCUUcAUuAGUCdTsdT
AD-48296.1	NEO1	4495	A-100614.1	GAAccAucAcAuucAcucAdTsdT	A-100615.1	UGAGUGAAUGUGAUGGUUCdTsdT
AD-48280.1	NEO1	5062	A-100640.1	GuGuAGAcAuuGGcAuuuAdTsdT	A-100641.1	uAAAUGCcAAUGUCuAcACdTsdT
AD-48275.1	NEO1	535	A-100560.1	cucAGuuAGAGGcucuucudTsdT	A-100561.1	AGAAGAGCCUCuAACUGAGdTsdT
AD-48276.1	NEO1	1283	A-100576.1	GAuGAuGcuGGGAcuuAuudTsdT	A-100577.1	AAuAAGUCCcAGcAUcAUCdTsdT
AD-48269.1	NEO1	533	A-100558.1	cucucAGuuAGAGGcucuudTsdT	A-100559.1	AAGAGCCUCuAACUGAGAGdTsdT
AD-48286.1	NEO1	5069	A-100642.1	cAuuGGcAuuuAuGuAcAAdTsdT	A-100643.1	UUGuAcAuAAAUGCcAAUGdTsdT
AD-48299.1	NEO1	791	A-100568.1	GcAGGucuuccAAGAuuuAdTsdT	A-100569.1	uAAAUCUUGGAAGACCUGCdTsdT
AD-48295.1	NEO1	2602	A-100598.1	ccuAGAuGAAAcucGuGuudTsdT	A-100599.1	AAcACGAGUUUcAUCuAGGdTsdT
AD-48292.1	NEO1	5329	A-100644.1	GcAuuGcuGuuuGuAAGcudTsdT	A-100645.1	AGCUuAcAAAcAGcAAUGCdTsdT
AD-48293.1	NEO1	686	A-100566.1	GuGGuGcAuuccAAAcAcAdTsdT	A-100567.1	UGUGUUUGGAAUGcACcACdTsdT
AD-48288.1	NEO1	1535	A-100580.1	GuuuuGGGucuGGuGAAAudTsdT	A-100581.1	AUUUcACcAGACCcAAAACdTsdT
AD-48307.1	NEO1	4066	A-100602.1	GccuGuGAuuAGuGcccAudTsdT	A-100603.1	AUGGGcACuAAUcAcAGGCdTsdT
AD-48270.1	NEO1	1282	A-100574.1	GGAuGAuGcuGGGAcuuAudTsdT	A-100575.1	AuAAGUCCcAGcAUcAUCCdTsdT
AD-48300.1	NEO1	1949	A-100584.1	GcucAAAAuAAGcAuGGcudTsdT	A-100585.1	AGCcAUGCUuAUUUUGAGCdTsdT
AD-48306.1	NEO1	2227	A-100586.1	ccGAGuGGuGGccuAcAAudTsdT	A-100587.1	AUUGuAGGCcACcACUCGGdTsdT

AD-48315.1	NEO1	5059	A-100636.1	GGAGuGuAGAcAuuGGcAudTsdT	A-100637.1	AUGCcAAUGUCuAcACUCCdTsdT
AD-48291.1	NEO1	4673	A-100628.1	GGAcuAAuGAAGGAccuAAdTsdT	A-100629.1	UuAGGUCCUUcAUuAGUCCdTsdT
AD-48272.1	NEO1	4096	A-100606.1	ccucGAuAAcccucAccAudTsdT	A-100607.1	AUGGUGAGGGUuAUCGAGGdTsdT
AD-48271.1	NEO1	2273	A-100590.1	GAuGuuGcuGuucGAAcAudTsdT	A-100591.1	AUGUUCGAAcAGcAAcAUCdTsdT
AD-48294.1	NEO1	1540	A-100582.1	GGGucuGGuGAAAucAGAudTsdT	A-100583.1	AUCUGAUUUcACcAGACCCdTsdT
AD-48278.1	NEO1	4123	A-100608.1	cuccAGcAGccucGcuucudTsdT	A-100609.1	AGAAGCGAGGCUGCUGGAGdTsdT
AD-48277.1	NEO1	2312	A-100592.1	GcuccucAGAAucuGuccudTsdT	A-100593.1	AGGAcAGAUUCUGAGGAGCdTsdT
AD-48313.1	NEO1	4086	A-100604.1	ccAuccAuucccucGAuAAdTsdT	A-100605.1	UuAUCGAGGGAAUGGAUGGdTsdT
AD-48289.1	NEO1	2484	A-100596.1	cucAGcuGAuuGAAGGucudTsdT	A-100597.1	AGACCUUcAAUcAGCUGAGdTsdT
AD-48290.1	NEO1	4179	A-100612.1	GGcccAuuGGcAcAuccAudTsdT	A-100613.1	AUGGAUGUGCcAAUGGGCCdTsdT
AD-48284.1	NEO1	4174	A-100610.1	cccAuGGcccAuuGGcAcAdTsdT	A-100611.1	UGUGCcAAUGGGCcAUGGGdTsdT
AD-48298.1	NEO1	6731	A-100646.1	GuAccuGGAuAcuGccAcAdTsdT	A-100647.1	UGUGGcAGuAUCcAGGuACdTsdT
AD-48311.1	NEO1	852	A-100572.1	cAAuucuGAAuuGuGAAGudTsdT	A-100573.1	ACUUcAcAAUUcAGAAUUGdTsdT
AD-48285.1	NEO1	4664	A-100626.1	cAccuGGAAGGAcuAAuGAdTsdT	A-100627.1	UcAUuAGUCCUUCcAGGUGdTsdT
AD-48282.1	NEO1	1448	A-100578.1	ccAAcuccAAcuGuGAAGudTsdT	A-100579.1	ACUUcAcAGUUGGAGUUGGdTsdT
AD-48302.1	NEO1	4542	A-100616.1	GAAGGAGccGGccuccuAudTsdT	A-100617.1	AuAGGAGGCCGGCUCCUUCdTsdT
AD-48303.1	NEO1	4767	A-100632.1	cuuGAAAAcAAGGAAuuGudTsdT	A-100633.1	AcAAUUCCUUGUUUUcAAGdTsdT
AD-48279.1	NEO1	4629	A-100624.1	GcuAuGAAccAGAuGAGcudTsdT	A-100625.1	AGCUcAUCUGGUUcAuAGCdTsdT
AD-48301.1	NEO1	3361	A-100600.1	GAuAcAuGAcuGGGuuAuudTsdT	A-100601.1	AAuAACCcAGUcAUGuAUCdTsdT
AD-48314.1	NEO1	4613	A-100620.1	GAAGAcuccGAGAGuAGcudTsdT	A-100621.1	AGCuACUCUCGGAGUCUUCdTsdT
AD-48312.1	NEO1	2236	A-100588.1	GGccuAcAAuAAAcAuGGudTsdT	A-100589.1	ACcAUGUUuAUUGuAGGCCdTsdT
AD-48304.1	NEO1	7033	A-100648.1	GuAcAcAcuuGuuuGGccudTsdT	A-100649.1	AGGCcAAAcAAGUGUGuACdTsdT
AD-48310.1	NEO1	7043	A-100650.1	GuuuGGccuuuuucuGuAGudTsdT	A-100651.1	ACuAcAGAAAAGGCcAAACdTsdT

It should be noted that unmodified versions of each of the modified sequences shown are included within the scope of the invention.

Table 19: SMAD4 Percent Inhibition

Table 19					
		0.1nM (% message remaining)		10nM (% message remaining)	
Target	ID	Avg	SD	Avg	SD
SMAD4	AD-48090	93.6	4.6	54.6	5.6
SMAD4	AD-48091	98.0	5.0	60.8	3.3
SMAD4	AD-48092	64.6	0.2	47.8	12.0
SMAD4	AD-48093	96.4	3.5	45.0	8.0
SMAD4	AD-48094	41.3	0.4	16.3	5.4
SMAD4	AD-48095	64.4	9.1	30.0	0.5
SMAD4	AD-48096	70.5	1.8	44.3	0.7
SMAD4	AD-48097	19.6	2.5	10.0	1.6
SMAD4	AD-48098	60.6	2.1	29.9	1.8
SMAD4	AD-48099	83.1	5.5	57.2	2.5
SMAD4	AD-48100	73.4	1.6	50.4	1.2
SMAD4	AD-48101	34.8	3.7	23.3	0.9
SMAD4	AD-48102	66.9	3.2	35.5	4.0
SMAD4	AD-48103	43.4	8.9	20.5	1.0
SMAD4	AD-48104	53.5	6.2	20.5	1.5
SMAD4	AD-48105	59.4	0.6	23.8	3.0
SMAD4	AD-48106	68.4	0.3	40.7	0.5
SMAD4	AD-48107	40.9	3.0	26.9	6.6
SMAD4	AD-48108	21.4	4.3	15.2	4.3
SMAD4	AD-48109	19.2	4.1	12.1	5.2
SMAD4	AD-48110	46.1	6.4	28.4	8.1
SMAD4	AD-48111	75.9	5.1	68.4	12.1
SMAD4	AD-48112	75.8	2.0	72.0	10.4
SMAD4	AD-48113	87.4	11.1	72.0	2.7

SMAD4	AD-48114	36.7	3.2	19.2	0.6
SMAD4	AD-48115	35.8	2.8	18.6	1.9
SMAD4	AD-48116	37.1	0.2	13.6	0.9
SMAD4	AD-48117	32.1	0.8	21.1	1.4
SMAD4	AD-48118	26.3	1.1	16.4	5.5
SMAD4	AD-48119	52.1	4.7	38.8	4.5
SMAD4	AD-48120	32.1	1.0	13.9	1.4
SMAD4	AD-48121	24.3	2.3	10.0	0.7
SMAD4	AD-48122	31.4	5.7	14.6	1.7
SMAD4	AD-48123	27.4	1.5	14.6	2.2
SMAD4	AD-48124	76.8	7.0	55.8	1.0
SMAD4	AD-48125	28.7	2.6	12.6	0.9
SMAD4	AD-48126	18.9	1.9	7.4	0.2
SMAD4	AD-48127	67.5	3.7	39.6	4.0
SMAD4	AD-48128	69.8	4.0	44.5	6.1
SMAD4	AD-48129	73.1	3.4	42.6	2.0
SMAD4	AD-48130	18.1	0.1	12.5	0.9
SMAD4	AD-48131	44.4	0.5	17.1	4.1
SMAD4	AD-48132	47.7	0.1	22.6	5.4
SMAD4	AD-48133	57.1	1.8	30.4	10.0
SMAD4	AD-48134	86.3	18.0	42.4	9.2

Table 20: NEO1 Percent Inhibition

Table 20					
		0.1nM (% message remaining)		10nM (% message remaining)	
Target	ID	Avg	SD	Avg	SD
Neo1	AD-48273	8.4	0.7	9.3	3.6
Neo1	AD-48287	8.6	5.5	10.4	2.7
Neo1	AD-48274	11.0	4.3	6.5	2.2
Neo1	AD-48309	11.0	0.6	6.5	0.8
Neo1	AD-48297	12.9	1.6	8.7	2.4
Neo1	AD-48296	14.0	6.9	7.6	0.1
Neo1	AD-48280	15.6	3.7	10.8	7.1
Neo1	AD-48275	17.7	6.9	8.4	3.8
Neo1	AD-48276	17.8	9.8	6.8	2.0
Neo1	AD-48269	18.4	5.5	10.9	4.4
Neo1	AD-48286	21.4	3.8	11.7	2.1
Neo1	AD-48299	22.9	3.0	11.7	3.8
Neo1	AD-48295	36.2	16.3	12.0	0.4
Neo1	AD-48292	44.3	6.8	14.8	2.2
Neo1	AD-48293	44.7	14.1	30.7	1.8
Neo1	AD-48288	46.9	21.9	31.9	5.2
Neo1	AD-48307	50.2	10.1	16.8	3.9
Neo1	AD-48270	54.2	10.6	65.9	42.5
Neo1	AD-48300	54.6	0.1	18.6	1.9
Neo1	AD-48306	56.6	19.5	16.0	2.3

Table 20					
		0.1nM (% message remaining)		10nM (% message remaining)	
Neo1	AD-48315	57.7	3.5	17.6	8.0
Neo1	AD-48291	60.2	12.0	35.2	6.4
Neo1	AD-48272	61.9	4.1	25.2	3.2
Neo1	AD-48271	62.6	4.7	35.4	6.8
Neo1	AD-48294	62.6	2.1	22.7	11.0
Neo1	AD-48278	62.9	13.8	27.4	1.3
Neo1	AD-48277	63.2	20.4	26.1	2.6
Neo1	AD-48313	68.2	18.7	43.7	2.2
Neo1	AD-48289	70.6	15.3	53.6	12.3
Neo1	AD-48290	73.8	22.6	60.0	3.9
Neo1	AD-48284	74.0	19.2	106.9	43.7
Neo1	AD-48298	76.0	6.9	75.4	19.3
Neo1	AD-48311	77.9	22.6	23.5	11.1
Neo1	AD-48285	81.0	11.5	65.3	14.2
Neo1	AD-48282	82.7	16.3	47.0	15.3
Neo1	AD-48302	83.3	3.1	32.8	6.7
Neo1	AD-48303	85.0	16.3	29.2	7.7
Neo1	AD-48279	90.2	6.2	51.7	14.3
Neo1	AD-48301	91.8	8.5	88.2	11.1
Neo1	AD-48314	96.7	16.7	128.8	37.8
Neo1	AD-48312	107.9	30.0	94.0	27.8
Neo1	AD-48304	111.6	22.3	91.6	33.2
Neo1	AD-48310	118.0	36.4	118.8	29.0

Table 21: BMP6 Duplexes

Table 21					
duplexName	sOligoSeq	SEQ ID NO	asOligoSeq	SEQ ID NO	Set
AD-47955.1	GcAGAAuuccGcAucuAcAdTsdT		UGuAGAUGCGGAAUUCUGCdTsdT		humanRhesus
AD-47957.1	GAAuAuGGuuGuAAGAGcudTsdT		AGCUCUuAcAACcAuAUUCdTsdT		humanRhesus
AD-47966.1	cucuucAuGcuGGAucuGudTsdT		AcAGAUCcAGcAUGAAGAGdTsdT		humanRhesus
AD-47989.1	GAGuucAAGuucAAcuuAudTsdT		AuAAGUUGAACUUGAACUCdTsdT		humanRhesus
AD-47993.1	cGuGAGuAGuuGuuGGucudTsdT		AGACcAAcAACuACUcACGdTsdT		humanRhesus
AD-47960.1	GGAcGAccAuGAGAGAuAAdTsdT		UuAUCUCUcAUGGUCGUCCdTsdT		humanRhesus
AD-47997.1	ccuAGAuuAcAucuGccuudTsdT		AAGGcAGAUGuAAUCuAGGdTsdT		humanRhesus
AD-47985.1	cAAcAGAGucGuAAucGcudTsdT		AGCGAUuACGACUCUGUUGdTsdT		humanRhesus
AD-47983.1	GucuAucAAAGGuAGAuuudTsdT		AAAUCuACCUUUGAuAGACdTsdT		humanRhesus

AD-47954.1	cccGGAcGAccAuG AGAGAdTsdT		UCUCUcAUGGUCGUC CGGGdTsdT		humanRhesus
AD-47972.1	cucGucAGcGAcAc cAcAAdTsdT		UUGUGGUGUCGCUG ACGAGdTsdT		humanRhesus
AD-47981.1	ccAcuAAcucGAAA ccAGAdTsdT		UCUGGUUUCGAGUuA GUGGdTsdT		humanRhesus
AD-47982.1	GuAAAuGAcGuGA GuAGuudTsdT		AACuACUcACGUcAUU uACdTsdT		humanRhesus
AD-47987.1	GGGGAcAcAcAuuc uGccudTsdT		AGGcAGAAUGUGUGU CCCCdTsdT		humanRhesus
AD-47994.1	cGGcuGcAGAAuuc cGcAudTsdT		AUGCGGAAUUCUGcA GCCGdTsdT		humanRhesus
AD-47973.1	GccGAcAAcAGAG ucGuAAdTsdT		UuACGACUCUGUUGU CGGCdTsdT		humanRhesus
AD-47975.1	GGAuGccAcuAAcu cGAAAdTsdT		UUUCGAGUuAGUGGc AUCCdTsdT		humanRhesus
AD-47979.1	ccGAcAAcAGAGuc GuAAudTsdT		AUuACGACUCUGUUG UCGGdTsdT		humanRhesus
AD-47996.1	cGuGcuGuGcGccA AcuAAdTsdT		UuAGUUGGCGcAcAGc ACGdTsdT		humanRhesus
AD-47968.1	cAAcGcAcAcAuGA AuGcAdTsdT		UGcAUUcAUGUGUGC GUUGdTsdT		humanRhesus
AD-47977.1	cuGucuAucAAAGG uAGAudTsdT		AUCuACCUUUGAuAG AcAGdTsdT		humanRhesus
AD-47995.1	GcGGGucuccAGu GcuucAdTsdT		UGAAGcACUGGAGAC CCGCdTsdT		humanRhesus
AD-47959.1	cuGAGuuuGGAuG ucuGuAdTsdT		uAcAGAcAUCcAAACUc AGdTsdT		humanRhesus
AD-47962.1	cAGGAAGcAuGAG cuGuAudTsdT		AuAcAGCUcAUGCUUC CUGdTsdT		humanRhesus
AD-47967.1	GGcuGGcuGGAAu uuGAcAdTsdT		UGUcAAAUUCcAGCcA GCCdTsdT		humanRhesus
AD-47986.1	GcAGAccuuGGuuc AccuudTsdT		AAGGUGAACcAAGGU CUGCdTsdT		humanRhesus
AD-47988.1	GAcGuGAGuAGuu GuuGGudTsdT		ACcAAcAACuACUcACG UCdTsdT		humanRhesus
AD-47990.1	cAGAGucGuAAuc GcucuAdTsdT		uAGAGCGAUuACGAC UCUGdTsdT		humanRhesus
AD-47991.1	cAGAccuuGGuucA ccuuAdTsdT		uAAGGUGAACcAAGG UCUGdTsdT		humanRhesus
AD-47956.1	GGGucuccAGuGcu ucAGAdTsdT		UCUGAAGcACUGGAG ACCCdTsdT		humanRhesus
AD-47974.1	GcAcAcAuGAAuGc AAccAdTsdT		UGGUUGcAUUcAUGU GUGCdTsdT		humanRhesus
AD-47976.1	GGuAAAuGAcGuG AGuAGudTsdT		ACuACUcACGUcAUUu ACCdTsdT		humanRhesus
AD-47980.1	cAcAcAuGAAuGcA AccAAdTsdT		UUGGUUGcAUUcAUG UGUGdTsdT		humanRhesus
AD-47984.1	cGAcAccAcAAAGA GuucAdTsdT		UGAACUCUUUGUGGU GUCGdTsdT		humanRhesus
AD-47964.1	cucAuuAAuAAuuu GcucAdTsdT		UGAGcAAAUuAUuAA UGAGdTsdT		humanRhesus
AD-47970.1	cAuuAAuAAuuuGc ucAcudTsdT		AGUGAGcAAAUuAUu AAUGdTsdT		humanRhesus
AD-47971.1	GuAcuGucuAucAA AGGuAdTsdT		uACCUUUGAuAGAcAG uACdTsdT		humanRhesus

AD-47963.1	cuuGuGGAuGccAcuAAcudTsdT		AGUuAGUGGcAUCcAcAAGdTsdT		humanRhesus
AD-47965.1	GuucAGuAcuGucuAucAAdTsdT		UUGAuAGAcAGuACUGAACdTsdT		humanRhesus
AD-47992.1	cuuGGAuuccuAGAuuAcAdTsdT		UGuAAUCuAGGAAUCcAAGdTsdT		humanRhesus
AD-47998.1	GGucuGuAGcAAGcuGAGudTsdT		ACUcAGCUUGCuAcAGACCdTsdT		humanRhesus
AD-47958.1	GAuuuuAAAGGAccucAuudTsdT		AAUGAGGUCCUUuAAAAUCdTsdT		humanRhesus
AD-47961.1	cAAAcuuuucuuAucAGcAdTsdT		UGCUGAuAAGAAAAGUUUGdTsdT		humanRhesus
AD-47969.1	GuGGAuGccAcuAAcucGAdTsdT		UCGAGUuAGUGGcAUCcACdTsdT		humanRhesus
AD-47978.1	GucAGcGAcAccAcAAAGAdTsdT		UCUUUGUGGUGUCGCUGACdTsdT		humanRhesus
AD-47305.1	ucAuGAGcuuuGuGAAccudTsdT		AGGUUcAcAAAGCUcAUGAdTsdT		humanRhesus Mouse
AD-47325.1	GAGAcGGcccuuAcGAcAAdTsdT		UUGUCGuAAGGGCCGUCUCdTsdT		humanRhesus Mouse
AD-47329.1	AcGGcccuuAcGAcAAGcAdTsdT		UGCUUGUCGuAAGGGCCGUdTsdT		humanRhesus Mouse
AD-47309.1	AAccuGGuGGAGuAcGAcAdTsdT		UGUCGuACUCcACcAGGUUdTsdT		humanRhesus Mouse
AD-47317.1	GcAGAGAcGGcccuuAcGAdTsdT		UCGuAAGGGCCGUCUCUGCdTsdT		humanRhesus Mouse
AD-47313.1	AccuGGuGGAGuAcGAcAAdTsdT		UUGUCGuACUCcACcAGGUdTsdT		humanRhesus Mouse
AD-47321.1	AGAGAcGGcccuuAcGAcAdTsdT		UGUCGuAAGGGCCGUCUCUdTsdT		humanRhesus Mouse
AD-47333.1	ucccAcucAAcGcAcAcAudTsdT		AUGUGUGCGUUGAGUGGGAdTsdT		humanRhesus Mouse
AD-48038.1	ucAAcGAcGcGGAcAuGGudTsdT		ACcAUGUCCGCGUCGUUGAdTsdT		mouseRat
AD-48010.1	GccAucucGGuucuuuAcudTsdT		AGuAAAGAACCGAGAUGGCdTsdT		mouseRat
AD-48042.1	AAuGccAucucGGuucuuuudTsdT		AAAGAACCGAGAUGGcAUUdTsdT		mouseRat
AD-48000.1	AAcGAcGcGGAcAuGGucAdTsdT		UGACcAUGUCCGCGUCGUUdTsdT		mouseRat
AD-48004.1	AuGccAucucGGuucuuuAdTsdT		uAAAGAACCGAGAUGGcAUdTsdT		mouseRat
It should be noted that unmodified versions of each of the modified sequences shown are included within the scope of the invention.					

CLAIMS

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of hepcidin antimicrobial peptide (HAMP), wherein said dsRNA is selected from the dsRNAs listed in Table 2, 3, 4, or 5 with a start position of 382, 380, 379, or 385.

2. The dsRNA of claim 1, wherein the dsRNA consists of AD-48141, wherein the sense strand of AD-48141 is GAAcAuAGGucuuGGAAuAdTdT and the antisense strand of AD-48141 is UAuUCcAAGACCuAuGuUCdTdT.

3. A dsRNA for inhibiting expression of HAMP, wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a HAMP mRNA transcript, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 2, 3, 4, or 5.

4. The dsRNA of claim 3, wherein the region of complementarity is at least 17 nucleotides in length.

5. The dsRNA of any one of claims 3-4, wherein the region of complementarity is between 19 and 21 nucleotides in length.

6. The dsRNA of any one of claims 3-5, wherein the region of complementarity is 19 nucleotides in length.

7. The dsRNA of any one of claims 3-6, wherein the region of complementarity consists of one of the antisense strand sequences of Table 2, 3, 4, or 5.

8. The dsRNA of any one of claims 3-7, wherein the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 2, 3, 4, or 5.

9. The dsRNA of any one of claims 3-8, wherein the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 2, 3, 4, or 5.

10. The dsRNA of any one of claims 3-9, wherein the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 2, 3, 4, or 5 and the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 2, 3, 4, or 5.

11. The dsRNA of any one of claims 3-10, wherein the sense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the sense strand sequences of Table 2, 3, 4, or 5

and the antisense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the antisense strand sequences of Table 2, 3, 4, or 5.

12. The dsRNA of any one of claims 3-11, wherein the sense strand comprises one of the sense strand sequences of Table 2, 3, 4, or 5.

13. The dsRNA of any one of claims 3-12, wherein the antisense strand comprises one of the antisense strand sequences of Table 2, 3, 4, or 5.

14. The dsRNA of any one of claims 3-13, wherein the sense strand comprises one of the sense strand sequences of Table 2, 3, 4, or 5 and the antisense strand comprises one of the antisense strand sequences of Table 2, 3, 4, or 5.

15. The dsRNA of any one of claims 3-14, wherein the sense strand consists of one of the sense strand sequences of Table 2, 3, 4, or 5 and the antisense strand consists of one of the antisense strand sequences of Table 2, 3, 4, or 5.

16. The dsRNA of any one of claims 3-15, wherein the dsRNA mediates degradation of HAMP mRNA.

17. The dsRNA of any one of claims 3 through 16, wherein said dsRNA further comprises at least one modified nucleotide.

18. The dsRNA of claim 17, wherein at least one of said modified nucleotides is chosen from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

19. The dsRNA of claim 17, wherein said modified nucleotide is chosen from the group consisting of: a 2'-fluoro modified nucleotide, a 2'-fluoro modified nucleoside, a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

20. The dsRNA of any one of claims 3 through 19, wherein each strand is no more than 30 nucleotides in length.

21. The dsRNA of any one of claims 3 through 20, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

22. The dsRNA of any one of claims 3-21, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

23. The dsRNA of any one of claims 3-22, wherein each strand comprises a 3' overhang of 2 nucleotides.

24. The dsRNA of any one of claims 1 through 23, further comprising a ligand.

25. The dsRNA of claim 24, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA.

26. The dsRNA of any one of claims 1 through 25, further comprising an N-Acetyl-Galactosamine (GalNac) conjugate.

27. The dsRNA of any one of claims 1 through 26, wherein the dsRNA is formulated in a nucleic acid lipid particle formulation.

28. The dsRNA of claim 27, wherein the nucleic acid lipid particle formulation is selected from Table A.

29. The dsRNA of claim 27, wherein the nucleic acid lipid particle formulation comprises MC3.

30. A cell comprising the dsRNA of any one of claims 1 through 29.

31. A vector encoding at least one strand of the dsRNA of any one of claims 1 through 29.

32. A cell comprising the vector of claim 31.

33. A pharmaceutical composition for inhibiting expression of a HAMP gene comprising the dsRNA of any one of claims 1 through 29.

34. The pharmaceutical composition of claim 33, further comprising a lipid formulation.

35. The pharmaceutical composition of claim 34, wherein the lipid formulation is a nucleic acid lipid particle formulation.

36. A dsRNA for inhibiting expression of hemojuvelin (HFE2), wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a HFE2 mRNA transcript, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10A.

37. The dsRNA of claim 36, wherein the region of complementarity is at least 17 nucleotides in length.

38. The dsRNA of any one of claims 36-37, wherein the region of complementarity is between 19 and 21 nucleotides in length.

39. The dsRNA of any one of claims 36-38, wherein the region of complementarity is 19 nucleotides in length.

40. The dsRNA of any one of claims 36-39, wherein the region of complementarity consists of one of the antisense strand sequences of Table 10A.

41. The dsRNA of any one of claims 36-40, wherein the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 10A.

42. The dsRNA of any one of claims 36-41, wherein the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 10A.

43. The dsRNA of any one of claims 36-42, wherein the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 10A and the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 10A.

44. The dsRNA of any one of claims 36-43, wherein the sense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the sense strand sequences of Table 10A and the antisense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the antisense strand sequences of Table 10A.

45. The dsRNA of any one of claims 36-44, wherein the sense strand comprises one of the sense strand sequences of Table 10A.

46. The dsRNA of any one of claims 36-45, wherein the antisense strand comprises one of the antisense strand sequences of Table 10A.

47. The dsRNA of any one of claims 36-46, wherein the sense strand comprises one of the sense strand sequences of Table 10A and the antisense strand comprises one of the antisense strand sequences of Table 10A.

48. The dsRNA of any one of claims 36-47, wherein the sense strand consists of one of the sense strand sequences of Table 10A and the antisense strand consists of one of the antisense strand sequences of Table 10A.

49. The dsRNA of any one of claims 36-48, wherein the dsRNA mediates degradation of HFE2 mRNA.

50. The dsRNA of any one of claims 36 through 49, wherein said dsRNA further comprises at least one modified nucleotide.

51. The dsRNA of claim 50, wherein at least one of said modified nucleotides is chosen from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

52. The dsRNA of claim 50, wherein said modified nucleotide is chosen from the group consisting of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

53. The dsRNA of any one of claims 36 through 52, wherein each strand is no more than 30 nucleotides in length.

54. The dsRNA of any one of claims 36 through 53, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

55. The dsRNA of claim 54, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

56. The dsRNA of any one of claims 36 through 55, further comprising a ligand.

57. The dsRNA of claim 56, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA.

58. The dsRNA of any one of claims 36 through 57, further comprising a GalNac conjugate.

59. The dsRNA of any one of claims 36 through 58, wherein the dsRNA is formulated in a nucleic acid lipid particle formulation.

60. The dsRNA of claim 59, wherein the nucleic acid lipid particle formulation is selected from Table A.

61. The dsRNA of claim 59, wherein the nucleic acid lipid particle formulation comprises MC3.

62. A cell comprising the dsRNA of any one of claims 36 through 61.

63. A vector encoding at least one strand of the dsRNA of any one of claims 36 through 61.

64. A cell comprising the vector of claim 63.

65. A pharmaceutical composition for inhibiting expression of a HFE2 gene comprising the dsRNA of any one of claims 36 through 61.

66. The pharmaceutical composition of claim 65, further comprising a lipid formulation.

67. The pharmaceutical composition of claim 66, wherein the lipid formulation is a nucleic acid lipid particle formulation.

68. A dsRNA for inhibiting expression of transferrin receptor 2 (TFR2), wherein said dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity to a TFR2 mRNA transcript, wherein said dsRNA is selected from the dsRNAs listed in Table 10B or 13 with a start position of 239 or 64; or wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10B or 13; or wherein the dsRNA is AD-52590.

69. The dsRNA of claim 68, wherein the region of complementarity is at least 17 nucleotides in length.

70. The dsRNA of any one of claims 68-69, wherein the region of complementarity is between 19 and 21 nucleotides in length.

71. The dsRNA of any one of claims 68-70, wherein the region of complementarity is 19 nucleotides in length.

72. The dsRNA of any one of claims 68-71, wherein the region of complementarity consists of one of the antisense strand sequences of Table 10B or 13.

73. The dsRNA of any one of claims 68-72, wherein the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 10B or 13.

74. The dsRNA of any one of claims 68-73, wherein the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 10B or 13.

75. The dsRNA of any one of claims 68-74, wherein the sense strand comprises 15 or more contiguous nucleotides of one of the sense strand sequences of Table 10B or 13 and the antisense strand comprises 15 or more contiguous nucleotides of one of the antisense strand sequences of Table 10B or 13.

76. The dsRNA of any one of claims 68-75, wherein the sense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the sense strand sequences of Table 10B or 13 and the antisense strand comprises 16, 17, 18, 19, 20, or more contiguous nucleotides of one of the antisense strand sequences of Table 10B or 13.

77. The dsRNA of any one of claims 68-76, wherein the sense strand comprises one of the sense strand sequences of Table 10B or 13.

78. The dsRNA of any one of claims 68-77, wherein the antisense strand comprises one of the antisense strand sequences of Table 10B or 13.

79. The dsRNA of any one of claims 68-78, wherein the sense strand comprises one of the sense strand sequences of Table 10B or 13 and the antisense strand comprises one of the antisense strand sequences of Table 10B or 13.

80. The dsRNA of any one of claims 68-79, wherein the sense strand consists of one of the sense strand sequences of Table 10B or 13 and the antisense strand consists of one of the antisense strand sequences of Table 10B or 13.

81. The dsRNA of any one of claims 68-80, wherein the dsRNA mediates degradation of TFR2 mRNA.

82. The dsRNA of any one of claims 68 through 81, wherein said dsRNA further comprises at least one modified nucleotide.

83. The dsRNA of claim 82, wherein at least one of said modified nucleotides is chosen from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

84. The dsRNA of claim 82, wherein said modified nucleotide is chosen from the group consisting of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

85. The dsRNA of any one of claims 68 through 84, wherein each strand is no more than 30 nucleotides in length.

86. The dsRNA of any one of claims 68 through 85, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

87. The dsRNA of any one of claims 68-86, wherein at least one strand comprises a 3' overhang of 2 nucleotides.

88. The dsRNA of any one of claims 68 through 87, further comprising a ligand.

89. The dsRNA of claim 88, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA.

90. The dsRNA of any one of claims 68 through 89, further comprising a GalNac conjugate.

91. The dsRNA of any one of claims 68 through 89, wherein the dsRNA is formulated in a nucleic acid lipid particle formulation.

92. The dsRNA of claim 91, wherein the nucleic acid lipid particle formulation is selected from Table A.

93. The dsRNA of claim 91, wherein the nucleic acid lipid particle formulation comprises MC3.

94. A cell comprising the dsRNA of any one of claims 68 through 93.

95. A vector encoding at least one strand of the dsRNA of any one of claims 68 through 93.

96. A cell comprising the vector of claim 95.

97. A pharmaceutical composition for inhibiting expression of a TFR2 gene comprising the dsRNA of any one of claims 68 through 93.

98. The pharmaceutical composition of claim 97, further comprising a lipid formulation.

99. The pharmaceutical composition of claim 98, wherein the lipid formulation is a nucleic acid lipid particle formulation.

100. A composition comprising a first dsRNA for inhibiting expression of a HAMP gene and a second dsRNA for inhibiting expression of an HFE2 gene, wherein the first dsRNA comprises a first sense strand and an first antisense strand, the first antisense strand comprising a region of complementarity to a HAMP mRNA transcript, wherein the first antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 2, 3, 4, or 5; and wherein the second dsRNA comprises a second sense strand and a second antisense strand, the second antisense strand comprising a region of complementarity to a HFE2 mRNA transcript, wherein the second antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10A.

101. A composition comprising a first dsRNA for inhibiting expression of a HAMP gene and a second dsRNA for inhibiting expression of an TFR2 gene, wherein said first dsRNA comprises a first sense strand and a first antisense strand, the first antisense strand comprising a region of complementarity to a HAMP mRNA transcript, wherein the first antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 2, 3, 4, or 5; and wherein said second dsRNA comprises a second sense strand and a second antisense strand, the second antisense strand

comprising a region of complementarity to a TFR2 mRNA transcript, wherein the second antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10B or 13.

102. A composition comprising a first dsRNA for inhibiting expression of a TFR2 gene and a second dsRNA for inhibiting expression of a HFE2 gene, wherein said first dsRNA comprises a first sense strand and a first antisense strand, the first antisense strand comprising a region of complementarity to a TFR2 mRNA transcript, wherein the first antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10B or 13; and wherein said second dsRNA comprises a second sense strand and a second antisense strand, the second antisense strand comprising a region of complementarity to a HFE2 mRNA transcript, wherein the second antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from one of the antisense strand sequences listed in Table 10A.

103. A composition comprising a plurality of dsRNAs selected from the dsRNAs of any one of claims 1 through 29, 36 through 61, and 68 through 93.

104. A method of inhibiting HAMP expression in a cell, the method comprising:

(a) introducing into the cell the dsRNA of any one of claims 1 through 29; and

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

105. The method of claim 104, wherein the HAMP expression is inhibited by at least 30%.

106. The method of any one of claims 104-105, wherein the HAMP expression is inhibited by at least 80%.

107. A method of treating a disorder associated with HAMP expression comprising administering to a subject in need of such treatment a therapeutically effective amount of the dsRNA of any of claims 1 through 29.

108. The method of claim 107, wherein the subject has anemia.

109. The method of any one of claims 107-108, wherein the subject has refractory anemia.

110. The method of any one of claims 107-109, wherein the subject has anemia of chronic disease (ACD).

111. The method of any one of claims 107-110, wherein the subject has iron-restricted erythropoiesis.

112. The method of any one of claims 107-111, wherein the subject is a human.

113. The method of any one of claims 107-112, wherein the dsRNA is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

114. The method of any one of claims 107-113, wherein the dsRNA is lipid formulated.

115. The method of any one of claims 107-114, wherein the dsRNA is lipid formulated in a formulation selected from Table A.

116. The method of any one of claims 107-115, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation.

117. The method of any one of claims 107-116, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously.

118. The method of any one of claims 107-117, wherein the dsRNA is conjugated to GalNac.

119. The method of any one of claims 107-118, wherein the dsRNA is conjugated to GalNac and administered subcutaneously.

120. The method of any one of claims 107-119, wherein the dsRNA is administered subcutaneously.

121. A method for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of the dsRNA of any of claims 1 through 29.

122. The method of claim 121, wherein the dsRNA is lipid formulated.

123. The method of any one of claims 121-122, wherein the dsRNA is lipid formulated in a nucleic acid lipid particle formulation.

124. The method of any one of claims 121-123, wherein the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously.

125. The method of any one of claims 121-124, wherein the dsRNA is administered intravenously.

126. The method of any one of claims 121-125, wherein the dsRNA is lipid formulated in a formulation selected from Table A.

127. The method of any one of claims 121-126, wherein the dsRNA is conjugated to GalNac.

128. The method of any one of claims 121-127, wherein the dsRNA is conjugated to GalNac and administered subcutaneously.

129. The method of any one of claims 121-128, wherein the dsRNA is administered subcutaneously.

130. The method of any one of claims 121-129, wherein the subject is a primate or a rodent.

131. The method of any one of claims 121-130, wherein the subject is a human.

132. The method of any one of claims 121-131, wherein the effective amount is a concentration of 0.01-5.0 mg/kg bodyweight of the subject.

133. The method of any one of claims 121-132, wherein the subject has fatigue, shortness of breath, headache, dizziness, or pale skin.

134. The method of any one of claims 121-133, wherein the subject has reduced iron levels compared to a subject without anemia.

135. The method of any one of claims 121-134, wherein the subject has haemoglobin (Hb) levels <9g/dL.

136. The method of any one of claims 121-135, wherein the subject has chronic kidney disease (CKD), cancer, chronic inflammatory disease, rheumatoid arthritis (RA), or iron-resistant iron-deficient anemia (IRIDA).

137. The method of any one of claims 121-136, wherein the subject has reduced renal erythropoietin (EPO) synthesis compared to a subject without CKD, a dietary deficiency, blood loss, or elevated hepcidin levels compared to a subject without CKD.

138. The method of any one of claims 121-137, wherein the subject has decreased renal excretion of hepcidin compared to a subject without CKD or low grade inflammation characterized by increased interleukin-6 (IL-6) levels compared to a subject without CKD.

139. The method of any one of claims 121-138, wherein the subject has a reticulocyte Hb of < 28 pg.

140. The method of any one of claims 121-139, wherein the subject has >10% hypochromic red blood cells (RBCs).

141. The method of any one of claims 121-140, further comprising determining the complete blood count (CBC), serum iron concentration, Transferrin (Tf) saturation, or ferritin levels of the subject.

142. The method of any one of claims 121-141, wherein administering results in an increase in iron levels in the subject.

143. The method of any one of claims 121-142, wherein administering results in a 2-fold increase in iron levels in the subject.

144. The method of any one of claims 121-143, wherein administering results in an increase in Tf saturation in the subject.

145. The method of any one of claims 121-144, further comprising determining the iron level in the subject.

146. The method of any one of claims 121-145, further comprising administering intravenous iron or ESAs to the subject.

147. A method of inhibiting HFE2 expression in a cell, the method comprising:

(a) introducing into the cell the dsRNA of any one of claims 36 through 61; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a HFE2 gene, thereby inhibiting expression of the HFE2 gene in the cell.

148. The method of claim 147, wherein the HFE2 expression is inhibited by at least 30%.

149. The method of any one of claims 147-148, wherein the HFE2 expression is inhibited by at least 80%.

150. A method of treating a disorder associated with HFE2 expression comprising administering to a subject in need of such treatment a therapeutically effective amount of the dsRNA of any of claims 36 through 61.

151. The method of claim 150, wherein the subject has anemia.

152. The method of any one of claims 150-151, wherein the subject has refractory anemia.

153. The method of any one of claims 150-152, wherein the subject has anemia of chronic disease (ACD).

154. The method of any one of claims 150-153, wherein the subject has iron-restricted erythropoiesis.

155. The method of any one of claims 150-154, wherein the subject is a human.

156. The method of any one of claims 150-155, wherein the dsRNA is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

157. The method of any one of claims 150-156, wherein the dsRNA is lipid formulated.

158. The method of any one of claims 150-157, wherein the dsRNA is lipid formulated in a formulation selected from Table A.

159. The method of any one of claims 150-158, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation.

160. The method of any one of claims 150-159, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously.

161. The method of any one of claims 150-160, wherein the dsRNA is conjugated to GalNac.

162. The method of any one of claims 150-161, wherein the dsRNA is conjugated to GalNac and administered subcutaneously.

163. The method of any one of claims 150-162, wherein the dsRNA is administered subcutaneously.

164. A method for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of the dsRNA of any of claims 36 through 61.

165. The method of claim 164, wherein the dsRNA is lipid formulated.

166. The method of any one of claims 164-165, wherein the dsRNA is lipid formulated in a formulation selected from Table A.

167. The method of any one of claims 164-166, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation.

168. The method of any one of claims 164-167, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously.

169. The method of any one of claims 164-168, wherein the dsRNA is conjugated to GalNac.

170. The method of any one of claims 164-169, wherein the dsRNA is conjugated to GalNac and administered subcutaneously.

171. The method of any one of claims 164-170, wherein the dsRNA is administered subcutaneously.

172. The method of any one of claims 164-171, wherein the subject is a primate or a rodent.

173. The method of any one of claims 164-172, wherein the subject is a human.

174. The method of any one of claims 164-173, wherein the effective amount is a concentration of 0.01-5.0 mg/kg bodyweight of the subject.

175. The method of any one of claims 164-174, wherein the subject has fatigue, shortness of breath, headache, dizziness, or pale skin.

176. The method of any one of claims 164-175, wherein the subject has reduced iron levels compared to a subject without anemia.

177. The method of any one of claims 164-176, wherein the subject has Hb levels <9g/dL.

178. The method of any one of claims 164-177, wherein the subject has chronic kidney disease (CKD), cancer, chronic inflammatory disease, rheumatoid arthritis (RA), or iron-resistant iron-deficient anemia (IRIDA).

179. The method of any one of claims 164-178, wherein the subject has reduced renal EPO synthesis compared to a subject without CKD, a dietary deficiency, blood loss, or elevated hepcidin levels compared to a subject without CKD.

180. The method of any one of claims 164-179, wherein the subject has decreased renal excretion of hepcidin compared to a subject without CKD or low grade inflammation characterized by increased interleukin-6 (IL-6) levels compared to a subject without CKD.

181. The method of any one of claims 164-180, wherein the subject has a reticulocyte Hb of < 28 pg.

182. The method of any one of claims 164-181, wherein the subject has >10% hypochromic RBCs.

183. The method of any one of claims 164-182, further comprising determining the CBC, serum iron concentration, Transferrin (Tf) saturation, or ferritin levels of the subject.

184. The method of any one of claims 164-183, wherein administering results in an increase in iron levels in the subject.

185. The method of any one of claims 164-184, wherein administering results in a 2-fold increase in iron levels in the subject.

186. The method of any one of claims 164-185, wherein administering results in an increase in Tf saturation in the subject.

187. The method of any one of claims 164-186, further comprising determining the iron level in the subject.

188. The method of any one of claims 164-187, further comprising administering intravenous iron or ESAs to the subject.

189. A method of inhibiting TFR2 expression in a cell, the method comprising:

(a) introducing into the cell the dsRNA of any one of claims 68 through 93; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a TFR2 gene, thereby inhibiting expression of the TFR2 gene in the cell.

190. The method of claim 189, wherein the TFR2 expression is inhibited by at least 30%.

191. The method of any one of claims 189-190, wherein the TFR2 expression is inhibited by at least 80%.

192. A method of treating a disorder associated with TFR2 expression comprising administering to a subject in need of such treatment a therapeutically effective amount of the dsRNA of any of claims 68 through 93.

193. The method of claim 192, wherein the subject has anemia.

194. The method of any one of claims 192-193, wherein the subject has refractory anemia.

195. The method of any one of claims 192-194, wherein the subject has anemia of chronic disease (ACD).

196. The method of any one of claims 192-195, wherein the subject has iron-restricted erythropoiesis.

197. The method of any one of claims 192-196, wherein the subject is a human.

198. The method of any one of claims 192-197, wherein the dsRNA is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

199. The method of any one of claims 192-198, wherein the dsRNA is lipid formulated.

200. The method of any one of claims 192-199, wherein the dsRNA is lipid formulated in a formulation selected from Table A.

201. The method of any one of claims 192-200, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation.

202. The method of any one of claims 192-201, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously.

203. The method of any one of claims 192-202, wherein the dsRNA is conjugated to GalNac.

204. The method of any one of claims 192-203, wherein the dsRNA is conjugated to GalNac and administered subcutaneously.

205. The method of any one of claims 192-204, wherein the dsRNA is administered subcutaneously.

206. A method for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of the dsRNA of any of claims 68 through 93.

207. The method of claim 206, wherein the dsRNA is lipid formulated.

208. The method of any one of claims 206-207, wherein the dsRNA is lipid formulated in a formulation selected from Table A.

209. The method of any one of claims 206-208, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation.

210. The method of any one of claims 206-209, wherein the the dsRNA is lipid formulated in a nucleic acid lipid particle formulation and administered intravenously.

211. The method of any one of claims 206-210, wherein the dsRNA is conjugated to GalNac.

212. The method of any one of claims 206-211, wherein the dsRNA is conjugated to GalNac and administered subcutaneously.

213. The method of any one of claims 206-212, wherein the dsRNA is administered subcutaneously.

214. The method of any one of claims 206-213, wherein the subject is a primate or a rodent.

215. The method of any one of claims 206-214, wherein the subject is a human.

216. The method of any one of claims 206-215, wherein the effective amount is a concentration of 0.01-5.0 mg/kg bodyweight of the subject.

217. The method of any one of claims 206-216, wherein the subject has fatigue, shortness of breath, headache, dizziness, or pale skin.

218. The method of any one of claims 206-217, wherein the subject has reduced iron levels compared to a subject without anemia.

219. The method of any one of claims 206-218, wherein the subject has Hb levels <9g/dL.

220. The method of any one of claims 206-219, wherein the subject has chronic kidney disease (CKD), cancer, chronic inflammatory disease, rheumatoid arthritis (RA), or iron-resistant iron-deficient anemia (IRIDA).

221. The method of any one of claims 206-220, wherein the subject has reduced renal EPO synthesis compared to a subject without CKD, a dietary deficiency, blood loss, or elevated hepcidin levels compared to a subject without CKD.

222. The method of any one of claims 206-221, wherein the subject has decreased renal excretion of hepcidin compared to a subject without CKD or low grade inflammation characterized by increased interleukin-6 (IL-6) levels compared to a subject without CKD.

223. The method of any one of claims 206-222, wherein the subject has a reticulocyte Hb of < 28 pg.

224. The method of any one of claims 206-223, wherein the subject has >10% hypochromic RBCs.

225. The method of any one of claims 206-224, further comprising determining the CBC, serum iron concentration, Transferrin (Tf) saturation, or ferritin levels of the subject.

226. The method of any one of claims 206-225, wherein administering results in an increase in iron levels in the subject.

227. The method of any one of claims 206-226, wherein administering results in a 2-fold increase in iron levels in the subject.

228. The method of any one of claims 206-227, wherein administering results in an increase in Tf saturation in the subject.

229. The method of any one of claims 206-228, further comprising determining the iron level in the subject.

230. The method of any one of claims 206-229, further comprising administering intravenous iron or ESAs to the subject.

231. A method of inhibiting HAMP, HFE2, and/or TFR2 expression in a cell, the method comprising:

(a) introducing into the cell a plurality of dsRNAs selected from the dsRNAs of any one of claims 1 through 29, 36 through 61, and 68 through 93; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain

degradation of the mRNA transcript of a HAMP, HFE2, and/or TFR2 gene, thereby inhibiting expression of the HAMP, HFE2, and/or TFR2 gene in the cell.

232. The method of claim 231, wherein the plurality of dsRNAs are introduced simultaneously.

233. The method of any one of claims 231-232, wherein the plurality of dsRNAs are introduced concurrently.

234. The method of any one of claims 231-233, wherein the plurality of dsRNAs are introduced individually.

235. The method of any one of claims 231-234, wherein the plurality of dsRNAs are introduced together.

236. The method of any one of claims 231-235, wherein the expression is inhibited by at least 30%.

237. The method of any one of claims 231-236, wherein the expression is inhibited by at least 80%.

238. A method of treating a disorder associated with HAMP, HFE2, and/or TFR2 expression comprising administering to a subject in need of such treatment a therapeutically effective amount of a plurality of dsRNAs selected from the dsRNAs of any one of claims 1 through 29, 36 through 61, and 68 through 93.

239. The method of claim 238, wherein the plurality of dsRNAs are administered to the subject simultaneously.

240. The method of any one of claims 238-239, wherein the plurality of dsRNAs are administered to the subject concurrently.

241. The method of any one of claims 238-240, wherein the plurality of dsRNAs are administered to the subject individually.

242. The method of any one of claims 238-241, wherein the plurality of dsRNAs are administered to the subject together.

243. The method of any one of claims 238-242, wherein the subject has anemia.

244. The method of any one of claims 238-243, wherein the subject has refractory anemia.

245. The method of any one of claims 238-244, wherein the subject has anemia of chronic disease (ACD).

246. The method of any one of claims 238-245, wherein the subject has iron-restricted erythropoiesis.

247. The method of any one of claims 238-246, wherein the subject is a human.

248. The method of any one of claims 238-247, wherein the dsRNA is administered at a concentration of 0.01 mg/kg-5 mg/kg bodyweight of the subject.

249. A method for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of a plurality of dsRNAs selected from the dsRNAs of any one of claims 1 through 29, 36 through 61, and 68 through 93.

250. The method of claim 249, wherein the plurality of dsRNAs are administered to the subject simultaneously.

251. The method of any one of claims 249-250, wherein the plurality of dsRNAs are administered to the subject concurrently.

252. The method of any one of claims 249-251, wherein the plurality of dsRNAs are administered to the subject individually.

253. The method of any one of claims 249-252, wherein the plurality of dsRNAs are administered to the subject together.

254. The method of any one of claims 249-253, wherein the dsRNA is lipid formulated.

255. The method of any one of claims 249-254, wherein the dsRNA is lipid formulated in a formulation selected from Table A.

256. The method of any one of claims 249-255, wherein the subject is a primate or a rodent.

257. The method of any one of claims 249-256, wherein the subject is a human.

258. The method of any one of claims 249-257, wherein the effective amount is a concentration of 0.01-5.0 mg/kg bodyweight of the subject.

259. The method of any one of claims 249-258, wherein the subject has fatigue, shortness of breath, headache, dizziness, or pale skin.

260. The method of any one of claims 249-259, wherein the subject has reduced iron levels compared to a subject without anemia.

261. The method of any one of claims 249-260, wherein the subject has Hb levels <9g/dL.

262. The method of any one of claims 249-261, wherein the subject has chronic kidney disease (CKD), cancer, chronic inflammatory disease, rheumatoid arthritis (RA), or iron-resistant iron-deficient anemia (IRIDA).

263. The method of any one of claims 249-262, wherein the subject has reduced renal EPO synthesis compared to a subject without CKD, a dietary deficiency, blood loss, or elevated hepcidin levels compared to a subject without CKD.

264. The method of any one of claims 249-263, wherein the subject has decreased renal excretion of hepcidin compared to a subject without CKD or low grade inflammation characterized by increased interleukin-6 (IL-6) levels compared to a subject without CKD.

265. The method of any one of claims 249-264, wherein the subject has a reticulocyte Hb of < 28 pg.

266. The method of any one of claims 249-265, wherein the subject has >10% hypochromic RBCs.

267. The method of any one of claims 249-266, further comprising determining the CBC, serum iron concentration, Transferrin (Tf) saturation, or ferritin levels of the subject.

268. The method of any one of claims 249-267, wherein administering results in an increase in iron levels in the subject.

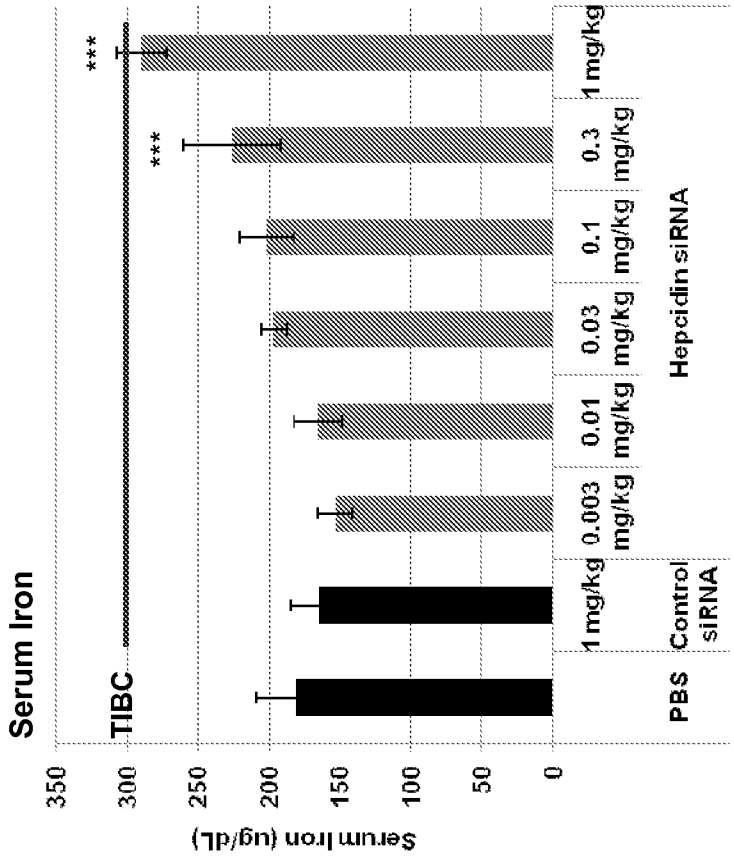
269. The method of any one of claims 249-268, wherein administering results in a 2-fold increase in iron levels in the subject.

270. The method of any one of claims 249-269, wherein administering results in an increase in Tf saturation in the subject.

271. The method of any one of claims 249-270, further comprising determining the iron level in the subject.

272. The method of any one of claims 249-271, further comprising administering intravenous iron or ESAs to the subject.

Figure 1


Serum Iron
TIBC
***
***
Serum Iron (ug/dL)
350
300
250
200
150
100
50
0
PBS
Control siRNA
1 mg/kg
0.003 mg/kg
0.01 mg/kg
0.03 mg/kg
0.1 mg/kg
0.3 mg/kg
1 mg/kg
Hepcidin siRNA
ANOVA, Dunnet's t post-hoc, compared to Control siRNA
* p < 0.05, *** p < 0.001

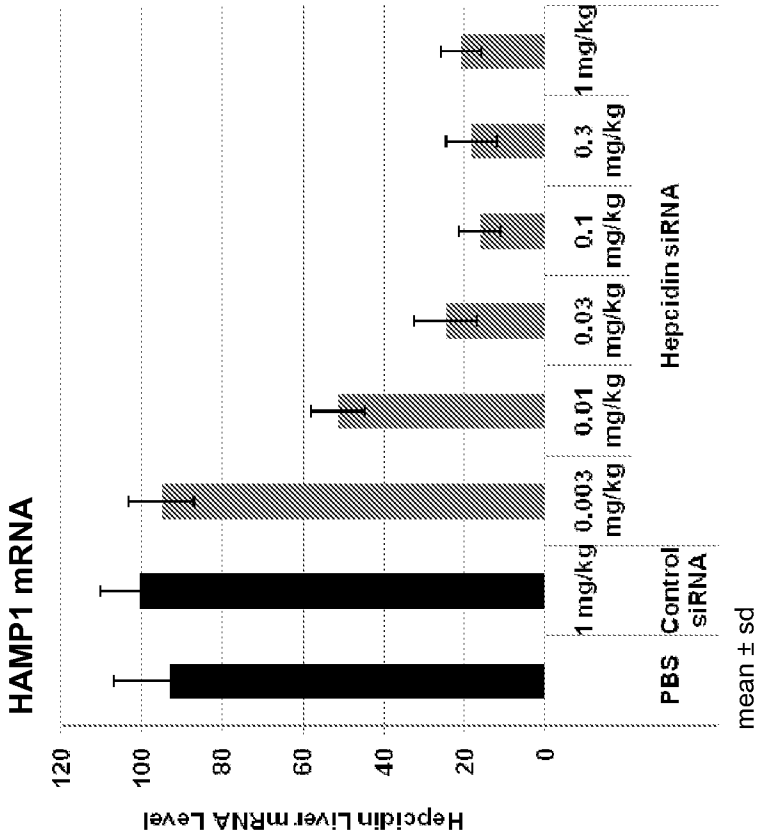

HAMP1 mRNA
Hepcidin Liver mRNA Level
120
100
80
60
40
20
0
PBS
Control siRNA
1 mg/kg
0.003 mg/kg
0.01 mg/kg
0.03 mg/kg
0.1 mg/kg
0.3 mg/kg
1 mg/kg
Hepcidin siRNA
mean ± sd

Figure 2

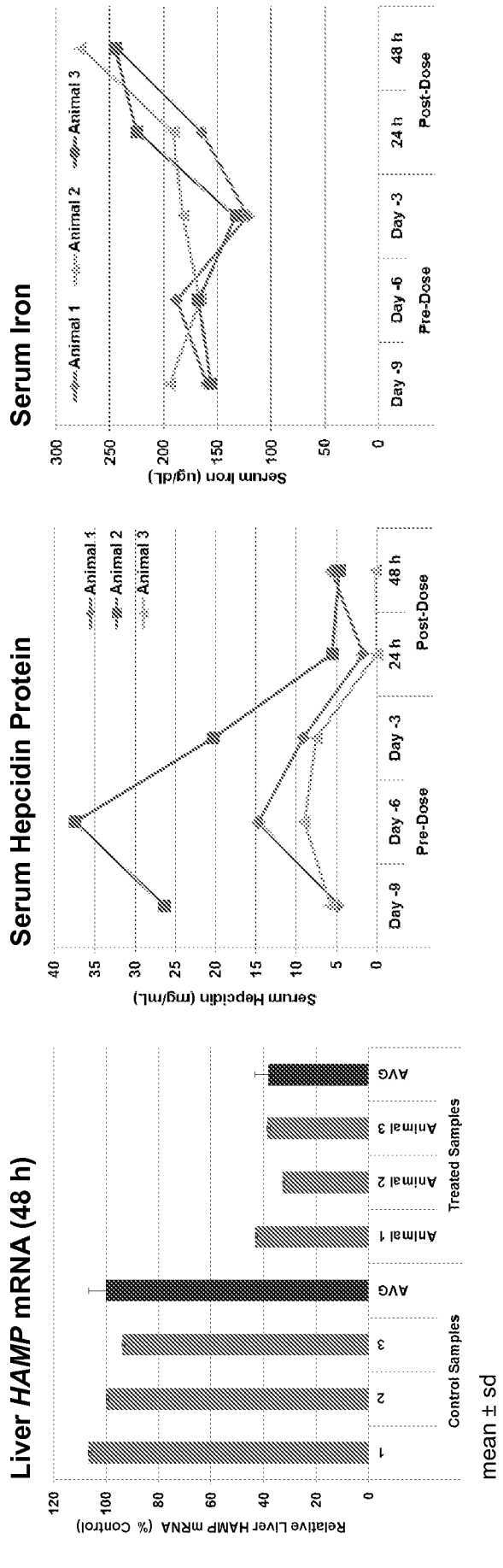
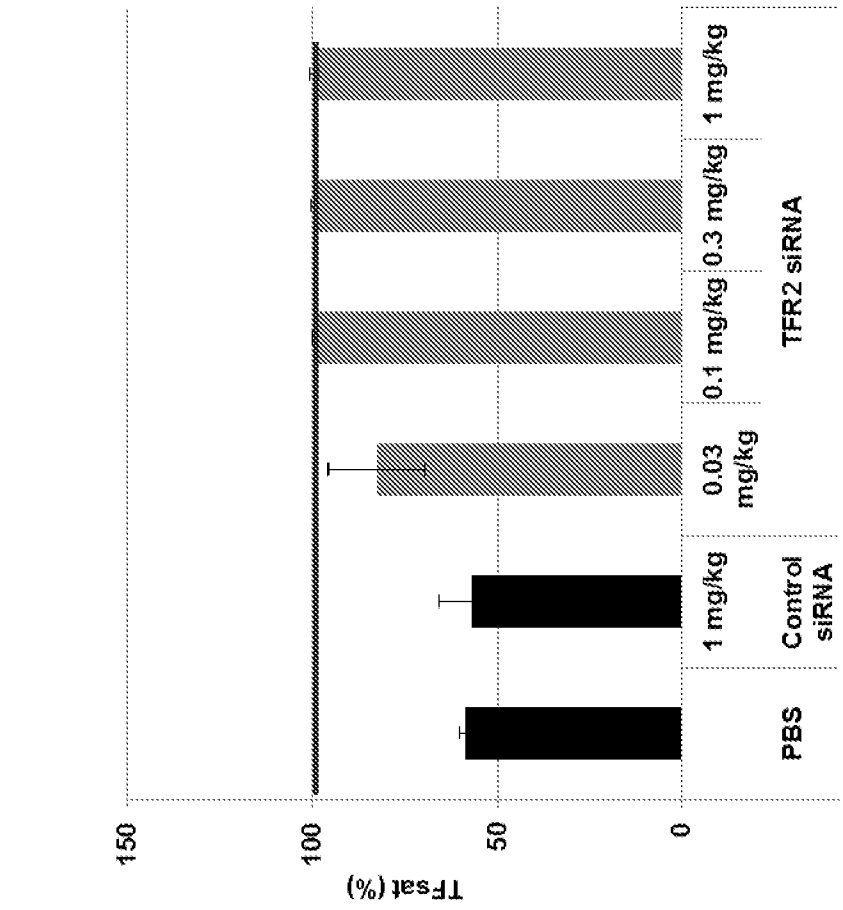
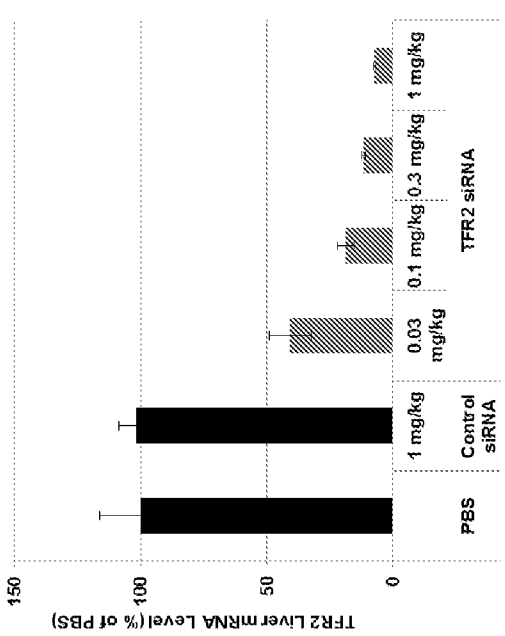

Liver HAMP mRNA (48 h)
Relative Liver HAMP mRNA (% Control)
120
100
80
60
40
20
0
1
2
3
AVG
Animal 1
Animal 2
Animal 3
AVG
Control Samples
Treated Samples
mean ± sd
Serum Hepcidin Protein
Serum Hepcidin (mg/mL)
40
35
30
25
20
15
10
5
0
Animal 1
Animal 2
Animal 3
Day -9
Day -6
Day -3
24 h
48 h
Pre-Dose
Post-Dose
Serum Iron
Serum Iron (ug/dL)
300
250
200
150
100
50
0
Animal 1
Animal 2
Animal 3
Day -9
Day -6
Day -3
24 h
48 h
Pre-Dose
Post-Dose

Figure 3


TFsat (%)
150
100
50
0
PBS
1 mg/kg
Control siRNA
0.03 mg/kg
0.1 mg/kg
0.3 mg/kg
1 mg/kg
TFR2 siRNA
C57BL6 mice, 48 hr post-administration
mean ± sd


TFR2 Liver mRNA Level (% of PBS)
150
100
50
0
PBS
1 mg/kg
Control siRNA
0.03 mg/kg
0.1 mg/kg
0.3 mg/kg
1 mg/kg
TFR2 siRNA

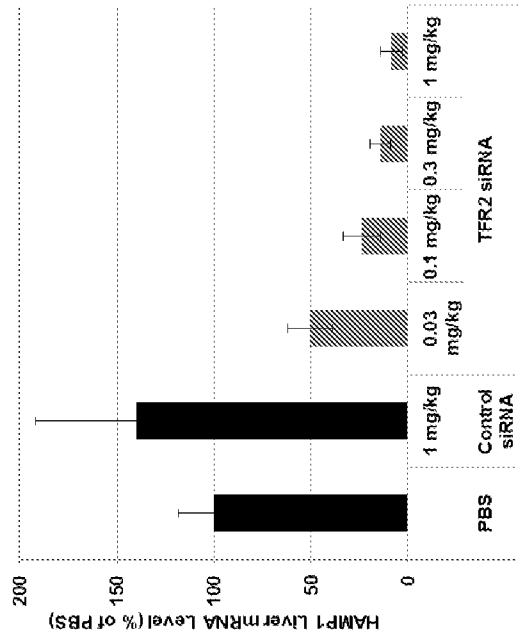

HAMP1 Liver mRNA Level (% of PBS)
200
150
100
50
0
PBS
1 mg/kg
Control siRNA
0.03 mg/kg
0.1 mg/kg
0.3 mg/kg
1 mg/kg
TFR2 siRNA

Figure 4

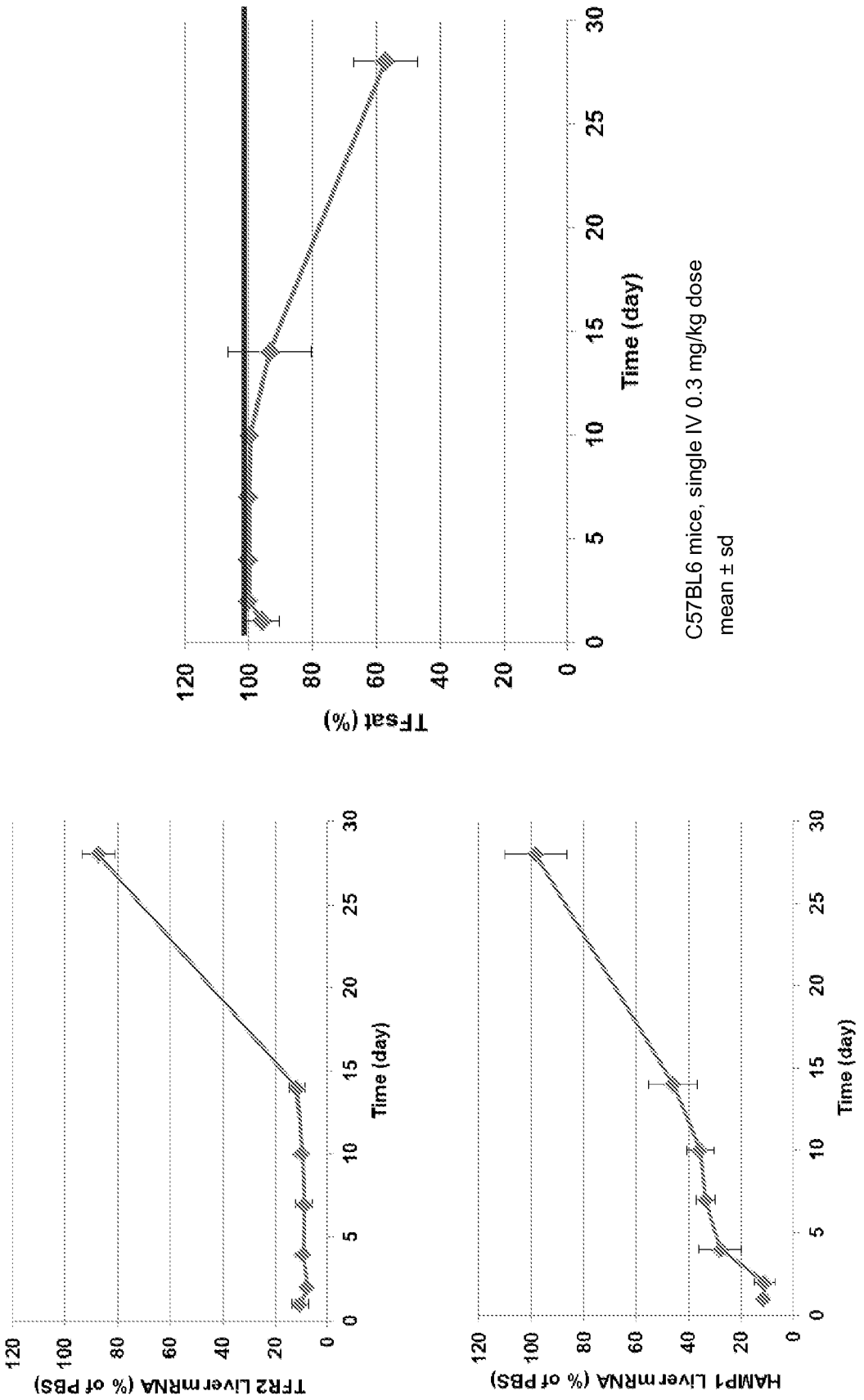

TFsat (%)
Time (day)
C57BL6 mice, single IV 0.3 mg/kg dose
mean ± sd
TFR2 Liver mRNA (% of PBS)
Time (day)
HAMP1 Liver mRNA (% of PBS)
Time (day)

Figure 5

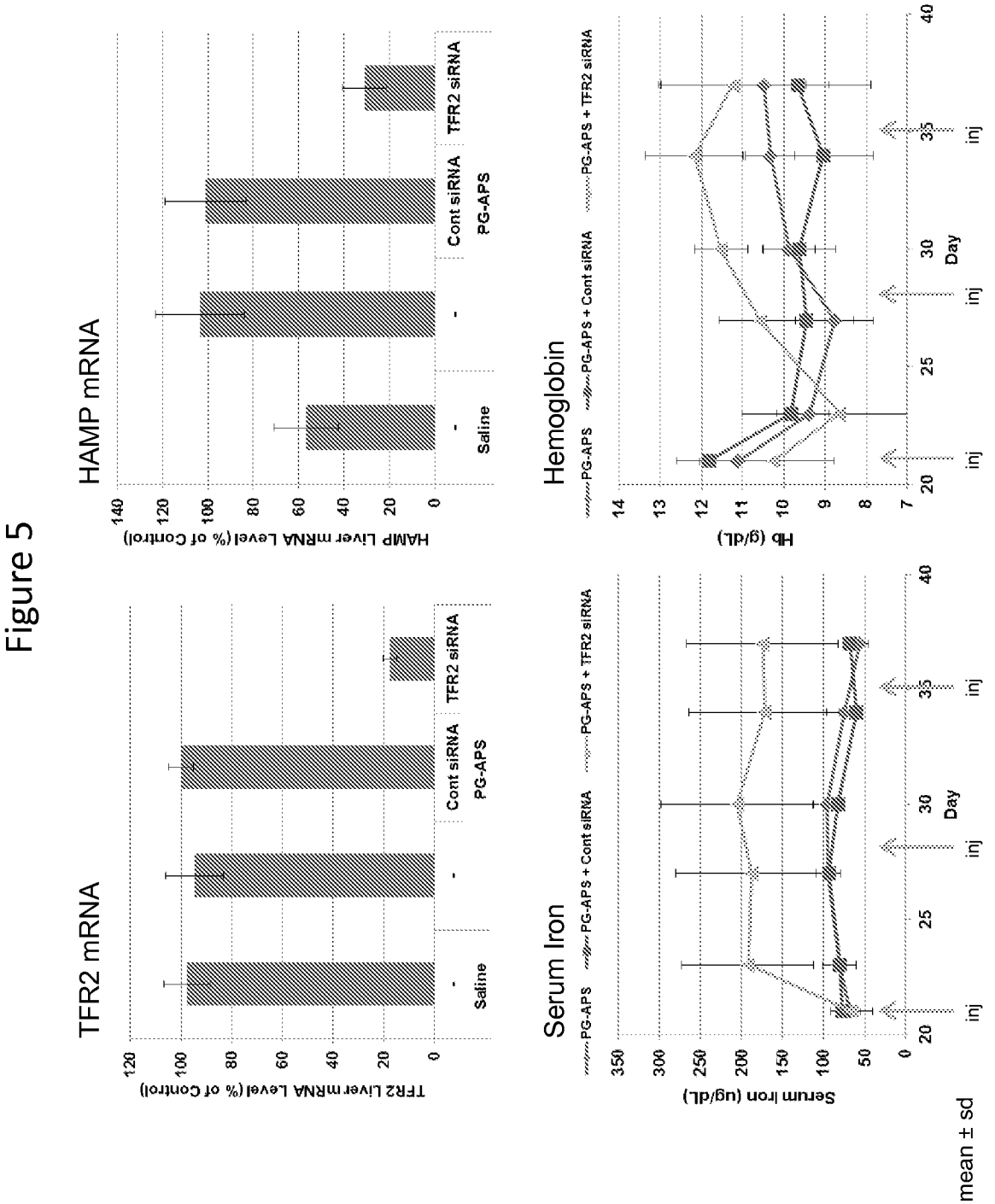

TFR2 mRNA
TFR2 Liver mRNA Level (% of Control)
120
100
80
60
40
20
0
-
-
Saline
Cont siRNA
TFR2 siRNA
PG-APS
HAMP mRNA
HAMP Liver mRNA Level (% of Control)
140
120
100
80
60
40
20
0
-
-
Saline
Cont siRNA
TFR2 siRNA
PG-APS
Hemoglobin
PG-APS
PG-APS + Cont siRNA
PG-APS + TFR2 siRNA
Hb (g/dL)
14
13
12
11
10
9
8
7
20
25
30
35
40
Day
inj
inj
inj
Serum Iron
PG-APS
PG-APS + Cont siRNA
PG-APS + TFR2 siRNA
Serum Iron (ug/dL)
350
300
250
200
150
100
50
0
20
25
30
35
40
Day
inj
inj
inj
mean ± sd

Figure 6

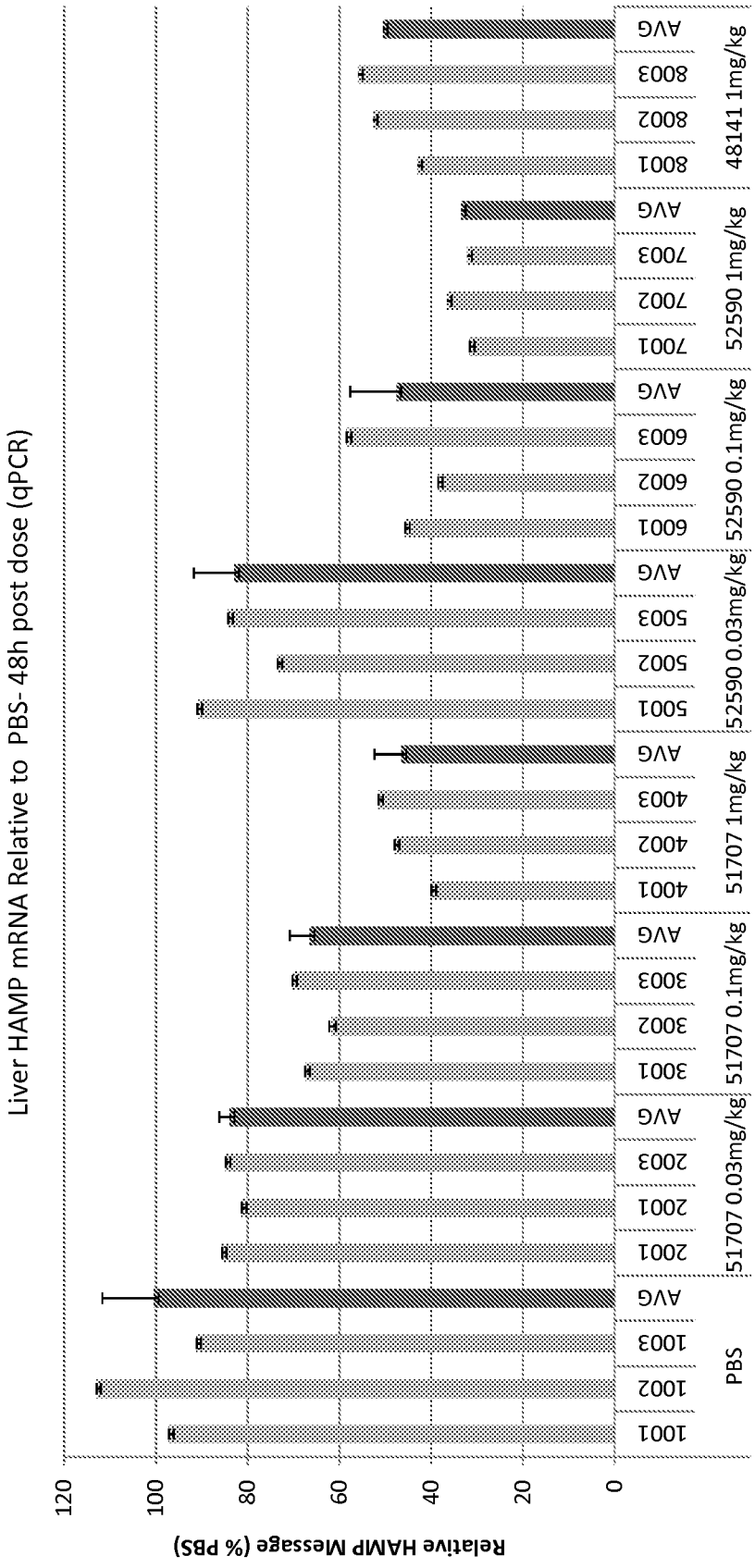

Liver HAMP mRNA Relative to PBS- 48h post dose (qPCR)
Relative HAMP Message (% PBS)
120
100
80
60
40
20
0
1001
1002
1003
AVG
2001
2001
2003
AVG
3001
3002
3003
AVG
4001
4002
4003
AVG
5001
5002
5003
AVG
6001
6002
6003
AVG
7001
7002
7003
AVG
8001
8002
8003
AVG
PBS
51707 0.03mg/kg
51707 0.1mg/kg
51707 1mg/kg
52590 0.03mg/kg
52590 0.1mg/kg
52590 1mg/kg
48141 1mg/kg

Figure 7

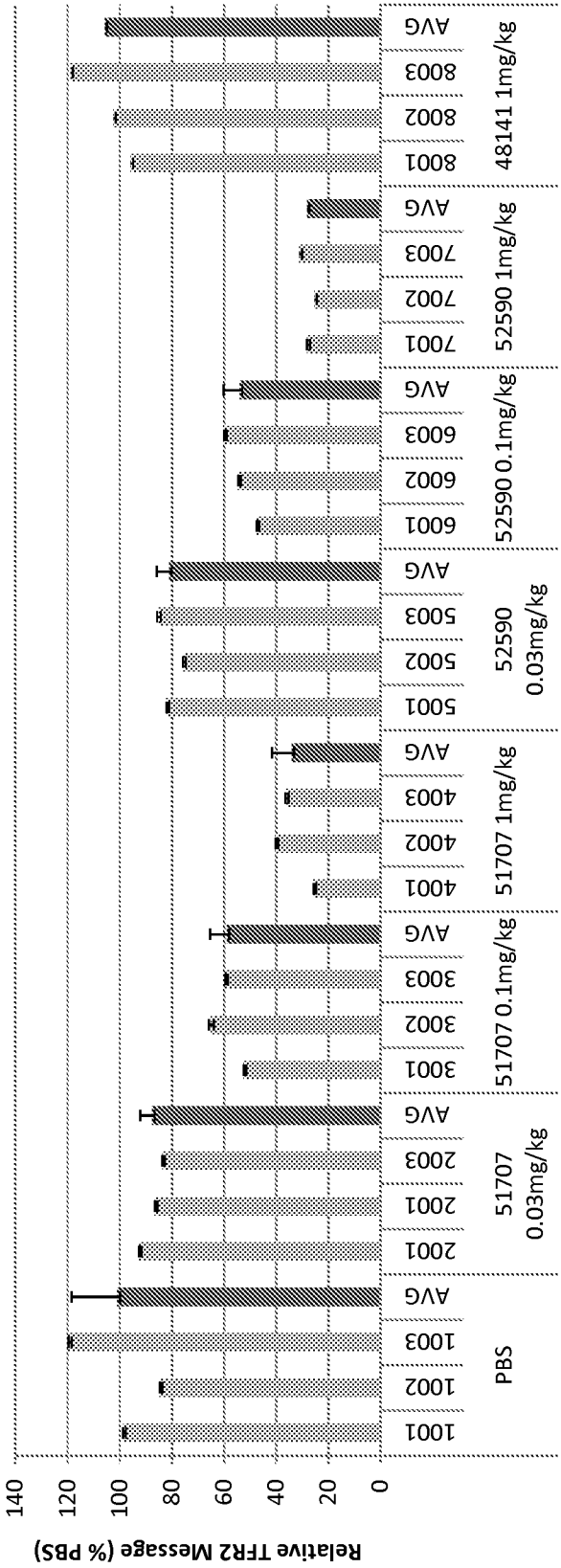

Liver TFR2mRNA Relative to PBS- 48h post dose (qPCR)
Relative TFR2 Message (% PBS)
140
120
100
80
60
40
20
0
PBS
1001
1002
1003
AVG
51707 0.03mg/kg
2001
2001
2003
AVG
51707 0.1mg/kg
3001
3002
3003
AVG
51707 1mg/kg
4001
4002
4003
AVG
52590 0.03mg/kg
5001
5002
5003
AVG
52590 0.1mg/kg
6001
6002
6003
AVG
52590 1mg/kg
7001
7002
7003
AVG
48141 1mg/kg
8001
8002
8003
AVG

Figure 8

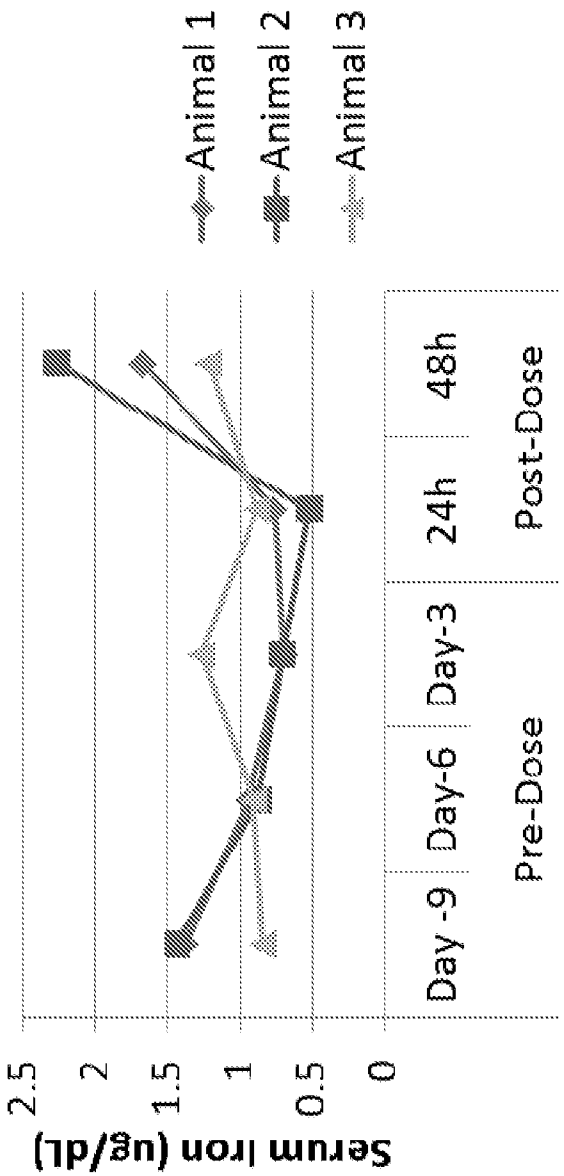

Serum Iron
Animal 1
Animal 2
Animal 3
Serum Iron (ug/dL)
2.5
2
1.5
1
0.5
0
Day -9
Day-6
Day-3
24h
48h
Pre-Dose
Post-Dose

Figure 9

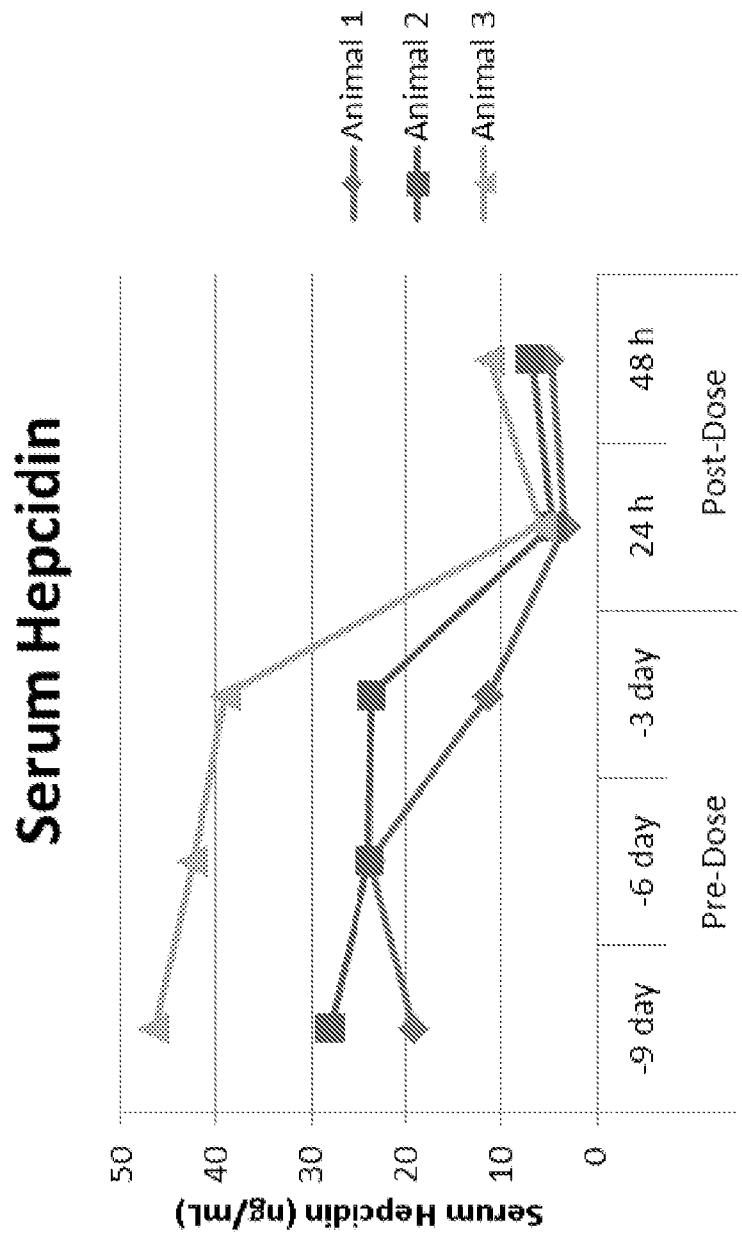
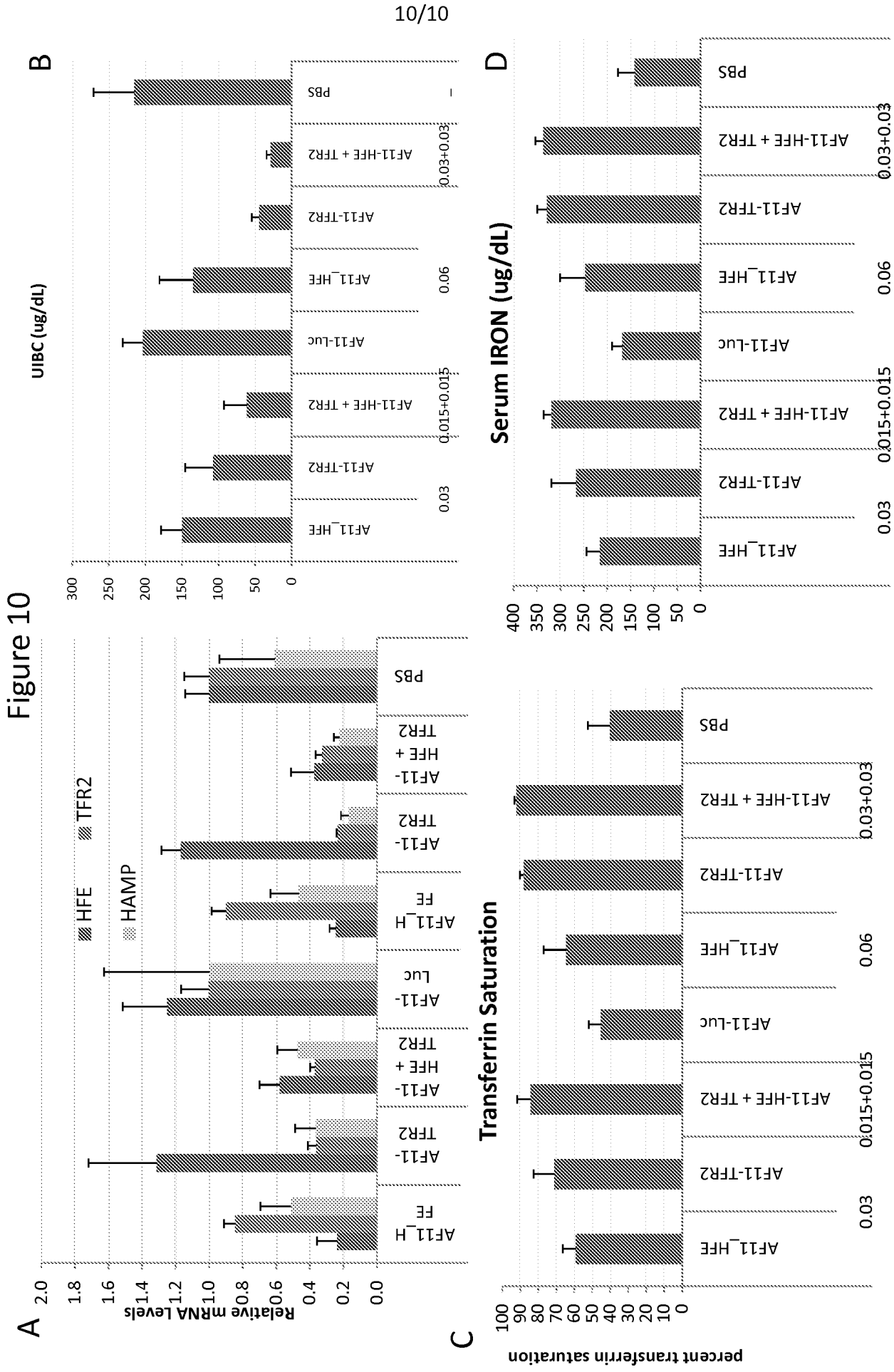

Serum Hepcidin
Animal 1
Animal 2
Animal 3
Serum Hepcidin (ng/mL)
50
40
30
20
10
0
-9 day
-6 day
-3 day
24 h
48 h
Pre-Dose
Post-Dose

Figure 10


A
Relative mRNA Levels
HFE
TFR2
HAMP
AF11_HFE
AF11-TFR2
AF11-HFE + TFR2
AF11-Luc
AF11_HFE
AF11-TFR2
AF11-HFE + TFR2
PBS
B
UIBC (ug/dL)
AF11_HFE
AF11-TFR2
AF11-HFE + TFR2
AF11-Luc
AF11_HFE
AF11-TFR2
AF11-HFE + TFR2
PBS
0.03
0.015+0.015
0.06
0.03+0.03
C
percent transferrin saturation
Transferrin Saturation
D
Serum IRON (ug/dL)