

CLAIMS

1. A method for selecting an oligonucleotide for reducing the expression of a target gene, the method comprising:
 - 5 i) scanning a target polynucleotide, or complement thereof, for a region with at least three continuous pyrimidine bases;
 - ii) producing one or more candidate oligonucleotides comprising the three continuous pyrimidine bases, wherein one or both of the following apply;
 - a) the candidate oligonucleotide comprises three continuous pyrimidine
10 bases within seven bases of the 5' end of the oligonucleotide, and
 - b) the candidate oligonucleotide comprises three continuous pyrimidine bases within seven bases of the 3' end of the oligonucleotide,
 - iii) testing the ability of the one or more candidate oligonucleotides to reduce expression of the target gene, and
 - 15 iv) selecting an oligonucleotide which reduces expression of the target gene.
2. The method of claim 1, wherein the three continuous pyrimidine bases of a candidate oligonucleotide have a modified base and/or a modified backbone.
- 20 3. The method of claim 1 or claim 2, wherein the oligonucleotide comprises:
 - a) a 5' region comprising bases which are modified and/or which have a modified backbone,
 - b) a middle region comprising ribonucleic acid, deoxyribonucleic acid, or combination thereof, bases, and
 - 25 c) a 3' region comprising bases which are modified and/or which have a modified backbone.
4. A method for selecting an oligonucleotide for reducing the expression of a target gene, the method comprising:
 - 30 i) scanning a target polynucleotide, or complement thereof, for a region with at least three continuous pyrimidine bases;
 - ii) producing one or more candidate oligonucleotides comprising a 5' region, a 3' region and a middle region comprising ribonucleic acid, deoxyribonucleic acid, or combination thereof, bases, wherein one or both of the 5' region and the 3' region
35 comprise bases which are modified and/or which have a modified backbone, and at least one of the following apply;

a) the 5' region comprises three continuous pyrimidine bases which are modified and/or which have a modified backbone,

b) the 5' region comprises bases which are modified and/or which have a modified backbone and the junction between the 5' region and middle region comprises
5 three continuous pyrimidine bases,

c) the 3' region comprises three continuous pyrimidine bases which are modified and/or which have a modified backbone, and

d) the 3' region comprises bases which are modified and/or which have a modified backbone and the junction between the 3' region and middle region comprises
10 three continuous pyrimidine bases,

iii) testing the ability of the one or more candidate oligonucleotides to reduce expression of the target gene, and

iv) selecting an oligonucleotide which reduces expression of the target gene.

15 5. The method of claim 3 or claim 4, wherein the middle region is about 10 bases in length.

6. The method according to any one of claims 3 to 5, wherein the 5' region and/or the 3' region are about 5 bases in length.

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7. The method according to any one of claims 1 to 6, wherein the three continuous pyrimidine bases are within five bases of the 5' and/or 3' end of the oligonucleotide.

8. The method according to any one of claims 1 to 7, wherein the three continuous
25 pyrimidine bases are within three bases of the 5' and/or 3' end of the oligonucleotide.

9. The method according to any one of claims 1 to 8, wherein the three continuous pyrimidine bases are at the 5' and/or 3' end of the oligonucleotide.

30 10. The method according to any one of claims 1 to 9, wherein the 5' three continuous pyrimidine bases have the sequence 5'-CUU-3', 5'-CUT-3', 5'-CCU-3', 5'-UUC-3', 5'-UUU-3' or 5'-CTT-3'.

11. The method of claim 10, wherein the 5' three continuous pyrimidine bases
35 comprise the sequence 5'-CUU-3'.

12. The method according to any one of claims 1 to 11, wherein the 3' three continuous pyrimidine bases have the sequence 5'-UUC-3', 5'-TUC-3' 5'-UCC-3', 5'-CUU-3', 5'-UUU-3' or 5'-TTC-3'.
13. The method of claim 12, wherein the 3' three continuous pyrimidine bases have the sequence 5'-UUC-3'.
14. The method of claim 13, wherein the 3' pyrimidine bases have the sequence 5'-CUUC-3'.
15. The method according to any one of claims 1 to 14, wherein one, two or all three of the pyrimidine bases are a modified base and/or have a modified backbone.
16. The method according to any one of claims 4 to 15, wherein the three continuous pyrimidine bases at the junction have the sequence 5'-mCmUT-3', 5'-mCTT-3', 5'-TmUmC-3' or 5'-TTmC-3', where m is a modified base and/or has a modified backbone.
17. The method according to any one of claims 1 to 16, wherein the modified base comprises a 2'-O-methyl, 2'-O-methoxyethoxy, 2'-fluoro, 2'-allyl, 2'-O-[2-(methylamino)-2-oxoethyl], 4'-thio, 4'-CH₂-O-2'-bridge, 4'-(CH₂)₂-O-2'-bridge, 2'-LNA, 2'-amino, fluoroarabinonucleotide, threose nucleic acid or 2'-O--(N-methylcarbamate).
18. The method according to any one of claims 2 to 17, wherein the modified backbone comprises a phosphorothioate, a non-bridging oxygen atom substituting a sulfur atom, a phosphonate such as a methylphosphonate, a phosphodiester, a phosphoromorpholidate, a phosphoropiperazidate, amides, methylene(methylamino), fromacetal, thioformacetal, a peptide nucleic acid or a phosphoroamidate such as a morpholino phosphorodiamidate (PMO), N^{3'}-P^{5'} phosphoramidite or thiophosphoroamidite.
19. The method according to any one of claims 1 to 18, wherein at least a portion of the oligonucleotide has/is a ribonucleic acid, deoxyribonucleic acid, DNA phosphorothioate, RNA phosphorothioate, 2'-O-methyl-oligonucleotide, 2'-O-methyl-oligodeoxyribonucleotide, 2'-O-hydrocarbyl ribonucleic acid, 2'-O-hydrocarbyl DNA, 2'-O-hydrocarbyl RNA phosphorothioate, 2'-O-hydrocarbyl DNA phosphorothioate, 2'-

F-phosphorothioate, 2'-F-phosphodiester, 2'-methoxyethyl phosphorothioate, 2-methoxyethyl phosphodiester, deoxy methylene(methylimino) (deoxy MMI), 2'-O-hydrocarby MMI, deoxy-methylphosphonate, 2'-O-hydrocarbyl methylphosphonate, morpholino, 4'-thio DNA, 4'-thio RNA, peptide nucleic acid, 3'-amidate, deoxy 3'-amidate, 2'-O-hydrocarbyl 3'-amidate, locked nucleic acid, cyclohexane nucleic acid, tricycle-DNA, 2'-fluoro-arabino nucleic acid, N3'-P5' phosphoroamidate, carbamate linked, phosphotriester linked, a nylon backbone modification and any combination thereof.

20. The method according to any one of claims 1 to 19, wherein the modified base comprises a 2'-O-methyl and a phosphorothioate backbone.

21. The method according to any one of claims 1 to 20, wherein the oligonucleotide is at least 10, at least about 18, at least about 20, at about least 25, between about 10 and about 50 nucleotides, between about 18 and about 50 nucleotides, between about 18 and about 30 nucleotides, between about 20 and about 30 nucleotides, between about 20 and 5,000 nucleotides, or about 20 bases in length.

22. The method according to any one of claims 1 to 21, wherein the oligonucleotide is an antisense oligonucleotide or a double stranded oligonucleotide for gene silencing.

23. The method of claim 21 or claim 22, wherein the oligonucleotide is a gapmer antisense oligonucleotide.

24. The method of claim 22 or claim 23, wherein one, two or all three of the three continuous pyrimidine bases are removed by an endonuclease *in vivo*.

25. The method of claim 22, wherein the double stranded oligonucleotide for gene silencing is an siRNA or an shRNA.

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26. The method of claim 21, wherein the oligonucleotide is between 10 and 16 bases in length and potentiates Toll-like receptor 8 (TLR8) activity when administered to an animal.

27. The method according to any one of claims 1 to 26, wherein the oligonucleotide does not inhibit Toll-like receptor 7 (TLR7) activity when administered to an animal.

28. The method of claim 27, wherein the animal is a human.
29. A method for selecting an oligonucleotide for reducing the expression of a target
 5 gene, the method comprising
- i) scanning a target polynucleotide, or complement thereof, for a region with one of the following sequences 5'-CUUGU-3', 5'-CCUAU-3', 5'-CAUUA-3', 5'-CGAAU-3' 5'-CUUAU-3', 5'-CUUUA-3', 5'-ACUGU-3', 5'-CUUCU-3' 5'-CAUAU-3' 5'-CUUCU-3' 5'-AAUUU-3' 5'-AAAUU-3' 5'-CCUUC-3', 5'-AAUCA-3' or 5'-
 10 CGUCU-3', wherein the U may be a T,
 - ii) producing one or more candidate oligonucleotides comprising
 - a) 5'-CUUGU-3', 5'-CCUAU-3', 5'-CAUUA-3', 5'-CGAAU-3' 5'-CUUAU-3', 5'-CUUUA-3' or 5'-ACUGU-3' at the 5' end, and/or
 - b) 5'-CUUCU-3' 5'-CAUAU-3' 5'-CUUCU-3' 5'-AAUUU-3' 5'-
 15 AAAUU-3' 5'-CCUUC-3', 5'-AAUCA-3' or 5'-CGUCU-3' at the 3' end,
 - iii) testing the ability of the one or more candidate oligonucleotides to reduce expression of the target gene, and
 - iv) selecting an oligonucleotide which reduces expression of the target gene.
- 20 30. A method for selecting an oligonucleotide for reducing the expression of a target gene, the method comprising:
- i) scanning a target polynucleotide, or complement thereof, for a region with at least two continuous cytosine bases;
 - ii) producing one or more candidate oligonucleotides comprising the two
 25 continuous cytosine bases, wherein the candidate oligonucleotide comprises two continuous cytosine bases at or towards the 5' end of the oligonucleotide,
 - iii) testing the ability of the one or more candidate oligonucleotides to reduce expression of the target gene, and
 - iv) selecting an oligonucleotide which reduces expression of the target gene.
- 30 31. The method of claim 30, wherein the two continuous cytosine bases of the oligonucleotide have a modified base and/or a modified backbone.
32. The method of claim 30 or claim 31, wherein the oligonucleotide comprises
 35 a) a 5' region comprising bases which are modified and/or which have a modified backbone,

b) a middle region comprising ribonucleic acid, deoxyribonucleic acid, or combination thereof, bases, and

c) a 3' region comprising bases which are modified and/or which have a modified backbone.

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33. A method for selecting an oligonucleotide for reducing the expression of a target gene, the method comprising

i) scanning a target polynucleotide, or complement thereof, for a region with at least two continuous cytosine bases;

10 ii) producing one or more candidate oligonucleotides comprising a 5' region, a 3' region and a middle region comprising ribonucleic acid, deoxyribonucleic acid, or combination thereof, bases, wherein one or both of the 5' region and the 3' region comprise bases which are modified and/or which have a modified backbone, and the 5' region comprises two continuous cytosine bases which are modified and/or which have
15 a modified backbone,

iii) testing the ability of the one or more candidate oligonucleotides to reduce expression of the target gene, and

iv) selecting an oligonucleotide which reduces expression of the target gene.

20 34. The method of claim 32 or claim 33, wherein the 5' region and/or the 3' region are about 3 bases in length.

35. The method of any one of claims 32 to 34, wherein the middle region is about 10 bases in length.

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36. The method of any one of claims 30 to 35, wherein the two continuous cytosine bases comprise a 2'-LNA and a phosphorothioate backbone.

37. The method according to any one of claims 1 to 36, which further comprises
30 testing the ability of the one or more candidate oligonucleotides to inhibit Toll-like receptor 7 (TLR7) activity, and selecting an oligonucleotide which does not inhibit TLR7 activity.

38. The method of any one of claims 29 to 37, wherein the oligonucleotide further
35 comprises one or more features according to any one of claims 2, 3 or 5 to 28.

39. A method for selecting an oligonucleotide for reducing the expression of a target gene, the method comprising
- i) scanning a target polynucleotide, or complement thereof, for a region comprising at least four of the bases are thymidine;
 - 5 ii) producing one or more candidate oligonucleotides comprising one or more modified bases and at least four thymidines,
 - iii) testing the ability of the one or more candidate oligonucleotides to reduce expression of the target gene and to potentiate Toll-like receptor 8 (TLR8) activity, and
 - iv) selecting an oligonucleotide which reduces expression of the target gene and
 - 10 which potentiates TLR8 activity.
40. The method of claim 39, wherein the oligonucleotide comprises:
- a) a 5' region at least five bases in length which are modified and/or which have a modified backbone,
 - 15 b) a middle region comprising a stretch of ten bases, wherein at least four of the bases are thymidine,
 - c) a 3' region at least five bases in length.
41. The method of claim 39 or claim 40, wherein
- 20 i) the at least four thymidine bases are in a continuous stretch, and/or
 - ii) one, two, three or four of the at least four thymidine bases are not in a continuous stretch.
42. A method for selecting an oligonucleotide which potentiates Toll-like receptor 8 (TLR8) activity, the method comprising
- i) scanning a target polynucleotide, or complement thereof, for a region with the sequence UC or CU and a stretch of ten bases, wherein at least two of the bases are thymidine;
 - 25 ii) producing one or more candidate oligonucleotides comprising;
 - 30 a) a 5' region at least five bases in length, wherein the 5' end consists of terminal 5'-mUmC-3' or terminal 5'-mCmU-3', where m is a modified base and/or has a modified backbone,
 - b) a middle region comprising a stretch of ten bases, wherein at least two of the bases are thymidine, and
 - 35 c) a 3' region at least five bases in length and/or has a modified backbone,

iii) testing the ability of the one or more candidate oligonucleotides to potentiate TLR8 activity, and

iv) selecting an oligonucleotide which potentiates TLR8 activity.

5 43. The method according to any one of claims 39 to 42, wherein the oligonucleotide further comprises one or more features according to any one of claims 2, 3 or 5 to 28.

44. A method of reducing the Toll-like receptor 7 (TLR7) inhibitory activity of an oligonucleotide, the method comprising modifying the oligonucleotide by adding a
10 sequence of nucleotides to the 5' and/or 3' end of the oligonucleotide such that the modified oligonucleotide comprises three continuous pyrimidine bases within seven bases of the 5' and/or 3' end of the oligonucleotide.

45. The method of claim 44, wherein one, two or all three of the pyrimidine bases are
15 a modified base and/or have a modified backbone.

46. A method of reducing the Toll-like receptor 7 (TLR7) inhibitory activity of an oligonucleotide, the method comprising modifying the oligonucleotide such that the modified oligonucleotide comprises at least one of the following;

20 a) a 5' region comprising three continuous pyrimidine bases which are modified and/or which have a modified backbone,

b) a 5' region comprising bases which are modified and/or which have a modified backbone and the junction between the 5' region and middle region comprises three continuous pyrimidine bases,

25 c) a 3' region comprising three continuous pyrimidine bases which are modified and/or which have a modified backbone, and

d) a 3' region comprising bases which are modified and/or which have a modified backbone and the junction between the 3' region and middle region comprises three continuous pyrimidine bases.

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47. The method according to any one of claims 44 to 46, wherein the three continuous pyrimidine bases are at the 5' and/or 3' end of the modified oligonucleotide.

48. A method of reducing the Toll-like receptor 7 (TLR7) inhibitory activity of an
35 oligonucleotide, the method comprising modifying the oligonucleotide by adding a sequence of nucleotides to the 5' end of the oligonucleotide such that the modified

oligonucleotide comprises two continuous cytosine bases at or towards the 5' end of the oligonucleotide.

49. The method of claim 48, wherein one or both of the two continuous cytosine bases
5 are a modified base and/or have a modified backbone.

50. A method of reducing the Toll-like receptor 7 (TLR7) inhibitory activity of an
oligonucleotide, the method comprising modifying the oligonucleotide such that the
modified oligonucleotide comprises a 5' region comprising two continuous cytosine
10 bases which are modified and/or which have a modified backbone.

51. The method according to any one of claims 48 to 50, wherein the two continuous
cytosine bases are at or towards the 5' end of the modified oligonucleotide.

15 52. The method of any one of claims 48 to 51, wherein the two continuous cytosine
bases comprise a 2'-LNA and a phosphorothioate backbone.

53. The method according to any one of claims 44 to 52, wherein the modified
oligonucleotide further comprises one or more features according to any one of claims 2,
20 3, 5 to 28, 31, 32 or 34 to 36.

54. The method according to any one of claims 44 to 53, which further comprises
testing the ability of the modified oligonucleotide to inhibit TLR7 activity, and selecting
an oligonucleotide which inhibits (TLR7) activity to a lesser extent than the unmodified
25 oligonucleotide.

55. An oligonucleotide selected using the method according to any one of claims 1 to
43, or modified using the method according to any one of claims 44 to 54.

30 56. An oligonucleotide comprising three continuous pyrimidine bases within seven
bases of the 5' and/or 3' end of the oligonucleotide.

57. An oligonucleotide comprising two continuous cytosine bases which are modified
and/or which have a modified backbone at or towards the 5' end of the oligonucleotide.

58. An oligonucleotide comprising a 5' region, a 3' region and a middle region comprising ribonucleic acid, deoxyribonucleic acid, or combination thereof, bases, wherein one or both of the 5' region and the 3' region comprise bases which are modified and/or which have a modified backbone, and at least one of the following apply;

5 a) the 5' region comprises three continuous pyrimidine bases which are modified and/or which have a modified backbone,

b) the 5' region comprises bases which are modified and/or which have a modified backbone and the junction between the 5' region and middle region comprises three continuous pyrimidine bases,

10 c) the 3' region comprises three continuous pyrimidine bases which are modified and/or which have a modified backbone,

d) the 3' region comprises bases which are modified and/or which have a modified backbone and the junction between the 3' region and middle region comprises three continuous pyrimidine bases, and

15 e) the 5' region comprises two continuous cytosine bases which are modified and/or which have a modified backbone.

59. The oligonucleotide of claim 57 or claim 58, wherein the two continuous cytosine bases comprise a 2'-LNA and a phosphorothioate backbone.

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60. The oligonucleotide of any one of claims 56 to 59, wherein at least one of the three continuous pyrimidine bases and/or the two continuous cytosine bases does not hybridize to a target polynucleotide.

25 61. The oligonucleotide of claim 60, wherein all three of the continuous pyrimidine bases do not hybridize to a target polynucleotide and/or both of the two continuous cytosine residues do not hybridize to a target polynucleotide.

62. An oligonucleotide comprising

30 i) 5'-CUUGU-3', 5'-CCUAU-3', 5'-CAUUA-3', 5'-CGAAU-3' 5'-CUUAU-3', 5'-CUUUA-3' or 5'-ACUGU-3' at the 5' end, and

ii) 5'-CUUCU-3' 5'-CAUAU-3' 5'-CUUCU-3' 5'-AAUUU-3' 5'-AAAUU-3' 5'-CCUUC-3', 5'-AAUCA-3' or 5'-CGUCU-3' at the 3' end.

63. An oligonucleotide comprising one or more modified bases and at least four thymidines, wherein the oligonucleotide potentiates Toll-like receptor 8 (TLR8) activity when administered to an animal.
- 5 64. The oligonucleotide of claim 63 which comprises
a) a 5' region at least five bases in length which are modified and/or which have a modified backbone,
b) a middle region comprising a stretch of ten bases, wherein at least four of the bases are thymidine,
10 c) a 3' region at least five bases in length.
65. The oligonucleotide of claim 63 or claim 64, wherein
i) the at least four thymidine bases are in a continuous stretch, and/or
ii) one, two, three or four of the at least four thymidine bases are not in a
15 continuous stretch.
66. An oligonucleotide comprising
a) a 5' region at least five bases in length, wherein the 5' end consists of terminal 5'-mUmC-3' or terminal 5'-mCmU-3', where m is a modified base and/or has a modified
20 backbone,
b) a middle region comprising a stretch of ten bases, wherein at least two of the bases are thymidine, and
c) a 3' region at least five bases in length and/or has a modified backbone,
wherein the oligonucleotide potentiates Toll-like receptor 8 (TLR8) activity when
25 administered to an animal.
67. The oligonucleotide according to any one of claims 63 to 66, which is also an oligonucleotide as defined in any one of claims 55 to 62.
- 30 68. The oligonucleotide according to any one of claims 55 to 67, which further comprises one or more features according to any one of claims 2, 3, 5 to 28, 31, 32, 34 to 36, 40 or 41.
69. An oligonucleotide comprising, consisting of or consisting essentially of a nucleic
35 acid sequence set forth in Tables 1 to 6 or a variant thereof.

70. A composition comprising an oligonucleotide according to any one of claims 55 to 69.

71. The composition of claim 70 which further comprises a pharmaceutically acceptable carrier.

72. The composition of claim 71 which further comprises an immune response modifier.

73. A method of reducing expression of a target gene in a cell, the method comprising contacting the cell with an oligonucleotide according to any one of claims 55 to 69.

74. A method of treating or preventing a disease in a subject, the method comprising administering to the subject an oligonucleotide according to any one of claims 55 to 69, wherein the oligonucleotide reduces the expression of a target gene involved in the disease.

75. The method of claim 74, wherein the animal has been, or will be, administered with an immune response modifier.

76. The method of claim 75, wherein the immune response modifier is a Toll-like receptor (TLR) agonist such as a base analog (including: a guanosine analog, a dezaadenosine analog, an imidazoquinoline or a derivative, a hydroxyadenine compound or a derivative, a thiazoloquinolone compound or a derivative, a benzoazepine compound or a derivative), or an RNA molecule.

77. The method of claim 76, wherein the TLR agonist is Resiquimod (R848), Loxoribine, Isatoribine, Imiquimod, CL075, CL097, CL264, CL307, 852A, or TL8-506.

78. Use of an oligonucleotide according to any one of claims 55 to 69 in the manufacture of a medicament for treating or preventing a disease in a subject, wherein the oligonucleotide reduces the expression of a target gene involved in the disease.

79. An oligonucleotide according to any one of claims 55 to 69 for use in treating or preventing a disease in a subject, wherein the oligonucleotide reduces the expression of a target gene involved in the disease.

80. The steps, features, integers, compositions and/or compounds disclosed herein or indicated in the specification of this application individually or collectively, and any and all combinations of two or more of said steps or features.