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(54) **VERFAHREN ZUR HEMMUNG DER EXPRESSION EINE ZIELGENS**

METHOD FOR INHIBITING THE EXPRESSION OF A TARGET GENE

PROCEDE POUR INHIBER L'EXPRESSION D'UN GENE CIBLE

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Claims

15 1. Method for inhibiting the expression of a target gene in a cell, comprising the following steps:

Introduction of at least one double-stranded ribonucleic acid (dsRNA I) in a quantity sufficient to inhibit expression of the target gene,

20 wherein dsRNA I exhibits a double-stranded structure made up from a maximum of 49 successive nucleotide pairs, and wherein one strand (as1) or at least one segment of said one strand (as1) of the double-stranded structure is complementary to the target gene,

wherein the dsRNA I exhibits an overhang consisting of 1 to 4 nucleotides at the 3'-end of said one strand (as1) and wherein the dsRNA I is blunt ended at an end (E1, E2) containing the 5'-end of said one strand (as1),

25 wherein the target gene is the MDR1 gene or exhibits one of the sequences SQ001 to SQ140, and wherein any method for the surgical or therapeutic treatment of a human or animal body, and any diagnostic method which is performed on the human or animal body, is excluded.

2. Method in accordance with claim 1, wherein the overhang is made up from 1 or 2 nucleotides.

30 3. Method in accordance with any one of the preceding claims, wherein at least one further double-stranded ribonucleic acid (dsRNA II) having a configuration according to the dsRNA I as defined in the preceding claims, is introduced into the cell, wherein said one strand (as1) or at least one segment of said one strand (as1) of dsRNA I is complementary to a first region (B1) of the target gene, and wherein another strand (as2) or at least one segment of the other strand (as2) of dsRNA II is complementary to a second region (B2) of the target gene.

35 4. Method in accordance with any one of the preceding claims, wherein dsRNA I and/or dsRNA II exhibit/s a length of fewer than 25, preferably 19 to 23 successive nucleotide pairs.

40 5. Method in accordance with claim 3 or 4, wherein the first (B1) and the second region (B2) overlap segmentally or adjoin each other.

6. Method in accordance with claim 3 or 4, wherein the first (B1) and the second region (B2) are separated from each other.

45 7. Method in accordance with any one of the preceding claims, wherein the target gene is selected from the following group: oncogene, cytokine gene, Id protein gene, prion gene; genes of angiogenesis-inducing molecules, of adhesion molecules, and of cell-surface receptors; genes of proteins involved in metastatic and/or invasive processes; genes of proteinases as well as of molecules that regulate apoptosis and the cell cycle.

50 8. Method in accordance with any one of the preceding claims, wherein a dsRNA construct being combined of antisense-(as1/2) and sense sequences (ss1/2) of the sequences SQ141-173 belonging together each are used as the dsRNA I/II.

55 9. Method in accordance with any one of the preceding claims, wherein expression is inhibited according to the principle of RNA interference.

10. Method in accordance with any one of the preceding claims, wherein the target gene is expressed in pathogenic organisms.

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11. Method in accordance with any one of the preceding claims, wherein the target gene is a component of a virus or viroid.
12. Method in accordance with claim 11, wherein the virus is a human pathogenic virus or viroid.
- 5 13. Method in accordance with claim 11, wherein the virus or viroid is a virus or viroid that is pathogenic in animals.
14. Method in accordance with any one of the preceding claims, wherein unpaired nucleotides are substituted by nucleoside thiophosphates.
- 10 15. Method in accordance with any one of the preceding claims, wherein at least one end (E1, E2) of dsRNA I/II is modified in order to counter degradation in the cell or dissociation in the individual strands.
16. Method in accordance with any one of the preceding claims, wherein the cohesion of the double-stranded structure effected by the complementary nucleotide pairs is increased by at least one chemical linkage.
- 15 17. Method in accordance with claim 16, wherein the chemical linkage is formed either by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably by means of van der Waals or stacking interactions, or by means of metal-ion coordination.
- 20 18. Method in accordance with claims 16 or 17, wherein the chemical linkage is formed in the vicinity of said one end (E1, E2).
19. Method in accordance with any one of claims 16 to 18, wherein the chemical linkage is created by one or several linkage groups, wherein the linkage groups are preferably poly-(oxyphosphinico-oxy-1,3-propanediol) and/or oligoethyleneglycol chains.
- 25 20. Method in accordance with any one of claims 16 to 18, wherein the chemical linkage is formed by using branched nucleotide analogs instead of nucleotides.
- 30 21. Method in accordance with any one of claims 16 to 18, wherein the chemical linkage is formed by purine analogs.
22. Method in accordance with any one of claims 16 to 18, wherein the chemical linkage is formed by azabenzene units.
- 35 23. Method in accordance with any one of claims 16 to 18, wherein at least one of the following groups is used in creating the chemical linkage: methylene blue; bifunctional groups, preferably bis-(2-chlorethyl)-amine; N-acetyl-N'-(p-glyoxyl-benzoyl)-cystamine; 4-thiouracil; psoralen.
- 40 24. Method in accordance with any one of claims 16 to 18, wherein the chemical linkage is formed by thiophosphoryl groups that are attached in the vicinity of the ends (E1, E2) of the double-stranded region.
25. Method in accordance with any one of claims 16 to 18, wherein the chemical linkage is generated by triple helix bonds that are present in the vicinity of the ends (E1, E2).
- 45 26. Method in accordance with any one of the preceding claims, wherein the dsRNA I/II is enclosed in micellar structures, most advantageously in liposomes.
27. Method in accordance with any one of the preceding claims, wherein the dsRNA I/II is bound to, associated with, or enclosed by at least one viral coat protein that stems from a virus, is derived from it, or is synthetically generated.
- 50 28. Method in accordance with claim 27, wherein the coat protein is derived from polyomavirus.
29. Method in accordance with claim 27 or 28, wherein the coat protein contains Virus Protein 1 (VP1) and/or Virus Protein 2 (VP2) of the polyomavirus.
- 55 30. Method in accordance with any one of claims 27 to 29, wherein at the formation of a capsid or capsid-like structure from the coat protein, the one side is turned toward the inside of the capsid or capsid-like structure.
31. Method in accordance with any one of the preceding claims, wherein the one strand (as1, as2) of dsRNA I/II is

complementary to the primary or processed RNA transcript of the target gene.

32. Method in accordance with any one of the preceding claims, wherein the cell is a vertebrate cell or a human cell.
- 5 33. Method in accordance with any one of the preceding claims, wherein the dsRNA I/II is taken up in a buffer solution for application.
34. Use of a double-stranded ribonucleic acid (dsRNA I) for the manufacture of a medicament,
10 wherein dsRNA I exhibits a double-stranded structure made up from a maximum of 49 successive nucleotide pairs, and wherein one strand (as1) or at least one segment of said one strand (as1) of the double-stranded structure is complementary to the target gene,
wherein the dsRNA I exhibits an overhang consisting of 1 to 4 nucleotides at the 3'-end of said one strand (as1) and wherein the dsRNA I is blunt ended at an end (E2) containing the 5'-end of said one strand (as1), wherein the target gene is the MDR1 gene or exhibits one of the sequences SQ001 to SQ140.
- 15 35. Use in accordance with claim 34, wherein the overhang is made up from 1 or 2 nucleotides.
36. Use in accordance with claim 34 or 35, wherein at least one further double-stranded ribonucleic acid (dsRNA II) having a configuration according to dsRNA I as defined in claim 34 or 35, is used for the manufacture of the medicament, wherein said one strand (as1) or at least one segment of said one strand (as1) of the dsRNA I is
20 complementary to a first region (B1) of the sense strand of the target gene, and wherein another strand (as2) or at least one segment of the other strand (as2) of dsRNA II is complementary to a second region (B2) of the target gene.
37. Use in accordance with any one of claims 34 to 36, wherein the dsRNA I and/or dsRNA II exhibit/s a length of fewer
25 than 25, preferably 19 to 23, successive nucleotide pairs.
38. Use in accordance with claim 36 or 37, wherein the first (B1) and the second region (B2) overlap segmentally or adjoin each other.
- 30 39. Use in accordance with claim 36 or 37, wherein the first (B1) and the second region (B2) are separated from each other.
40. Use in accordance with any one of claims 34 to 39, wherein the target gene is selected from the following group: oncogene, cytokine gene, Id protein gene, prion gene; genes of angiogenesis-inducing molecules, of adhesion molecules, and of cell-surface receptors; genes of proteins involved in metastatic and/or invasive processes; genes
35 of proteinases as well as of molecules that regulate apoptosis and the cell cycle.
41. Use in accordance with any one of claims 34 to 40, wherein a dsRNA construct being combined of antisense-(as1/2) and sense sequences (ssl/2) of the sequences SQ141-173 belonging together each are used as the dsRNA I/II.
- 40 42. Use in accordance with any one of claims 34 to 41, wherein expression is inhibited according to the principle of RNA interference.
43. Use in accordance with any one of claims 34 to 42, wherein the target gene is expressed in pathogenic organisms.
- 45 44. Use in accordance with any one of claims 34 to 43, wherein the target gene is a component of a virus or viroid.
45. Use in accordance with claim 44, wherein the virus is a human pathogenic virus or viroid.
46. Use in accordance with claim 44, wherein the virus or viroid is a virus or viroid that is pathogenic in animals.
- 50 47. Use in accordance with any one of claims 34 to 46, wherein unpaired nucleotides are substituted by nucleoside thiophosphates.
48. Use in accordance with any one of claims 34 to 47, wherein at least one end (E1, E2) of the dsRNA is modified in order to counter degradation in the cell or dissociation in the individual strands.
- 55 49. Use in accordance with any one of claims 34 to 48, wherein the cohesion of the double-stranded structure effected by the complementary nucleotide pairs is increased by at least one chemical linkage.

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50. Use in accordance with claim 49, wherein the chemical linkage is formed either by a covalent or ionic bond, a hydrogen bond, hydrophobic interactions, preferably by means of van der Waals or stacking interactions, or by means of metal-ion coordination.
- 5 51. Use in accordance with claim 49 or 50, wherein the chemical linkage is formed in the vicinity of said one end (E1, E2).
52. Use in accordance with any one of claims 49 to 51, wherein the chemical linkage is created by one or several linkage groups, wherein the linkage groups are preferably poly-(oxyphosphinico-oxy-1,3-propanediol) and/or oligoethyleneglycol chains.
- 10 53. Use in accordance with any one of claims 49 to 51, wherein the chemical linkage is formed by using branched nucleotide analogs instead of nucleotides.
54. Use in accordance with any one of claims 49 to 51, wherein the chemical linkage is formed by purine analogs.
- 15 55. Use in accordance with any one of claims 49 to 51, wherein the chemical linkage is formed by azabenzene units.
56. Use in accordance with any one of claims 49 to 51, wherein at least one of the following groups is used in creating the chemical linkage: methylene blue; bifunctional groups, preferably bis-(2-chlorethyl)-amine; N-acetyl-N'-(p-glyoxyl-benzoyl)-cystamine; 4-thiouracil; psoralen.
- 20 57. Use in accordance with any one of claims 49 to 51, wherein the chemical linkage is formed by thiophosphoryl groups that are attached in the vicinity of the ends (E1, E2) of the double-stranded region.
- 25 58. Use in accordance with any one of claims 49 to 51, wherein the chemical linkage is generated by triple helix bonds that are present in the vicinity of the ends (E1, E2).
59. Use in accordance with any one of claims 34 to 58, wherein the dsRNA I/II is enclosed in micellar structures, most advantageously in liposomes.
- 30 60. Use in accordance with any one of claims 34 to 59, wherein the dsRNA I/II is bound to, associated with, or enclosed by at least one viral coat protein that stems from a virus, is derived from it, or is synthetically generated.
61. Use in accordance with claim 60, wherein the coat protein is derived from polyomavirus.
- 35 62. Use in accordance with claims 60 or 61, wherein the coat protein contains Virus Protein 1 (VP1) and/or Virus Protein 2 (VP2) of the polyomavirus.
63. Use in accordance with any one of claims 60 to 62, wherein at the formation of a capsid or capsid-like structure from the coat protein, the one side is turned toward the inside of the capsid or capsid-like structure.
- 40 64. Use in accordance with any one of claims 34 to 63, wherein the one strand (as1, as2) of dsRNA I/II is complementary to the primary or processed RNA transcript of the target gene.
65. Use in accordance with any one of claims 34 to 64, wherein the cell is a vertebrate cell or a human cell.
- 45 66. Use in accordance with any one of claims 34 to 65, wherein the medicament is suitable for the delivery of a dose of at most 5 mg per kg body weight per day of dsRNA I/II to a mammal, preferably a human.
- 50 67. Use in accordance with any one of claims 34 to 66, wherein the dsRNA I/II is taken up in a buffer solution for application.
68. Use in accordance with any one of claims 34 to 67, wherein the medicament is suitable for application of dsRNA I/II orally, by inhalation, or by injection or infusion intravenously, intratumorally, or intraperitoneally.
- 55 69. Medicament for inhibiting the expression of a target gene in a cell, containing a double-stranded ribonucleic acid (dsRNA I) in a dosage sufficient to inhibit the expression of the target gene, wherein dsRNA I exhibits a double-stranded structure made up from a maximum of 49 successive nucleotide pairs, and wherein one strand (as1) or at least one segment of said one strand (as1) of the double-stranded structure is

complementary to the target gene,
 wherein the dsRNA I exhibits an overhang consisting of 1 to 4 nucleotides at the 3'-end of said one strand (as1)
 and wherein the dsRNA I is blunt ended at an end (E2) containing the 5'-end of said one strand (as1), wherein the
 target gene is the MDR1 gene or exhibits one of the sequences SQ001 to SQ140.

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- 70.** Medicament in accordance with claim 69, wherein the overhang is made up from 1 or 2 nucleotides.
- 71.** Medicament in accordance with any one of claims 69 or 70 containing at least one further double-stranded ribonucleic
 acid (dsRNA II) having a configuration according to the dsRNA I as defined in claims 69 or 70, wherein said one
 10 strand (as1) or at least one segment of said one strand (as1) of dsRNA I is complementary to a first region (31) of
 the target gene, and wherein another strand (as2) or at least one segment of the other strand (as2) of dsRNA II is
 complementary to a second region (B2) of the target gene.
- 72.** Medicament in accordance with any one of claims 69 to 71, wherein the dsRNA I and/or dsRNA II exhibit/s a length
 15 of fewer than 25, preferably in 19 to 23, successive nucleotide pairs.
- 73.** Medicament in accordance with claim 71 or 72, wherein the first (B1) and the second region (B2) overlap segmentally
 or adjoin each other.
- 74.** Medicament in accordance with any one of claims 69 to 73, wherein the target gene is selected from the following
 20 group: oncogene, cytokine gene, Id protein gene, prion gene; genes of angiogenesis-inducing molecules, of adhesion
 molecules, and of cell-surface receptors; genes of proteins involved in metastatic and/or invasive processes; genes
 of proteinases as well as of molecules that regulate apoptosis and the cell cycle.
- 75.** Medicament in accordance with any one of claims 69 to 74, wherein a dsRNA construct being combined of anti-
 25 sense-(as1/2) and sense sequences (ss1/2) of the sequences SQ141-173 belonging together each are used as the
 dsRNA.
- 76.** Medicament in accordance with any one of claims 69 to 75, wherein expression is inhibited according to the principle
 30 of RNA interference.
- 77.** Medicament in accordance with any one of claims 69 to 76, wherein the target gene can be expressed in pathogenic
 organisms.
- 78.** Medicament in accordance with any one of claims 69 to 77, wherein the target gene is a component of a virus or viroid.
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- 79.** Medicament in accordance with claim 78, wherein the virus is a human pathogenic virus or viroid.
- 80.** Medicament in accordance with claim 78, wherein the virus or viroid is a virus or viroid that is pathogenic in animals.
 40
- 81.** Medicament in accordance with any one of claims 69 to 80, wherein unpaired nucleotides are substituted by nucl-
 eoside thiophosphates.
- 82.** Medicament in accordance with any one of claims 69 to 81, wherein at least one end (E1, E2) of the dsRNA is
 45 modified in order to counter degradation in the cell or dissociation in the individual strands.
- 83.** Medicament in accordance with any one of claims 69 to 82, wherein the cohesion of the double-stranded structure
 effected by the complementary nucleotide pairs is increased by at least one chemical linkage.
- 84.** Medicament in accordance with claim 83, wherein the chemical linkage is formed either by a covalent or ionic bond,
 50 a hydrogen bond, hydrophobic interactions, preferably by means of van der Waals or stacking interactions, or by
 means of metal-ion coordination.
- 85.** Medicament in accordance with claim 83 or 84, wherein the chemical linkage is formed in the vicinity of said one
 55 end (E1, E2).
- 86.** Medicament in accordance with any one of claims 83 to 85, wherein the chemical linkage is created by one or
 several linkage groups, wherein the linkage groups are preferably poly-(oxyphosphinico-oxy-1,3-propandiol) and/or

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oligoethyleneglycol chains.

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- 87.** Medicament in accordance with any one of claims 83 to 85, wherein the chemical linkage is formed by using branched nucleotide analogs instead of nucleotides.
- 88.** Medicament in accordance with any one of claims 83 to 85, wherein the chemical linkage is formed by purine analogs.
- 89.** Medicament in accordance with any one of claims 83 to 85, wherein the chemical linkage is formed by azabenzene units.
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- 90.** Medicament in accordance with any one of claims 83 to 85, wherein at least one of the following groups is used in creating the chemical linkage: methylene blue; bifunctional groups, preferably bis-(2-chlorethyl)-amine; N-acetyl-N'-(p-glyoxyl-benzoyl)-cystamine; 4-thiouracil; psoralen.
- 91.** Medicament in accordance with any one of claims 83 to 85, wherein the chemical linkage is formed by thiophosphoryl groups that are attached in the vicinity of the ends (E1, E2) of the double-stranded region.
- 92.** Medicament in accordance with any one of claims 83 to 85, wherein the chemical linkage is generated by triple helix bonds that are present in the vicinity of the ends (E1, E2).
- 20
- 93.** Medicament in accordance with any one of claims 69 to 92, wherein the dsRNA I/II is enclosed in micellar structures, most advantageously in liposomes.
- 94.** Medicament in accordance with any one of claims 69 to 93, wherein the dsRNA I is bound to, associated with, or enclosed by at least one viral coat protein that stems from a virus, is derived from it, or is synthetically generated.
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- 95.** Medicament in accordance with claim 94, wherein the coat protein is derived from polyomavirus.
- 96.** Medicament in accordance with claims 94 or 95, wherein the coat protein contains Virus Protein 1 (VP1) and/or Virus Protein 2 (VP2) of the polyomavirus.
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- 97.** Medicament in accordance with any one of claims 94 to 96, wherein at the formation of a capsid or capsid-like structure from the coat protein, the one side is turned toward the inside of the capsid or capsid-like structure.
- 98.** Medicament in accordance with any one of claims 69 to 97, wherein the one strand (as1, as2) of dsRNA I is complementary to the primary or processed RNA transcript of the target gene.
- 35
- 99.** Medicament in accordance with any one of claims 69 to 98, wherein the cell is a vertebrate cell or a human cell.
- 100.** Medicament in accordance with any one of claims 71, 72 or 74 to 99, wherein the first (B1) and the second region (B2) are separated from each other.
- 101.** Medicament in accordance with any one of claims 69 to 100, wherein a maximum amount of 5 mg of the dsRNA is contained in each dosing unit.
- 45
- 102.** Medicament in accordance with any one of claims 69 to 101, wherein the dsRNA is taken up in a buffer solution.
- 103.** Medicament in accordance with any one of claims 69 to 102, wherein the dsRNA is suitable to be administered orally, by inhalation, or by injection or infusion intravenously, intratumorally, or intraperitoneally.
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