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(71) Applicant : AVENTIS PHARMA DEUTSCHLAND GMBH [DE/DE]; Brüningstrasse 50, D-65929 Frankfurt (DE).

(72) Inventors: UHLMANN, Eugen; Zum Talblick 31, D-61479 Glashütten (DE). GREINER, Beate; Königsteinerstrasse 45, D-65812 Bad Soden (DE). UNGER, Eberhard; Rosenweg 19, D-07751 Jena-Cospeda (DE). GOTHE, Gislinde; Falkenweg 4, D-07751 Jena-Cospeda (DE). SCHWERDEL, Marc; Kahlaische Strasse 27, D-07745 Jena (DE).

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(54) Title: OLIGONUCLEOTIDES FOR INHIBITING THE EXPRESSION OF HUMAN EG5

(54) Bezeichnung: OLIGONUKLEOTIDE ZUR INHIBIERUNG DER EXPRESSION VON HUMANEM EG5

(57) Abstract: The invention relates to an oligonucleotide or one of its derivatives that is characterized by a sequence that corresponds to a nucleotide sequence that encodes a defined part of human eg5 or a mutated form thereof. The invention further relates to a method of producing said oligonucleotide and to its use.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft ein Oligonukleotid oder eines seiner Derivate, dadurch gekennzeichnet, dass es eine Sequenz aufweist, die einem bestimmten Teil einer humanen eg5 oder einer davon mutierten Form kodierenden Nukleinsäuresequenz entspricht; darüberhinaus betrifft die Erfindung ein Verfahren zur Herstellung des Oligonukleotids und dessen Verwendung.

Description

Oligonucleotides for the inhibition of human eg5 expression

The present invention relates to an oligonucleotide or a derivative thereof which has a sequence that corresponds to a particular part of a nucleic acid sequence which encodes human eg5 or a mutant form thereof, and the invention further relates to a method of making the oligonucleotide and the use thereof.

During mitosis a microtubule-based spindle apparatus helps distribute the duplicated chromosomes equally to the daughter cells. Kinesin-related motor proteins are part of the forces which are required for spindle assembly and chromosome segregation. The formation of a bipolar mitotic spindle involves the activity of many different motor proteins. One human kinesin-related motor protein is human eg5, which interacts with the mitotic centrosomes and has been shown to be essential for bipolar spindle formation (Blangy et al., Cell (1995) 83, 1159). Microinjection of specific anti-human-eg5 antibodies blocks centrosome migration and causes cells to arrest in mitosis.

Another possibility to block bipolar spindle formation would be the inhibition of eg5 expression. One way to specifically inhibit eg5 expression is by the use of antisense oligonucleotides, which can be optionally modified in order to improve their properties (E. Uhlmann and A. Peyman, Chemical Reviews 90, 543 (1990); S. Agrawal, TIBTECH 1996, 376). Antisense oligonucleotides are thought to bind to specific sequences of the mRNA, resulting in degradation of the mRNA and/or inhibition of protein synthesis.

The present invention provides an oligonucleotide or a derivative thereof, which corresponds to a part of the eg5 encoding sequence - preferably human eg5 or a pathogen organism's eg5, e.g. Plasmodium falciparum (malaria). The oligonucleotide preferably corresponds to 8 to 100 nucleotides, particularly preferably 8 to 20 nucleotides of the eg5 sequence. The oligonucleotide or derivative

thereof binds to said sequence and inhibits the formation of the eg5 protein. The human eg5 sequence has been reported (Blangy et al., Cell (1995) 83, 1159). SEQ ID NO.: 20 gives an example for a sequence which encodes human eg5. SEQ ID NO.: 21 gives an example for Plasmodium falciparum eg 5 sequence.

Preferably, the oligonucleotide has a sequence that corresponds to a part of a nucleic acid which encodes human eg5 or Plasmodium falciparum eg5. The phrase "corresponds to" means that the base sequence of the oligonucleotide is complementary to a part of a nucleic acid sequence that encodes eg5 (e.g. gene, cDNA, mRNA) and therefore allows the oligonucleotide to hybridize to (bind to) that "sense part " of the eg5 encoding nucleic acid. This is why it is called "antisense oligonucleotide". Therefore, in a preferred embodiment of the invention, the oligonucleotide is an antisense oligonucleotide. In another preferred embodiment of the invention the oligonucleotide is a ribozyme. A ribozyme is a catalytic nucleic acid which cleaves mRNA. Preferably the ribozyme is selected from the group of hammerhead ribozymes (Vaish et al., Nucleic Acids Res. (1998) 26, 5237).

An oligonucleotide according to the invention binds to a part of the eg5 mRNA which is appropriate for hybridization and inhibits formation of the eg5 protein. Oligonucleotides which are appropriate for binding to eg5 mRNA and inhibit expression are e.g. directed against the translational starter region of eg5.

The part of the eg5 encoding nucleic acid sequence corresponding to the oligonucleotide has a length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleotides, and, preferably, the oligonucleotide corresponds to a length of 12 nucleotides or 19 nucleotides of an eg5 encoding sequence. Therefore, an oligonucleotide according to the invention has a length of 10 (10mer), 11 (11mer), 12 (12mer), 13 (13mer), 14 (14mer), 15 (15mer), 16 (16mer), 17 (17mer), 18 (18mer) or 19 (19mer) nucleotides.

In a preferred embodiment of the invention, the oligonucleotide has a length of 12 or 19 nucleotides; such oligonucleotides might for example have one of the sequences

SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9 or a part thereof, wherein

SEQ ID NO. 1: 3-'CTTAAGGCAGTACCGCAGC-5'; 5'CGACGCCATGACGGAATTC-3'
SEQ ID NO. 2: 3'-ACCACTCTACGTCTGGTAA-5'; 5'-AATGGTCTGCATCTCACCA-3'
SEQ ID NO. 3: 3'-GGCAGTACCGCAGCGTCGG-5'; 5'-GGCTGCGACGCCATGACGG-3'
SEQ ID NO. 4: 3'-CTTAAGGCAGTA-5'; 5'-ATGACGGAATTC-3'
SEQ ID NO. 5: 3'-TAAGGCAGTACC-5'; 5'-CCATGACGGAAT-3'
SEQ ID NO. 6: 3'-GGCAGTACCGCA-5'; 5'-ACGCCATGACGG-3'
SEQ ID NO. 7: 3'-AGTACCGCAGCG-5'; 5'-GCGACGCCATGA-3'
SEQ ID NO. 8: 3'-CCGCAGCGTCGG-5'; 5'-GGCTGCGACGCC-3'
SEQ ID NO. 9: 3'-GCAGCGTCGGTT-5'; 5'-TTGGCTGCGACG-3'.

Very particularly preferably the oligonucleotide is modified in order to improve its properties, e.g. in order to increase its resistance to nucleases or to make it resistant to nucleases, to improve its binding affinity to a complementary eg5 encoding nucleic acid, e.g. mRNA, or in order to increase its cellular uptake.

Therefore, the present invention preferably relates to an oligonucleotide which has a particular sequence as outlined above and which has in addition one or more chemical modifications in comparison to a "natural" DNA, which is composed of the "natural" nucleosides deoxyadenosine (adenine + ß-D-2´-deoxyribose), deoxyguanosine (guanine + ß-D-2´-deoxyribose), deoxycytidine (cytosine + ß-D-2´-deoxyribose) and thymidine (thymine + ß-D-2´-deoxyribose) linked via phosphodiester internucleoside bridges. The oligonucleotides can have one or more modifications of the same type and/or modifications of a different type; each type of modification can be independently selected from the other types of modifications known to be used for modifying oligonucleotides.

The invention also relates to derivatives of the oligonucleotides, for example their salts, in particular their physiologically tolerated salts. Salts and physiologically tolerated salts are e.g. described in Remingtons Pharmaceuticals Science (1985) Mack Publishing Company, Easton, PA (page 1418). Derivatives also relate to modified oligonucleotides which have one or more modifications (e.g. at particular

nucleotide positions and/or at particular internucleoside bridges, oligonucleotide analogs (e.g. polyamide-nucleic acids (PNAs), phosphomonoester nucleic acids (PHONAs = PMENAs), oligonucleotide chimeras (e.g. consisting of a DNA and a PNA part or consisting of a DNA and a PHONA part)). Derivatives also relate to oligonucleotides which correspond to alleles and/or mutant forms of a normal or natural eg5, e.g. alleles and/or mutants of human eg5 (e.g. with respect to SEQ ID NO. 20) and alleles and/or mutants of Plasmodium falciparum eg5 (e.g. with respect to SEQ ID NO. 21).

Examples of chemical modifications are known to the skilled person and are described, for example, in E. Uhlmann and A. Peyman, Chemical Reviews 90 (1990) 543 and "Protocols for Oligonucleotides and Analogs" Synthesis and Properties & Synthesis and Analytical Techniques, S. Agrawal, Ed, Humana Press, Totowa, USA 1993 and S.T. Crooke, F. Bennet, Ann. Rev. Pharmacol. Toxicol. 36 (1996) 107-129; J. Hunziker and C. Leuman (1995) Mod. Synt. Methods, 7, 331-417.

For example, in comparison to natural DNA a phosphodiester internucleoside bridge, a ß-D-2´-deoxyribose unit and/or a natural nucleoside base (adenine, guanine, cytosine, thymine) can be modified or replaced, respectively. An oligonucleotide according to the invention can have one or more modifications, wherein each modification is located at a particular phosphodiester internucleoside bridge and/or at a particular ß-D-2´-deoxyribose unit and/or at a particular natural nucleoside base position in comparison to an oligonucleotide of the same sequence which is composed of natural DNA.

For example, the invention relates to an oligonucleotide which comprises one or more modifications and wherein each modification is independently selected from:

a) the replacement of a phosphodiester internucleoside bridge located at the 3´- and/or the 5´-end of a nucleoside by a modified internucleoside bridge,

b) the replacement of a phosphodiester bridge located at the 3´- and/or the 5´-end of a nucleoside by a dephospho bridge,

c) the replacement of a sugar phosphate unit from the sugar phosphate backbone by another unit,

d) the replacement of a ß-D-2´-deoxyribose unit by a modified sugar unit,

e) the replacement of a natural nucleoside base by a modified nucleoside base,

f) the conjugation to a molecule which influences the properties of the oligonucleotide,

g) the conjugation to a 2'5'-linked oligoadenylate or a derivative thereof, optionally via an appropriate linker, and

h) the introduction of a 3´-3´ and/or a 5´-5´ inversion at the 3´- and/or the 5´-end of the oligonucleotide.

More detailed examples for the chemical modification of an oligonucleotide are

a) the replacement of a phosphodiester internucleoside bridge located at the 3´- and/or the 5´-end of a nucleoside by a modified internucleoside bridge, wherein the modified internucleoside bridge is for example selected from phosphorothioate, phosphorodithioate, $NR^1R^{1'}$-phosphoramidate, boranophosphate, phosphate-(C_1-C_{21})-O-alkyl ester, phosphate-[(C_6-C_{12})-aryl-((C_1-C_{21}) -O-alkyl]ester, (C_1-C_8)-alkyl-phosphonate and/or (C_6-C_{12})arylphosphonate bridges and (C_7-C_{12})-• -hydroxymethyl-aryl (e.g. disclosed in WO 95/01363), wherein (C_6-C_{12})-aryl, (C_6-C_{20})-aryl and (C_6-C_{14})-aryl are optionally substituted by halogen, alkyl, alkoxy, nitro or cyano, and

where R^1 and $R^{1'}$ are, independently of each other, hydrogen, (C_1-C_{18})-alkyl, (C_6-C_{20})-aryl, (C_6-C_{14})-aryl-(C_1-C_8)-alkyl, preferably hydrogen, (C_1-C_8)-alkyl, preferably (C_1-C_4)-alkyl and/or methoxyethyl,

or

R^1 and $R^{1'}$ form, together with the nitrogen atom carrying them, a 5- to 6-membered heterocyclic ring which can additionally contain a further heteroatom from the group O, S and N,

b) the replacement of a phosphodiester bridge located at the 3´- and/or the 5´-end of a nucleoside by a dephospho bridge (dephospho bridges are described, for example, in Uhlmann, E. and Peyman, A. in "Methods in Molecular Biology", Vol. 20, "Protocols for Oligonucleotides and Analogs", S. Agrawal, Ed., Humana

Press, Totowa 1993, Chapter 16, 355ff), wherein a dephospho bridge is for example formacetal, 3'-thioformacetal, methylhydroxylamine, oxime, methylenedimethyl-hydrazo, dimethylenesulfone and/or a silyl group;

c) the replacement of a sugar phosphate unit (ß-D-2'-deoxyribose and phosphodiester internucleoside bridge together form a sugar phosphate unit) from the sugar phosphate backbone (sugar phosphate backbone is composed of sugar phosphate units) by another unit, wherein the other unit is for example suitable to build up

- a "morpholino-derivative" oligomer (as described, for example, in E.P. Stirchak et al., Nucleic Acids Res. 17 (1989) 6129), that is e.g. the replacement by a morpholino-derivative unit;
- a polyamide nucleic acid ("PNA") (as described for example in P.E. Nielsen et al., Bioconj. Chem. 5 (1994) 3 and in EP 0672677 A2); that is e.g. the replacement by a PNA backbone unit, e.g. by 2-aminoethylglycine;
- a phosphonic acid monoester nucleic acid ("PHONA") (as described e.g. in Peyman et al., Angew. Chem. Int. Ed. Engl. 35 (1996) 2632-2638 and in EP 0739898 A2); that is e.g. the replacement by a PHONA backbone unit;

d) the replacement of a ß-D-2´-deoxyribose unit by a modified sugar unit, wherein the modified sugar unit is for example selected from β-D-ribose, α-D-2'-deoxyribose, L-2'-deoxyribose, 2'-F-2'-deoxyribose, 2'-O-(C_1-C_6)-alkylribose, the preferred 2'-O-(C_1-C_6)-alkylribose being 2'-O-methylribose, 2'-O-(C_2-C_6)-alkenylribose, 2'-[O-(C_1-C_6)-alkyl-O-(C_1-C_6)-alkyl]ribose, 2'-NH_2-2'-deoxyribose, β-D-xylo-furanose, α-arabinofuranose, 2,4-dideoxy-β-D-erythro-hexo-pyranose, and carbocyclic (described, for example, in Froehler, J. Am. Chem. Soc. 114 (1992) 8320) and/or open-chain sugar analogs (described, for example, in Vandendriessche et al., Tetrahedron 49 (1993) 7223) and/or bicyclosugar analogs (described, for example, in M. Tarkov et al., Helv. Chim. Acta 76 (1993) 481);

e) the replacement of a natural nucleoside base by a modified nucleoside base, wherein the modified nucleoside base is for example selected from uracil,

hypoxanthine, 5-(hydroxymethyl)uracil, N^2-dimethylguanosine, pseudouracil, 5-(hydroxymethyl)uracil, 5-aminouracil, dihydrouracil, 5-fluorouracil, 5-fluorocytosine, 5-chlorouracil, 5-chlorocytosine, 5-bromouracil, 5-bromocytosine, 2,4-diaminopurine, 8-azapurine, a substituted 7-deazapurine, preferably 7-deaza-7-substituted and/or 7-deaza-8-substituted purine or other modifications of natural nucleoside bases, (modified nucleoside bases are e.g. described in EP 0 710 667 A2 and EP 0 680 969 A2);

f) the conjugation to a molecule which influences the properties of the oligonucleotide, wherein the conjugation of the oligonucleotide to one or more molecules which (favorably) influence the properties of the oligonucleotide (for example the ability of the oligonucleotide to penetrate the cell membrane or to enter a cell, the stability toward nucleases, the affinity for an eg5 encoding target sequence, the pharmacokinetics of the oligonucleotide, the ability of an antisense oligonucleotide/ribozyme or a molecule conjugated to the oligonucleotide respectively to attack the eg5 encoding target sequence, e.g. the ability to bind to and/or to crosslink, when the oligonucleotide hybridizes with the eg5 encoding target sequence), wherein examples for molecules that can be conjugated to an oligonucleotide are polylysine, intercalating agents such as pyrene, acridine, phenazine or phenanthridine, fluorescent agents such as fluorescein, crosslinking agents such as psoralen or azidoproflavin, lipophilic molecules such as $(C_{12}$-$C_{20})$-alkyl, lipids such as 1,2-dihexadecyl-rac-glycerol, steroids such as cholesterol or testosterone, vitamins such as vitamin E, poly- or oligoethylene glycol, preferably linked to the oligonucleotide via a phosphate group (e.g. triethylene glycol phosphate, hexaethylene glycol phosphate), $(C_{12}$-$C_{18})$-alkyl phosphate diesters and/or O-CH_2-CH(OH)-O-$(C_{12}$-$C_{18})$alkyl, these molecules can be conjugated at the 5'-end and/or the 3'-end and/or within the sequence, e.g. to a nucleoside base in order to generate an oligonucleotide conjugate; processes for preparing an oligonucleotide conjugate are known to the skilled person and are described, for example, in Uhlmann, E. & Peyman, A., Chem. Rev. 90 (1990) 543, M. Manoharan in "Antisense Research and Applications", Crooke and Lebleu, Eds., CRC Press, Boca Raton, 1993, Chapter 17, p. 303ff. and EP-A 0 552 766;

g) the conjugation to a 2'5'-linked oligoadenylate, preferably via an appropriate linker molecule, wherein the 2´5´-linked oligoadenylate is for example selected from 2'5'-linked triadenylate, 2'5'-linked tetraadenylate, 2'5'-linked pentaadenylate, 2'5'-linked hexaadenyltate or 2'5'-linked heptaadenylate molecules and derivatives thereof, wherein a 2´5´-linked oligoadenylate derivative is for example Cordycepin (2'5'-linked 3'-deoxyadenylate) and wherein an example for an appropriate linker is triethylene glycol and wherein the 5'-end of the 2'5'-linked oligoadenylate must bear a phosphate, diphosphate or triphosphate residue in which one or more oxygen atoms can be replaced e.g. by sulfur atoms, wherein the substitution by a phosphate or thiophosphate residue is preferred; and

h) the introduction of a 3´-3´ and/or a 5´-5´ inversion at the 3´- and/or the 5´-end of the oligonucleotide, wherein this type of chemical modification is known to the skilled person and is described, for example, in M. Koga et al., J. Org. Chem. 56 (1991) 3757, EP 0 464 638 and EP 0 593 901.

The replacement of a sugar phosphate unit from the sugar phosphate backbone by another unit, which can be e.g. a PNA backbone unit or a PHONA backbone unit, is preferably the replacement of a nucleotide by e.g. a PNA unit or a PHONA unit, which already comprises natural nucleoside bases and/or modified nucleoside bases, e.g. one of the modified nucleoside bases from the group of uracil, hypoxanthine, 5-(hydroxy-methyl)uracil, N^2-dimethylguanosine, pseudouracil, 5-(hydroxymethyl)uracil, 5-aminouracil, pseudouracil, dihydrouracil, 5-fluorouracil, 5-fluorocytosine, 5-chlorouracil, 5-chlorocytosine, 5-bromouracil, 5-bromocytosine, 2,4-diamino-purine, 8-azapurine, a substituted 7-deazapurine, preferably 7-deaza-7-substituted and/or 7-deaza-8-substituted purine or other modifications of a natural nucleoside base (modified nucleotide bases are e.g. described in EP 0 710 667 A2 and EP 0 680 969 A2).

The oligonucleotide modifications described in EP 0 710 667 A2, EP 0 680 969 A2, EP 0 464 638, EP 0 593 901, WO 95/01363, EP 0 672 677 A2, EP 0 739 898 A2 and EP 0 552 766 are hereby incorporated by reference.

In a special embodiment of the invention, one or more phosphodiester internucleoside bridges within the oligonucleotide sequence are modified; preferably one or more phosphodiester internucleoside bridges are replaced by phosphorothioate internucleoside bridges and/or (C_6-C_{12})aryl phosphonate internucleoside bridges, preferably by α-hydroxybenzyl phosphonate bridges in which the benzyl group is preferably substituted, e.g. with nitro, methyl, halogen. In an all-phosphorothioate oligonucleotide, all phosphodiester internucleoside bridges are modified by phosphorothioate. Preferably, the invention relates to an oligonucleotide in which not all phosphodiester internucleoside bridges are modified uniformly with phosphorothioate (phosphorothioate internucleoside bridges). Preferably, at least one internucleoside bridge has a different type of modification or is not modified. In particular the invention relates to an oligonucleotide which comprises in addition at least one other type of modification.

In another special embodiment of the invention, one or more nucleosides (ß-D-2´-deoxyribose and/or nucleoside base) within the oligonucleotide sequence are modified; preferably the ß-D-2´-deoxyribose is substituted by 2´-O-(C_1-C_6)alkyl-ribose, preferably by 2´-O-methylribose and/or the nucleoside base is substituted by 8-azapurine, 7-deaza-7-substituted purine and/or 7-deaza-8-substituted purine (purine: adenine, guanine). Preferably, the invention relates to an oligonucleotide in which not all nucleosides are modified uniformly. Preferably the invention relates to an oligonucleotide which comprises in addition at least one other type of modification.

In another special embodiment of the invention, one or more sugar phosphate units from the sugar phosphate backbone are replaced by PNA backbone units, preferably by 2-aminoethylglycine units. Preferably the sugar phosphate units which are replaced are connected together at least to a certain extent. Preferably, the invention relates to an oligonucleotide in which not all sugar phosphate units are uniformly replaced. In particular the invention relates to chimeric oligonucleotides, e.g. composed of one or more PNA parts and one or more DNA parts. For such chimeric oligonucleotides, for example the following non-limiting examples of

modification patterns are possible: DNA-PNA, PNA-DNA, DNA-PNA-DNA, PNA-DNA-PNA, DNA-PNA-DNA-PNA, PNA-DNA-PNA-DNA. Comparable patterns would be possible for chimeric molecules composed of DNA parts and PHONA parts, e.g. DNA-PHONA, PHONA -DNA, DNA- PHONA -DNA, PHONA -DNA- PHONA, DNA-PHONA -DNA- PHONA, PHONA -DNA- PHONA -DNA. In addition of course, chimeric molecules comprising three different parts like DNA part(s), PHONA part(s) and PNA part(s) are possible. Preferably the invention relates to an oligonucleotide which comprises in addition at least one other type of modification.

In another special embodiment of the invention, the oligonucleotide is connected at its 3´-end and/or at its 5´-end to a (C_{12}-C_{18})alkyl residue, preferably a C_{16} alkyl residue, a triethylene glycol residue or a hexaethylene glycol residue – these residues are preferably connected to the oligonucleotide via a phosphate group. Preferably, the invention relates to an oligonucleotide in which not both ends (3´-and 5´-end) are (uniformly) modified. Preferably, the invention relates to an oligonucleotide which comprises in addition at least one other type of modification.

In a preferred embodiment of the invention only particular positions within an oligonucleotide sequence are modified (e.g. partially modified oligonucleotide). Partially modified oligonucleotides are also named minimal modified oligonucleotides in some documents. Within the sequence a modification can be located at particular positions (at particular nucleotides, at particular nucleosides, at particular nucleoside bases, at particular internucleoside bridges).

In a particular embodiment of the invention, a partially modified oligonucleotide is prepared by only replacing some of the phosphodiester bridges with modified internucleoside bridges, e.g. phosphorothioate bridges and/or α-hydroxybenzyl phosphonate bridges. In particular, the invention comprises such oligonucleotides which are only modified to a certain extent.

In particular the invention relates to an oligonucleotide wherein 1 to 5 terminal nucleotide units at the 5´-end and/or at the 3´-end are protected by modifying internucleoside bridges located at the 5´- and/or the 3´-end of the corresponding

nucleoside, preferably by replacement of the phosphodiester internucleoside bridges by phosphorothioate bridges and/or α-hydroxybenzyl phosphonate bridges. Very particularly preferably the 1 to 5 terminal nucleotide units at the 3´-end of the oligonucleotide are protected by modified internucleoside bridges located at the 5´- and/or the 3´-end of the corresponding nucleosides. Optionally, the 1 to 5 terminal nucleotide units at the 5´-end of the oligonucleotide are in addition protected by modified internucleoside bridges located at the 5´- and/or the 3´-end of the corresponding nucleoside. Optionally, the oligonucleotide may comprise additional modifications at other positions.

Furthermore, the invention relates to an oligonucleotide wherein at least one internal pyrimidine nucleoside and/or an internucleoside bridge located at the 5´-end and/or the 3´-end of this pyrimidine nucleoside (a nucleoside with a pyrimidine base like cytosine, uracil, thymine) is modified, preferably by replacement of the phosphodiester internucleoside bridge(s) by (a) phosphorothioate bridge(s) and/or (an) α-hydroxybenzyl phosphonate bridge(s).

In a preferred embodiment of the invention terminal 1 to 5 nucleotide units at the 5´-end and/or at the 3´-end of the oligonucleotide are protected by modifying internucleoside bridges located at the 5´- and/or the 3´-end of the corresponding nucleoside and wherein in addition at least one internal pyrimidine nucleoside and/or an internucleoside bridge located at the 5´-end of this pyrimidine nucleoside and/or located at the 3´-end of this pyrimidine nucleoside is modified.

The principle of partially modified oligonucleotides is described e.g. in A. Peyman, E. Uhlmann, Biol. Chem. Hoppe-Seyler, 377 (1996) 67-70 and in EP 0 653 439. These documents are hereby incorporated by reference. In this case, 1-5 terminal nucleotide units at the 5'-end/or and at the 3'-end are protected, e.g. the phosphodiester internucleoside bridges located at the 3´- and/or the 5´-end of the corresponding nucleosides are for example replaced by phosphorothioate internucleoside bridges. In addition, preferably at least one internal pyrimidine nucleoside (or nucleotide respectively) position is modified; preferably the 3´- and/or the 5´-internucleoside bridge(s) of a pyrimidine nucleoside is/are modified/replaced,

for example by (a) phosphorothioate internucleoside bridge(s). Partially modified oligonucleotides exhibit particularly advantageous properties; for example they exhibit a particularly high degree of nuclease stability in association with minimal modification. They also have a significantly reduced propensity for non-antisense effects which are often associated with the use of all-phosphorothioate oligonucleotides (Stein and Krieg (1994) Antisense Res. Dev. 4, 67). Partially modified oligonucleotides also show a higher binding affinity than all-phosphorothioates.

The invention relates in particular to partially/minimally modified oligonucleotides.

SEQ ID NO. 10: 3-'C*T*T*A A G G C*A G T*A C*C G*C A G*C-5', **(K3)**
5'-C G A C*G*C*C*A*T G A*C G G A A*T*T*C-3';

SEQ ID NO. 11: 3'-A*C*C*A C*T C*T A C*G T*C*T G G*T A*A-5', **(K4)**
5'-A*AT*GGT*C*TG*CAT*CT*CA*C*C*A-3';

SEQ ID NO. 12: 3'-G*G*C*A G*T A C*C G C*A G*C G T*C G*G-5', **(K6)**
5'-G*G C*T G C*G A*C G C*C A T*G A*C*G*G-3';

SEQ ID NO. 13: 3'-C*T*T*A A G G*C A G*T*A-5',
5'-A*T*G A C*G G A A*T*T*C-3';

SEQ ID NO. 14: 3'-T*A*A G G C*A G*T A*C*C-5',
5'-C*C*A T*G A*C G G A*A*T-3';

SEQ ID NO. 15: 3'-G*G*C A G*T A C*C*G C*A-5',
5'-A*C G*C*C A T*G A C*G*G-3';

SEQ ID NO. 16: 3'-A*G*T A C*C G*C A G*C*G-5',
5'-G*C*G A C*G C*C A T*G*A-3';

SEQ ID NO. 17: 3'-C*C*G*C A G*C G T*C G*G-5',
5'-G*G C*T G C*G A C*G*C*C-3';

SEQ ID NO. 18 3'-G*C*A G C*G T*C G G*T*T-5',
5'-T*T*G G C*T G C*G A*C*G-3'.

wherein " * " denotes the position of an internucleoside bridge modification; preferably " * " is a phosphorothioate internucleoside bridge.

Another example for a special embodiment of the invention relates to a partially modified oligonucleotide wherein a nucleoside is modified, e.g. a modification of a

nucleoside base and/or a modification of a ß-D-2´-deoxyribose unit. Preferably a ß-D-2´-deoxyribose is replaced by 2'-O-(C_1-C_6)-alkylribose; very particularly preferred is the replacement by 2'-O-methylribose (replacement of a ß-D-2´-deoxyribonucleoside by a 2´-O-methylribonucleoside).

According to the invention, the oligonucleotide can have, in addition to one type of modification, also other types of modification.

Therefore, in another embodiment of the invention the oligonucleotide comprises modified internucleoside bridges at particular positions and in addition modifications of a nucleoside at particular positions, preferably the replacement of ß-D-2´-deoxyribose. In a preferred embodiment of the invention, the internucleoside modification is the replacement of a phosphodiester bridge by a phosphorothioate bridge and the modification of the ß-D-2´-deoxyribose is the replacement by 2´-O-methylribose; in this case, the oligonucleotide is a chimeric oligonucleotide, which is composed of modified and unmodified DNA and RNA parts - which comprise the 2'-O-methylribonucleosides and ß-D-2´-deoxyribonucleosides and phosphoro- diester and phosphorothioate internucleoside bridges.

A further preferred embodiment of the invention provides an oligonucleotide which has one or more (C_{12}-C_{18})-alkyl residues, preferably a C_{16}-alkyl residue at its 3'- and/or its 5'-end. A (C_{12}-C_{18})-alkyl residue can e.g. be bound as a phosphodiester as described in EP 0 552 766 A2 (EP 0 552 766 A2 is hereby incorporated by reference) or as a 3'-phosphodiester of O-CH_2-CH(OH)-O-(C_{12}-C_{18})-alkyl. Preferred is an oligonucleotide that has a C_{16}-alkyl residue bound to its 3'- and/or 5'-end.

The invention also relates to an oligonucleotide in which the 3'- and/or the 5´-end is connected to an oligoethylene glycol residue, preferably a triethylene glycol or a hexaethylene glycol, very particularly preferably via a phosphodiester (tri- or hexaethylene glycol phosphate ester). Of course, such an oligonucleotide may also comprise additional modifications.

In another specific embodiment of the invention the oligonucleotide is connected via a linker to a 2'5'-linked oligoadenylate-5'-(thio)phosphate. The linker can e.g. be an oligo-ethylene glycol phosphate, preferably triethylene glycol phosphate, tetra-ethylene glycol phosphate or hexa-ethylene glycol phosphate residue. The 2'5'-linked oligoadenylate is preferably attached via its 2'-end as a tetra- or as a penta-adenylate whose 5'-hydroxy function is substituted by a phosphate or thiophosphate residue. The 2'5'-oligoadenylate is known to induce RNase L to cleave the target mRNA (Torrence et al., Proc. Natl. Acad. Sci. U.S.A. (1993) 90, 1300). The 2'5'-oligoadenylate serves to activate ribonuclease L (RNase L) which then degrades the eg5 mRNA. Instead of a 2'5'-linked adenylate, e.g. a 2'5'-linked 3'-deoxy adenylate, derived from the nucleoside analog cordycepin, can be introduced. In this case, the oligonucleotide part, which is complementary to the target nucleic acid, is preferably modified at particular positions by 2'-O-(C_1-C_6)-alkylribonucleoside (preferably 2'-O-methylribonucleoside) or by PNA.

Another preferred embodiment of the invention involves the replacement of one or more natural nucleoside base(s) by non-natural or modified nucleoside bases respectively, preferably by 8-azapurines and/or 7-deaza-7-substituted purines and/or 7-deaza-8-substituted purine, e.g. as described in EP 0 171 066 and EP 0 680 969.

In another preferred embodiment of the invention, the oligonucleoside can exhibit 3'3' and / or 5'5'-inversions at the 3'- and/or 5'-end, e.g. as described in EP 0 464 638 and EP 0 593 901.

Another preferred embodiment of the invention relates to the replacement of one or more phosphodiester bridges by • -hydroxybenzyl phosphonate bridges as described in WO 95/01363.

In another preferred embodiment of the invention the oligonucleotide comprises a modification of the sugar phosphate backbone, preferably by PNA units.

Also other patterns of modification are possible e.g. DNA-PNA-DNA, PNA-DNA. Comparable patterns of modification are also possible for PHONA/DNA chimeras. These modification patterns can be combined with any other type of modification

and, of course, similar patterns of modification are also possible for other oligonucleotides according to the invention.

The above concrete oligonucleotides – particular sequence, particular type(s) of modification(s) at particular positions (specific "pattern of modification") are only examples for different embodiments of the invention. The invention is not limited to these concrete oligonucleotides. Also other combinations of sequence and pattern of modification are possible.

An oligonucleotide according to the invention specifically inhibits the expression of the target protein (which is eg5) or the target sequence (a nucleic acid which encodes eg5, preferably eg5 mRNA) respectively. Preferably, an oligonucleotide according to the invention specifically inhibits the expression of eg5. This results in a reduction in the eg5 protein level in comparison to untreated expression. The specificity can for example be demonstrated by determining the effect of an oligonucleotide according to the invention upon eg5 expression in comparison to the effect of the same oligonucleotide upon beta actin expression, on the mRNA and/or the protein level. Upon treatment with an oligonucleotide according to the invention only the eg5 mRNA and/or eg5 protein level is reduced, while e.g. beta actin (a house-keeping protein) mRNA and/or beta-actin protein level remaines unchanged.

Preferably an oligonucleotide according to the invention can efficiently inhibit the expression of eg5 in human cells and/or has the ability to inhibit tumor growth in vertebrates. Preferably, an oligonucleotide according to the invention reduces the eg5 mRNA and/or protein level in tumors of treated individuals relative to untreated individuals. Preferably, an oligonucleotide according to the invention reduces tumor volume in a vertebrate, e.g in mice compared to untreated mice or relative to the tumor volume of the same animal determined before treatment.

The invention also relates to a method for the preparation of an oligonucleotide according to the invention. A method for preparation comprises the chemical synthesis of the oligonucleotide. Preferably the chemical synthesis is performed by a standard method known to be used for the synthesis of oligonucleotides, e.g. the phoshoramidite method according to Caruthers (1983) Tetrahedron Letters 24, 245,

the H-phosphonate methode (Todd et al. (1957) J. Chem. Soc. 3291) or the phosphotriester methode (Sonveaux (1986) Bioorg. Chem. 14,274; Gait, M.J. "Oilgonucleotide Synthesis, A practical Approach", IRL Press, Oxford,1984) or improved or varied methods derived from these standard methods. An oligonucleotide according to the invention can for example be prepared as described in Example 1. Preferably an oligonucleotide according to the invention is synthesized on a solid phase by condensing suitably protected monomers (e.g. nucleosides) in order to form internucleoside bridges between these monomers.

The invention relates e.g. to a method for preparing an oligonucleotide or a derivative thereof, where a nucleotide unit with a 3´- or a 2´-terminal phosphorus (V) group and a free 5´-hydroxyl or mercapto grouping is reacted with a further nucleotide unit with a phosphorus (III) or a phosphorus (V) grouping in the 3´-position, or its activated derivatives, and wherein optionally protective groups are used, which can be temporarily introduced in the oligonucleotide in order to protect other functions and which are removed after synthesis, and the oligonucleotide which has been cleaved from the solid phase can optionally be converted into a physiologically tolerated salt. In order to synthesize a modified oligonucleotide, standard methods are varied to a certain extent. Those variations are known to a person of skill in the art and are e.g. described in Agrawal S. "Protocols for oligonucleotides and analogs" (1993, Human Press Inc., Totowa, New Jersey). The preparation of modified oligonucleotides is also described in EP 0 710 667, EP 0 680 969, EP 0 464 638, EP 0 593 901, WO 95/01363, EP 0 672 677, EP 0 739 898 and EP 0 552 766. The methods of preparing modified oligonucleotides described in the above documents are hereby incorporated by reference.

The invention further relates to a method of inhibiting the expression of eg5 and/or modulating the expression of an eg5 encoding nucleic acid, wherein an oligonucleotide according to the invention is brought into contact with an eg5 encoding nucleic acid (e.g. mRNA, cDNA) and the oligonucleotide is hybridized with this eg5 encoding nucleic acid.

Therefore, the invention also relates to a method wherein the oligonucleotide is brought into contact with an eg5 encoding nucleic acid (e.g. mRNA; cDNA), for

example by introducing the oligonucleotide into a cell by known methods, for example by incubation of cells with said oligonucleotide or a formulation thereof – such a formulation may comprise uptake enhancers, such as lipofectin, lipofectamine, cellfectin or polycations (e.g. polylysine).

For example, an oligonucleotide which was incubated previously with cellfectin for e.g. 30 minutes at room temperature is then incubated about 5 hours or less with a cell in order to introduce the oligonucleotide into the cell.

The invention further relates to the use of the oligonucleotide, preferably as antisense oligonucleotide (binding of the oligonucleotide to an eg5 encoding mRNA) or as ribozyme (binding to an eg5 encoding mRNA and cleavage of this mRNA). In another special embodiment of the invention, the oligonucleotide can be used to induce RNAse H cleavage of the eg5 encoding mRNA, thus resulting in a reduction in eg5 expression.

The invention relates to the use of an oligonucleotide for inhibiting formation of a bipolar mitotic spindle and therefore for inhibiting cell proliferation, especially tumor growth.

The invention furthermore relates to the use of the oligonucleotide as pharmaceutical and to the use of the oligonucleotide for preparing a pharmaceutical composition. In particular, the oligonucleotide can be used in a pharmaceutical composition which is employed for preventing and/or treating diseases which are associated with the expression of eg5, or which can be cured by the inhibition of eg5 expression.

The invention furthermore relates to a pharmaceutical composition which comprises an oligonucleotide and/or its physiologically tolerated salts in addition to pharmaceutically unobjectable excipients or auxiliary substances.

The invention relates to a pharmaceutical composition which comprises at least one oligonucleotide according to the invention that can be used for the treatment of diseases which can be cured by inhibition of eg5 expression, such as restenosis and cancer.

The invention further relates to a method for preparing a pharmaceutical composition, which comprises mixing of one or more oligonucleotides according to the invention with physiologically acceptable excipients and optionally additional substances, e.g. if appropiate with suitable additives and/or auxiliaries.

The invention relates in particular to the use of an oligonucleotide or a pharmaceutical composition prepared therefrom for the treatment of cancer, e.g. for inhibiting tumor growth and tumor metastasis. For example the oligonucleotide or a pharmaceutical composition prepared therefrom may be used for the treatment of solid tumors, like breast cancer, lung cancer, head and neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophagus cancer, gastrointestinal cancer, glioma, liver cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, Wilm's tumor, multiple myeloma and for the treatment of skin cancer, like melanoma, for the treatment of lymphomas and blood cancer. The invention further relates to the use of an oligonucleotide according to the invention or a pharmaceutical composition prepared therefrom for inhibiting eg5 expression and/or for inhibiting accumulation of ascites fluid and pleural effusion in different types of cancer, e.g. breast cancer, lung cancer, head cancer, neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophagus cancer, gastrointestinal cancer, glioma, liver cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, Wilm's tumor, multiple myeloma, skin cancer, melanoma, lymphomas and blood cancer. Owing to the inhibitory effect on eg5 expression, an oligonucleotide according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life.

The invention furthermore relates to the use of an oligonucleotide or a pharmaceutical composition thereof, e.g. for treating cancer or for preventing tumor metastasis, in combination with other pharmaceuticals and/or other therapeutic methods, e.g. with known pharmaceuticals and/or known therapeutic methods, such as for example those which are currently employed for treating cancer and/or for preventing tumor metastasis. Preference is given to a combination with radiation

therapy and chemotherapeutic agents, such as cisplatin, cyclophosphamide, 5-fluorouracil, adriamycin, daunorubicin or tamoxifen.

The oligonucleotide and/or its physiologically tolerated salt can be administered to an animal, preferably a mammal, and in particular a human, on its own, in a mixture with another oligonucleotide (or its physiologically tolerated salt), or in the form of a pharmaceutical composition which permits topical, percutaneous, parenteral or enteral use and which comprises, as the active constituent, an effective dose of at least one oligonucleotide, in addition to customary pharmaceutically unobjectionable excipients and auxiliary substances. Such a pharmaceutical composition normally comprises from about 0.1 to 90% by weight of the therapeutically active oligonucleotide(s). The dose can vary within wide limits and is to be adjusted to the individual circumstances in each individual case. In order to treat psoriasis, preference is given to a topical use. In the case of cancer, preference is given to infusions, oral and rectal administration, or nasal application in an aerosol, preferable in the case of lung cancer, while in the case of diabetic retinopathy, preference is given to a topical, intravitreal and oral administration.

A pharmaceutical composition can be prepared in a manner known per se (e.g. Remingtons Pharmaceutical Sciences, Mack Publ. Co., Easton, PA (1985)), with pharmaceutically inert inorganic and/or organic excipients being used. Lactose, corn starch and/or derivatives thereof, talc, stearic acid and/or its salts, etc. can, for example, be used for preparing pills, tablets, film-coated tablets and hard gelatin capsules. Examples of excipients for soft gelatin capsules and/or suppositories are fats, waxes, semisolid and liquid polyols, natural and/or hardened oils, etc. Examples of suitable excipients for preparing solutions and/or syrups are water, sucrose, invert sugar, glucose, polyols, etc. Suitable excipients for preparing injection solutions are water, alcohols, glycerol, polyols, vegetable oils, etc. Suitable excipients for microcapsules, implants and/or rods are mixed polymers of glycolic acid and lactic acid. In addition, there are liposome formulations which are e.g. described in N. Weiner (Drug Develop Ind Pharm 15 (1989) 1523), "Liposome Dermatics" (Springer Verlag 1992) and Hayashi (Gene Therapy 3 (1996) 878). The pharmaceutical composition may also comprise a formulation which enhances the

oral availability of the oligonucleotide, such as enhancers of intestinal absorption, e.g. mannitol, urea, bile salts, such as CDCA (chenodeoxycholate) (2 %). Dermal administration can also be effected, for example, using ionophoretic methods and/or by means of electroporation. Furthermore, use can be made of lipofectins and other carrier systems, for example those which are used in gene therapy. Systems which can be used to introduce oligonucleotides in a highly efficient manner into eukaryotic cells or into the nuclei of eukaryotic cells are particularly suitable. A pharmaceutical composition may also comprise two or more different oligonucleotides and/or their physiologically tolerated salts and, furthermore, in addition to at least one oligonucleotide, one or more different therapeutic active ingredients.

In addition to the active ingredients and excipients, a pharmaceutical composition can also comprise additives, such as fillers, extenders, disintegrants, binders, lubricants, wetting agents, stabilizing agents, emulsifiers, preservatives, sweeteners, dyes, flavorings or aromatizing agents, thickeners, diluents or buffering substances, and, in addition, solvents and/or solubilizing agents and/or agents for achieving a slow release effect, and also salts for altering the osmotic pressure, coating agents and/or antioxidants.

Examples:

Example 1: Oligonucleotide synthesis

Oligonucleotides (ON s) were synthesized using an Applied Biosystems 394 DNA synthesizer (Perkin Elmer Applied Biosystems, Inc., Foster City, USA) and standard phosphoramidite chemistry. After coupling, phosphorothioate linkages were introduced by sulfurization using the Beaucage reagent followed by capping with acetic anhydride and N-methylimidazole. After cleavage from the solid support and final deprotection by treatment with concentrated ammonia, ON s were purified by polyacrylamide gel electrophoresis. The *2'-O-methyl* modified ON s were prepared by replacing the standard phosphoramidites in the corresponding cycle with 2'-O-

methyl ribonucleoside phophoramidites. All ON s were analyzed by negative ion electrospray mass spectroscopy (Fisons Bio-Q) which in all cases confirmed the calculated mass. The C16-modified oligonucleotides were synthesised using hexadecyloxy(cyanoethoxy)-N,N-diisopropyl-aminophosphane as phosphitylating reagent in the last step of oligonucleotide synthesis in place of a standard amidite, or by starting from a correspondingly derivatized solid support. The triethylene glycol linker is commercially available from Glen Research Corporation. The 2'-phosphoramidites of adenosin or cordycepin were obtained from Chem. Genes Corporation and Chemogen Corporation, respectively. The introduction of 5'-phosphates or thiophosphate residues was carried out as described previously (Uhlmann and Engels (1986) Tetrahedron Lett. 27, 1023). The PNA-DNA chimeras are prepared as described in EP 0 672 677.

Analysis of the oligonucleotides was done by

a) analytical gel electrophoresis in 20% acrylamide, 8M urea, 45μM tris-borate buffer, pH 7.0 and/or

b) HPLC analysis: Waters GenPak FAX column, gradient CH_3CN (400ml), H_2O (1.6l), NaH_2PO_4 (3.1g), NaCl (11.7g), pH6.8 (0.1M NaCl) after CH_3CN (400ml), H_2O (1.6l), NaH_2PO_4 (3.1g), NaCl (17.53g), pH6.8 (1.5M NaCl) and/or

c) capillary electrophoresis using a Beckmann eCAP^{TM}, U100P gel capillary column, 65 cm length, 100 mm Internal Diameter, window 15 cm from one end, buffer 140 μM Tris, 360mM borate, 7M urea and/or

d) negative ion electrospray mass spectrometry which in all cases confirmed the expected mass values.

The methods for analyzing oligonucleotides according to a), b), c) and d) are known to a person of skill in the art. These methods are for example described in Schweitzer and Engels "Analysis of oligonucleotides" (in "Antisense – from technology to therapy", a laboratrory manual and textbook, Schlingensiepen et al. eds., Biol. Science Vol. 6 (1997) p. 78 – 103).

The following oligonucleotides were prepared (see description)

and tested:

ON1: 3-'C*T*T*A A G G C*A G T*A C*C G*C A G*C (K3) SEQ ID NO. 10

ON2: 3'-A*C*C*A C*T C*T A C*G T*C*T G G*T A*A (K4) SEQ ID NO. 11

ON3: 3'-A*A*G*A G*T C*A C*T C*T C*C*T A G G*C (K5) SEQ. ID NO. 19

ON4: 3-'C*T*T*A A G G C*A G T*A C*C G*C A G*C-FITC-5' (K6) SEQ ID NO. 10

ON5: 3'-G*G*C A G*T A C*C G*C A G C*G SEQ ID NO. 22

ON6: 3'-C*T*T*A A G G*C A G*T*A SEQ ID NO. 13

ON7: 3'-T*A*A G G C*A G*T A*C*C SEQ ID NO. 14

ON8: 3'-G*G*C A G*T A C*C*G C*A SEQ ID NO. 15

ON9: 3'-C*A*G*T A C*C G*C A G*C SEQ ID NO. 23

ON10: 3'-A*G*T A C*C G*C A G*C*G SEQ ID NO. 16

ON11: 3'-C*C*G*C A G*C G T*C G*G SEQ ID NO. 17

ON12: 3'-G*C*A G C*G T*C G G*T*T SEQ ID NO. 18

ON13: 3'-A*A*G*A G*T C*A C*T C*T C*C*T A G G*C-Flu-5' (Vergleich 1) SEQ ID NO. 19

ON14: 3'-G*G*C*A G*T A C*C G C*A G*C G T*C G*G SEQ ID NO. 12

ON15: 3'-C*T*T*A A G G*C A G*T*A-FITC SEQ ID NO. 13

wherein

"*" is a phosphorothioate internucleoside bridge,

and FITC is a fluorescence label.

ON1 to ON 12 were tested in a cell-based assay for their effectiveness in inhibiting the proliferation of REH leukemia cells. ON1, ON2, ON4-ON12, ON 14, ON15 are

antisense oligonucleotides directed against the translational start region of eg5 mRNA. ON4 is the 5'-fluoresceine labeled analog of ON1. ON3 is a comparison oligonucleotide.

The results of the proliferation inhibiton experiment are shown in Figure 1.

Example 2: Determination of the antiproliferative activity of the eg5 antisense oligonucleotides

The REH cells (human pre-B leukemia cells, DSM ACC 22) or the A549 tumor cells were cultivated inOptiMEM (Gibco BRL) with 10% fetal calf serum (FCS, GIBCO-BRL) at 37°C under 5% CO_2. The cell density for the assay was about 1 x 106/ml. The oligonucleotides (0.17mM) were mixed with cellfectin (0.83 mg/ml; Gibco-BRL) for complex formation to improve cellular uptake. The oligonucleotide/cellfectin complex was incubated with the cells in 24-well plates for 4 hours in the absence of serum. The oligonucleotide/cellfectin complex was then removed and serum was added to a final concentration of 10%. After 96 hours' incubation at 37 °C under 5% CO_2 the cell density was measured with Casy 1 (from Schärfe). For this, the cells in each well were mixed thoroughly and immediately diluted 1:100 with Casyton. Mean values of cell density were determined in each case from 3 individual wells of the same oligonucleotide concentration. The results of the antiproliferative activity are depicted in Figure 1.

Table 1: Nucleotide sequence of human eg5 (SEQ ID NO. 20)

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  1  GAATTCCGTC ATGGCGTCGC AGCCAAATTC GTCTGCGAAG AAGAAAGAGG
 51  AGAAGGGGAA GAACATCCAG GTGGTGGTGA GATGCAGACC ATTTAATTTG
101  GCAGAGCGGA AAGCTAGCGC CCATTCAATA GTAGAATGTG ATCCTGTACG
151  AAAAGAAGTT AGTGTACGAA CTGGAGGATT GGCTGACAAG AGCTCAAGGA
201  AAACATACAC TTTTGATATG GTGTTTGGAG CATCTACTAA ACAGATTGAT
251  GTTTACCGAA GTGTTGTTTG TCCAATTCTG GATGAAGTTA TTATGGGCTA
301  TAATTGCACT ATCTTTGCGT ATGGCCAAAC TGGCACTGGA AAAACTTTTA
351  CAATGGAAGG TGAAAGGTCA CCTAATGAAG AGTATACCTG GGAAGAGGAT
401  CCCTTGGCTG GTATAATTCC ACGTACCCTT CATCAAATTT TTGAGAAACT
451  TACTGATAAT GGTACTGAAT TTTCAGTCAA AGTGTCTCTG TTGGAGATCT
501  ATAATGAAGA GCTTTTTGAT CTTCTTAATC CATCATCTGA TGTTTCTGAG
551  AGACTACAGA TGTTTGATGA TCCCCGTAAC AAGAGAGGAG TGATAATTAA
601  AGGTTTAGAA GAAATTACAG TACACAACAA GGATGAAGTC TATCAAATTT
651  TAGAAAAGGG GGCAGCAAAA AGGACAACTG CAGCTACTCT GATGAATGCA
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 701  TACTCTAGTC GTTCCCACTC AGTTTTCTCT GTTACAATAC ATATGAAAGA
 751  AACTACGATT GATGGAGAAG AGCTTGTTAA AATCGGAAAG TTGAACTTGG
 801  TTGATCTTGC AGGAAGTGAA AACATTGGCC GTTCTGGAGC TGTTGATAAG
 851  AGAGCTCGGG AAGCTGGAAA TATAAATCAA TCCCTGTTGA CTTTGGGAAG
 901  GGTCATTACT GCCCTTGTAG AAAGAACACC TCATGTTCCT TATCGAGAAT
 951  CTAAACTAAC TAGAATCCTC CAGGATTCTC TTGGAGGGCG TACAAGAACA
1001  TCTATAATTG CAACAATTTC TCCTGCATCT CTCAATCTTG AGGAAACTCT
1051  GAGTACATTG GAATATGCTC ATAGAGCAAA GAACATATTG AATAAGCCTG
1101  AAGTGAATCA GAAACTCACC AAAAAAGCTC TTATTAAGGA GTATACGGAG
1151  GAGATAGAAC GTTTAAAACG AGATCTTGCT GCAGCCCGTG AGAAAAATGG
1201  AGTGTATATT TCTGAAGAAA ATTTTAGAGT CATGAGTGGA AAATTAACTG
1251  TTCAAGAAGA GCAGATTGTA GAATTGATTG AAAAAATTGG TGCTGTTGAG
1301  GAGGAGCTGA ATAGGGTTAC AGAGTTGTTT ATGGATAATA AAAATGAACT
1351  TGACCAGTGT AAATCTGACC TGCAAAATAA AACACAAGAA CTTGAAACCA
1401  CTCAAAAACA TTTGCAAGAA ACTAAATTAC AACTTGTTAA AGAAGAATAT
1451  ATCACATCAG CTTTGGAAAG TACTGAGGAG AAACTTCATG ATGCTGCCAG
1501  CAAGCTGCTT AACACAGTTG AAGAAACTAC AAAAGATGTA TCTGGTCTCC
1551  ATTCCAAACT GGATCGTAAG AAGGCAGTTG ACCAACACAA TGCAGAAGCT
1601  CAGGATATTT TTGGCAAAAA CCTGAATAGT CTGTTTAATA ATATGGAAGA
1651  ATTAATTAAG GATGGCAGCT CAAAGCAAAA GGCCATGCTA GAAGTACATA
1701  AGACCTTATT TGGTAATCTG CTGTCTTCCA GTGTCTCTGC ATTAGATACC
1751  ATTACTACAG TAGCACTTGG ATCTCTCACA TCTATTCCAG AAAATGTGTC
1801  TACTCATGTT TCTCAGATTT TTAATATGAT ACTAAAAGAA CAATCATTAG
1851  CAGCAGAAAG TAAAACTGTA CTACAGGAAT TGATTAATGT ACTCAAGACT
1901  GATCTTCTAA GTTCACTGGA AATGATTTTA TCCCCAACTG TGGTGTCTAT
1951  ACTGAAAATC AATAGTCAAC TAAAGCATAT TTTCAAGACT TCATTGACAG
2001  TGGCCGATAA GATAGAAGAT CAAAAAAAAA GGAACTCAGA TGGCTTTCTC
2051  AGTATACTGT GTAACAATCT ACATGAACTA CAAGAAAATA CCATTTGTTC
2101  CTTGGTTGAG TCACAAAAGC AATGTGGAAA CCTAACTGAA GACCTGAAGA
2151  CAATAAAGCA GACCCATTCC CAGGAACTTT GCAAGTTAAT GAATCTTTGG
2201  ACAGAGAGAT TCTGTGCTTT GGAGGAAAAG TGTGAAAATA TACAGAAACC
2251  ACTTAGTAGT GTCCAGGAAA ATATACAGCA GAAATCTAAG GATATAGTCA
2301  ACAAAATGAC TTTTCACAGT CAAAAATTTT GTGCTGATTC TGATGGCTTC
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2351 TCACAGGAAC TCAGAAATTT TAACCAAGAA GGTACAAAAT TGGTTGAAGA

2401 ATCTGTGAAA CACTCTGATA AACTCAATGG CAACCTGGAA AAAATATCTC

2451 AAGAGACTGA ACAGAGATGT GAATCTCTGA ACACAAGAAC AGTTTATTTT

2501 TCTGAACAGT GGGTATCTTC CTTAAATGAA AGGGAACAGG AACTTCACAA

2551 CTTATTGGAG GTTGTAAGCC AATGTTGTGA GGCTTCAAGT TCAGACATCA

2601 CTGAGAAATC AGATGGACGT AAGGCAGCTC ATGAGAAACA GCATAACATT

2651 TTTCTTGATC AGATGACTAT TGATGAAGAT AAATTGATAG CACAAAATCT

2701 AGAACTTAAT GAAACCATAA AAATTGGTTT GACTAAGCTT AATTGCTTTC

2751 TGGAACAGGA TCTGAAACTG GATATCCCAA CAGGTACGAC ACCACAGAGG

2801 AAAAGTTATT TATACCCATC AACACTGGTA AGAACTGAAC CACGTGAACA

2851 TCTCCTTGAT CAGCTGAAAA GGAAACAGCC TGAGCTGTTA ATGATGCTAA

2901 ACTGTTCAGA AAACAACAAA GAAGAGACAA TTCCGGATGT GGATGTAGAA

2951 GAGGCAGTTC TGGGGCAGTA TACTGAAGAA CCTCTAAGTC AAGAGCCATC

3001 TGTAGATGCT GGTGTGGATT GTTCATCAAT TGGCGGGGTT CCATTTTTCC

3051 AGCATAAAAA ATCACATGGA AAAGACAAAG AAAACAGAGG CATTAACACA

3101 CTGGAGAGGT CTAAAGTGGA AGAAACTACA GAGCACTTGG TTACAAAGAG

3151 CAGATTACCT CTGCGAGCCC AGATCAACCT TTAATTCACT TGGGGGTTGG

3201 CAATTTTATT TTTAAAGAAA AACTTAAAAA TAAAACCTGA AACCCCAGAA

3251 CTTGAGCCTT GTGTATAGAT TTTAAAAGAA TATATATATC AGCCGGGCGC

3301 GTGGCTCTAG CTGTAATCCC AGCTAACTTT GGAGGCTGAG GCGGGTGGAT

3351 TGCTTGAGCC CAGGAGTTTG AGACCAGCCT GGCCAACGTG CGCTAAAACC

3401 TTCGTCTCTG TTAAAAATTA GCCGGGCGTG GTGGGCACAC TCCTGTAATC

3451 CCAGCTACTG GGGAGGCTGA GGCACGAGAA TCACTTGAAC CCAGAAGCGG

3501 GGTTGCAGTG AGCCAAAGGT ACACCACTAC ACTCCAGCCT GGGCAACAGA

3551 GCAAGACTCG GTCTCAAAAA TAAAATTTAA AAAAGATATA AGGCAGTACT

3601 GTAAATTCAG TTGAATTTTG ATATCTACCC ATTTTTCTGT CATCCCTATA

3651 GTTCACTTTG TATTAAATTG GGTTTCATTT GGGATTTGCA ATGTAAATAC

3701 GTATTTCTAG TTTTCATATA AAGTAGTTCT TTTAGGAATT C

Table 2: SEQ ID NO. 21: Sequence of P. falciparum (partial sequence; Genbank, ID Z98551).

5'

TTTTTTTTTTTTTATTCCTTGGATGTTCTTGGTAGTTTAAATTTTTTATTTTTGTAGTTTTC
TTCTTTTATACGTTTTAAAGCAGGGGATGCCTTTTTAGGAAATGCCCTATTTTCAATAGCTT
TAATTTTTGTAGATTGAAATTTATTATTATTATTATTATTGTTGTTGTTGTTGTTGTTG
TTGTTGTTGTTATTATTTGAATAATTATTTGTTATATGAACATTTTGAACATTTATATTTCT
CTTTCTTTCATATTCTTTTAAACTTGTTACACTCATATTTTCTGTATTTACATCAAATCTTT
TATTATGTTGATTGTTATTTAAATAATTTAATTCTTGATATGTTTCATCTATTGGTTGTATA
GGATTATCCGTTGTATTCTTATTATATAGCATATATTCATTTAAGGGTAGATTATTGTGATT
AGTTTTTACATTTAATTTATTTTTATCACCTTTATTATTTATATTATGAGGTATACTACTAT
TCGTTGTATGATCATTTAAACTATTGTAACGAGAGTAATTATTTTCATGCGCTACAATTTTA
TCATCTTGAATAAGAAATTGGAAGTTTTCATCGATTTGTTCAAATACTTTACTTAAATCTAT
ATCATGTGTTGTTGTAATTTGTTCTATCTCTTTCATCAAGGTATTTTTAACTTCCAAGTATA
AATTTTGTCTTATGATATCATCATTATAAAGATAATAATTATGATGATCACCTTGATCTATT
TTATTATCATCATTATAAAGATAATAATTATGATGATCACCTTGATCCATTTTATTATCATC
ATTATAAAGATAATTATTATGATCATGACCTTGATCCATTTTATTATCATCATTATAAAGAT
AATTATTATGATCATGACCTTGATCCATTTTATTATCATCATTATAAAGATAATTATTATGA
TCATGACCTTGATCCATTTTATTATCATCATAATTATTATTGTCACCATTTTTATTATTGTC
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GATTATTTTTATTATTATCATGATTATTTTTATTATTATCATCATTTTTATTATTATCATAA
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Table 3:

Sequence homology:. Comparison of human eg5 sequence with Plasmodium falciparum -eg5 sequence

```
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human.SEQ       GAATTCCGTCAT.........GGCGTC....GCAGCC.AAATTC...GTCTGCGAAGAAG
PLASMO.SEQ      TTTTTTTTTTTTTATTCCTTGGATGTTCTTGGTAGTTTAAATTTTTTATTTTTGTAGTTT

               61                                                   120
human.SEQ       .........AAAGA....GGAGAAGGGGAAGAACATCCAGGTGGTGGTGAGATGCAGACC
PLASMO.SEQ      TCTTCTTTTATACGTTTTAAAGCAGGGGATGCCTTTTTAGGAAATGCCCTATTTTCAATA

               121                                                  180
human.SEQ       A.TTTAATTTGGCAGAGCGGAAAGCTAGCGCCCAT.TCAATAGTAGAATGTGATCCTGTA
PLASMO.SEQ      GCTTTAATTTTTGTAGATTGAAATTTATTATTATTATTATTATTATTGT.TGTTGTTGTT

               181                                                  240
human.SEQ       CGAAAAGAAGTTAGTGT.ACGAACTGGAGGATTGGCTG..ACAAGAGCTCAAGGAAAACA
PLASMO.SEQ      GTTGTTGTTGTTGTTGTTATTATTTGAATAATTATTTGTTATATGAACATTTTGAACATT

               241                                                  300
human.SEQ       TACACTTTTGAT......ATGGTGTTTGGAGC.............ATCTACTAAAC..AG
PLASMO.SEQ      TATATTTCTCTTTCTTTCATATTCTTTTAAACTTGTTACACTCATATTTTCTGTATTTAC

               301                                                  360
human.SEQ       ATTGA..TGTTTACCG....AAGTGTTGTTTG.....TCCAATTCTGGATGAAGTT.AT.
PLASMO.SEQ      ATCAAATCTTTTATTATGTTGATTGTTATTTAAATAATTTAATTCTTGATATGTTTCATC
```

```
            361                                                    420
human.SEQ   TATGGGCTATA....ATTGCAC....TATCTTTGC.GTATGGC.CAAACT........GG
PLASMO.SEQ  TATTGGTTGTATAGGATTATCCGTTGTATTCTTATTATATAGCATATATTCATTTAAGGG

            421                                                    480
human.SEQ   CA.....CTG.GAAAAACTTTTACAATGGA...AGGTGAAAGGTC......ACCTA....
PLASMO.SEQ  TAGATTATTGTGATTAGTTTTTACATTTAATTTATTTTTATCACCTTTATTATTTATATT
```

Figure 1:

This figure summarizes the results of Examples 1 + 2:

The effect of oligonucleotides ON1 to ON12 (eg5 antisense) on the inhibition of proliferation of REH cells (in percent) is shown.

■ 1st experiment

◆ 2nd experiment

CF : cellfectin comparison

Claims:

1. An antisense oligonucleotide or a derivative thereof, which corresponds to a part of an eg5 encoding sequence.

2. An antisense nucleotide or a derivative thereof as claimed in claim 1, which corresponds to 8-100 nucleotides of an eg5 encoding sequence.

3. An antisense oligonucleotide or a derivative thereof as claimed in either of claims 1 and 2, which has a length of 8 to 20 nucleotides.

4. An antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 3, which corresponds to a part of a human eg5 and/or Plasmodium falciparum eg5 encoding sequence.

5. An antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 4, which has one of the sequences SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3 , SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8 or SEQ ID NO. 9,
wherein
SEQ ID NO. 1 is 5'-CGACGCCATGACGGAATTC-3'
SEQ ID NO. 2 is 5'-AATGGTCTGCATCTCACCA-3'
SEQ ID NO. 3 is 5'-GGCTGCGACGCCATGACGG-3'
SEQ ID NO. 4 is 5'-ATGACGGAATTC-3'
SEQ ID NO. 5 is 5'-CCATGACGGAAT-3'
SEQ ID NO. 6 is 5'-ACGCCATGACGG-3'
SEQ ID NO. 7 is 5'-GCGACGCCATGA-3'
SEQ ID NO. 8 is 5'-GGCTGCGACGCC-3' and
SEQ ID NO. 9 is 5'-TTGGCTGCGACG-3'.

6. An antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 5, wherein the oligonucleotide has one or more modifications and wherein each modification is located at a particular phosphodiester

internucleoside bridge and/or a particular ß-D-2´-deoxyribose unit and/or a particular natural nucleoside base position in comparison to an oligonucleotide of the same sequence which is composed of natural DNA.

7. An antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 6, wherein 1 to 5 terminal nucleotide units at the 5´-end and/or at the 3´-end of the oligonucleotide are protected by modified internucleoside bridges located at the 5´- and/or the 3´-end of the corresponding nucleoside(s).

8. An antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 7, wherein at least one internal pyrimidine nucleoside and/or an internucleoside bridge located at the 5´-end and/or the 3´-end of this pyrimidine nucleoside is modified.

9. An antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 8, wherein each modification is independently selected from

a) the replacement of the phosphodiester bridge at the 3´- and/or the 5´-end of a nucleoside by a modified internucleoside bridge,

b) the replacement of the phosphodiester bridge at the 3´- and/or the 5´-end of a nucleoside by a dephospho bridge,

c) the replacement of a sugar phosphate residue from the sugar phosphate backbone by another residue,

d) the replacement of a ß-D-2´-deoxyribose unit by a modified sugar unit,

e) the replacement of a natural nucleoside base by a modified nucleoside base,

f) the conjugation to a molecule which influences the properties of the oligonucleotide,

g) the conjugation to a 2'5'-linked oligoadenylate molecule or a derivative thereof, optionally via an appropriate linker molecule, and

h) the introduction of a 3´-3´ and/or a 5´-5´ inversion at the 3´- and/or the 5´-end of the oligonucleotide.

10. A method of making an antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 9 by condensing suitably protected monomers on a solid phase.

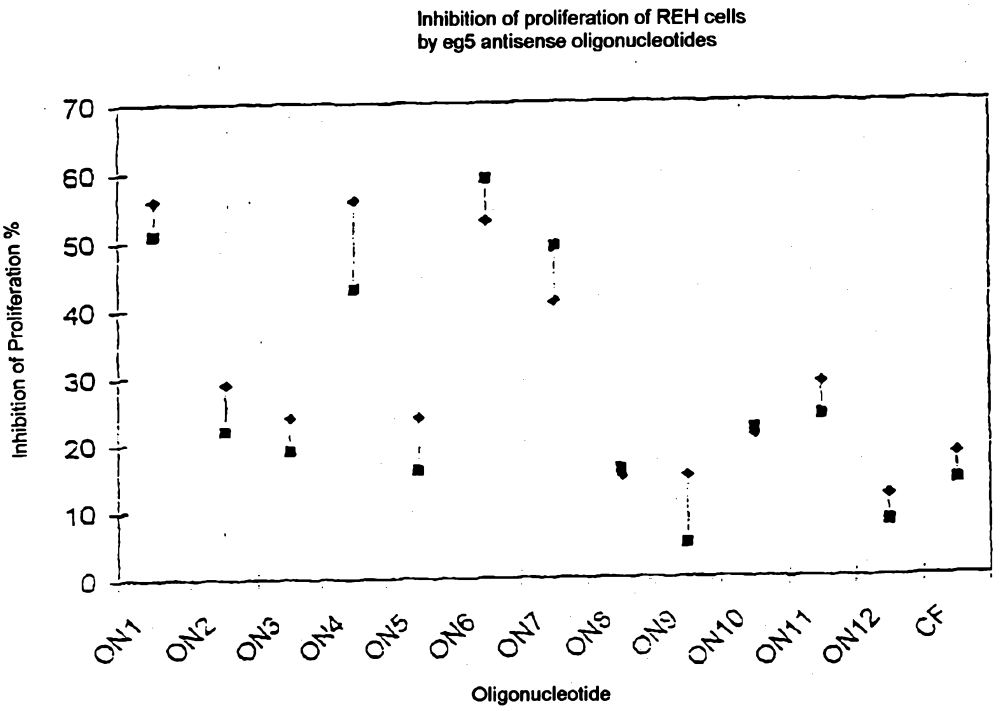
11. The use of an antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 9 for inhibition of the expression of eg5.

12. A method of inhibition of the expression of eg5, wherein an antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 9 is brought into contact with an eg5 encoding nucleic acid and binds with the latter.

13. A method of making a pharmaceutical composition by mixing one or more antisense oligonucleotides or a derivative thereof as claimed in one or more of claims 1 to 9 with a physiologically acceptable excipient and optionally additional substances.

14. A pharmaceutical composition which comprises at least one antisense oligonucleotide or a derivative thereof as claimed in one or more of claims 1 to 9.

Fig: 1


Inhibition of proliferation of REH cells
by eg5 antisense oligonucleotides
Inhibition of Proliferation %
70
60
50
40
30
20
10
0
ON1
ON2
ON3
ON4
ON5
ON6
ON7
ON8
ON9
ON10
ON11
ON12
CF
Oligonucleotide

SEQUENCE LISTING

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<120> Oligonucleotides for the inhibition of human eg5 expression

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