The present disclosure provides meroduplex ribonucleic acid molecules (mdRNA) capable of decreasing or silencing TLR (e.g., TLR3, TLR7, TLR8, or TLR9) 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 TLR3, TLR7, TLR8, or TLR9 mRNA. In addition, the meroduplex may have at least one uridine is a 5-methyluridine, a nucleoside is a locked nucleic acid, or optionally other modifications, and any combination thereof. Also provided are methods of decreasing expression of a TLR gene in a cell or in a subject to treat a TLR-related disease.
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- as to applicant’s entitlement to apply for and be granted a patent (Rule 4.17(H))
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WHAT IS CLAIMED IS:

1. A meroduplex ribonucleic acid (mdRNA) molecule that down regulates the expression of any one of a human toll-like receptor 3 (TLR3) mRNA, a human toll-like receptor 7 (TLR7) mRNA, a human toll-like receptor 8 (TLR8) mRNA, or a human toll-like receptor 9 (TLR9) mRNA, the mdRNA molecule comprising a first strand of 15 to 40 nucleotides in length that is complementary to a portion of any one of human TLR3 nucleic acid sequence as set forth in SEQ ID NO: 1158, human TLR7 nucleic acid sequence as set forth in SEQ ID NO: 1454, human TLR8 nucleic acid sequence as set forth in SEQ ID NO: 1939 or 1940, or human TLR9 nucleic acid sequence as set forth in SEQ ID NO: 2681, 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 any one of human TLR3, human TLR7, human TLR8, or human TLR9 mRNA, the mdRNA molecule comprising a first strand of 15 to 40 nucleotides in length that is
complementary to a portion of any one of human TLR3 nucleic acid sequence as set forth in SEQ ID NO:1158, human TLR7 nucleic acid sequence as set forth in SEQ ID NO:1454, human TLR8 nucleic acid sequence as set forth in SEQ ID NO:1939 or 1940, or human TLR9 nucleic acid sequence as set forth in SEQ ID NO:2681, 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:

R₁ and R₂ are each independently a -H, -OH, -OCH₃, -OCH₂OCH₂CH₃, -OCH₂CH₂OCH₃, halogen, substituted or unsubstituted Ci-Ci₉ alkyl, alkoxy, alkoxyalkyl, hydroxyalkyl, carboxyalkyl, alkylsulfonylamino, aminoalkyl, dialkylamino, alkylaminoalkyl, dialkylaminoalkyl, haloalkyl, trifluoromethyl, cycloalkyl, (cycloalkyl)alkyl, substituted or unsubstituted C₂-Ci₉ alkenyl, substituted or unsubstituted -O-allyl, -0-CH₂CH=CH₂, -0-CH=CHCH₃, substituted or unsubstituted C₂-Ci₉ alkynyl, carbamoyl, carbamyl, carboxy, carbonylamino, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, -NH₂, -NO₂, -C≡, or heterocyclo group,

R₃ and R₄ are each independently a hydroxyl, a protected hydroxyl, a phosphate, or an internucleoside linking group, and

R₅ and R₈ 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 R² is methyl and R² is -OH or -O-methyl.

11. The mdRNA molecule of claim 7 wherein at least one R² is selected from the group consisting of 2′-O-(C₁-C₅) alkyl, 2′-O-methyl, 2′-OCH₂OCH₂CH₃, 2′-OCH₂CH₂OCH₃, 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 any one of human TLR3, human TLR7, human TLR8, or human TLR9, the mdRNA molecule comprising a first strand of 15 to 40 nucleotides in length that is complementary to a portion of any one of human TLR3 nucleic acid sequence as set forth in SEQ ID NO: 1158, human TLR7 nucleic acid sequence as set forth in SEQ ID NO: 1454, human TLR8 nucleic acid sequence as set forth in SEQ ID NO: 1939 or 1940, or human TLR9 nucleic acid sequence as set forth in SEQ ID NO:2681, 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 TLR3 nucleic acid sequence as set forth in any one of SEQ ID NOS: 1159-1453, or human TLR7 nucleic acid sequence as set forth in any one of SEQ ID NOS: 1455-1938, or human TLR8 nucleic acid sequence as set forth in any one of SEQ ID NOS: 1941-2680, or human TLR9 nucleic acid sequence as set forth in any one of SEQ ID NOS:2682-3058.

20. The mdRNA molecule of claim 15 wherein the first strand is 25 to 29 nucleotides in length and is complementary to a human TLR3 nucleic acid sequence as set forth in any one of SEQ ID NOS: 1159-1453, or human TLR7 nucleic acid sequence as set forth in any one of SEQ ID NOS: 1455-1938, or human TLR8 nucleic acid sequence as set forth in any one of SEQ ID NOS: 1941-2680, or human TLR9 nucleic acid sequence as set forth in any one of SEQ ID NOS:2682-3058.

21. A method for reducing the expression of a human TLR gene, comprising administering an mdRNA molecule according to any one of claims 1-20 to a cell expressing a human TLR3, TLR7, TLR8, or TLR9 gene, wherein the mdRNA molecule reduces the expression of the human TLR3, TLR7, TLR8, or TLR9 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 any one of a human toll-like receptor 3 (TLR3) mRNA, a
human toll-like receptor 7 (TLR7) mRNA, a human toll-like receptor 8 (TLR8)
mRNA, or a human toll-like receptor 9 (TLR9) mRNA, the dsRNA molecule
comprising a first strand of 26 to 40 nucleotides in length that is complementary to a
portion of any one of human TLR3 nucleic acid sequence as set forth in SEQ ID
NO: 1158, human TLR7 nucleic acid sequence as set forth in SEQ ID NO: 1454,
human TLR8 nucleic acid sequence as set forth in SEQ ID NO: 1939 or 1940, or
human TLR9 nucleic acid sequence as set forth in SEQ ID NO: 2681, 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 of any one of
human TLR3, human TLR7, human TLR8, or human TLR9, the dsRNA molecule
comprising a first strand of 26 to 40 nucleotides in length that is complementary to a portion of any one of human TLR3 nucleic acid sequence as set forth in SEQ ID NO: 1158, human TLR7 nucleic acid sequence as set forth in SEQ ID NO: 1454, human TLR8 nucleic acid sequence as set forth in SEQ ID NO: 1939 or 1940, or human TLR9 nucleic acid sequence as set forth in SEQ ID NO: 2681, 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:

R¹ and R² are each independently a -H, -OH, -OCH₃, -OCH₂OCH₂CH₃, -OCH₂CH₂OCH₃, halogen, substituted or unsubstituted Ci-Cio alkyl, alkoxy, alkoxyalkyl, hydroxyalkyl, carboxyalkyl, alkylsulfonylamino, aminoalkyl, dialkylamino, alkylaminoalkyl, dialkylaminoalkyl, haloalkyl, trifluoromethyl, cycloalkyl, (cycloalkyl)alkyl, substituted or unsubstituted C₂-Cio alkenyl, substituted or unsubstituted -O-allyl, -O-CH₂CH=CH₂, -O-CH=CHCH₃, substituted or unsubstituted C₂-Cio alkynyl, carbamoyl, carbamyl, carboxy, carbonylamino, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, -NH₂, -NO₂, -C≡N, or heterocyclo group.

R³ and R⁴ are each independently a hydroxyl, a protected hydroxyl, a phosphate, or an internucleoside linking group, and

R⁵ and R⁸ 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 R¹ is methyl and R² is -OH or -O-methyl.
33. The dsRNA molecule of claim 30 wherein at least one R² is selected from the group consisting of 2'-0-(Ci-C₅) alkyl, 2'-O-methyl, 2'-OCH₂OCH₂CH₃, 2'-OCH₂CH₂OCH₃, 2'-0-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 IL6 gene, comprising administering a dsRNA molecule according to any one of claims 24-36 to a cell expressing the IL6 gene, wherein the dsRNA molecule reduces the expression of the IL6 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.