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(54) Title: NUCLEIC ACID COMPOUNDS FOR INHIBITING CD19 GENE EXPRESSION AND USES THEREOF

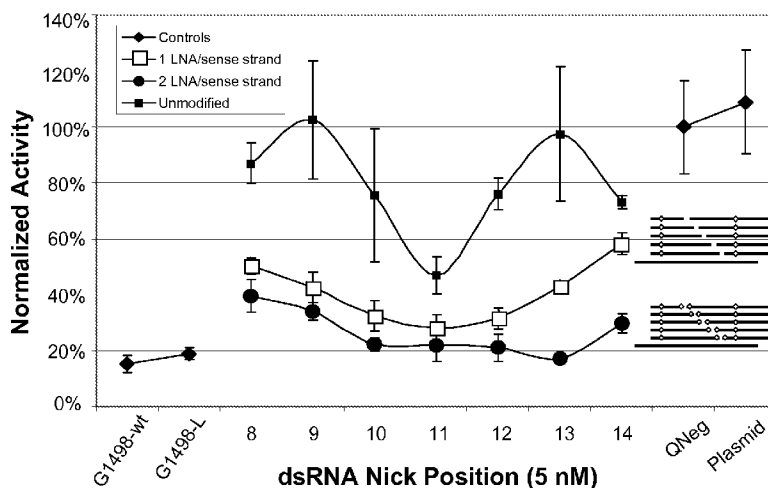


Fig. 9

(57) Abstract: The present disclosure provides meroduplex ribonucleic acid molecules (mdRNA) capable of decreasing or silencing CD 19 gene expression. An mdRNA of this disclosure comprises at least three strands that combine to form at least two non-overlapping double-stranded regions separated by a nick or gap wherein one strand is complementary to a CD 19 mRNA. In addition, the meroduplex may have at least one uridine substituted with a 5-methyluridine, a nucleoside replaced with a locked nucleic acid, or optionally other modifications, and any combination thereof. Also provided are methods of decreasing expression of a CD 19 gene in a cell or in a subject to treat a CD19-related disease.

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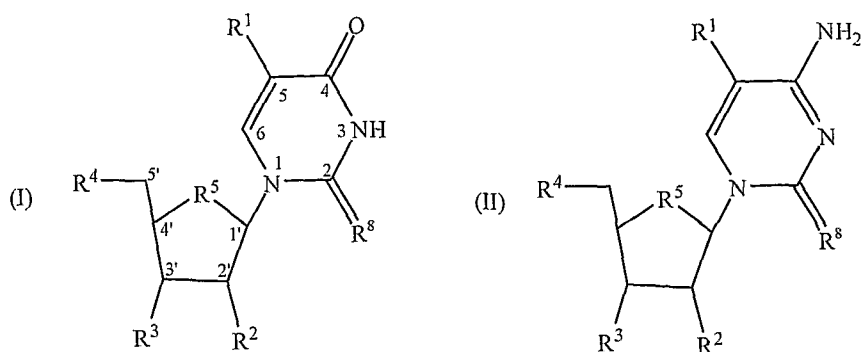
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**AMENDED CLAIMS****received by the International Bureau on 17 November 2008 (17.11.08)**

1. A meroduplex ribonucleic acid (mdRNA) molecule that down regulates the expression of a human CD19 molecule (CD19) mRNA, comprising a first strand of 15 to 40 nucleotides in length that is complementary to a portion of the human CD19 mRNA as set forth in SEQ ID NO:1158, and a second strand and a third strand that is each complementary to non-overlapping regions of the first strand, wherein the second strand and third strand can anneal with the first strand to form at least two double-stranded regions spaced apart by a nick or a gap.
2. The mdRNA molecule of claim 1 wherein the first strand is 15 to 25 nucleotides in length or 26 to 40 nucleotides in length.
3. The mdRNA molecule of claim 1 wherein the gap comprises from 1 to 10 unpaired nucleotides.
4. The mdRNA molecule of claim 1 wherein the mdRNA molecule comprises at least one 5-methyluridine, 2-thioribothymidine, or 2'-O-methyl-5-methyluridine.
5. The mdRNA molecule of claim 1 wherein the mdRNA molecule comprises at least one locked nucleic acid (LNA) molecule, deoxy nucleotide, G clamp, 2'-sugar modification, modified internucleoside linkage, or any combination thereof.
6. The mdRNA molecule of claim 1 wherein the mdRNA contains an overhang of one to four nucleotides on at least one 3'-end that is not part of the gap or has a blunt end at one or both ends of the mdRNA.
7. An mdRNA molecule that down regulates the expression of a human CD19 mRNA, the mdRNA molecule comprising a first strand of 15 to 40 nucleotides in length that is complementary to the human CD19 mRNA as set forth in SEQ ID NO:1158, and a second strand and a third strand that is each complementary to non-overlapping regions of the first strand, wherein the second strand and third strand can anneal with the first strand to form at least two double-stranded regions spaced apart by a nick or a gap, and wherein at least one pyrimidine of the mdRNA molecule is a pyrimidine nucleoside according to Formula I or II:



wherein:

$R^1$  and  $R^2$  are each independently a -H, -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>OCH<sub>2</sub>CH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, halogen, substituted or unsubstituted C<sub>1</sub>-C<sub>10</sub> alkyl, alkoxy, alkoxyalkyl, hydroxyalkyl, carboxyalkyl, alkylsulfonylamino, aminoalkyl, dialkylamino, alkylaminoalkyl, dialkylaminoalkyl, haloalkyl, trifluoromethyl, cycloalkyl, (cycloalkyl)alkyl, substituted or unsubstituted C<sub>2</sub>-C<sub>10</sub> alkenyl, substituted or unsubstituted -O-allyl, -O-CH<sub>2</sub>CH=CH<sub>2</sub>, -O-CH=CHCH<sub>3</sub>, substituted or unsubstituted C<sub>2</sub>-C<sub>10</sub> alkynyl, carbamoyl, carbamyl, carboxy, carbonylamino, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, -NH<sub>2</sub>, -NO<sub>2</sub>, -C≡, or heterocyclo group,

$R^3$  and  $R^4$  are each independently a hydroxyl, a protected hydroxyl, a phosphate, or an internucleoside linking group, and

$R^5$  and  $R^8$  are each independently O or S.

8. The mdRNA molecule of claim 7 wherein the first strand is 15 to 25 nucleotides in length or 26 to 40 nucleotides in length.

9. The mdRNA molecule of claim 7 wherein the gap comprises from 1 to 10 unpaired nucleotides.

10. The mdRNA molecule of claim 7 wherein at least one nucleoside is according to Formula I and in which  $R^1$  is methyl and  $R^2$  is -OH or -O-methyl.

11. The mdRNA molecule of claim 7 wherein at least one  $R^2$  is selected from the group consisting of 2'-O-(C<sub>1</sub>-C<sub>5</sub>) alkyl, 2'-O-methyl, 2'-OCH<sub>2</sub>OCH<sub>2</sub>CH<sub>3</sub>, 2'-OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, 2'-O-allyl, and fluoro.

12. The mdRNA molecule of claim 7 wherein the mdRNA molecule comprises at least one 5-methyluridine, 2-thioribothymidine, or 2'-O-methyl-5-methyluridine.
13. The mdRNA molecule of claim 7 wherein the mdRNA molecule comprises at least one locked nucleic acid (LNA) molecule, deoxy nucleotide, G clamp, 2'-sugar modification, modified internucleoside linkage, or any combination thereof.
14. The mdRNA molecule of claim 7 wherein contains an overhang of one to four nucleotides on at least one 3'-end that is not a part of the gap or the dsRNA molecule has a blunt end on one or both ends of the mdRNA molecule.
15. An mdRNA molecule that down regulates the expression of a human CD19 mRNA, the mdRNA molecule comprising a first strand of 15 to 40 nucleotides in length that is complementary to the human CD19 mRNA as set forth in SEQ ID NO:1158, and a second strand and a third strand that is each complementary to non-overlapping regions of the first strand, wherein the second strand and third strand can anneal with the first strand to form at least two double-stranded regions spaced apart by a nick or a gap, and wherein the double-stranded regions have a combined length of about 15 base pairs to about 40 base pairs.
16. The mdRNA molecule of claim 15 wherein the first strand is 15 to 25 nucleotides in length or 26 to 40 nucleotides in length.
17. The mdRNA molecule of claim 15 wherein the gap comprises from 1 to 10 unpaired nucleotides.
18. The mdRNA molecule of claim 15 wherein the mdRNA molecule comprises at least one 5-methyluridine, 2-thioribothymidine, or 2'-O-methyl-5-methyluridine.
19. The mdRNA molecule of claim 15 wherein the first strand is 19 to 23 nucleotides in length and is complementary to a human CD19 nucleic acid sequence as set forth in any one of SEQ ID NOS:1159-1347.

20. The mdRNA molecule of claim 15 wherein the first strand is 25 to 29 nucleotides in length and is complementary to a human CD19 nucleic acid sequence as set forth in any one of SEQ ID NOS:1159-1347.

21. A method for reducing the expression of a human CD19 gene, comprising administering an mdRNA molecule according to any one of claims 1-20 to a cell expressing a human CD19 gene, wherein the mdRNA molecule reduces the expression of the human CD19 gene in the cell.

22. The method according to claim 21 wherein the cell is a human cell.

23. Use of an mdRNA as defined in any one of the preceding claims for the manufacture of a medicament for use in the therapy of a hyperproliferative or inflammatory disease.

24. A double-stranded ribonucleic acid (dsRNA) molecule that down regulates the expression of a human CD19 molecule (CD19) mRNA, the dsRNA molecule comprising a first strand of 26 to 40 nucleotides in length that is complementary to a portion of the human CD19 mRNA as set forth in SEQ ID NO:1158, and a second strand that is complementary to the first strand, and wherein upon annealing of the first strand and the second strand the dsRNA has a 3' overhang and a blunt end.

25. The dsRNA molecule of claim 24 wherein the first strand is from 27 to 35 nucleotides in length.

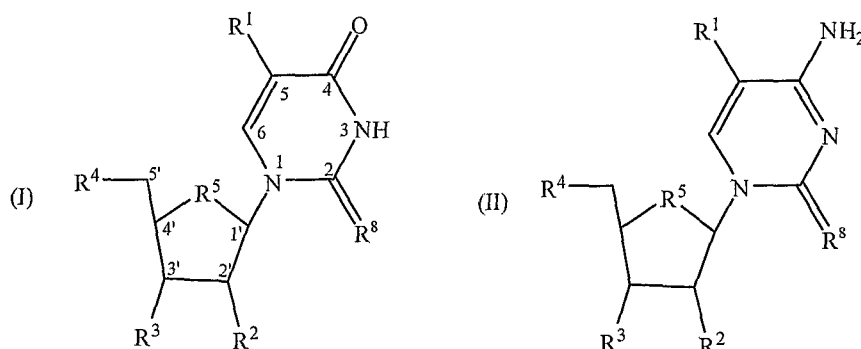
26. The dsRNA molecule of claim 24 wherein the dsRNA molecule comprises at least one 5-methyluridine, 2-thioribothymidine, or 2'-O-methyl-5-methyluridine.

27. The dsRNA molecule of claim 24 wherein the dsRNA molecule comprises at least one locked nucleic acid (LNA) molecule, deoxy nucleotide, G clamp, 2'-sugar modification, modified internucleoside linkage, or any combination thereof.

28. The dsRNA molecule of claim 24 wherein the 3'-overhang has from one to four nucleotides and is on the first strand.

29. The dsRNA molecule of claim 24 wherein the dsRNA molecule has a 5'-terminal end comprising a hydroxyl or a phosphate.

30. A dsRNA molecule that down regulates the expression a human CD19 mRNA, the dsRNA molecule comprising a first strand of 26 to 40 nucleotides in length that is complementary to a portion of the human CD19 mRNA as set forth in SEQ ID NO:1158, and a second strand that is complementary to the first strand, and wherein upon annealing of the first strand and the second strand the dsRNA has a 3' overhang and a blunt end, and wherein at least one pyrimidine of the dsRNA molecule comprises a pyrimidine nucleoside according to Formula I or II:



wherein:

$R^1$  and  $R^2$  are each independently a -H, -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>OCH<sub>2</sub>CH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, halogen, substituted or unsubstituted C<sub>1</sub>-C<sub>10</sub> alkyl, alkoxy, alkoxyalkyl, hydroxyalkyl, carboxyalkyl, alkylsulfonylamino, aminoalkyl, dialkylamino, alkylaminoalkyl, dialkylaminoalkyl, haloalkyl, trifluoromethyl, cycloalkyl, (cycloalkyl)alkyl, substituted or unsubstituted C<sub>2</sub>-C<sub>10</sub> alkenyl, substituted or unsubstituted -O-allyl, -O-CH<sub>2</sub>CH=CH<sub>2</sub>, -O-CH=CHCH<sub>3</sub>, substituted or unsubstituted C<sub>2</sub>-C<sub>10</sub> alkynyl, carbamoyl, carbamyl, carboxy, carbonylamino, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, -NH<sub>2</sub>, -NO<sub>2</sub>, -C≡N, or heterocyclo group,

$R^3$  and  $R^4$  are each independently a hydroxyl, a protected hydroxyl, a phosphate, or an internucleoside linking group, and

$R^5$  and  $R^8$  are each independently O or S.

31. The dsRNA molecule of claim 30 wherein the first strand is from 27 to 35 nucleotides in length.

32. The dsRNA molecule of claim 30 wherein at least one nucleoside is according to Formula I and in which R<sup>1</sup> is methyl and R<sup>2</sup> is -OH or -O-methyl.

33. The dsRNA molecule of claim 30 wherein at least one R<sup>2</sup> is selected from the group consisting of 2'-O-(C<sub>1</sub>-C<sub>5</sub>) alkyl, 2'-O-methyl, 2'-OCH<sub>2</sub>OCH<sub>2</sub>CH<sub>3</sub>, 2'-OCH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, 2'-O-allyl, and 2'-fluoro.

34. The dsRNA molecule of claim 30 wherein the dsRNA molecule comprises at least one 5-methyluridine, 2-thioribothymidine, or 2'-O-methyl-5-methyluridine.

35. The dsRNA molecule of claim 30 wherein the dsRNA molecule comprises at least one LNA, deoxy nucleotide, G clamp, 2'-sugar modification, modified internucleoside linkage, or any combination thereof.

36. The dsRNA molecule of claim 30, wherein the 3'-overhang has from one to four nucleotides and is on the first strand.

37. A method for reducing the expression of a human CD19 gene, comprising administering a dsRNA molecule according to any one of claims 24-36 to a cell expressing a human CD19 gene, wherein the dsRNA molecule reduces the expression of the human CD19 gene in the cell.

38. The method according to claim 37 wherein the cell is a human cell.

39. Use of a dsRNA molecule as defined in any one of claims 24-38 for the manufacture of a medicament for use in the therapy of a hyperproliferative or inflammatory disease.