

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 January 2010 (07.01.2010)

PCT

(10) International Publication Number
WO 2010/001349 A8

(51) International Patent Classification:
A61K 31/7088 (2006.01) C12N 15/11 (2006.01)

(21) International Application Number:
PCT/IB2009/052860

(22) International Filing Date:
1 July 2009 (01.07.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/077,588 2 July 2008 (02.07.2008) US
08104612.0 2 July 2008 (02.07.2008) EP

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(48) Date of publication of this corrected version:
11 March 2010

(15) Information about Correction:
see Notice of 11 March 2010

(54) Title: RNA ANTAGONISTS TARGETING HSP27

(57) Abstract: The present invention relates to oligomer compounds (oligomers), which target Hsp27 mRNA in a cell, leading to reduced expression of Hsp27. Reduction of Hsp27 expression is beneficial for the treatment of certain medical disorders, such as cancer. The invention provides therapeutic compositions comprising oligomers and methods for modulating the expression of Hsp27 using said oligomers, including methods of treatment.



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RNA ANTAGONISTS TARGETING HSP27

FIELD OF INVENTION

The invention provides compounds, compositions and methods for modulating the expression of Hsp27. In particular, this invention relates to oligomeric compounds (oligomers), which target Hsp27 mRNA in a cell, leading to reduced expression of Hsp27. Reduction of Hsp27 expression is beneficial for a range of medical disorders, such as cancer.

RELATED CASES

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Serial No. 61/077,588 filed 2 July 2008, the disclosure of which is incorporated herein by reference in its entirety. The present application also claims priority from EP 08104612 filed on 2 July 2008.

BACKGROUND

Hsp27 is a molecular chaperone that is constitutively expressed in several mammalian cells, but particularly in pathological conditions. In addition, these proteins share anti-apoptotic properties and are tumorigenic when expressed in cancer cells. Hsp27 expression is associated with a poor prognosis in virtually all cancer forms such as leukemias, breast, gastric, liver, and prostate cancer, and osteosarcomas. Increased Hsp27 expression may also predict the response to some anticancer treatments. For example, expression of Hsp27 is implicated in resistance to chemotherapy in breast cancer and predicts a poor response to chemotherapy in leukemia patients.

Besides cancer modulation, the expression of Hsp27 proteins have also been considered to be of importance for treatment of myopathies and asthma.

Oncogenex are developing an antisense oligonucleotide which targets the Hsp27 mRNA – referred to as the OGX-427 antisense compound. WO2007/025229 and US 7,101,991 disclose antisense oligonucleotides which target Hsp27.

There is a need for improved antisense oligomers targeting Hsp27.

SUMMARY OF INVENTION

The invention provides an oligomer of from 10 – 50 monomers, such as 10 - 30 monomers, which comprises a contiguous sequence (a first region) of 10 – 50 monomers, such as 10 – 30 monomers, wherein the contiguous sequence (the first region) is at least 80% (e.g., 85%, 90%, 95%, 98%, or 99%) identical (homologous) to a region corresponding to a mammalian Hsp27 gene or mRNA or to the reverse complement of a target region of a

nucleic acid which encodes a mammalian Hsp27, such as a mammalian Hsp27 gene or mRNA, such as a nucleic acid having the sequence set forth in SEQ ID NO: 137, or naturally occurring variants thereof. Thus, for example, the oligomer hybridizes to a region of a single-stranded nucleic acid molecule having the sequence shown in SEQ ID NO: 137.

5 The invention provides an oligomer of from 10 – 50 monomers, such as 10 - 30 monomers, which comprises a contiguous sequence (a first region) of 10 – 50 monomers, such as 10 – 30 monomers, wherein the contiguous sequence (the first region) is at least 80% (e.g., 85%, 90%, 95%, 98%, or 99%) identical (homologous) to a region corresponding to a mammalian Hsp27 gene or mRNA, or to the reverse complement of a target region of a
10 nucleic acid which encodes a mammalian Hsp27, such as a mammalian Hsp27 gene or mRNA, such as a nucleic acid having the sequence set forth in SEQ ID NO: 137, or naturally occurring variants thereof; and wherein at least one monomer in the first region is a nucleoside analogue wherein the nucleoside analogue is a Locked Nucleic Acid (LNA) monomer. Thus, for example, the oligomer hybridizes to a region of a single-stranded
15 nucleic acid molecule having the sequence shown in SEQ ID NO: 137.

 The invention provides for a conjugate comprising the oligomer according to the invention, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to the oligomer.

 The invention provides for a pharmaceutical composition comprising the oligomer or
20 the conjugate according to the invention, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

 The invention provides for the oligomer or the conjugate according to the invention, for use as a medicament, such as for the treatment of a disease or a medical disorder as disclosed herein, such as a hyperproliferative disorder, such as cancer or other
25 hyperproliferative disorder.

 The invention provides for the use of an oligomer or the conjugate according to the invention, for the manufacture of a medicament for the treatment of a disease or disorder as disclosed herein, such as a hyperproliferative disorder, such as cancer.

 The invention provides for a method of treating a disease or disorder as disclosed
30 herein, such as a hyperproliferative disorder, such as cancer, the method comprising administering, e.g. an effective amount of, an oligomer, a conjugate or a pharmaceutical composition according to the invention to an animal suffering from or susceptible to the disease or disorder (such as a patient suffering from or susceptible to the disease or disorder).

35 In one embodiment, the disease or disorder or condition is associated with overexpression of a Hsp27 gene or mRNA.

The invention provides for a method for the inhibition of Hsp27 in a cell which is expressing Hsp27, the method comprising contacting the cell with an oligomer, or a conjugate according to the invention so as to affect the inhibition of Hsp27 expression (e.g. to cause an inhibitory effect on Hsp27 expression) in said cell.

5 The invention provides an oligomer of from 10 - 50 monomers, which comprises a first region of 10 - 50 contiguous monomers, wherein the sequence of the first region is at least 80% identical to a region corresponding to a mammalian Hsp27 gene or to the reverse complement of a target region of a nucleic acid which encodes a mammalian Hsp27.

10 The invention further provides a conjugate comprising the oligomer according to the invention, which comprises at least one non-nucleotide or non-polynucleotide moiety ("conjugated moiety") covalently attached to the oligomer of the invention.

The invention provides for pharmaceutical compositions comprising an oligomer or conjugate of the invention, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

15 The invention further provides for an oligomer according to the invention, for use in medicine.

20 The invention further provides for the use of the oligomer of the invention for the manufacture of a medicament for the treatment of one or more of the diseases referred to herein, such as a disease selected from the group consisting of cancer, myopathies and asthma.

The invention further provides for an oligomer according to the invention, for use for the treatment of one or more of the diseases referred to herein, such as a disease selected from the group consisting of cancer, myopathies and asthma.

25 Pharmaceutical and other compositions comprising an oligomer of the invention are also provided. Further provided are methods of down-regulating the expression of Hsp27 in cells or tissues comprising contacting said cells or tissues, *in vitro* or *in vivo*, with an effective amount of one or more of the oligomers, conjugates or compositions of the invention.

30 Also disclosed are methods of treating an animal (a non-human animal or a human) suspected of having, or susceptible to, a disease or condition, associated with expression, or over-expression of Hsp27 by administering to the non-human animal or human a therapeutically or prophylactically effective amount of one or more of the oligomers, conjugates or pharmaceutical compositions of the invention. Further, methods of using oligomers for the inhibition of expression of Hsp27, and for treatment of diseases associated with activity of Hsp27 are provided.

35 The invention provides for a method for treating a disease selected from the group consisting of: cancer, myopathies and asthma, the method comprising administering an

effective amount of one or more oligomers, conjugates, or pharmaceutical compositions thereof to an animal in need thereof (such as a patient in need thereof).

The invention provides for methods of inhibiting (*e.g.*, by down-regulating) the expression of Hsp27 in a cell or a tissue, the method comprising the step of contacting the cell or tissue, *in vitro* or *in vivo*, with an effective amount of one or more oligomers, conjugates, or pharmaceutical compositions thereof, to effect down-regulation of expression of Hsp27.

BRIEF DESCRIPTION OF FIGURES

- Figure 1.** Real-time Quantitative PCR showing Hsp27 mRNA normalized to GAPDH, 24h after transfection of PC3 cells with the indicated oligonucleotides.
- Figure 2.** MTS cell proliferation after transfection of PC3 cells with the indicated oligonucleotides
- Figure 3.** Hsp27 cDNA (mRNA) sequence – human – SEQ ID NO 137. GenBank accession number NM_001540.
- Figure 4. IC₅₀ determination of oligomers in A549 cells.** QPCR data from A549 cells 24h after transfection with Hsp27 oligomers (which may be referred to as oligos). The data have been normalized with GAPDH mRNA expression and are compared to target expression in mock (100%). Mock transfected cells are transfected with the transfection agent only (negative control).
- Figure 5. IC₅₀ determination of oligomers in PC3 cells.** QPCR data from PC3 cells 24h after transfection with Hsp27 oligos. The data have been normalized with GAPDH mRNA expression and are compared to target expression in mock (100%). Mock transfected cells are transfected with the transfection agent only (negative control).
- Figure 6.** Serum stability assay -digestion at 37 °C with aliquots taken at 0, 24, 48 and 120 h. The results are visualized by gel electrophoresis in PAGE. A DNA phosphorothioate has been used as a positive control.
- Figure 7.** Hsp27 mRNA down-regulation in mouse liver of mice treated with Hsp27 oligomers.
- Figure 8.** ALT levels in mouse blood serum in mice treated with Hsp27 oligomers.
- Figure 9.** AST levels in mouse blood serum in mice treated with Hsp27 oligomers.
- Figure 10.** Long-term culture with the oligomer having the sequence set forth in SEQ ID NO: 135.
- Figure 11.** Long-term culture with the oligomer having the sequence set forth in SEQ ID NO: 135 down-modulates Hsp27 mRNA and protein.

Figure 12. Example of combination effects of anti-Hsp27 LNA-based oligonucleotide plus cytotoxic agent: Effect of combination with TNF• on cellular growth.

Figure 13. Anti-tumor effects of the oligomer having the sequence set forth in SEQ ID NO: 135 in PC3 Xenograft.

5 **Figure 14.** Target Knockdown in Tumors by anti-Hsp27 Oligonucleotides in Animal Models. The size of the bands in the marker are 43KDa, 34KDa and 23 KDa.

DETAILED DESCRIPTION OF INVENTION

The Oligomer

10 The invention employs oligomeric compounds (referred herein as oligomers), for use in modulating the function of nucleic acid molecules encoding mammalian Hsp27, such as the Hsp27 nucleic acid shown in SEQ ID NO: 137, and naturally occurring variants of such nucleic acid molecules encoding mammalian Hsp27. The term “oligomer” in the context of the invention, refers to a molecule formed by covalent linkage of two or more monomers (*i.e.* an oligonucleotide). In some embodiments, the oligomer comprises or consists of from 10 –
15 50 covalently linked monomers, such as from 10-30 covalently linked monomers, such as 10-24 covalently linked monomers, such as 10-18 covalently linked monomers, such as 10-16 covalently linked monomers.

In some embodiments, the terms “nucleoside”, “nucleotide”, “unit” and “monomer” are used interchangeably. It will be recognised that when referring to a sequence of nucleotides
20 or monomers, what is referred to is the sequence of bases, such as A, T, G, C or U.

The term “nucleotide” as used herein, refers to a glycoside comprising a sugar moiety, a base moiety and a covalently linked group (linkage group) such as a phosphate or phosphorothioate internucleotide linkage group, and covers both naturally occurring nucleotides, such as DNA or RNA, and non-naturally occurring nucleotides comprising
25 modified sugar and/or base moieties, which are also referred to as “nucleotide analogues” herein. Herein, a single nucleotide (unit) may also be referred to as a monomer or nucleic acid unit.

In the field of biochemistry, the term “nucleoside” is commonly used to refer to a glycoside comprising a sugar moiety and a base moiety, and may therefore be used when
30 referring to the “nucleotide” units, which are covalently linked by the internucleotide linkages between the nucleotides of the oligomer. In the field of biotechnology, the term “nucleotide” is often used to refer to a nucleic acid monomer or unit, and as such in the context of an oligonucleotide may refer to the base – such as the “nucleotide sequence”, typically refers to the nucleobase sequence (*i.e.* the presence of the sugar backbone and internucleoside
35 linkages are implicit). Likewise, particularly in the case of oligonucleotides where one or

more of the internucleoside linkage groups are modified, the term "nucleotide" may refer to a "nucleoside" for example the term "nucleotide" may be used, even when specifying the presence or nature of the linkages between the nucleosides.

5 The person having ordinary skill in the art would understand that, in the context of the present invention, the 5' terminal nucleotide of an oligonucleotide (oligomer) does not comprise a 5' internucleotide linkage group, although it may or may not comprise a 5' terminal group.

10 The term "monomer" includes both nucleosides and deoxynucleosides (collectively, "nucleosides") that occur naturally in nucleic acids and that do not contain either modified sugars or modified nucleobases, *i.e.*, compounds in which a ribose sugar or deoxyribose sugar is covalently bonded to a naturally-occurring, unmodified nucleobase (base) moiety (*i.e.*, the purine and pyrimidine heterocycles adenine, guanine, cytosine, thymine or uracil) and "nucleoside analogues," which are nucleosides that either do occur naturally in nucleic acids or do not occur naturally in nucleic acids, wherein either the sugar moiety is other than
15 a ribose or a deoxyribose sugar (such as bicyclic sugars or 2' modified sugars, such as 2' substituted sugars), or the base moiety is modified (*e.g.*, 5-methylcytosine), or both.

An "RNA monomer" is a nucleoside containing a ribose sugar and an unmodified nucleobase.

20 A "DNA monomer" is a nucleoside containing a deoxyribose sugar and an unmodified nucleobase.

A "Locked Nucleic Acid monomer," "locked monomer," or "LNA monomer" is a nucleoside analogue having a bicyclic sugar, as further described herein below.

25 The terms "corresponding to" and "corresponds to" refer to the comparison between the nucleotide/nucleoside sequence (*i.e.* the nucleobase or base sequence) of the oligomer or contiguous nucleotide/nucleoside sequence (a first region) and the equivalent contiguous nucleotide/nucleoside sequence of a further sequence selected from either i) a sub-sequence of the reverse complement of the nucleic acid target, and/or ii) the sequence of nucleotides/nucleosides provided herein. Nucleotide/nucleoside analogues are compared directly to their equivalent or corresponding nucleotides/nucleosides. A first region which
30 corresponds to a further sequence under i) or ii) typically is identical to that sequence over the length of the first region (such as the contiguous nucleotide/nucleoside sequence) or, as described herein may, in some embodiments, be at least 80% homologous to a corresponding sequence, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% homologous, at least 97% homologous,
35 at least 98% homologous, at least 99% homologous, such as 100% homologous (identical).

The terms “corresponding nucleoside analogue” and “corresponding nucleoside” indicate that the base moiety in the nucleoside analogue and the base moiety in the nucleoside are identical. For example, when the “nucleoside” contains a 2'-deoxyribose sugar linked to an adenine, the “corresponding nucleoside analogue” contains, for example, a modified sugar linked to an adenine base moiety.

The terms “oligomer”, “oligomeric compound” and “oligonucleotide” are used interchangeably in the context of the invention, and refer to a molecule formed by covalent linkage of two or more monomers by, for example, a phosphate group (forming a phosphodiester linkage between nucleosides) or a phosphorothioate group (forming a phosphorothioate linkage between nucleosides). The oligomer consists of, or comprises, 10 – 50 monomers, such as 10 – 30 monomers, such as 10 - 24 monomers, such as 10 - 18 monomers, such as 10 - 16 monomers. The oligomer consists of or comprises a first region (a contiguous sequence) which, for example, consists of 9 - 30 contiguous monomers, such as 9 - 24 monomers, such as 9 - 18 monomers, such as 9 - 16 monomers.

In some embodiments, the terms “contiguous sequence”, “contiguous monomers” and “region” are interchangeable.

In some embodiments, an oligomer comprises nucleosides, or nucleoside analogues, or mixtures thereof as referred to herein. An “LNA oligomer” or “LNA oligonucleotide” refers to an oligonucleotide containing one or more LNA monomers.

Nucleoside analogues that are optionally included within oligomers may function similarly to corresponding nucleosides, or may have specific improved functions. Oligomers wherein some or all of the monomers are nucleoside analogues are often preferred over native forms because of several desirable properties of such oligomers, such as the ability to penetrate a cell membrane, good resistance to extra- and/or intracellular nucleases and high affinity and specificity for the nucleic acid target. LNA monomers are particularly preferred, for example, for conferring one or more of the above-mentioned properties.

In various embodiments, one or more nucleoside analogues present within the oligomer are “silent” or “equivalent” in function to the corresponding natural nucleoside, *i.e.*, have no functional effect on the way the oligomer functions to inhibit target gene expression.

Such “equivalent” nucleoside analogues are nevertheless useful if, for example, they are easier or cheaper to manufacture, or are more stable under storage or manufacturing conditions, or can incorporate a tag or label. Typically, however, the analogues will have a functional effect on the way in which the oligomer functions to inhibit expression; for example, by producing increased binding affinity to the target region of the target nucleic acid and/or increased resistance to nucleases, such as intracellular nucleases, and/or increased ease of transport into the cell.

Thus, in various embodiments, oligomers according to the invention comprise nucleoside monomers and at least one nucleoside analogue monomer, such as an LNA monomer, or other nucleoside analogue monomers.

The term "at least one" comprises the integers larger than or equal to 1, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 and so forth. In various embodiments, such as when referring to the nucleic acid or protein targets of the oligomers of the invention, the term "at least one" includes the terms "at least two" and "at least three" and "at least four." Likewise, in some embodiments, the term "at least two" comprises the terms "at least three" and "at least four."

In some embodiments, the oligomer comprises or consists of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous monomers in the first region.

In some embodiments, the oligomer comprises or consists of 10 - 24 contiguous monomers, such as 10 – 22 contiguous monomers, such as 10 – 18 contiguous monomers, such as 10 – 16 contiguous monomers, such as 12 – 18 contiguous monomers, such as 13 – 17 or 12 – 16 contiguous monomers, such as 13, 14, 15, 16 or 24 contiguous monomers. It should be understood that when a range is given for an oligomer, or contiguous nucleotide sequence length it includes the lower and upper lengths provided in the range, for example from (or between) 10 – 30, includes both 10 and 30.

In certain embodiments, the oligomer comprises or consists of 10, 11, 12, 13, or 14 contiguous monomers.

In various embodiments, the oligomer according to the invention consists of no more than 24 monomers, such as no more than 22 monomers, such as no more than 20 monomers, such as no more than 18 monomers, such as 15, 16 or 17 monomers. In some embodiments, the oligomer of the invention comprises less than 20 monomers.

In various embodiments, the oligomers of the invention do not comprise RNA monomers.

In various embodiments, the oligomers according to the invention are linear molecules or are linear as synthesised. The oligomer, in such embodiments, is a single stranded molecule, and typically does not comprise short regions of, for example, at least 3, 4 or 5 contiguous monomers, which are complementary to another region within the same oligomer such that the oligomer forms an internal duplex. In some embodiments, the oligomer is essentially not double stranded, *i.e.*, is not a siRNA.

In some embodiments, the oligomer of the invention consists of a contiguous stretch of monomers (a first region), the sequence of which is identified by a SEQ ID NO disclosed herein (*see, e.g.*, Tables 1-3). In other embodiments, the oligomer comprises a first region,

the region consisting of a contiguous stretch of monomers of the nucleic acid molecule encoding the target, and one or more additional regions which consist of at least one additional monomer. In some embodiments, the sequence of the first region is identified by a SEQ ID NO disclosed herein.

5 **Gapmer Design**

Typically, the oligomer of the invention is a gapmer.

A "gapmer" is an oligomer which comprises a contiguous stretch of monomers capable of recruiting an RNase (*e.g.*, such as RNaseH) as further described herein below, such as a region of at least 6 or 7 DNA monomers, referred to herein as region B. Region B is flanked
10 both on its 5' and 3' ends by regions respectively referred to as regions A and C, each of regions A and C comprising or consisting of nucleoside analogues, such as affinity-enhancing nucleoside analogues, such as 1 – 6 nucleoside analogues. The RNase is preferably RNaseH, such as *E. coli* or human RNaseH. The capability of an oligomer to recruit RNaseH is determined when the oligomer is formed in a duplex with a
15 complementary RNA molecule (such as a mRNA target).

In some embodiments, the monomers which are capable of recruiting RNase are selected from the group consisting of DNA monomers, alpha-L-LNA monomers, C4' alkylated DNA monomers (see PCT/EP2009/050349 and Vester *et al.*, *Bioorg. Med. Chem. Lett.* 18 (2008) 2296 – 2300, hereby incorporated by reference), and UNA (unlocked nucleic acid) nucleotides (see Fluiter *et al.*, *Mol. Biosyst.*, 2009, 10, 1039 hereby incorporated by
20 reference). UNA is unlocked nucleic acid, typically where the C2' – C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed, forming an unlocked "sugar" residue.

Typically, the gapmer comprises regions, from 5' to 3', A-B-C, or optionally A-B-C-D or
25 D-A-B-C, wherein: region A (A) consists of or comprises at least one nucleoside analogue, such as at least one LNA monomer, such as 1-6 nucleoside analogues, such as LNA monomers, and region B (B) consists of or comprises at least five contiguous monomers which are capable of recruiting RNase (when formed in a duplex with a complementary target region of the target RNA molecule, such as the mRNA target), such as DNA
30 monomers; region C (C) consists of or comprises at least one nucleoside analogue, such as at least one LNA monomer, such as 1-6 nucleoside analogues, such as LNA monomers; and region D (D), when present, consists of or comprises 1, 2 or 3 monomers, such as DNA monomers.

In various embodiments, region A consists of 1, 2, 3, 4, 5 or 6 nucleoside analogues,
35 such as LNA monomers, such as 2-5 nucleoside analogues, such as 2-5 LNA monomers, such as 3 or 4 nucleoside analogues, such as 3 or 4 LNA monomers; and/or region C

consists of 1, 2, 3, 4, 5 or 6 nucleoside analogues, such as LNA monomers, such as 2-5 nucleoside analogues, such as 2-5 LNA monomers, such as 3 or 4 nucleoside analogues, such as 3 or 4 LNA monomers.

In certain embodiments, region B consists of or comprises 5, 6, 7, 8, 9, 10, 11 or 12
5 contiguous monomers (e.g. consecutive nucleotides) which are capable of recruiting RNase, such as RNaseH, or 6-10, or 7-9 contiguous monomers, such as 10 or 9 or 8 contiguous monomers which are capable of recruiting RNase. In certain embodiments, region B consists of or comprises at least one DNA monomer, such as 1-12 DNA monomers, preferably 4-12 DNA monomers, more preferably 6-10 DNA monomers, such as 7-10 DNA
10 monomers, most preferably 8, 9 or 10 DNA monomers.

In various embodiments, region A consists of 3 or 4 nucleoside analogues, such as LNA monomers, region B consists of 7, 8, 9 or 10 DNA monomers, and region C consists of 3 or 4 nucleoside analogues, such as LNA monomers. Such designs include (A-B-C) 3-10-3, 3-10-4, 4-10-3, 3-9-3, 3-9-4, 4-9-3, 3-8-3, 3-8-4, 4-8-3, 3-7-3, 3-7-4, 4-7-3, and may
15 further include region D, which may have one or 2 monomers, such as DNA monomers.

Further gapmer designs are disclosed in WO2004/046160, which is hereby incorporated by reference.

WO2008/113832, which claims priority from US provisional application 60/977,409 hereby incorporated by reference, refers to 'shortmer' gapmer oligomers. In some
20 embodiments, oligomers presented here may be such shortmer gapmers.

In certain embodiments, the oligomer consists of 10, 11, 12, 13, 14, 15 or 16 monomers, wherein the regions of the oligomer have the pattern (5' – 3'), A-B-C, or optionally A-B-C-D or D-A-B-C, wherein: region A consists of 1, 2 or 3 nucleoside analogue monomers, such as LNA monomers; region B consists of 7, 8, 9 or 10 contiguous monomers
25 which are capable of recruiting RNase, such as RNaseH; and region C consists of 1, 2 or 3 nucleoside analogue monomers, such as LNA monomers. When present, region D consists of a single DNA monomer.

In certain embodiments, region A consists of 1 LNA monomer. In certain embodiments, region A consists of 2 LNA monomers. In certain embodiments, region A
30 consists of 3 LNA monomers. In certain embodiments, region C consists of 1 LNA monomer. In certain embodiments, region C consists of 2 LNA monomers. In certain embodiments, region C consists of 3 LNA monomers. In certain embodiments, region B consists of 7 nucleoside monomers. In certain embodiments, region B consists of 8 nucleoside monomers. In certain embodiments, region B consists of 9 nucleoside
35 monomers. In certain embodiments, region B consists of 10 nucleoside monomers. In certain embodiments, region B comprises 1 – 10 DNA monomers, such as 2, 3, 4, 5, 6, 7, 8

or 9 DNA monomers. In certain embodiments, region B comprises 1 – 9 DNA monomers, such as 2, 3, 4, 5, 6, 7 or 8 DNA monomers. In certain embodiments, region B consists of DNA monomers. In certain embodiments, region B comprises at least one LNA monomer which is in the alpha-L configuration, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 LNA monomers in the alpha-L-configuration. In certain embodiments, region B comprises at least one alpha-L-oxy LNA monomer. In certain embodiments, all the LNA monomers in region B that are in the alpha-L- configuration are alpha-L-oxy LNA units. In certain embodiments, the number of monomers present in the A-B-C regions are selected from the group consisting of (nucleoside analogue monomers – region B – nucleoside analogue monomers): 1-8-1, 1-8-2, 2-8-1, 2-8-2, 3-8-3, 2-8-3, 3-8-2, 4-8-1, 4-8-2, 1-8-4, 2-8-4, or; 1-9-1, 1-9-2, 2-9-1, 2-9-2, 2-9-3, 3-9-2, 1-9-3, 3-9-1, 3-9-3, 4-9-1, 1-9-4, or; 1-10-1, 1-10-2, 2-10-1, 2-10-2, 1-10-3, 3-10-1, 2-10-3, 3-10-2, or 3-10-3. In certain embodiments, the number of monomers present in the A-B-C regions of the oligomer of the invention respectively is selected from the group consisting of: 2-7-1, 1-7-2, 2-7-2, 3-7-3, 2-7-3, 3-7-2, 3-7-4, and 4-7-3. In certain embodiments, each of regions A and C consists of three LNA monomers, and region B consists of 8 or 9 or 10 nucleoside monomers, preferably DNA monomers. In certain embodiments, each of regions A and C consists of two LNA monomers, and region B consists of 8 or 9 nucleoside monomers, preferably DNA monomers.

In various embodiments, other gapmer designs include those where regions A and/or C consists of 3, 4, 5 or 6 nucleoside analogues, such as monomers containing a 2'-O-methoxyethyl-ribose sugar (2'-MOE) or monomers containing a 2'-fluoro-deoxyribose sugar, and region B consists of 8, 9, 10, 11 or 12 nucleosides, such as DNA monomers, where regions A-B-C have 3-9-3, 3-10-3, 5-10-5 or 4-12-4 monomers. Further gapmer designs are disclosed in WO 2007/146511A2, hereby incorporated by reference.

25 ***Internucleoside Linkages***

The monomers of the oligomers described herein are coupled together via linkage groups. Suitably, each monomer is linked to the 3' adjacent monomer via a linkage group.

The person having ordinary skill in the art would understand that, in the context of the present invention, the 5' monomer at the end of an oligomer does not comprise a 5' linkage group, although it may or may not comprise a 5' terminal group.

The terms "linkage group" and "internucleoside linkage" mean a group capable of covalently coupling together two contiguous monomers. Specific and preferred examples include phosphate groups (forming a phosphodiester between adjacent nucleoside monomers) and phosphorothioate groups (forming a phosphorothioate linkage between adjacent nucleoside monomers).

Suitable linkage groups include those listed in WO2007/031091, for example in the first paragraph of page 34 of WO2007/031091 (hereby incorporated by reference).

It is, in various embodiments, preferred to modify the linkage group from its normal phosphodiester to one that is more resistant to nuclease attack, such as phosphorothioate or boranophosphate – these two being cleavable by RNase H, thereby permitting RNase-mediated antisense inhibition of expression of the target gene.

In some embodiments, suitable sulphur (S) containing linkage groups as provided herein are preferred. In various embodiments, phosphorothioate linkage groups are preferred, particularly for the gap region (B) of gapmers. In certain embodiments, phosphorothioate linkages are used to link together monomers in the flanking regions (A and C). In various embodiments, phosphorothioate linkages are used for linking regions A or C to region D, and for linking together monomers within region D.

In various embodiments, regions A, B and C, comprise linkage groups other than phosphorothioate, such as phosphodiester linkages, particularly, for instance when the use of nucleoside analogues protects the linkage groups within regions A and C from endonuclease degradation – such as when regions A and C comprise LNA monomers.

In various embodiments, adjacent monomers of the oligomer are linked to each other by means of phosphorothioate groups.

It is recognised that the inclusion of phosphodiester linkages, such as one or two linkages, into an oligomer with a phosphorothioate backbone, particularly with phosphorothioate linkage groups between or adjacent to nucleoside analogue monomers (typically in region A and/or C), can modify the bioavailability and/or bio-distribution of an oligomer – see WO2008/053314, hereby incorporated by reference.

In some embodiments, such as the embodiments referred to above, where suitable and not specifically indicated, all remaining linkage groups are either phosphodiester or phosphorothioate, or a mixture thereof.

In some embodiments all the internucleoside linkage groups are phosphorothioate.

When referring to specific gapmer oligonucleotide sequences, such as those provided herein, it will be understood that, in various embodiments, when the linkages are phosphorothioate linkages, alternative linkages, such as those disclosed herein may be used, for example phosphate (phosphodiester) linkages may be used, particularly for linkages between nucleoside analogues, such as LNA monomers. Likewise, in various embodiments, when referring to specific gapmer oligonucleotide sequences, such as those provided herein, when one or more monomers in region A or C, such as LNA monomers, comprises a 5-methylcytosine base, other monomers in that region may contain unmodified cytosine bases.

Target Nucleic Acid

The terms “nucleic acid” and “polynucleotide” are used interchangeably herein, and are defined as a molecule formed by covalent linkage of two or more monomers, as above-described. Including 2 or more monomers, “nucleic acids” may be of any length, and the
 5 term is generic to “oligomers”, which have the lengths described herein. The terms “nucleic acid” and “polynucleotide” include single-stranded, double-stranded, partially double-stranded, and circular molecules.

The term “target nucleic acid”, as used herein, refers to DNA or RNA (*e.g.*, mRNA or pre-mRNA) encoding a mammalian Hsp27 polypeptide, such as human Hsp27, such as the
 10 nucleic acid having the sequence shown in SEQ ID NO: 137, and naturally occurring allelic variants of such nucleic acids. In certain embodiments, the mammalian Hsp27 is a mouse Hsp27. In some embodiments, for example when used in research or diagnostics, the “target nucleic acid” is a cDNA or a synthetic oligonucleotide derived from the above DNA or
 15 RNA nucleic acid targets. The oligomers according to the invention are typically capable of hybridising to the target nucleic acid.

Exemplary target nucleic acids include mammalian Hsp27-encoding nucleic acids having the GenBank Accession numbers shown in the table below, along with their corresponding protein sequences:

	GenBank Accession Number Nucleic acid (mRNA/cDNA sequence)	GenBank Accession Number Polypeptide (deduced)
Human	NM_001540 (SEQ ID No 137)	NP_001531
Mouse	NM_024441	NP_077761

It is recognised that the above-disclosed GenBank Accession numbers for nucleic acids
 20 refer to cDNA sequences and not to mRNA sequences per se. The sequence of a mature mRNA can be derived directly from the corresponding cDNA sequence with thymine bases (T) being replaced by uracil bases (U).

The term “naturally occurring variant thereof” refers to variants of the Hsp27
 25 polypeptide or nucleic acid sequence which exist naturally within the defined taxonomic group, such as mammals, such as mouse, monkey, and preferably human Hsp27. Typically, when referring to “naturally occurring variants” of a polynucleotide the term also encompasses any allelic variant of the Hsp27 encoding genomic DNA which is found at Chromosome 7: 75.77 - 75.77 Mb by chromosomal translocation or duplication, and the RNA, such as mRNA derived therefrom. “Naturally occurring variants” may also include
 30 variants derived from alternative splicing of the Hsp27 mRNA. When referenced to a specific polypeptide sequence, *e.g.*, the term also includes naturally occurring forms of the protein

which may therefore be processed, *e.g.* by co- or post-translational modifications, such as signal peptide cleavage, proteolytic cleavage, glycosylation, etc.

In certain embodiments, oligomers described herein bind to a region of the target nucleic acid (the "target region") by either Watson-Crick base pairing, Hoogsteen hydrogen bonding, or reversed Hoogsteen hydrogen bonding, between the monomers of the oligomer and monomers of the target nucleic acid. Such binding is also referred to as "hybridisation." Unless otherwise indicated, binding is by Watson-Crick pairing of complementary bases (*i.e.*, adenine with thymine (DNA) or uracil (RNA), and guanine with cytosine), and the oligomer binds to the target region because the sequence of the oligomer is identical to, or partially- identical to, the sequence of the reverse complement of the target region; for purposes herein, the oligomer is said to be "complementary" or "partially complementary" to the target region, and the percentage of "complementarity" of the oligomer sequence to that of the target region is the percentage "identity" (homology) to the reverse complement of the sequence of the target region.

The terms "reverse complement", "reverse complementary" and "reverse complementarity" as used herein are interchangeable with the terms "complement", "complementary" and "complementarity".

Unless otherwise made clear by context, the "target region" herein will be the region of the target nucleic acid having the sequence that best aligns with the reverse complement of the sequence of the specified oligomer (or region thereof), using the alignment program and parameters described herein below.

In determining the degree of "complementarity" between oligomers of the invention (or regions thereof) and the target region of the nucleic acid which encodes mammalian Hsp27, such as those disclosed herein, the degree of "complementarity" (also, "homology" or "identity") is expressed as the percentage identity (or percentage homology) between the sequence of the oligomer (or region thereof) and the sequence of the target region (or the reverse complement of the target region) that best aligns therewith. The percentage is calculated by counting the number of aligned bases that are identical between the 2 sequences, dividing by the total number of contiguous monomers in the oligomer, and multiplying by 100. In such a comparison, if gaps exist, it is preferable that such gaps are merely mismatches rather than areas where the number of monomers within the gap differs between the oligomer of the invention and the target region.

As used herein, the terms "homologous" and "homology" are interchangeable with the terms "identity" and "identical".

Amino acid and polynucleotide alignments, percentage sequence identity, and degree of complementarity may be determined for purposes of the invention using the ClustalW

algorithm using standard settings: see <http://www.ebi.ac.uk/emboss/align/index.html>, Method: EMBOSS::water (local): Gap Open = 10.0, Gap extend = 0.5, using Blosum 62 (protein), or DNFull for nucleotide/nucleobase sequences.

5 As will be understood, depending on context, "mismatch" refers to a non-identity in sequence (as, for example, between the nucleobase sequence of an oligomer and the reverse complement of the target region to which it binds; as for example, between the base sequence of two aligned Hsp27 encoding nucleic acids), or to noncomplementarity in sequence (as, for example, between an oligomer and the target region to which it binds).

10 In some embodiments, the oligomers according to the invention are capable of inhibiting (such as, by down-regulating) the expression of one or more Hsp27 target genes in a cell which is expressing, or is capable of expressing (*i.e.* by alleviating Hsp27 repression of the Hsp27 target gene in a cell) an Hsp27 target gene.

15 The oligomers which target Hsp27 mRNA, may hybridize to any site along the target mRNA nucleic acid, such as the 5' untranslated leader, exons, introns and 3' untranslated tail. However, it is preferred that the oligomers which target Hsp27 mRNA hybridise to the mature mRNA form of the target nucleic acid.

Suitably, the oligomer of the invention or conjugate thereof is capable of down-regulating (e.g. reducing or removing) expression of the Hsp27 gene. In various
20 embodiments, the oligomer (or conjugate) of the invention can effect the inhibition of Hsp27, typically in a mammalian cell, such as a human cell. In certain embodiments, the oligomers of the invention, or conjugates thereof, bind to the target nucleic acid and affect inhibition of Hsp27 mRNA expression of at least 10% or 20% compared to the expression level in the absence of the oligomer(s) or conjugate(s), more preferably of at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% as compared to the Hsp27 expression level in the absence of
25 the oligomer(s) or conjugate(s). In some embodiments, such inhibition is seen when using from about 0.04 nM to about 25nM, such as from about 0.8 nM to about 20nM of the oligomer or conjugate. As illustrated herein the cell type is, in some embodiments, a human cell, such as a cancer cell, such as a human lung cancer cell or a human prostate cancer cell (e.g. *in vitro* – transfected cells). The oligomer concentration used is, in some
30 embodiments, 5nM. The oligomer concentration used is, in some embodiments 25nM. The oligomer concentration used is, in some embodiments 1nM. It should be noted that the concentration of oligomer used to treat the cell is in various typical embodiments performed in an *in vitro* cell assay, using transfection (Lipofecton), as illustrated in the Examples. In the absence of a transfection agent, the oligomer concentration required to obtain the down-
35 regulation of the target is typically between 1 and 25µM, such as 5µM.

In various embodiments, the inhibition of mRNA expression is less than 100% (*i.e.*, less than complete inhibition of expression), such as less than 98% inhibition, less than 95% inhibition, less than 90% inhibition, less than 80% inhibition, such as less than 70% inhibition. In various embodiments, modulation of gene expression can be determined by measuring protein levels, *e.g.* by methods such as SDS-PAGE followed by western blotting using suitable antibodies raised against the target protein. Alternatively, modulation of expression levels can be determined by measuring levels of mRNA, *e.g.* by northern blotting or quantitative RT-PCR. When measuring via mRNA levels, the level of down-regulation when using an appropriate dosage, such as from about 0.04 nM to about 25nM, such as from about 0.8 nM to about 20nM, is, in various embodiments, typically to a level of 10-20% of the normal levels in the absence of the oligomer, conjugate or composition of the invention.

The invention therefore provides a method of down-regulating or inhibiting the expression of Hsp27 protein and/or mRNA in a cell which is expressing Hsp27 protein and/or mRNA, the method comprising contacting the cell with an effective amount of the oligomer or conjugate according to the invention to down-regulate or inhibit the expression of the Hsp27 protein and/or mRNA in the cell. Suitably the cell is a mammalian cell, such as a human cell. The contacting may occur, in some embodiments, *in vitro*. The contacting may occur, in some embodiments, *in vivo*.

20 **Oligomer Sequences**

In some embodiments, the oligomers of the invention have sequences that are identical to a sequence selected from the group consisting of SEQ ID NOs: 1 to 128.

Further provided are target nucleic acids (*e.g.*, DNA or mRNA encoding Hsp27) that contain target regions that are (fully or perfectly) complementary or partially-complementary to one or more of the oligomers of the invention. In certain embodiments, the oligomers bind to variants of Hsp27 target regions, such as allelic variants (such as an Hsp27 gene present at gene locus Chromosome 7: 75.77 – 75.77 Mb). In some embodiments, a variant of an Hsp27 target region has at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the target region having a sequence set forth in SEQ ID NO: 137. Thus, in other embodiments, the oligomers of the invention have sequences that differ in 1, 2 or 3 bases when compared to a sequence selected from the group consisting of SEQ ID NOs: 1 to 128. Typically, an oligomer of the invention that binds to a variant of an Hsp27 target region is capable of inhibiting (*e.g.*, by down-regulating) Hsp27.

In other embodiments, oligomers of the invention are LNA oligomers, for example, those oligomers having the sequences shown in SEQ ID NOs: 129-136. In various embodiments, the oligomers of the invention are potent inhibitors of Hsp27 mRNA and protein expression. In some embodiments, the phrase "potent inhibitor" refers to an oligomer with an IC50 of less than 5nM as determined by the lipofectamine transfection assay of Example 5. In some embodiments, the IC50 is less than 4nM, such as less than 2nM.

In various embodiments, oligomers of the invention are LNA oligomers having the sequences of SEQ ID NO: 131 or SEQ ID NO: 135.

In various embodiments, the oligomer comprises or consists of a first region having a base sequence sequence which is identical or partially identical to the sequence of the reverse complement of a target region in SEQ ID NO: 137. In various embodiments, the oligomer comprises or consists of a first region having a sequence selected from the group consisting of SEQ ID NOS: 1-128.

In certain embodiments, the oligomer comprises or consists of a first region having a base sequence which is fully complementary (perfectly complementary) to the sequence of a target region of a nucleic acid which encodes a mammalian Hsp27.

However, in some embodiments, the oligomer includes 1, 2, 3, or 4 (or more) mismatches as compared to the best-aligned target region of an Hsp27 target nucleic acid, and still sufficiently binds to the target region to effect inhibition of Hsp27 mRNA or protein expression. The destabilizing effect of mismatches on Watson-Crick hydrogen-bonded duplex may, for example, be compensated by increased length of the oligomer and/or an increased number of nucleoside analogues, such as LNA monomers, present within the oligomer.

In various embodiments, the oligomer base sequence comprises no more than 3, such as no more than 2 mismatches compared to the base sequence of the best-aligned target region of, for example, a target nucleic acid which encodes a mammalian Hsp27.

In some embodiments, the oligomer base sequence comprises no more than a single mismatch when compared to the base sequence of the best-aligned target region of a nucleic acid which encodes a mammalian Hsp27.

In various embodiments, the base sequence of the oligomer of the invention, or of a first region thereof, is preferably at least 80% identical to a base sequence selected from the group consisting of SEQ ID NOS: 1-15 and 121, 16-30 and 122, 31-45 and 123, 46-60 and 124, 61-75 and 125, 76-90 and 126, 91-105, and 127, and 106-120 and 128, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

95%, at least 96%, at least 97%, at least 98%, at least 99% identical, such as 100% identical.

In certain embodiments, the base sequence of the oligomer of the invention or of a first region thereof is at least 80% identical to the base sequence of the reverse complement of a target region present in SEQ ID NO: 137, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, such as 100% identical.

In various embodiments, the base sequence of the oligomer of the invention, or of a first region thereof, is preferably at least 80% complementary to a target region of SEQ ID NO: 137, such as at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% complementary, at least 97% complementary, at least 98% complementary, at least 99% complementary, such as 100% complementary (perfectly complementary).

In some embodiments the oligomer (or a first region thereof) has a base sequence selected from the group consisting of SEQ ID NOs: 1, 16, 31, 46, 61, 76, 91, and 106, or is selected from the group consisting of at least 9 or 10 contiguous monomers of SEQ ID NOs: 1, 16, 31, 46, 61, 76, 91, and 106. In other embodiments, the sequence of the oligomer of the invention or a first region thereof comprises one, two, or three base moieties that differ (e.g. are mismatches) from those in oligomers having sequences of SEQ ID NOs: 1, 16, 31, 46, 61, 76, 91, and 106, or the sequences of at least 9 or 10 contiguous monomers thereof, when optimally aligned with the selected sequence or region thereof.

In some embodiments, the term "first region" as used herein refers to a portion (sub-sequence) of an oligomer. For example, the 16 monomer sequence set forth in SEQ ID NO: 1 is a subsequence of the 24 monomer sequence set forth in SEQ ID NO: 121, *i.e.*, the sequence set forth in SEQ ID NO: 121 comprises the sequence set forth in SEQ ID NO: 1.

In some embodiments the oligomer (or a first region thereof) has a base sequence selected from the group consisting of SEQ ID NOs: 121-128, or the sequences of at least 9 or 10 contiguous monomers thereof. In other embodiments, the sequence of the oligomer (or a first region thereof) comprises one, two, or three base moieties that differ from those in oligomers having sequences of SEQ ID NOs: 121-128, or the sequences of at least 9 or 10 contiguous monomers thereof, when optimally aligned with the selected sequence or region thereof.

In various embodiments, the oligomers comprise a region of 9, 10, 11, 12, 13, 14, 15 or 16 contiguous monomers, such as 12 – 16, having a base sequence identically present in a sequence selected from the group consisting of SEQ ID No 1, 16, 31, 46, 61, 76, 91, and 106. In other embodiments, the oligomers include a region which comprises one, two, or

three base moieties that differ from those in oligomers having sequences of SEQ ID NOs: 1, 16, 31, 46, 61, 76, 91, and 106.

In some embodiments the first region consists of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 contiguous monomers, such as 9-22, such as 12
5 -24, such as 12 -22, such as 12-18, such as 12 -16 monomers. Suitably, in some
embodiments, the first region is of the same length as the oligomer of the invention.

In some embodiments the oligomer comprises additional monomers at the 5' and/or 3' ends of the first region, such as, independently, 1, 2, 3, 4 or 5 additional monomers at the 5' end and/or the 3' end of the oligomer, which are non-complementary to the target region. In
10 various embodiments, the oligomer of the invention comprises a first region that is
complementary to the target, which is flanked 5' and/or 3' by additional monomers which are
complementary to the target region. In some embodiments the additional 5' or 3' monomers
are nucleosides, such as DNA or RNA monomers. In various embodiments, the 5' or 3'
monomers represent region D as referred to in the context of gapmer oligomers herein.

15 In some embodiments the oligomer according to the invention consists of or comprises
contiguous monomers (a first region) having a nucleobase sequence according to SEQ ID
NO:121, or at least 9 contiguous monomers thereof such as 10, 11, 12, 13, 14, 15 or 16
contiguous monomers thereof, such as SEQ ID NOs 1-15.

In some embodiments the oligomer according to the invention consists of or comprises
20 contiguous monomers (a first region) having a nucleobase sequence according to SEQ ID
NO:122, or at least 9 contiguous monomers thereof such as 10, 11, 12, 13, 14, 15 or 16
contiguous monomers thereof, such as SEQ ID NOs 16-30.

In some embodiments the oligomer according to the invention consists of or comprises
contiguous monomers (a first region) having a nucleobase sequence according to SEQ ID
25 NO:123, or at least 9 contiguous monomers thereof such as 10, 11, 12, 13, 14, 15 or 16
contiguous monomers thereof, such as SEQ ID NOs 31-45.

In some embodiments the oligomer according to the invention consists of or comprises
contiguous monomers (a first region) having a nucleobase sequence according to SEQ ID
NO:124, or at least 9 contiguous monomers thereof such as 10, 11, 12, 13, 14, 15 or 16
30 contiguous monomers thereof, such as SEQ ID NOs 46-60.

In some embodiments the oligomer according to the invention consists of or comprises
contiguous monomers (a first region) having a nucleobase sequence according to SEQ ID
NO:125, or at least 9 contiguous monomers thereof such as 10, 11, 12, 13, 14, 15 or 16
contiguous monomers thereof, such as SEQ ID NOs 61-75.

35 In some embodiments the oligomer according to the invention consists of or comprises
contiguous monomers (a first region) having a nucleobase sequence according to SEQ ID

NO:126, or at least 9 contiguous monomers thereof such as 10, 11, 12, 13, 14, 15 or 16 contiguous monomers thereof, such as SEQ ID NOs 76-90.

In some embodiments the oligomer according to the invention consists of or comprises contiguous monomers (a first region) having a nucleobase sequence according to SEQ ID
5 NO:127, or at least 9 contiguous monomers thereof such as 10, 11, 12, 13, 14, 15 or 16 contiguous monomers thereof, such as SEQ ID NOs 91-105.

In some embodiments the oligomer according to the invention consists of or comprises contiguous monomers (a first region) having a nucleobase sequence according to SEQ ID
10 NO:128, or at least 9 contiguous monomers thereof such as 10, 11, 12, 13, 14, 15 or 16 contiguous monomers thereof, such as SEQ ID NO 106-120.

Nucleosides and Nucleoside analogues

In some embodiments, the terms “nucleoside analogue” and “nucleotide analogue” are used interchangeably.

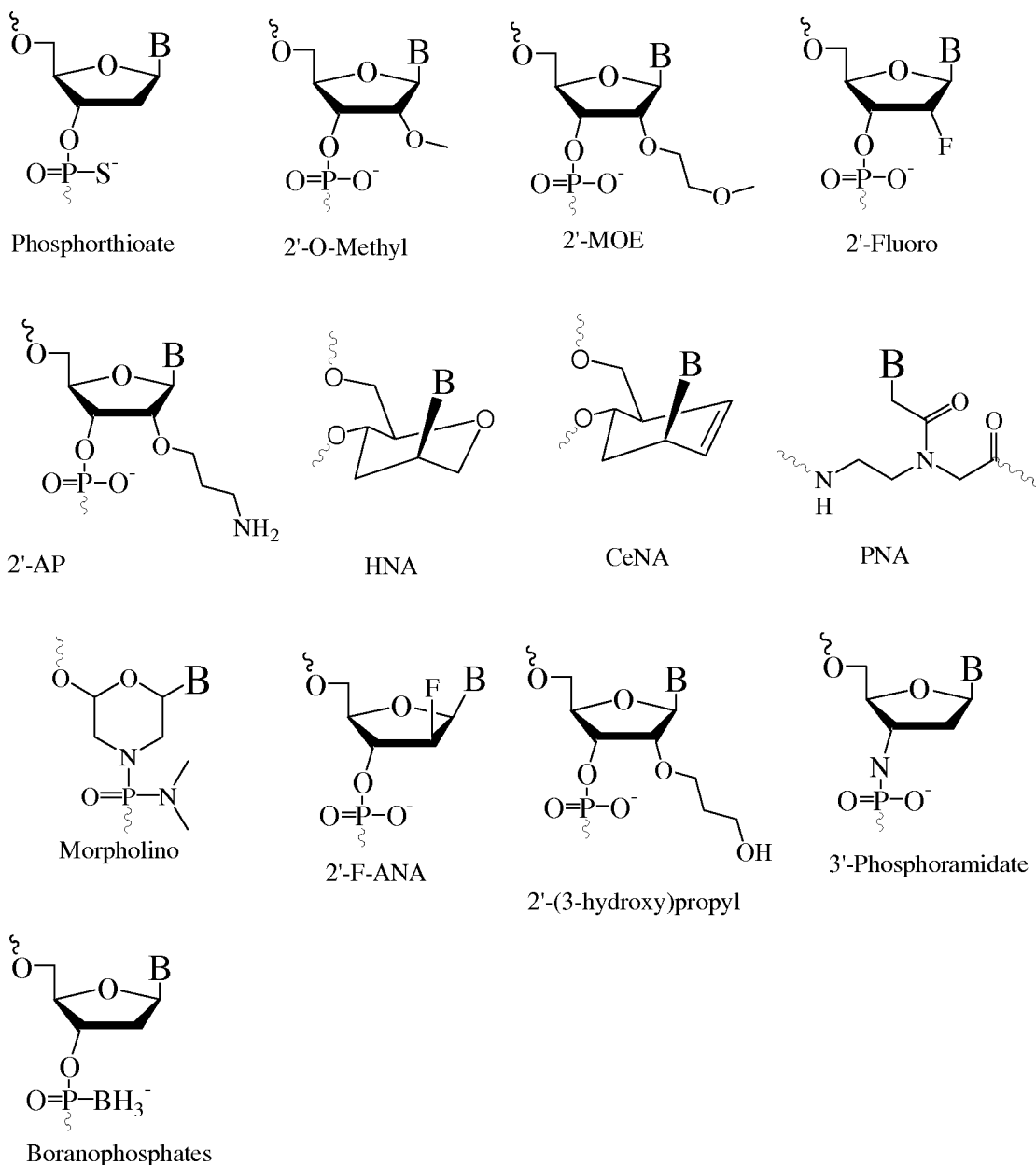
In various embodiments, at least one of the monomers present in the oligomer is a
15 nucleoside analogue that contains a modified base, such as a base selected from 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, 2-chloro-6-aminopurine, xanthine and hypoxanthine.

In various embodiments, at least one of the monomers present in the oligomer is a
20 nucleoside analogue that comprises a modified sugar.

In some embodiments, the linkage between at least 2 contiguous monomers of the oligomer is other than a phosphodiester linkage.

In certain embodiments, the oligomer includes at least one monomer that has a modified base, at least one monomer (which may be the same monomer) that has a
25 modified sugar, and at least one inter-monomer linkage that is non-naturally occurring.

Specific examples of nucleoside analogues are described by *e.g.* Freier & Altmann; *Nucl. Acid Res.*, 1997, 25, 4429-4443 and Uhlmann; *Curr. Opinion in Drug Development*, 2000, 3(2), 293-213, and in Scheme 1 (in which some nucleoside analogues are shown as nucleotides)



Scheme 1

The oligomer may thus comprise or consist of a simple sequence of naturally occurring nucleosides – preferably DNA monomers, but also possibly RNA monomers, or a combination of nucleosides and one or more nucleoside analogues. In some embodiments, such nucleoside analogues suitably enhance the affinity of the oligomer for the target region of the target nucleic acid.

Examples of suitable and preferred nucleoside analogues are described in WO2007/031091, or are referenced therein.

In some embodiments, the nucleoside analogue comprises a sugar moiety modified to provide a 2'-substituent group, such as 2'-O-alkyl-ribose sugars, 2'-amino-deoxyribose sugars, and 2'-fluoro-deoxyribose sugars.

In some embodiments, the nucleoside analogue comprises a bicyclic sugar (LNA), which enhances binding affinity and may also provide some increased nuclease resistance. In various embodiments, the LNA monomer is selected from oxy-LNA (such as beta-D-oxy-LNA, and alpha-L-oxy-LNA), and/or amino-LNA (such as beta-D-amino-LNA and alpha-L-amino-LNA) and/or thio-LNA (such as beta-D-thio-LNA and alpha-L-thio-LNA) and/or ENA (such as beta-D-ENA and alpha-L-ENA). In certain embodiments, the LNA monomers are beta-D-oxy-LNA. LNA monomers are further described below.

In various embodiments, incorporation of affinity-enhancing nucleoside analogues in the oligomer, such as LNA monomers or monomers containing 2'-substituted sugars, or incorporation of modified linkage groups provides increased nuclease resistance. In various embodiments, incorporation of affinity-enhancing nucleoside analogues allows the size of the oligomer to be reduced, and also reduces the size of the oligomer that binds specifically to a target region of a target sequence.

In some embodiments, the oligomer comprises at least 1 nucleoside analogue. In some embodiments, the oligomer comprises at least 2 nucleoside analogues. In some embodiments, the oligomer comprises from 3-8 nucleoside analogues, *e.g.* 6 or 7 nucleoside analogues. In various embodiments, at least one of the nucleoside analogues is a locked nucleic acid (LNA) monomer; for example at least 3 or at least 4, or at least 5, or at least 6, or at least 7, or 8, nucleoside analogues are LNA monomers. In some embodiments, all the nucleoside analogues are LNA monomers.

It will be recognised that when referring to a preferred oligomer base sequence, in certain embodiments, the oligomers comprise a corresponding nucleoside analogue, such as a corresponding LNA monomer or other corresponding nucleoside analogue, which raises the duplex stability (T_m) of the oligomer/target region duplex (*i.e.* affinity enhancing nucleoside analogues).

In various embodiments, any mismatches (*i.e.*, non-complementarities) between the base sequence of the oligomer and the base sequence of the target region, if present, are preferably located other than in the regions of the oligomer that contain affinity-enhancing nucleoside analogues (*e.g.*, regions A or C), such as within region B as referred to herein, and/or within region D as referred to herein, and/or in regions consisting of DNA monomers, and/or in regions which are 5' or 3' to the region of the oligomer that is complementary to the target region.

In some embodiments the nucleoside analogues present within the oligomer of the invention (such as in regions A and C mentioned herein) are independently selected from, for example: monomers containing 2'-O-alkyl-ribose sugars, monomers containing 2'-amino-deoxyribose sugars, monomers containing 2'-fluoro-deoxyribose sugars, LNA monomers,

monomers containing arabinose sugars ("ANA monomers"), monomers containing 2'-fluoro-arabinose sugars, monomers containing d-arabino-hexitol sugars ("HNA monomers"), intercalating monomers as defined in Christensen (2002) Nucl. Acids. Res. 30: 4918-4925, hereby incorporated by reference, and 2'-O-methoxyethyl-ribose (2'MOE) sugars. In some
5 embodiments, there is only one of the above types of nucleoside analogues present in the oligomer of the invention, or region thereof.

In certain embodiments, the nucleoside analogues contain 2'MOE sugars, 2'-fluoro-deoxyribose sugars, or LNA sugars, and as such the oligomer of the invention may comprise nucleoside analogues which are independently selected from these three types. In certain
10 oligomer embodiments containing nucleoside analogues, at least one of said nucleoside analogues contains a 2'-MOE-ribose sugar, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleoside analogues containing 2'-MOE-ribose sugars. In some embodiments, at least one nucleoside analogue contains a 2'-fluoro-deoxyribose sugar, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleoside analogues containing 2'-fluoro-DNA nucleotide sugars.

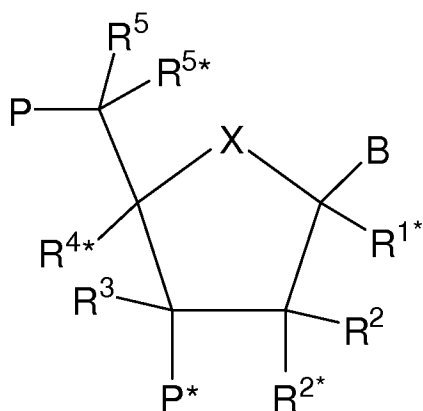
In various embodiments, the oligomer according to the invention comprises at least one Locked Nucleic Acid (LNA) monomer, such as 1, 2, 3, 4, 5, 6, 7, or 8 LNA monomers, such as 3 – 7 or 4 to 8 LNA monomers, or 3, 4, 5, 6 or 7 LNA monomers. In various
15 embodiments, all the nucleoside analogues are LNA monomers. In certain embodiments, the oligomer comprises both beta-D-oxy-LNA monomers, and one or more of the following LNA monomers: thio-LNA monomers, amino-LNA monomers, oxy-LNA monomers, and/or ENA monomers in either the beta-D or alpha-L configurations, or combinations thereof. In certain embodiments, the cytosine base moieties of all LNA monomers in the oligomer are 5-methylcytosines. In certain embodiments of the invention, the oligomer comprises both LNA and DNA monomers. Typically, the combined total of LNA and DNA monomers is 10-25,
20 preferably 10-24, preferably 10-20, preferably 10-18, even more preferably 12-16. In some embodiments of the invention, the oligomer or region thereof consists of at least one LNA monomer, and the remaining monomers are DNA monomers. In certain embodiments, the oligomer comprises only LNA monomers and nucleosides (such as RNA or DNA monomers, most preferably DNA monomers) optionally with modified linkage groups such as
30 phosphorothioate.

In various embodiments, at least one of the nucleoside analogues present in the oligomer has a modified base selected from the group consisting of 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

LNA

The term "LNA" or "LNA monomer" refers to a bicyclic nucleoside analogue, known as "Locked Nucleic Acid". When used in the context of an "LNA oligonucleotide", LNA refers to an oligonucleotide containing one or more such bicyclic nucleoside analogues. LNA nucleosides are characterised by the presence of a linker group (such as a bridge) between C2' and C4' of the ribose sugar ring to form a bicyclic system – for example between the R^{4*} and R^{2*} groups as described below.

The LNA used in the oligonucleotide compounds (oligomers) of the invention preferably has the structure of the general formula I



Formula I

wherein for all chiral centers, asymmetric groups may be found in either R or S orientation;

wherein X is selected from -O-, -S-, -N(R^{N*})- and -C(R⁶R^{6*})-, more preferably -O-;

B is selected from hydrogen, optionally substituted C₁₋₄-alkoxy, optionally substituted C₁₋₄-alkyl, optionally substituted C₁₋₄-acyloxy, nucleobases including naturally occurring and nucleobase analogues, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; preferably, B is a nucleobase or nucleobase analogue;

P designates an internucleoside linkage to an adjacent monomer, or a 5'-terminal group, said internucleoside linkage or 5'-terminal group optionally including the substituent R⁵ or equally applicable the substituent R^{5*};

P* designates an internucleoside linkage to an adjacent monomer, or a 3'-terminal group;

R^{4*} and R^{2*} together form a bivalent linker group, such as, for example, a biradical consisting of 1 - 4 groups/atoms each independently selected from -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)₂-, -S-, -SO₂-, -N(R^a)-, and >C=Z, wherein Z is selected from -O-, -S-, and -N(R^a)-, and R^a and R^b are each independently selected from hydrogen, optionally substituted C₁₋₁₂-alkyl, optionally substituted C₂₋₁₂-alkenyl, optionally substituted C₂₋₁₂-alkynyl,

hydroxy, optionally substituted C₁₋₁₂-alkoxy, C₂₋₁₂-alkoxyalkyl, C₂₋₁₂-alkenyloxy, carboxy, C₁₋₁₂-alkoxycarbonyl, C₁₋₁₂-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkyl-carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphonyloxy, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphanyl, C₁₋₆-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where each aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may form an optionally substituted methylene (=CH₂), wherein for all chiral centers, asymmetric groups may be found in either *R* or *S* orientation, and;

each of the substituents R^{1*}, R², R³, R⁵, R^{5*}, R⁶ and R^{6*} is independently selected from hydrogen, optionally substituted C₁₋₁₂-alkyl, optionally substituted C₂₋₁₂-alkenyl, optionally substituted C₂₋₁₂-alkynyl, hydroxy, C₁₋₁₂-alkoxy, C₂₋₁₂-alkoxyalkyl, C₂₋₁₂-alkenyloxy, carboxy, C₁₋₁₂-alkoxycarbonyl, C₁₋₁₂-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkyl-carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphonyloxy, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphanyl, C₁₋₆-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where each aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene; wherein R^N is selected from hydrogen and C₁₋₄-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and basic salts and acid addition salts thereof. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

Where the definitions used herein refer to substituted C₁₋₄-alkyl, substituted C₁₋₆ alkyl, substituted C₁₋₁₂-alkyl, substituted C₂₋₁₂-alkenyl, substituted C₂₋₆ alkenyl, substituted C₂₋₁₂-alkynyl, substituted C₂₋₆ alkynyl, substituted C₁₋₁₂-alkoxy, substituted C₁₋₆ alkoxy, substituted C₁₋₄-alkoxy, substituted C₁₋₄-acyloxy, substituted aryl, substituted heteroaryl, substituted methylene, substituted acyl, substituted C₁₋₆ aminoalkyl or substituted amide, suitable substituents preferably include one or more R^g groups, wherein each R^g is independently selected from halogen, C₁₋₆ alkyl, substituted C₁₋₆ alkyl, C₂₋₆ alkenyl, substituted C₂₋₆ alkenyl, C₂₋₆ alkynyl, substituted C₂₋₆ alkynyl, CN, OJ₁, SJ₁, NJ₁J₂, N₃, COOJ₁, C(=X)J₁, C(=X)NJ₁J₂, CN, O-C(=O)NJ₁J₂, O-C(=X)J₁, NJ₁C(=NH)NJ₁J₂ and NJ₁C(=X)NJ₁J₂ wherein X is O or S;

and each J_1 and J_2 is independently selected from H, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} aminoalkyl, substituted C_{1-6} aminoalkyl and a protecting group. Preferably, each R^g is independently selected from halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, CN, OJ_1 , SJ_1 , NJ_1J_2 , N_3 , $COOJ_1$, $C(=X)J_1$, $C(=X)NJ_1J_2$, CN, $O-C(=O)NJ_1J_2$, $O-C(=X)J_1$, $NJ_1C(=NH)NJ_1J_2$ and $NJ_1C(=X)NJ_1J_2$ wherein X is O or S; and each J_1 and J_2 is independently selected from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl and a protecting group. Suitable protecting groups are described in "Protective Groups in Organic Synthesis" by Theodora W Greene and Peter G M Wuts, 3rd edition (John Wiley & Sons, 1999).

10 In some embodiments, R^{4*} and R^{2*} together form a linker group selected from $C(R^aR^b)-$, $C(R^aR^b)-O-$, $C(R^aR^b)-NR^a-$, $C(R^aR^b)-S-$, and $C(R^aR^b)-C(R^aR^b)-O-$, wherein R^a and R^b are as defined above. In some embodiments, R^a and R^b are each independently selected from hydrogen and C_{1-6} alkyl, and are more preferably each independently selected from hydrogen and methyl.

15 In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are each independently selected from hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl and substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

20 In some preferred embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are all hydrogen.

In some embodiments, R^{1*} , R^2 , R^3 are each independently selected from hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl and substituted C_{1-6} aminoalkyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some preferred embodiments, R^{1*} , R^2 , R^3 are all hydrogen.

In some embodiments, R^5 and R^{5*} are each independently selected from H, $-CH_3$, $-CH_2-CH_3$, $-CH_2-O-CH_3$, and $-CH=CH_2$. Preferably, in some embodiments, either R^5 or R^{5*} is hydrogen, and the other group (R^5 or R^{5*} respectively) is selected from C_{1-5} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{1-6} alkyl, substituted C_{2-6} alkenyl, substituted C_{2-6} alkynyl and substituted acyl ($-C(=O)-$); wherein each substituted group is mono or poly substituted with substituent groups independently selected from halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, OJ_1 , SJ_1 , NJ_1J_2 , N_3 , $COOJ_1$, CN, $O-C(=O)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$ and $N(H)C(=X)N(H)J_2$ wherein X is O or S; and each J_1 and J_2 is independently selected from H, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6}

alkenyl, substituted C₂₋₆ alkenyl, C₂₋₆ alkynyl, substituted C₂₋₆ alkynyl, C₁₋₆ aminoalkyl, substituted C₁₋₆ aminoalkyl and a protecting group. In some embodiments either R⁵ or R^{5*} is substituted C₁₋₆ alkyl. In some embodiments either R⁵ or R^{5*} is substituted methylene, wherein preferred substituent groups include one or more groups independently selected from F, NJ₁J₂, N₃, CN, OJ₁, SJ₁, O-C(=O)NJ₁J₂, N(H)C(=NH)NJ₁J₂ and N(H)C(O)N(H)J₂. In some embodiments each J₁ and J₂ is independently H or C₁₋₆ alkyl. In some embodiments either R⁵ or R^{5*} is methyl, ethyl or methoxymethyl. In some embodiments either R⁵ or R^{5*} is methyl. In some embodiments either R⁵ or R^{5*} is ethylenyl. In some embodiments either R⁵ or R^{5*} is substituted acyl. In some embodiments either R⁵ or R^{5*} is C(=O)NJ₁J₂. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Examples of such 5' modified bicyclic nucleotides are disclosed in WO 2007/134181, which is hereby incorporated by reference in its entirety.

In some embodiments B is a nucleobase, including nucleobase analogues and naturally occurring nucleobases, such as a purine or pyrimidine, or a substituted purine or substituted pyrimidine, or a nucleobase selected from adenine, cytosine, thymine, adenine, uracil, and/or a modified or substituted nucleobase, such as 5-thiazolo-uracil, 2-thio-uracil, 5-propynyl-uracil, 2'thio-thymine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine and 2,6-diaminopurine.

In some embodiments, R^{4*} and R^{2*} together form a linker group selected from - C(R^aR^b)-O-, -C(R^aR^b)-C(R^cR^d)-O-, -C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-O-, -C(R^aR^b)-O-C(R^cR^d)-, -C(R^aR^b)-O-C(R^cR^d)-O-, -C(R^aR^b)-C(R^cR^d)-, -C(R^aR^b)-C(R^cR^d)-C(R^eR^f)-, C(R^a)=C(R^b)-C(R^cR^d)-, -C(R^aR^b)-N(R^c)-, -C(R^aR^b)-C(R^cR^d)-N(R^e)-, -C(R^aR^b)-N(R^c)-O-, -C(R^aR^b)-S- and -C(R^aR^b)-C(R^cR^d)-S-, wherein R^a, R^b, R^c, R^d, R^e, and R^f are each independently selected from hydrogen, optionally substituted C₁₋₁₂-alkyl, optionally substituted C₂₋₁₂-alkenyl, optionally substituted C₂₋₁₂-alkynyl, hydroxy, C₁₋₁₂-alkoxy, C₂₋₁₂-alkoxyalkyl, C₂₋₁₂-alkenyloxy, carboxy, C₁₋₁₂-alkoxycarbonyl, C₁₋₁₂-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkyl-carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphanyl, C₁₋₆-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where each aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH₂). For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments R^{4*} and R^{2*} together designate a linker group selected from -CH₂-O-, -CH₂-S-, -CH₂-NH-, -CH₂-N(CH₃)-, -CH₂-CH₂-O-, -CH₂-CH(CH₃)-, -CH₂-CH₂-S-, -CH₂-CH₂-NH-, -CH₂-CH₂-CH₂-, -CH₂-CH₂-CH₂-O-, -CH₂-CH₂-CH(CH₃)-, -CH=CH-CH₂-, -CH₂-O-CH₂-O-, -CH₂-NH-O-, -CH₂-N(CH₃)-O-, -CH₂-O-CH₂-, -CH(CH₃)-O-, -CH(CH₂-O-CH₃)-O-, -CH₂-CH₂- and -CH=CH-. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In some embodiments, R^{4*} and R^{2*} together form a linker group C(R^aR^b)-N(R^c)-O-, wherein R^a and R^b are each independently selected from hydrogen, halogen, C₁₋₆ alkyl, substituted C₁₋₆ alkyl, C₂₋₆ alkenyl, substituted C₂₋₆ alkenyl, C₂₋₆ alkynyl, substituted C₂₋₆ alkynyl, C₁₋₆ alkoxy, substituted C₁₋₆ alkoxy, acyl, substituted acyl, C₁₋₆ aminoalkyl and substituted C₁₋₆ aminoalkyl, more preferably R^a and R^b are hydrogen, and; wherein R^c is selected from hydrogen, halogen, C₁₋₆ alkyl, substituted C₁₋₆ alkyl, C₂₋₆ alkenyl, substituted C₂₋₆ alkenyl, C₂₋₆ alkynyl, substituted C₂₋₆ alkynyl, C₁₋₆ alkoxy, substituted C₁₋₆ alkoxy, acyl, substituted acyl, C₁₋₆ aminoalkyl, substituted C₁₋₆ aminoalkyl, and more preferably R^c is hydrogen.

In some embodiments, R^{4*} and R^{2*} together form a linker group C(R^aR^b)-O-C(R^cR^d)-O-, wherein R^a, R^b, R^c, and R^d are each independently selected from hydrogen, halogen, C₁₋₆ alkyl, substituted C₁₋₆ alkyl, C₂₋₆ alkenyl, substituted C₂₋₆ alkenyl, C₂₋₆ alkynyl, substituted C₂₋₆ alkynyl, C₁₋₆ alkoxy, substituted C₁₋₆ alkoxy, acyl, substituted acyl, C₁₋₆ aminoalkyl, substituted C₁₋₆ aminoalkyl, and more preferably R^a, R^b, R^c, and R^d are hydrogen.

In some embodiments, R^{4*} and R^{2*} form a linker group -CH(Z)-O-, wherein Z is selected from C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, substituted C₁₋₆ alkyl, substituted C₂₋₆ alkenyl, substituted C₂₋₆ alkynyl, acyl, substituted acyl, substituted amide, thiol and substituted thiol; and wherein each of the substituted groups, is, independently, mono or poly substituted with optionally protected substituent groups independently selected from halogen, oxo, hydroxyl, OJ₁, NJ₁J₂, SJ₁, N₃, OC(=X)J₁, OC(=X)NJ₁J₂, NJ³C(=X)NJ₁J₂ and CN, wherein each J₁, J₂ and J₃ is, independently, H or C₁₋₆ alkyl, and X is O, S or NJ₁. In some embodiments Z is C₁₋₆ alkyl or substituted C₁₋₆ alkyl. In some embodiments Z is methyl. In some embodiments Z is substituted C₁₋₆ alkyl. In some embodiments said substituent group is C₁₋₆ alkoxy. In some embodiments Z is CH₃OCH₂-. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Examples of such bicyclic nucleotides are disclosed in US 7,399,845 which is hereby incorporated by reference in its entirety. In some embodiments, R^{1*}, R², R³, R⁵, R^{5*} are all hydrogen. In some embodiments, R^{1*}, R², R^{3*} are hydrogen, and one or both of R⁵, R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181.

In some embodiments, R^{4*} and R^{2*} together form a linker group which comprises a substituted amino group, for example, R^{4*} and R^{2*} together form a linker group that consists of, or comprises, the group $-\text{CH}_2-\text{N}(\text{R}^c)-$, wherein R^c is C_{1-12} alkyloxy. In some

embodiments R^{4*} and R^{2*} together form a linker group $-\text{Cq}_3\text{q}_4-\text{NOR}-$, wherein q_3 and q_4 are

5 each independently selected from hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl and substituted C_{1-6} aminoalkyl; wherein each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, OJ_1 , SJ_1 , NJ_1J_2 , COOJ_1 , CN , $\text{O}-\text{C}(=\text{O})\text{NJ}_1\text{J}_2$,

10 $\text{N}(\text{H})\text{C}(=\text{NH})\text{N J}_1\text{J}_2$ and $\text{N}(\text{H})\text{C}(=\text{X}=\text{N}(\text{H})\text{J}_2$ wherein X is O or S ; and each of J_1 and J_2 is independently selected from H , C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl and a protecting group. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Examples of such bicyclic nucleotides are disclosed in WO2008/150729 which is hereby incorporated by reference in its entirety. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*}

15 are each independently selected from hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl and substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are all hydrogen. In some embodiments, R^{1*} , R^2 , R^3 are all hydrogen and one or both of R^5 , R^{5*} may be other than hydrogen as referred to above and in

20 WO 2007/134181. In some embodiments R^{4*} and R^{2*} together form a linker group $\text{C}(\text{R}^a\text{R}^b)-\text{O}-$, wherein R^a and R^b are each independently selected from halogen, C_{1-12} alkyl, substituted C_{1-12} alkyl, C_{2-12} alkenyl, substituted C_{2-12} alkenyl, C_{2-12} alkynyl, substituted C_{2-12} alkynyl, C_{1-12} alkoxy, substituted C_{1-12} alkoxy, OJ_1 , SJ_1 , SOJ_1 , SO_2J_1 , NJ_1J_2 , N_3 , CN , $\text{C}(=\text{O})\text{OJ}_1$, $\text{C}(=\text{O})\text{NJ}_1\text{J}_2$, $\text{C}(=\text{O})\text{J}_1$, $\text{O}-\text{C}(=\text{O})\text{NJ}_1\text{J}_2$, $\text{N}(\text{H})\text{C}(=\text{NH})\text{NJ}_1\text{J}_2$,

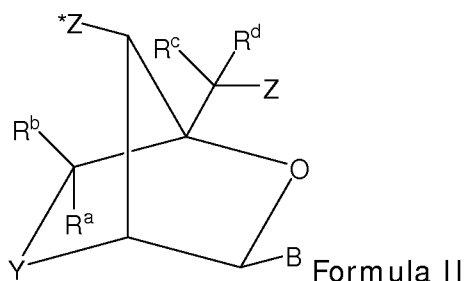
25 $\text{N}(\text{H})\text{C}(=\text{O})\text{NJ}_1\text{J}_2$ and $\text{N}(\text{H})\text{C}(=\text{S})\text{NJ}_1\text{J}_2$; or R^a and R^b together are $=\text{C}(\text{q}_3)(\text{q}_4)$; q_3 and q_4 are each, independently, H , halogen, C_{1-12} alkyl or substituted C_{1-12} alkyl; each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, OJ_1 , SJ_1 , NJ_1J_2 , N_3 , CN , $\text{C}(=\text{O})\text{OJ}_1$,

30 $\text{C}(=\text{O})\text{NJ}_1\text{J}_2$, $\text{C}(=\text{O})\text{J}_1$, $\text{O}-\text{C}(=\text{O})\text{NJ}_1\text{J}_2$, $\text{N}(\text{H})\text{C}(=\text{O})\text{NJ}_1\text{J}_2$ and $\text{N}(\text{H})\text{C}(=\text{S})\text{NJ}_1\text{J}_2$; each J_1 and J_2 is independently selected from H , C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} aminoalkyl, substituted C_{1-6} aminoalkyl and a protecting group. Such compounds are disclosed in WO2009/006478, hereby incorporated in its entirety by reference.

35 In some embodiments, R^{4*} and R^{2*} form a linker group - Q -, wherein Q is $\text{C}(\text{q}_1)(\text{q}_2)\text{C}(\text{q}_3)(\text{q}_4)$, $\text{C}(\text{q}_1)=\text{C}(\text{q}_3)$, $\text{C}[\text{C}(\text{q}_1)(\text{q}_2)]-\text{C}(\text{q}_3)(\text{q}_4)$ or $\text{C}(\text{q}_1)(\text{q}_2)-\text{C}[\text{C}(\text{q}_3)(\text{q}_4)]$; q_1 , q_2 , q_3 ,

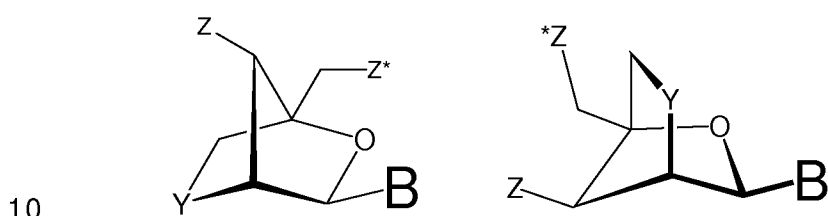
q_4 are each independently selected from H, halogen, C_{1-12} alkyl, substituted C_{1-12} alkyl, C_{2-12} alkenyl, substituted C_{1-12} alkoxy, OJ_1 , SJ_1 , SOJ_1 , SO_2J_1 , NJ_1J_2 , N_3 , CN, $C(=O)OJ_1$, $C(=O)-NJ_1J_2$, $C(=O)J_1$, $-C(=O)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ and $N(H)C(=S)NJ_1J_2$; each J_1 and J_2 is independently selected from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl and a protecting group; and, optionally wherein when Q is $C(q_1)(q_2)(q_3)(q_4)$ and one of q_3 or q_4 is CH_3 then at least one of the other of q_3 or q_4 or one of q_1 and q_2 is other than H. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are all hydrogen. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Examples of such bicyclic nucleotides are disclosed in WO2008/154401 which is hereby incorporated by reference in its entirety. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are each independently selected from hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{2-6} alkynyl, C_{1-6} alkoxy, substituted C_{1-6} alkoxy, acyl, substituted acyl, C_{1-6} aminoalkyl and substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*} , R^2 , R^3 , R^5 , R^{5*} are all hydrogen. In some embodiments, R^{1*} , R^2 , R^3 are all hydrogen and one or both of R^5 , R^{5*} may be other than hydrogen as referred to above and in WO 2007/134181 or WO2009/067647 (alpha-L-bicyclic nucleic acids analogs).

In some preferred embodiments the LNA monomer present in the oligonucleotide compounds of the invention preferably has the structure of the general formula II:

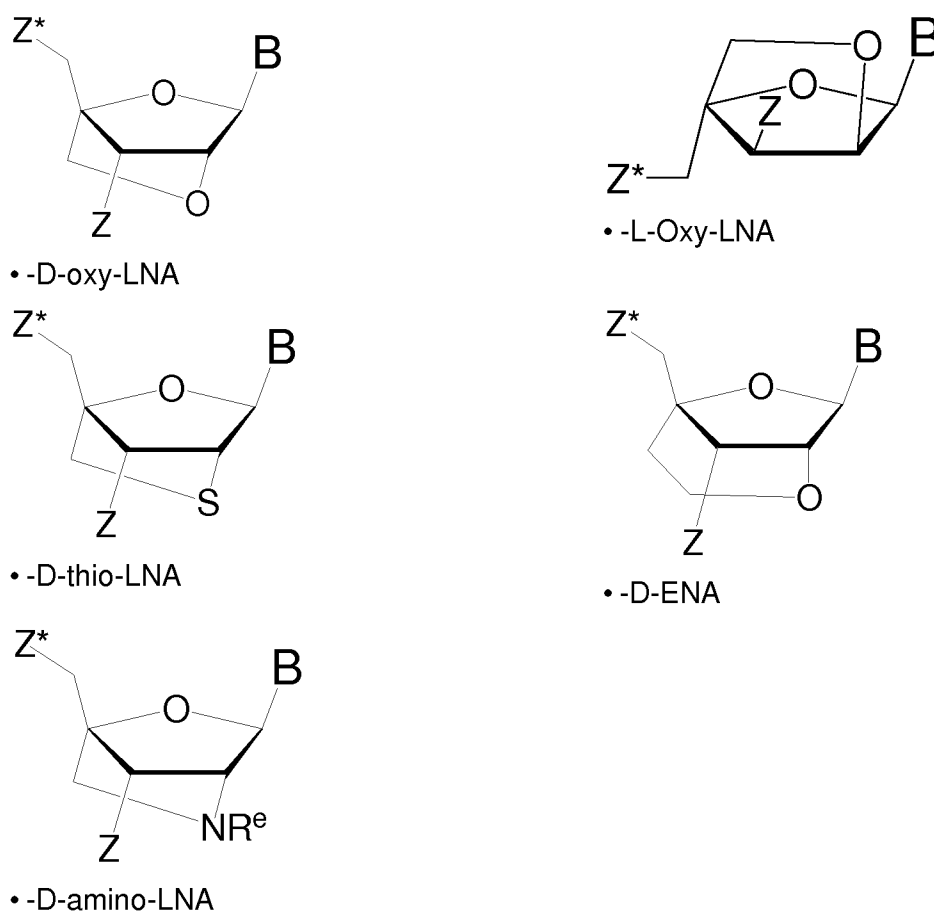


wherein Y is selected from $-O-$, $-CH_2O-$, $-S-$, $-NH-$, $N(R^e)$ and $-CH_2-$; Z and Z^* are each independently selected from an internucleoside linkage, R^H , a terminal group and a protecting group; B constitutes a natural or non-natural nucleotide base moiety (nucleobase), and R^H is selected from hydrogen and C_{1-4} -alkyl; R^a , R^b , R^c , R^d and R^e are each independently selected from hydrogen, optionally substituted C_{1-12} -alkyl, optionally substituted C_{2-12} -alkenyl, optionally substituted C_{2-12} -alkynyl, hydroxy, C_{1-12} -alkoxy, C_{2-12} -alkoxyalkyl, C_{2-12} -alkenyloxy, carboxy, C_{1-12} -alkoxycarbonyl, C_{1-12} -alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6} -alkyl)amino, carbamoyl, mono- and di(C_{1-6} -alkyl)-amino-carbonyl, amino- C_{1-6} -alkyl-aminocarbonyl, mono- and di(C_{1-6} -alkyl)amino- C_{1-6} -alkyl-aminocarbonyl, C_{1-6} -alkyl-carbonylamino, carbamido, C_{1-6} -alkanoyloxy, sulphonyloxy, C_{1-6} -

alkylsulphonyloxy, nitro, azido, sulphonyl, C₁₋₆-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where each aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH₂); and R^H is selected from hydrogen and C₁₋₄-alkyl. In some preferred embodiments R^a, R^b, R^c, R^d and R^e are each independently selected from hydrogen and C₁₋₆ alkyl, more preferably methyl. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation, for example, two exemplary stereochemical isomers include the beta-D and alpha-L isoforms, which may be illustrated as follows:



Specific exemplary LNA units are shown below (in Scheme 2):



Scheme 2

The term "thio-LNA" comprises a locked nucleoside in which Y in the general formula above is selected from S or -CH₂-S-. Thio-LNA can be in both beta-D and alpha-L-configuration.

5 The term "amino-LNA" comprises a locked nucleoside in which Y in the general formula above is selected from -N(H)-, N(R)-, CH₂-N(H)-, and -CH₂-N(R)- where R is selected from hydrogen and C₁₋₄-alkyl. Amino-LNA can be in both beta-D and alpha-L-configuration.

10 The term "oxy-LNA" comprises a locked nucleoside in which Y in the general formula above represents -O-. Oxy-LNA can be in both beta-D and alpha-L-configuration.

The term "ENA" comprises a locked nucleoside in which Y in the general formula above is -CH₂-O- (where the oxygen atom of -CH₂-O- is attached to the 2'-position relative to the base B). R^e is hydrogen or methyl.

15 In some exemplary embodiments LNA is selected from beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA and beta-D-thio-LNA, in particular beta-D-oxy-LNA.

RNase H recruitment

In some embodiments, an oligomer functions via non-RNase-mediated degradation of a target mRNA, such as by steric hindrance of translation, or other mechanisms; however, in various embodiments, oligomers of the invention are capable of recruiting one or more
20 RNase enzymes or complexes, such as endo-ribonuclease (RNase), such as RNase H.

Typically, the oligomer, comprises a region of at least 6, such as at least 7 contiguous monomers, such as at least 8 or at least 9 contiguous monomers, including 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 contiguous monomers, which, when forming a duplex with the target region of the target RNA, is capable of recruiting RNase. The region of the oligomer which
25 is capable of recruiting RNase may be region B, as referred to in the context of a gapmer as described herein. In some embodiments, the region of the oligomer which is capable of recruiting RNase, such as region B, consists of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 monomers.

EP 1 222 309 provides *in vitro* methods for determining RNaseH activity, which may
30 be used to determine the ability of the oligomers of the invention to recruit RNaseH. An oligomer is deemed capable of recruiting RNaseH if, when contacted with the complementary region of the RNA target, it has an initial rate, as measured in pmol/l/min, of at least 1%, such as at least 5%, such as at least 10% or more than 20% of the initial rate determined using an oligonucleotide having the same base sequence but containing only
35 DNA monomers, with no 2' substitutions, with phosphorothioate linkage groups between all

monomers in the oligonucleotide, using the methodology provided by Examples 91 - 95 of EP 1 222 309, incorporated herein by reference.

In some embodiments, an oligomer is deemed essentially incapable of recruiting RNaseH if, when contacted with the complementary target region of the RNA target, and RNaseH, the RNaseH initial rate, as measured in pmol/l/min, is less than 1%, such as less than 5%, such as less than 10% or less than 20% of the initial rate determined using an oligonucleotide having the same base sequence, but containing only DNA monomers, with no 2' substitutions, with phosphorothioate linkage groups between all monomers in the oligonucleotide, using the methodology provided by Examples 91 - 95 of EP 1 222 309.

In other embodiments, an oligomer is deemed capable of recruiting RNaseH if, when contacted with the complementary target region of the RNA target, and RNaseH, the RNaseH initial rate, as measured in pmol/l/min, is at least 20%, such as at least 40%, such as at least 60%, such as at least 80% of the initial rate determined using an oligonucleotide having the same base sequence, but containing only DNA monomers, with no 2' substitutions, with phosphorothioate linkage groups between all monomers in the oligonucleotide, using the methodology provided by Examples 91 - 95 of EP 1 222 309.

Typically, the region of the oligomer which forms the duplex with the complementary target region of the target RNA and is capable of recruiting RNase contains DNA monomers and optionally LNA monomers and forms a DNA/RNA-like duplex with the target region. The LNA monomers are preferably in the alpha-L configuration, particularly preferred being alpha-L-oxy LNA.

In various embodiments, the oligomer of the invention comprises both nucleosides and nucleoside analogues, and is in the form of a gapmer, a headmer or a mixmer.

A "headmer" is defined as an oligomer that comprises a region X and a region Y that is contiguous thereto, with the 5'-most monomer of region Y linked to the 3'-most monomer of region X. Region X comprises a contiguous stretch of non-RNase recruiting nucleoside analogues and region Y comprises a contiguous stretch (such as at least 7 contiguous monomers) of DNA monomers or nucleoside analogue monomers recognizable and cleavable by the RNase.

A "tailmer" is defined as an oligomer that comprises a region X and a region Y that is contiguous thereto, with the 5'-most monomer of region Y linked to the 3'-most monomer of the region X. Region X comprises a contiguous stretch (such as at least 7 contiguous monomers) of DNA monomers or nucleoside analogue monomers recognizable and cleavable by the RNase, and region Y comprises a contiguous stretch of non-RNase recruiting nucleoside analogues.

Other “chimeric” oligomers, called “mixmers”, consist of an alternating composition of (i) DNA monomers or nucleoside analogue monomers recognizable and cleavable by RNase, and (ii) non-RNase recruiting nucleoside analogue monomers.

5 In some embodiments, in addition to enhancing affinity of the oligomer for the target region, some nucleoside analogues also mediate RNase (*e.g.*, RNaseH) binding and cleavage. Since •-L-LNA monomers recruit RNaseH activity to a certain extent, in some embodiments, gap regions (*e.g.*, region B as referred to herein) of oligomers containing •-L-LNA monomers consist of fewer monomers recognizable and cleavable by the RNaseH, and more flexibility in the mixmer construction is introduced.

10 **Conjugates**

In the context of this disclosure, the term “conjugate” indicates a compound formed by the covalent attachment (“conjugation”) of an oligomer as described herein, to one or more moieties that are not themselves nucleic acids or monomers (“conjugated moieties”). Examples of such conjugated moieties include macromolecular compounds such as
15 proteins, fatty acid chains, sugar residues, glycoproteins, polymers, or combinations thereof. Typically proteins may be antibodies for a target protein. Typical polymers may be polyethylene glycol.

Accordingly, provided herein are conjugates comprising an oligomer as herein described, and at least one conjugated moiety that is not a nucleic acid or monomer,
20 covalently attached to said oligomer. Therefore, in certain embodiments where the oligomer of the invention consists of contiguous monomers having a specified sequence of bases, as herein disclosed, the conjugate may also comprise at least one conjugated moiety that is covalently attached to the oligomer.

In various embodiments of the invention, the oligomer is conjugated to a moiety that
25 increases the cellular uptake of oligomeric compounds. WO2007/031091 provides suitable ligands and conjugates (moieties), which are hereby incorporated by reference.

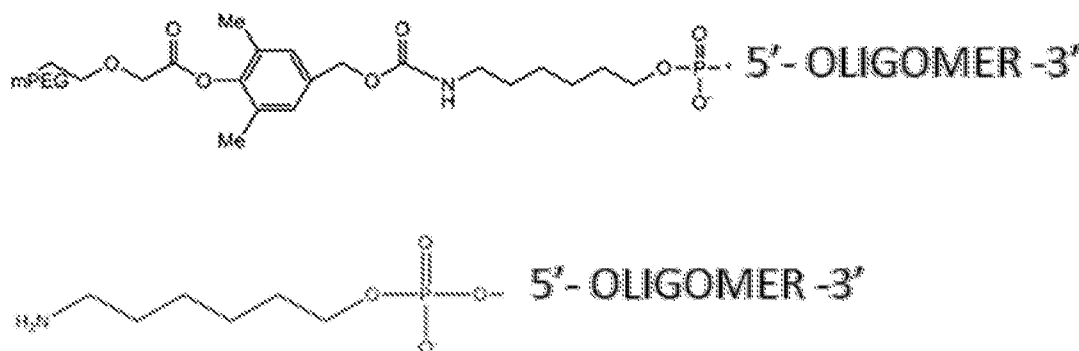
In various embodiments, conjugation (to a conjugated moiety) may enhance the activity, cellular distribution or cellular uptake of the oligomer of the invention. Such moieties include, but are not limited to, antibodies, polypeptides, lipid moieties such as a cholesterol
30 moiety, cholic acid, a thioether, *e.g.* Hexyl-s-tritylthiol, a thiocholesterol, an aliphatic chain, *e.g.*, dodecandiol or undecyl residues, a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-o-hexadecyl-rac-glycero-3-h-phosphonate, a polyamine or a polyethylene glycol chain, an adamantane acetic acid, a palmityl moiety, an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety.

In certain embodiments, the oligomers of the invention are conjugated to active drug substances, for example, aspirin, ibuprofen, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

In certain embodiments the conjugated moiety is a sterol, such as cholesterol.

5 In various embodiments, the conjugated moiety comprises or consists of a positively charged polymer, such as a positively charged peptide of, for example 1 -50, such as 2 – 20 such as 3 – 10 amino acid residues in length, and/or polyalkylene oxide such as polyethylene glycol (PEG) or polypropylene glycol – see WO 2008/034123, hereby incorporated by reference. Suitably the positively charged polymer, such as a polyalkylene
10 oxide may be attached to the oligomer of the invention via a linker such as the releasable linker described in WO 2008/034123.

By way of example, the following moieties may be used in the conjugates of the invention:



15 **Activated oligomers**

The term “activated oligomer,” as used herein, refers to an oligomer of the invention that is covalently linked (i.e., functionalized) to at least one functional moiety that permits covalent linkage of the oligomer to one or more conjugated moieties, i.e., moieties that are not themselves nucleic acids or monomers, to form the conjugates herein described.

20 Typically, a functional moiety will comprise a chemical group that is capable of covalently bonding to the oligomer via, e.g., a 3'-hydroxyl group or the exocyclic NH₂ group of the adenine base, a spacer that is preferably hydrophilic and a terminal group that is capable of binding to a conjugated moiety (e.g., an amino, sulfhydryl or hydroxyl group). In some embodiments, this terminal group is not protected, e.g., is an NH₂ group. In other
25 embodiments, the terminal group is protected, for example, by any suitable protecting group such as those described in “Protective Groups in Organic Synthesis” by Theodora W. Greene and Peter G. M. Wuts, 3rd edition (John Wiley & Sons, 1999). Examples of suitable hydroxyl protecting groups include esters such as acetate ester, aralkyl groups such as benzyl, diphenylmethyl, or triphenylmethyl, and tetrahydropyranyl. Examples of suitable

amino protecting groups include benzyl, alpha-methylbenzyl, diphenylmethyl, triphenylmethyl, benzyloxycarbonyl, tert-butoxycarbonyl, and acyl groups such as trichloroacetyl or trifluoroacetyl.

In some embodiments, the functional moiety is self-cleaving. In other embodiments, the functional moiety is biodegradable. See e.g., U.S. Patent No. 7,087,229, which is incorporated by reference herein in its entirety.

In some embodiments, oligomers of the invention are activated (i.e. functionalized) at the 5' end in order to allow covalent attachment of the conjugated moiety to the 5' end of the oligomer. In other embodiments, oligomers of the invention can be functionalized at the 3' end. In still other embodiments, oligomers of the invention can be functionalized along the backbone or on the heterocyclic base moiety. In yet other embodiments, oligomers of the invention can be functionalized at more than one position independently selected from the 5' end, the 3' end, the backbone and the base.

In some embodiments, activated oligomers of the invention are synthesized by incorporating during the synthesis one or more monomers that is covalently attached to a functional moiety. In other embodiments, activated oligomers of the invention are synthesized with monomers that have not been functionalized, and the oligomer is functionalized upon completion of synthesis.

In some embodiments, the oligomers are functionalized with a hindered ester containing an aminoalkyl linker, wherein the alkyl portion has the formula $(\text{CH}_2)_w$, wherein w is an integer ranging from 1 to 10, preferably about 6, wherein the alkyl portion of the alkylamino group can be straight chain or branched chain, and wherein the functional group is attached to the oligomer via an ester group $(-\text{O}-\text{C}(\text{O})-(\text{CH}_2)_w\text{NH})$.

In other embodiments, the oligomers are functionalized with a hindered ester containing a $(\text{CH}_2)_w$ -sulfhydryl (SH) linker, wherein w is an integer ranging from 1 to 10, preferably about 6, wherein the alkyl portion of the alkylamino group can be straight chain or branched chain, and wherein the functional group attached to the oligomer via an ester group $(-\text{O}-\text{C}(\text{O})-(\text{CH}_2)_w\text{SH})$. In some embodiments, sulfhydryl-activated oligonucleotides are conjugated with polymer moieties such as polyethylene glycol or peptides (via formation of a disulfide bond).

Activated oligomers containing hindered esters as described above can be synthesized by any method known in the art, and in particular, by methods disclosed in PCT Publication No. WO 2008/034122 and the examples therein, which is incorporated herein by reference in its entirety.

Activated oligomers covalently linked to at least one functional moiety can be synthesized by any method known in the art, and in particular, by methods disclosed in U.S.

Patent Publication No. 2004/0235773, which is incorporated herein by reference in its entirety, and in Zhao *et al.* (2007) *J. Controlled Release* 119:143-152; and Zhao *et al.* (2005) *Bioconjugate Chem.* 16:758-766.

In still other embodiments, the oligomers of the invention are functionalized by
5 introducing sulfhydryl, amino or hydroxyl groups into the oligomer by means of a
functionalizing reagent substantially as described in U.S. Patent Nos. 4,962,029 and
4,914,210, i.e., a substantially linear reagent having a phosphoramidite at one end linked
through a hydrophilic spacer chain to the opposing end which comprises a protected or
unprotected sulfhydryl, amino or hydroxyl group. Such reagents primarily react with hydroxyl
10 groups of the oligomer. In some embodiments, such activated oligomers have a
functionalizing reagent coupled to a 5'-hydroxyl group of the oligomer. In other
embodiments, the activated oligomers have a functionalizing reagent coupled to a 3'-
hydroxyl group. In still other embodiments, the activated oligomers of the invention have a
functionalizing reagent coupled to a hydroxyl group on the backbone of the oligomer. In yet
15 further embodiments, the oligomer of the invention is functionalized with more than one of
the functionalizing reagents as described in U.S. Patent Nos. 4,962,029 and 4,914,210,
incorporated herein by reference in their entirety. Methods of synthesizing such
functionalizing reagents and incorporating them into monomers or oligomers are disclosed in
U.S. Patent Nos. 4,962,029 and 4,914,210.

20 In some embodiments, the 5'-terminus of a solid-phase bound oligomer is
functionalized with a dienyl phosphoramidite derivative, followed by conjugation of the
deprotected oligomer with, e.g., an amino acid or peptide via a Diels-Alder cycloaddition
reaction.

In various embodiments, the incorporation of monomers containing 2'-sugar
25 modifications, such as a 2'-carbamate substituted sugar or a 2'-(O-pentyl-N-phthalimido)-
deoxyribose sugar into the oligomer facilitates covalent attachment of conjugated moieties to
the sugars of the oligomer. In other embodiments, an oligomer with an amino-containing
linker at the 2'-position of one or more monomers is prepared using a reagent such as, for
example, 5'-dimethoxytrityl-2'-O-(e-phthalimidylaminopentyl)-2'-deoxyadenosine-3'-- N,N-
30 diisopropyl-cyanoethoxy phosphoramidite. See, e.g., Manoharan, et al., *Tetrahedron Letters*,
1991, 34, 7171.

In still further embodiments, the oligomers of the invention have amine-containing
functional moieties on the nucleobase, including on the N6 purine amino groups, on the
exocyclic N2 of guanine, or on the N4 or 5 positions of cytosine. In various embodiments,
35 such functionalization may be achieved by using a commercial reagent that is already
functionalized in the oligomer synthesis.

Some functional moieties are commercially available, for example, heterobifunctional and homobifunctional linking moieties are available from the Pierce Co. (Rockford, Ill.). Other commercially available linking groups are 5'-Amino-Modifier C6 and 3'-Amino-Modifier reagents, both available from Glen Research Corporation (Sterling, Va.). 5'-Amino-Modifier
5 C6 is also available from ABI (Applied Biosystems Inc., Foster City, Calif.) as Aminolink-2, and 3'-Amino-Modifier is also available from Clontech Laboratories Inc. (Palo Alto, Calif.).

Compositions

In various embodiments, the oligomer of the invention is used in pharmaceutical formulations and compositions. Suitably, such compositions comprise a pharmaceutically
10 acceptable diluent, carrier, salt or adjuvant. WO2007/031091 provides suitable and preferred pharmaceutically acceptable diluents, carriers and adjuvants - which are hereby incorporated by reference. Suitable dosages, formulations, administration routes, compositions, dosage forms, combinations with other therapeutic agents, pro-drug
15 formulations are also provided in WO2007/031091 - which are also hereby incorporated by reference. Details on techniques for formulation and administration also may be found in the latest edition of "REMINGTON'S PHARMACEUTICAL SCIENCES" (Maack Publishing Co, Easton Pa.).

In some embodiments, an oligomer of the invention is covalently linked to a conjugated moiety to aid in delivery of the oligomer across cell membranes. An example of
20 a conjugated moiety that aids in delivery of the oligomer across cell membranes is a lipophilic moiety, such as cholesterol. In various embodiments, an oligomer of the invention is formulated with lipid formulations that form liposomes, such as Lipofectamine 2000 or Lipofectamine RNAiMAX, both of which are commercially available from Invitrogen. In some embodiments, the oligomers of the invention are formulated with a mixture of one or
25 more lipid-like non-naturally occurring small molecules ("lipidoids"). Libraries of lipidoids can be synthesized by conventional synthetic chemistry methods and various amounts and combinations of lipidoids can be assayed in order to develop a vehicle for effective delivery of an oligomer of a particular size to the targeted tissue by the chosen route of administration. Suitable lipidoid libraries and compositions can be found, for example in
30 Akinc *et al.* (2008) Nature Biotechnol., available at <http://www.nature.com/nbt/journal/vaop/ncurrent/abs/nbt1402.html>, which is incorporated by reference herein.

As used herein, the term "pharmaceutically acceptable salts" refers to salts that retain the desired biological activity of the herein identified oligomers and exhibit acceptable levels
35 of undesired toxic effects. Non-limiting examples of such salts can be formed with organic

amino acid and base addition salts formed with metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with a cation formed from ammonia, N,N'-dibenzylethylene-diamine, D-glucosamine, tetraethylammonium, or ethylenediamine; or (c) combinations of
5 (a) and (b); e.g., a zinc tannate salt or the like.

In certain embodiments, the pharmaceutical compositions according to the invention comprise other active ingredients in addition to an oligomer or conjugate of the invention, including active agents useful for the treatment of cancer, myopathies and/or asthma.

10 In some embodiments, the additional active agent is selected from the group consisting of docetaxel, vincristine, 5-fluorouracil, TRAIL, irinotecan, 17-AAG, platin compounds, and irradiation.

In one embodiment, the invention provides for a combined therapy, characterised in that the therapy comprises administering the pharmaceutical composition according to the invention, and an additional active agent (e.g. docetaxel), which in certain embodiments are
15 administered prior to, during or subsequent to the administration of the pharmaceutical compositions of the invention.

The invention also provides a kit of parts wherein a first part comprises at least one oligomer, conjugate and/or the pharmaceutical composition according to the invention and a further part comprises one or more active agents (e.g. docetaxel) useful for the treatment of
20 cancer, myopathies and/or asthma. It is therefore envisaged that the kit of parts may be used in a method of treatment, as referred to herein, where the method comprises administering both the first part and the further part, either simultaneously or one after the other.

Applications

25 The term "treatment" as used herein refers to both treatment of an existing disease (e.g., a disease or disorder as referred to herein below), or prevention of a disease, *i.e.*, prophylaxis. It will therefore be recognised that, in certain embodiments, "treatment" includes prophylaxis.

In various embodiments, the oligomers of the invention may be utilized as research
30 reagents for, for example, diagnostics, therapeutics and prophylaxis.

In some embodiments, such oligomers may be used for research purposes to specifically inhibit the expression of Hsp27 protein (typically by degrading or inhibiting the Hsp27 mRNA and thereby preventing protein formation) in cells and experimental animals, thereby facilitating functional analysis of the target or an appraisal of its usefulness as a
35 target for therapeutic intervention.

In certain embodiments, the oligomers may be used in diagnostics to detect and/or to quantify Hsp27 expression in cells and tissues by Northern blotting, in-situ hybridisation or similar techniques.

5 In various therapeutic embodiments, a non-human animal or a human suspected of having a disease or disorder which can be treated by modulating the expression of Hsp27 is treated by administering an effective amount of an oligomer in accordance with this invention. Further provided are methods of treating a mammal, such as treating a human, suspected of having or being prone to a disease or condition, associated with expression of Hsp27 by administering a therapeutically or prophylactically effective amount of one or more
10 of the oligomers, conjugates or compositions of the invention.

In certain embodiments, the invention also provides for the use of the oligomers or conjugates of the invention as described for the manufacture of a medicament for the treatment of a disorder as referred to herein, or for a method of the treatment of a disorder as referred to herein.

15 In various embodiments, the invention also provides for a method for treating a disorder as referred to herein, said method comprising administering an oligomer according to the invention as herein described, and/or a conjugate according to the invention, and/or a pharmaceutical composition according to the invention to an animal in need thereof (such as a patient in need thereof).

20 ***Medical Indications***

In certain therapeutic embodiments, the disorder to be treated is a hyperproliferative disorders (*e.g.*, cancer), such as prostate cancer, lung cancer, leukemia, gastric cancer, breast cancer, ovarian cancer, bladder cancer, renal cancer, pancreatic cancer, multiple myeloma, brain tumours, fibrosarcoma osteosarcomas and liver cancer. In various
25 embodiments, the treatment of such a disease or condition according to the invention may be combined with one or more other anti-cancer treatments, such as radiotherapy, chemotherapy or immunotherapy.

In certain other embodiments, the disorder to be treated is a myopathy and/or asthma.

30 In various embodiments, the disease or disorder is associated with a mutation of the Hsp27 gene or a gene whose protein product is associated with or interacts with Hsp27. Therefore, in various embodiments, the target mRNA is a mutated form of the Hsp27 sequence, for example, it comprises one or more single point mutations or triplet repeats.

In various embodiments, the disease or disorder is associated with abnormal levels of Hsp27.

The term "abnormal" as used herein refers to over-expression (e.g. up-regulation) of the Hsp27 gene in a cell compared to the expression level in a cell of an animal which does not have a disease, disorder or condition mentioned herein.

5 In some embodiments, an oligomer, a conjugate or a composition according to the invention can be used for the treatment of conditions associated with over-expression (e.g. up-regulation) of the Hsp27 gene.

In other embodiments, the disease or disorder is associated with abnormal levels of a mutated form of Hsp27.

10 The terms "mutation" and "mutated form" as used herein refer to a variant of Hsp27 nucleic acid shown in SEQ ID NO: 137. Said variant may be associated with a disease, disorder or condition as referred to herein. In some embodiments, the term "variant" as used herein refers to a nucleotide sequence having a base sequence which differs from SEQ ID NO: 137 by one or more nucleotide additions and/or substitutions and/or deletions. In some
15 embodiments the variant has at least 80%, 85%, 90% or 95% sequence homology (identity) with SEQ ID NO: 137. In the same or different embodiment, the variant has no more than 60 additional nucleotides and/or substituted nucleotides and/or deleted nucleotides over the whole of SEQ ID NO: 137; such as no more than 30 additional nucleotides and/or substituted nucleotides and/or deleted nucleotides; such as no more than 15 additional
20 nucleotides and/or substituted nucleotides and/or deleted nucleotides over the whole of SEQ ID NO: 137.

Hsp27 is a binding partner of numerous polypeptides. Hsp27 may act as a chaperone by binding unfolded proteins for trafficking in either refolding pathways or cellular degradation pathways. Examples of polypeptides which Hsp27 may bind to include annexin II, DAXX, F-actin, cytochrome c, caspase-3, Akt1, AR, I κ B α , FAS and MDM2. In various
25 embodiments, the invention relates to methods of modulating the expression of a gene encoding a polypeptide capable of binding to Hsp27. In other embodiments, the invention relates to methods of modulating the activity of a polypeptide capable of binding to Hsp27. In some embodiments, the binding of Hsp27 to a polypeptide results in increased expression or activity of the gene encoding the polypeptide to which Hsp27 is bound. In other
30 embodiments, the binding of Hsp27 to a polypeptide results in decreased expression or activity of the gene encoding the polypeptide which is bound to Hsp27. In some embodiments, the binding of Hsp27 to a polypeptide results in increased activity of the polypeptide which is bound to Hsp27. In other embodiments, the binding of Hsp27 to a polypeptide results in decreased activity of the polypeptide which is bound to Hsp27.

35 The invention further provides use of an oligomer of the invention in the manufacture of a medicament for the treatment of any and all conditions disclosed herein.

In various embodiments, the invention is directed to a method of treating a mammal suffering from or susceptible to a condition associated with abnormal levels of Hsp27 mRNA or protein, comprising administering to the mammal a therapeutically effective amount of an oligomer of the invention, or a conjugate thereof, that comprises one or more LNA monomers.

An interesting aspect of the invention is directed to the use of an oligomer (compound) as defined herein or a conjugate as defined herein for the preparation of a medicament for the treatment of a condition as disclosed herein above.

In various embodiments, the invention encompasses a method of preventing or treating a disease comprising administering a therapeutically effective amount of an oligomer according to the invention, or a conjugate thereof, to a non-human animal or a human in need of such therapy.

In certain embodiments, the LNA oligomers of the invention, or conjugates thereof, are administered for a short period time rather than continuously.

In certain embodiments of the invention, the oligomer (compound) is linked to a conjugated moiety, for example, in order to increase the cellular uptake of the oligomer. In one embodiment the conjugated moiety is a sterol, such as cholesterol.

In various embodiments, the invention is directed to a method for treating abnormal levels of Hsp27, the method comprising administering an oligomer of the invention, or a conjugate or a pharmaceutical composition thereof, to an animal (such as a patient) in need of such treatment, and optionally further comprising the administration of a further chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is conjugated to the oligomer, is present in the pharmaceutical composition, or is administered in a separate formulation.

The invention also relates to an oligomer, a composition or a conjugate as defined herein for use as a medicament.

The invention further relates to use of an oligomer, composition, or a conjugate as defined herein for the manufacture of a medicament for the treatment of abnormal levels of Hsp27 or expression of mutant forms of Hsp27 (such as allelic variants, such as those associated with one of the diseases referred to herein).

Moreover, in various embodiments, the invention relates to a method of treating an animal (such as a patient) suffering from a disease or condition selected from the group consisting of cancer, myopathies and asthma, the method comprising the step of administering a pharmaceutical composition as defined herein to the animal (such as a patient) in need thereof.

In certain embodiments, the methods of the invention are employed for treatment or prophylaxis against diseases caused by abnormal levels of Hsp27.

In some embodiments, the invention is directed to a method for treating abnormal levels of Hsp27, said method comprising administering a oligomer of the invention, or a conjugate of the invention or a pharmaceutical composition of the invention to an animal (such as a patient) in need thereof.

Moreover, the invention relates to a method of treating an animal (such as a human) suffering from a disease or condition such as those referred to herein.

An animal (such as a patient) who is in need of treatment is an animal (such as a patient) suffering from or likely to suffer from the disease or disorder.

Suitable animals include human and non-human animals.

In some embodiments, the animal is a mammal. Examples include humans, rodents (such as rats and mice), rabbits, primates, non-human primates (such as chimpanzees and monkeys), horses, cattle, sheep, pigs, dogs and cats.

Suitable dosages, formulations, administration routes, compositions, dosage forms, combinations with other therapeutic agents, pro-drug formulations are also provided in WO2007/031091 - which is hereby incorporated by reference.

The invention also provides for a pharmaceutical composition comprising an oligomer or a conjugate as herein described, and a pharmaceutically acceptable diluent, carrier or adjuvant. WO2007/031091 provides suitable and preferred pharmaceutically acceptable diluents, carriers and adjuvants - which are hereby incorporated by reference.

EMBODIMENTS

The following embodiments of the invention may be used in combination with the other embodiments described herein.

1. An oligomer of between 10 - 30 nucleotides in length which comprises a contiguous nucleotide sequence of a total of between 10 – 30 nucleotides, wherein said contiguous nucleotide sequence is at least 80% homologous to a region corresponding to a mammalian Hsp27 gene or the reverse complement of an mRNA, such as SEQ ID NO: 137 or a naturally occurring variant thereof.
2. The oligomer according to embodiment 1, wherein the contiguous nucleotide sequence is at least 80% homologous to a region corresponding to any of SEQ ID NO: 1-15 and 121, 16-30 and 122, 31-45 and 123, 46-60 and 124, 61-75 and 125, 76-90 and 126, 91-105, and 127, and 106-120 and 128.
3. The oligomer according to embodiment 1 or 2, wherein the contiguous nucleotide sequence comprises no mismatches or no more than one or two mismatches with the reverse complement of the corresponding region of 137.

4. The oligomer according to any one of embodiments 1 to 3, wherein the nucleotide sequence of the oligomer consists of the contiguous nucleotide sequence.
5. The oligomer according to any one of embodiments 1 to 4, wherein the contiguous nucleotide sequence is between 10 – 18 nucleotides in length.
- 5 6. The oligomer according to any one of embodiments 1 to 5, wherein the contiguous nucleotide sequence comprises nucleotide analogues.
7. The oligomer according to embodiment 6, wherein the nucleotide analogues are sugar modified nucleotides, such as sugar modified nucleotides selected from the group consisting of: Locked Nucleic Acid (LNA) units; 2'-O-alkyl-RNA units, 2'-OMe-RNA units,
10 2'-amino-DNA units, and 2'-fluoro-DNA units.
8. The oligomer according to embodiment 6, wherein the nucleotide analogues are LNA.
9. The oligomer according to any one of embodiments 6 to 8 which is a gapmer.
10. The oligomer according to any one of embodiments 1 to 9, which inhibits the expression of Hsp27 gene or mRNA in a cell which is expressing Hsp27 gene or mRNA.
- 15 11. The oligomer according to any one of embodiments 1 to 10 which is selected from the group consisting of SEQ ID NO 129, 130, 131, 132, 133, 134, 135 and 136.
12. A conjugate comprising the oligomer according to any one of embodiments 1 to 11, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to said oligomer.
- 20 13. A pharmaceutical composition comprising the oligomer according to any one of embodiments 1 to 10, or the conjugate according to embodiment 12, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.
14. The oligomer according to any one of embodiments 1 to 11, or the conjugate according to embodiment 12, for use as a medicament, such as for the treatment of cancer,
25 myopathies and asthma.
15. The use of an oligomer according to any one of the embodiments 1 to 11, or a conjugate as defined in embodiment 12, for the manufacture of a medicament for the treatment of cancer, myopathies and asthma.
16. A method of treating cancer, myopathies and asthma, said method comprising
30 administering an oligomer according to any one of the embodiments 1 to 11, or a conjugate according to embodiment 12, or a pharmaceutical composition according to embodiment 13, to a patient suffering from, or likely to suffer from cancer, myopathies and asthma.
17. A method for the inhibition of Hsp27 in a cell which is expressing Hsp27, said method
35 comprising administering an oligomer according to any one of the embodiments 1 to 11,

or a conjugate according to embodiment 12 to said cell so as to inhibit Hsp27 in said cell.

EXAMPLES

Example 1: Monomer synthesis

5 The LNA monomer building blocks and derivatives were prepared following published procedures and references cited therein - see WO07/031081 and the references cited therein.

Example 2: Oligonucleotide synthesis

10 Oligonucleotides (oligomers) were synthesized according to the method described in WO07/031081. Table 1 shows examples of antisense oligonucleotide motifs and of the invention.

Example 3: Design of the oligonucleotides

15 In accordance with the invention, a series of oligonucleotides (oligomers) were designed to target different regions of the human Hsp27 mRNA using the published sequence GenBank accession number NM_001540, presented herein as SEQ ID NO: 137 (Figure 3)

Table 1: Antisense oligonucleotide sequences of the invention. SEQ ID NOS: 1-120 and SEQ ID NOS: 121-128 (shown in Table 2) are oligomer sequences designed to target human Hsp27 mRNA.

SEQ ID NO	Sequence (5'-3')	Length (bases)
SEQ ID NO: 1	GAGATGTAGCCATGCT	16
SEQ ID NO: 2	GAGATGTAGCCATGC	15
SEQ ID NO: 3	AGATGTAGCCATGCT	15
SEQ ID NO: 4	GAGATGTAGCCATG	14
SEQ ID NO: 5	AGATGTAGCCATGC	14
SEQ ID NO: 6	GATGTAGCCATGCT	14
SEQ ID NO: 7	GAGATGTAGCCAT	13
SEQ ID NO: 8	AGATGTAGCCATG	13
SEQ ID NO: 9	GATGTAGCCATGC	13
SEQ ID NO: 10	ATGTAGCCATGCT	13
SEQ ID NO: 11	GAGATGTAGCCA	12
SEQ ID NO: 12	AGATGTAGCCAT	12

SEQ ID NO	Sequence (5'-3')	Length (bases)
SEQ ID NO: 13	GATGTAGCCATG	12
SEQ ID NO: 14	ATGTAGCCATGC	12
SEQ ID NO: 15	TGTAGCCATGCT	12
SEQ ID NO: 16	ACGGTCAGTGTGCCCT	16
SEQ ID NO: 17	ACGGTCAGTGTGCC	15
SEQ ID NO: 18	CGGTCAGTGTGCCCT	15
SEQ ID NO: 19	ACGGTCAGTGTGCC	14
SEQ ID NO: 20	CGGTCAGTGTGCC	14
SEQ ID NO: 21	GGTCAGTGTGCCCT	14
SEQ ID NO: 22	GTCAGTGTGCCCT	13
SEQ ID NO: 23	GGTCAGTGTGCC	13
SEQ ID NO: 24	CGGTCAGTGTGCC	13
SEQ ID NO: 25	ACGGTCAGTGTGC	13
SEQ ID NO: 26	ACGGTCAGTGTG	12
SEQ ID NO: 27	CGGTCAGTGTGC	12
SEQ ID NO: 28	GGTCAGTGTGCC	12
SEQ ID NO: 29	GTCAGTGTGCC	12
SEQ ID NO: 30	TCAGTGTGCCCT	12
SEQ ID NO: 31	CGTGTATTTCCGCGTG	16
SEQ ID NO: 32	CGTGTATTTCCGCGT	15
SEQ ID NO: 33	GTGTATTTCCGCGTG	15
SEQ ID NO: 34	CGTGTATTTCCGCG	14
SEQ ID NO: 35	GTGTATTTCCGCGT	14
SEQ ID NO: 36	TGTATTTCCGCGTG	14
SEQ ID NO: 37	CGTGTATTTCCGC	13
SEQ ID NO: 38	GTGTATTTCCGCG	13
SEQ ID NO: 39	TGTATTTCCGCGT	13
SEQ ID NO: 40	GTATTTCCGCGTG	13
SEQ ID NO: 41	CGTGTATTTCCG	12

SEQ ID NO	Sequence (5'-3')	Length (bases)
SEQ ID NO: 42	GTGTATTTCCGC	12
SEQ ID NO: 43	TGTATTTCCGCG	12
SEQ ID NO: 44	GTATTTCCGCGT	12
SEQ ID NO: 45	TATTTCCGCGTG	12
SEQ ID NO: 46	TGCGTGGCTAGCTTGG	16
SEQ ID NO: 47	TGCGTGGCTAGCTTG	15
SEQ ID NO: 48	GCGTGGCTAGCTTGG	15
SEQ ID NO: 49	TGCGTGGCTAGCTT	14
SEQ ID NO: 50	GCGTGGCTAGCTTG	14
SEQ ID NO: 51	CGTGGCTAGCTTGG	14
SEQ ID NO: 52	TGCGTGGCTAGCT	13
SEQ ID NO: 53	GCGTGGCTAGCTT	13
SEQ ID NO: 54	CGTGGCTAGCTTG	13
SEQ ID NO: 55	GTGGCTAGCTTGG	13
SEQ ID NO: 56	TGCGTGGCTAGC	12
SEQ ID NO: 57	GCGTGGCTAGCT	12
SEQ ID NO: 58	CGTGGCTAGCTT	12
SEQ ID NO: 59	GTGGCTAGCTTG	12
SEQ ID NO: 60	TGGCTAGCTTGG	12
SEQ ID NO: 61	ATGGTGATCTCGTTGG	16
SEQ ID NO: 62	ATGGTGATCTCGTTG	15
SEQ ID NO: 63	TGGTGATCTCGTTGG	15
SEQ ID NO: 64	ATGGTGATCTCGTT	14
SEQ ID NO: 65	TGGTGATCTCGTTG	14
SEQ ID NO: 66	GGTGATCTCGTTGG	14
SEQ ID NO: 67	ATGGTGATCTCGT	13
SEQ ID NO: 68	TGGTGATCTCGTT	13
SEQ ID NO: 69	GGTGATCTCGTTG	13
SEQ ID NO: 70	GTGATCTCGTTGG	13

SEQ ID NO	Sequence (5'-3')	Length (bases)
SEQ ID NO: 71	ATGGTGATCTCG	12
SEQ ID NO: 72	TGGTGATCTCGT	12
SEQ ID NO: 73	GGTGATCTCGTT	12
SEQ ID NO: 74	GTGATCTCGTTG	12
SEQ ID NO: 75	TGATCTCGTTGG	12
SEQ ID NO: 76	ATTTTGCAGCTTCTGG	16
SEQ ID NO: 77	ATTTTGCAGCTTCTG	15
SEQ ID NO: 78	TTTTGCAGCTTCTGG	15
SEQ ID NO: 79	ATTTTGCAGCTTCT	14
SEQ ID NO: 80	TTTTGCAGCTTCTG	14
SEQ ID NO: 81	TTTGCAGCTTCTGG	14
SEQ ID NO: 82	ATTTTGCAGCTTC	13
SEQ ID NO: 83	TTTTGCAGCTTCT	13
SEQ ID NO: 84	TTTGCAGCTTCTG	13
SEQ ID NO: 85	TTGCAGCTTCTGG	13
SEQ ID NO: 86	ATTTTGCAGCTT	12
SEQ ID NO: 87	TTTTGCAGCTTC	12
SEQ ID NO: 88	TTTGCAGCTTCT	12
SEQ ID NO: 89	TTGCAGCTTCTG	12
SEQ ID NO: 90	TGCAGCTTCTGG	12
SEQ ID NO: 91	GGCACAGCCAGTGGCG	16
SEQ ID NO: 92	GGCACAGCCAGTGGC	15
SEQ ID NO: 93	GCACAGCCAGTGGCG	15
SEQ ID NO: 94	GGCACAGCCAGTGG	14
SEQ ID NO: 95	GCACAGCCAGTGGC	14
SEQ ID NO: 96	CACAGCCAGTGGCG	14
SEQ ID NO: 97	GGCACAGCCAGTG	13
SEQ ID NO: 98	GCACAGCCAGTGG	13
SEQ ID NO: 99	CACAGCCAGTGGC	13
SEQ ID NO: 100	ACAGCCAGTGGCG	13

SEQ ID NO	Sequence (5'-3')	Length (bases)
SEQ ID NO: 101	GGCACAGCCAGT	12
SEQ ID NO: 102	GCACAGCCAGTG	12
SEQ ID NO: 103	CACAGCCAGTGG	12
SEQ ID NO: 104	ACAGCCAGTGGC	12
SEQ ID NO: 105	CAGCCAGTGGCG	12
SEQ ID NO: 106	TATCAAAAGAACACAC	16
SEQ ID NO: 107	TATCAAAAGAACACA	15
SEQ ID NO: 108	ATCAAAAGAACACAC	15
SEQ ID NO: 109	TATCAAAAGAACAC	14
SEQ ID NO: 110	ATCAAAAGAACACA	14
SEQ ID NO: 111	TCAAAAGAACACAC	14
SEQ ID NO: 112	TATCAAAAGAACA	13
SEQ ID NO: 113	ATCAAAAGAACAC	13
SEQ ID NO: 114	TCAAAAGAACACA	13
SEQ ID NO: 115	CAAAGAACACAC	13
SEQ ID NO: 116	TATCAAAAGAAC	12
SEQ ID NO: 117	ATCAAAAGAACA	12
SEQ ID NO: 118	TCAAAAGAACAC	12
SEQ ID NO: 119	CAAAGAACACA	12
SEQ ID NO: 120	AAAGAACACAC	12

Table 2 shows 24mer sequence motifs from which oligomers of the invention may be designed – the bold type represents 16mer sequence motifs as shown in Table 1.

Table 2 – 24mers Sequence motifs		
16mer SEQ IDs	Corresponding 24mer sequence comprising 16mer	24mer SEQ ID
SEQ ID NO: 1	ccgggagatgtagccatgctcgtc	SEQ ID NO: 121
SEQ ID NO: 16	ctccacggtgcagtggtgccctcagg	SEQ ID NO: 122
SEQ ID NO: 31	gcagcgtgtatttccgcgtgaagc	SEQ ID NO: 123
SEQ ID NO: 46	ggactgctggttagcttgggcat	SEQ ID NO: 124
SEQ ID NO: 61	tgggatggtgatctcgttggactg	SEQ ID NO: 125

SEQ ID NO: 76	tcggattttgcagcttctgggcc	SEQ ID NO: 126
SEQ ID NO: 91	gggaggcacagccagtggcgcgag	SEQ ID NO: 127
SEQ ID NO: 106	aatgatcaaaaagaacacacaggt	SEQ ID NO: 128

Table 3: Oligonucleotide designs of the invention

In SEQ ID NOs: 129-136, upper case letters indicates nucleotide (nucleoside) analogue monomers (e.g. β-D-oxy LNA monomers) and subscript “s” represents phosphorothioate linkage groups between the monomers. In some embodiments, all cytosine bases in LNA monomers are 5-methylcytosines. Lower case letters represent nucleotide (DNA) monomers. “s” may be substituted with any other internucleoside linkage, such as those described herein.

Table 3 - Oligomer designs	
SEQ ID NO	Sequence (5'-3')
SEQ ID NO: 129	G _s A _s G _s a _s t _s g _s t _s a _s g _s c _s c _s a _s t _s G _s C _s T
SEQ ID NO: 130	A _s C _s G _s g _s t _s c _s a _s g _s t _s g _s t _s g _s c _s C _s C _s T
SEQ ID NO: 131	C _s G _s T _s g _s t _s a _s t _s t _s c _s c _s g _s c _s G _s T _s G
SEQ ID NO: 132	T _s G _s C _s g _s t _s g _s g _s c _s t _s a _s g _s c _s t _s T _s G _s G
SEQ ID NO: 133	A _s T _s G _s g _s t _s g _s a _s t _s c _s t _s c _s g _s t _s T _s G _s G
SEQ ID NO: 134	A _s T _s T _s t _s t _s g _s c _s a _s g _s c _s t _s c _s T _s G _s G
SEQ ID NO: 135	G _s G _s C _s a _s c _s a _s g _s c _s c _s a _s g _s t _s g _s G _s C _s G
SEQ ID NO: 136	T _s A _s T _s c _s a _s a _s a _s g _s a _s a _s c _s a _s C _s A _s C

Example 4: *In vitro* model: Cell culture

The effect of antisense oligonucleotides on target nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. The target can be expressed endogenously or by transient or stable transfection of a nucleic acid encoding said target nucleic acid. The expression level of target nucleic acid can be routinely determined using, for example, Northern blot analysis, Real-Time PCR, Ribonuclease protection assays. The following cell types are provided for illustrative purposes, but other cell types can be routinely used, provided that the target is expressed in the cell type chosen. Cells were cultured in the appropriate medium as described below and maintained at 37°C at 95-98% humidity and 5% CO₂. Cells were routinely passaged 2-3 times weekly.

A549 The human lung cancer cell line A549 was cultured in DMEM (Sigma) + 10% fetal bovine serum (FBS) + 2 mM Glutamax I + gentamicin (25µg/ml).

PC3 The human prostate cancer cell line PC3 was cultured in DMEM (Sigma) + 10% fetal bovine serum (FBS) + 2 mM Glutamax I + gentamicin (25µg/ml).

Example 5: *In vitro* model: Treatment with antisense oligonucleotide

The cells were treated with an oligomer (oligonucleotide) using the cationic liposome formulation LipofectAMINE 2000 (Gibco) as transfection vehicle. Cells were seeded in 6-well cell culture plates (NUNC) and treated when 75-90% confluent. Oligonucleotide concentrations used ranged from 1 nM to 16 nM final concentration. Formulation of oligonucleotide-lipid complexes were carried out essentially as described by the manufacturer using serum-free OptiMEM (Gibco) and a final lipid concentration of 5 µg/mL LipofectAMINE 2000. Cells were incubated at 37°C for 4 hours and treatment was stopped by removal of oligonucleotide-containing culture medium. Cells were washed and serum-containing media was added. After oligonucleotide treatment, cells were allowed to recover for 20 hours before they were harvested for RNA analysis.

Example 6: *In vitro* model: Extraction of RNA and cDNA synthesis

For RNA isolation from the cell lines, the RNeasy mini kit (Qiagen cat. no. 74104) was used according to the protocol provided by the manufacturer. First strand synthesis was performed using Reverse Transcriptase reagents from Ambion according to the manufacturer's instructions.

For each sample, 0.5 • g total RNA was adjusted to 10.8 • l with RNase free H₂O and mixed with 2 • l random decamers (50 • M) and 4 • l dNTP mix (2.5 mM each dNTP) and heated to 70°C for 3 min after which the samples were rapidly cooled on ice. After cooling the samples on ice, 2 • l 10x Buffer RT, 1 • l MMLV Reverse Transcriptase (100 U/• l) and 0.25 • l RNase inhibitor (10 U/• l) was added to each sample, followed by incubation at 42°C for 60 min, heat inactivation of the enzyme at 95°C for 10 min and then the sample was cooled to 4°C.

Example 7: *In vitro* model: Analysis of Oligonucleotide Inhibition of Hsp27 Expression by Real-time PCR

Antisense modulation of Hsp27 mRNA expression can be assayed in a variety of ways known in the art. For example, Hsp27 mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR. Real-time quantitative PCR is presently preferred. RNA analysis can be performed on total cellular RNA or mRNA. Methods of RNA isolation and RNA analysis such as Northern blot analysis is routine in the art and is taught in, for example, Current Protocols in Molecular Biology, John Wiley and Sons. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available Multi-Color Real Time PCR Detection System, available from Applied Biosystem.

Real-time Quantitative PCR Analysis of Hsp27 mRNA Levels

The content of human Hsp27 mRNA in the samples was quantified using the human Hsp27 ABI Prism Pre-Developed TaqMan Assay Reagent (Applied Biosystems cat. no. Hs03044127_g1) according to the manufacturer's instructions.

5 Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA quantity was used as an endogenous control for normalizing any variance in sample preparation. The sample content of human GAPDH mRNA was quantified using the human GAPDH ABI Prism Pre-Developed TaqMan Assay Reagent (Applied Biosystems cat. no. 4310884E) according to the manufacturer's instructions.

10 Real-time Quantitative PCR is a technique well known in the art and is taught in for example Heid et al. Real time quantitative PCR, Genome Research (1996), 6: 986-994.

Real time PCR

15 The cDNA from the first strand synthesis performed as described in Example 6 was diluted 2-20 times, and analyzed by real time quantitative PCR using Taqman 7500 FAST from Applied Biosystems. The primers and probe were mixed with 2 x Taqman Fast Universal PCR master mix (2x) (Applied Biosystems Cat.# 4364103) and added to 4 μ l cDNA to a final volume of 10 μ l. Each sample was analysed in triplicate. Standard curves were generated by assaying 2-fold dilutions of a cDNA that had been prepared on material purified from a cell line expressing the RNA of interest. Sterile H₂O was used instead of cDNA for the no template control. PCR program: 95° C for 30 seconds, followed by 40 cycles of 95°C, 3
20 seconds, 60° C, 30 seconds. Relative quantities of target mRNA sequence were determined from the calculated Threshold cycle using the Applied Biosystems Fast System SDS Software Version 1.3.1.21.

Example 8: *In vitro* analysis: Antisense Inhibition of Human Hsp27 Expression by oligonucleotides

25 Oligonucleotides presented in Table 4 were evaluated for their potential to knockdown Hsp27 mRNA expression at concentrations of 1, 4 and 16 nM in PC3 cells (see Figure 1). The data are presented in Table 4 as percentage down-regulation of Hsp27 mRNA relative to mock transfected cells at 4 nM. Mock transfected cells are transfected with lipid, but without oligo (a negative control). Lower case letters represent DNA units, bold upper case
30 letters represent LNA such as β -D-oxy-LNA units. All cytosine bases in the LNA monomers are 5-methylcytosine. Subscript "s" represents phosphorothioate linkage.

Table 4

Specific compound SEQ ID	Oligomer SEQ ID No.	Sequence (5'-3')	Hsp27 mRNA (% inhib.)
SEQ ID NO:138	SEQ ID NO:129	G _S A _S G _S a _S t _S g _S t _S a _S g _S c _S c _S a _S t _S G _S C _S T	78%
SEQ ID NO:139	SEQ ID NO:130	A _S C _S G _S g _S t _S c _S a _S g _S t _S g _S t _S g _S c _S C _S C _S T	79%
SEQ ID NO:140	SEQ ID NO:131	C _S G _S T _S g _S t _S a _S t _S t _S c _S c _S g _S c _S G _S T _S G	78%
SEQ ID NO:141	SEQ ID NO:132	T _S G _S C _S g _S t _S g _S g _S c _S t _S a _S g _S c _S t _S T _S G _S G	93%
SEQ ID NO:142	SEQ ID NO:133	A _S T _S G _S g _S t _S g _S a _S t _S c _S t _S g _S c _S t _S T _S G _S G	79%
SEQ ID NO:143	SEQ ID NO:134	A _S T _S T _S t _S t _S g _S c _S a _S g _S c _S t _S c _S T _S G _S G	79%
SEQ ID NO:144	SEQ ID NO:135	G _S G _S C _S a _S c _S a _S g _S c _S c _S a _S g _S t _S g _S G _S C _S G	94%
SEQ ID NO:145	SEQ ID NO:136	T _S A _S T _S c _S a _S a _S a _S a _S g _S a _S a _S c _S a _S C _S A _S C	78%

As shown in Table 4, oligonucleotides of SEQ ID NOs: 129, 130, 131, 132, 133, 134, 135 and 136 demonstrated about 75% or greater inhibition of Hsp27 mRNA expression at 4 nM in these experiments and are therefore preferred. Also preferred are oligonucleotides based on the illustrated antisense oligonucleotide sequences, for example varying the length (shorter or longer) and/or nucleobase content (e.g. the type and/or proportion of analogue units), which also provide good inhibition of Hsp27 mRNA expression.

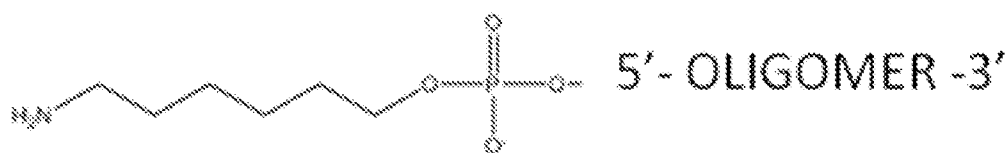
Example 9: Measurement of proliferating viable cells (MTS assay)

PC3 cells were seeded to a density of 200,000 cells per well in a 6 well plate in 2 ml medium DMEM (Sigma D5671) + 2mM Glutamax I (Gibco 35050-038) + 10% FBS (Brochom #193575010) + 25 µg/µl Gentamicin (Sigma G1397 50 mg/ml) the day prior to transfection. The next day cells were transfected as described in Example 5. The final oligonucleotide concentrations were 4 and 16 nM. After 4 hours of treatment, media was removed and cells were trypsinized and seeded to a density of 5000 cells per well in clear 96 well plates (Scientific Orange no. 1472030100) in 100 µl DMEM medium (Sigma D5671) + 2mM Glutamax I (Gibco 35050-038) + 10% FBS (Brochom #193575010) + 25 µg/µl Gentamicin (Sigma G1397 50 mg/ml). Viable cells were measured at the times indicated by adding 10 µl the tetrazolium compound [3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine ethosulfate; PES) (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega).

Viable cells were measured at 490 nm in a Powerwave (Biotek Instruments). The OD490 nm was plotted against time/h. (See Figure 2).

Example 10: Preparation of a conjugate of SEQ ID NO: 129, 130, 131, 132, 133, 134, 135 and 136 and polyethylene glycol

- 5 An oligomer is functionalized on the 5' terminus by attaching an aminoalkyl group, such as hexan-1-amine blocked with a blocking group such as Fmoc to the 5' phosphate group of the oligomer using routine phosphoramidite chemistry, oxidizing the resultant compound, deprotecting it and purifying it to achieve the functionalized oligomer (an activated oligomer) having the formula (I):

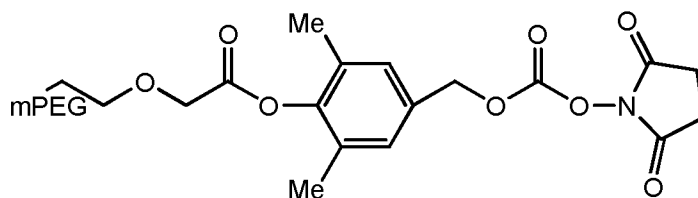


10

(I)

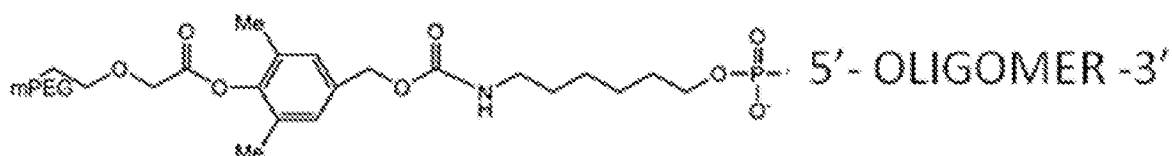
The term oligomer in formula (I) or (III) refers to an oligomer of the invention – such as an oligomer selected from the group consisting of SEQ ID NO: 129, 130, 131, 132, 133, 134, 135 and 136.

- 15 A solution of activated PEG, such as the one shown in formula (II):



(II)

- 20 wherein the PEG moiety has an average molecular weight of 12,000, and the compound of formula (I) in PBS buffer is stirred at room temperature for 12 hours. The reaction solution is extracted three times with methylene chloride and the combined organic layers are dried over magnesium sulphate and filtered and the solvent is evaporated under reduced pressure. The residue is dissolved in double distilled water and loaded onto an anion exchange column. Unreacted PEG linker is eluted with water and the product is eluted with NH₄HCO₃ solution. Fractions containing pure product are pooled and lyophilized to yield the
- 25 conjugate of formula (III):



(III)

wherein the oligomer (for example, SEQ ID NO: 129, 130, 131, 132, 133, 134, 135 and 136) is attached to a PEG polymer having average molecular weight of 12,000 via a releasable linker.

5 **Example 11: IC₅₀ determination**

The experimental procedure used is as described above in Examples 4, 5, 6 and 7. All six Hsp27 oligomers (oligos) tested have IC₅₀ • 4 nM in both cell lines A549 (figure 4) and PC3 (figure 5).

Example 12: Plasma Stability

10 The stability of the Hsp27 oligomers was investigated after incubation in mouse plasma at 37°C for 24h (1 day), 48h (two days) and 120h (five days). All oligomers showed *in-vitro* stability whereby more than 90% of active compound remained after 24 h when incubated with mouse plasma at 37°C h. For oligomers 131 and 132, a weaker band appeared after 24-48 h, which became especially prominent after 120 h. Methodology: Mouse plasma
15 (Lithium heparin plasma from BomTac:NMRI mice, collected 14-09-05, Taconic Europe) was defrosted and aliquoted into tubes with 45 • l plasma/tube. Following, 5 • l oligomer (200 • M) was added to the 45 • l plasma to a final concentration of 20 • M. After thorough mixing, the samples were incubated at 37°C for 0-120 hrs. At different time points (0h, 24h, 48h and 120h) samples were collected and the reaction was quenched by snap freezing the samples
20 in liquid nitrogen. For analysis, samples were added to loading buffer and analysed by electrophoresis on a PAGE-sequencing gel under denaturing conditions. The results are shown in Figure 6.

Example 13: T_m Determination

The melting temperature of the LNA-containing oligomer/RNA duplexes was determined
25 using a UV-spectrometry system with corresponding software (Perkin Elmer, Fremont, USA). The LNA oligomer and its complementary RNA were added in final concentrations of 1.5 • M to the T_m-buffer (200 nM NaCl, 0.2 nM EDTA, 20 mM NaP, pH 7.0). Duplex formation was prepared by heating the samples to 95°C for 3 min followed by cooling at room temperature for 30 min. Melting temperature (T_m) values were measured in a Lambda 25
30 UV/VIS spectrometer (Perkin Elmer) and data were collected and analysed using the TempLab software (Perkin Elmer). The instrument was programmed to heat the oligomer duplex sample from 20-95°C and afterwards cooling the sample to 25°C. During this process the absorbance at 260 nm was recorded. The melting curves were used to calculate T_m values. The T_m's of the oligomers against RNA were determined (Table 5). SEQ IDs 129,
35 130, 131, 132 and 135 have T_m's around 70°C. SEQ ID No 136 has a T_m of 44.4°C.

Table 5. T_m determination against complementary RNA.

Oligo	Tm/RNA °C
129	67.6 °C
130	78.1 °C
131	68.1 °C
132	73.8 °C
135	76.4 °C
136	44.4 °C

Example 14: *In vivo* analysis: Down-regulation of mouse HSP27 in mouse liver after *in vivo* (*i.v.*) administration of HSP27 oligonucleotides.

Female NMRI mice received *i.v.* injection of oligonucleotides having the sequences of SEQ ID NO: 129, 130, 131, 132 and 135 on three consecutive days at a dosage of 25mg/kg. Animals were sacrificed 24h after last dosing. The liver was stored in RNA/ater stabilizing solution until use. Total RNA was extracted from liver tissue and Hsp27 mRNA levels were analyzed with qPCR (quantitative PCR). Data were compared to Hsp27 expression in saline treated control animals. The results are shown in Figure 7.

Example 15: *In vivo* analysis: ALT and AST determination in mouse liver after *i.v.* administration of HSP27 oligonucleotides.

Female NMRI mice received *i.v.* injection of oligonucleotides having the sequences of SEQ ID NO: 129, 130, 131, 132 and 135 on day 0, 3, 6 and 9 at a dosage of 10mg/kg. Animals were sacrificed 24h after last dosing. ALT and AST levels were determined in the blood serum, free from red blood cells, obtained from the mice at the time of sacrifice. The activity of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) in mouse serum was determined using an enzymatic ALT assay (ABX Pentra A11A01627 (ALT) or A11A01629 (AST), Horiba ABX Diagnostics, France) according to the manufacturer's instruction but adjusted to 96-well format. In short, serum samples were diluted 2.5 fold with H₂O and assayed in duplicate. After addition of 50 µl diluted sample or standard (multical from ABX Pentra, A11A01652) to each well, 200 µl of 37 °C ALT reagent mix was added to each well. Kinetic measurements were performed at 340nm and 37 °C for 5 min with an interval of 30s. Data were correlated to the 2-fold diluted standard curve and results were presented as ALT activity in U/L. The results are shown in figures 8 and 9.

Example 16. Long-term cell culture without lipofection

The capacity of the anti-HSP27 oligonucleotide having the sequence set forth in SEQ ID NO: 135 to exhibit inhibition of cell proliferation in long-term culture in the absence of lipofection or other transfection agents was investigated. Over twenty cell lines were examined in cell cultures incubated in standard growth media with a range of doses, i.e., 0, 0.32, 0.63, 1.25,

2.5, 5, 10, and 20 micromolar, for the oligonucleotides for a period of 1 to 7 days. Typical media and the cell proliferation assay method are described in Example 9.

In the absence of lipofection, the following cell lines demonstrated no significant inhibition at any oligonucleotide concentration: 15PC3 (a prostate cancer cell line), PC3 (a human

5 prostate cancer cell line), A549 (a human lung cancer cell line), DLD-1 (a human colon cancer cell line), SW480 (a human colon adenocarcinoma cell line), 518A2 (a human melanoma cell line), Calu-6 (a human pulmonary carcinoma cell line), 22RV1 (a human prostate carcinoma cell line), and Hep3B (a human hepatoma cell line). The following cell lines exhibited 20% to 70% inhibition of cell proliferation at 2.5 to 20 micromolar

10 concentrations of SEQ ID NO 135: H1581 (a large cell carcinoma cell line), U87MG (a human glioblastoma-astrocytoma, epithelial-like cell line), A427 (a human lung adenocarcinoma cell line), LNCaP (a human prostate adenocarcinoma cell line), BxPC3 (a human pancreatic cancer cell line), HCC827 (a human esophageal squamous cell carcinoma cell line), DU-145 (a human prostate cancer cell line), H1975 (a non-small cell

15 lung carcinoma cell line), SKBR3 (a human breast carcinoma cell line), Huh7 (a human hepatoma cell line), HT1080 (a human fibrosarcoma cell line), Jimt (a human breast carcinoma cell line), MB231 (a human breast adenocarcinoma cell line), HCC827R (derived from the lung cancer cell line HCC827), and 786-O (a human human renal cell cancer cell line). Example data after 7 days of cell culture are shown in Figure 10. Control cell cultures

20 that were not exposed to the oligomer having SEQ ID NO: 135 correspond to 100% cell growth. It is noted that both colon cancer lines tested were unresponsive whereas all three breast cancer cell lines tested were responsive to growth inhibition. Further titration experiments with HT1080 cells and SEQ ID NOs: 135 and 131 demonstrated IC50 values for growth inhibition of 2 micromolar for the oligomers having the sequence set forth in SEQ

25 ID NO: 135 and 0.6 micromolar for SEQ ID NO: 131. In conclusion, the oligomer having the sequence of SEQ ID NO: 135 demonstrated moderate growth inhibition in some, but not all, cell lines under the growth conditions tested.

Example 17. Long-term culture with the oligomer having the sequence of SEQ ID NO: 135 down-modulates HSP27 mRNA and protein

30 The biological effects of incubation of antisense oligonucleotides in the cell culture media has been examined. Using higher oligonucleotide concentrations (micromolar) compared to lipofection methods (nanomolar), we evaluated the treatment of cells with these compounds with regard to target down-modulation by measuring the relative levels of the target HSP27 mRNA with standard real-time quantitative polymerase chain reaction methods including

35 measurement of housekeeping genes such as GAPDH to normalize the data. Cell lines were plated in 6-well microwell dishes using the recommended commercial media (ATCC) plus

various doses of oligonucleotides. RT-PCR analysis was conducted on an ABI 7500 system and data were analyzed with ABI 7500 Fast System SDS software. Gene-specific probe-primers were designed using ABI software. A PCR example program is as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C 15 sec, 60 °C 1 min. The Western blot analysis was analyzed on a Fuji Film LAS-1000 for quantification of chemiluminescence with anti-HSP27 antibody reagents, and anti-tubulin antibodies (R&D Systems). Several cell lines were investigated for the capability of anti-HSP27 oligonucleotides to down-modulate target mRNA and protein levels under conditions of long-term incubation in the absence of lipofection or other transfection agents as described in Example 15. Following 1 to 7 days of cell culture with the oligomer having the sequence of SEQ ID NO: 135 in a 0 to 5 micromolar dosing range, HSP27 mRNA levels were measured by quantitative real-time PCR as described in Example 7. HSP27 protein was measured by Western blot immunoassays using commercial antibody horseradish peroxidase conjugates versus HSP27 or control proteins including alpha-tubulin. Evaluation of the specificity of the down-modulation of HSP27 by the oligomer having the sequence of SEQ ID NO: 135 was performed by assessing the effects of scrambled or mismatched oligonucleotides.

Figure 11 shows the dose-dependent down-modulation of either HSP27 mRNA (Figure 11A) or HSP27 protein (Figure 11B). The approximate IC₅₀ for target down-modulation is 300 nanomolar for HSP27 mRNA or HSP27 protein in the cell line 15PC3 (a prostate cancer cell line) after 6 days. Similar results were obtained for additional cell lines including HT1080 (a human fibrosarcoma cell line), which exhibited a 10-fold knockdown of HSP27 mRNA and HSP27 protein at 2-5 micromolar doses after 3-4 days. The effect on HSP27 mRNA appeared to be specific since an antisense against HER3 failed to knock down HSP27 mRNA. In conclusion, incubation of LNA-based antisense oligonucleotides in cell cultures of tumor cell lines, can exhibit a dose-dependent and sequence-specific reduction in the levels of the target HSP27 mRNA and protein.

Example 18. Combination effects of anti-HSP27 LNA-based oligonucleotides plus TNF α on cellular growth

Several cell lines were investigated for the capability of anti-HSP27 oligonucleotides to inhibit cell growth when combined with another cytotoxic agent under conditions of long-term incubation in the absence of lipofection or other transfection agents.

Long-term culture of 15PC3 cells (prostate cancer cell line) with 10 micromolar of the oligomer having the sequence of SEQ ID NO: 135 for 5 days followed by 3 days of incubation with a dose range (0 – 50 ng/mL) of TNF-alpha resulted in additive effects of the two agents (Figure 12, top). Long-term culture of BxPC3 cells (a human pancreatic cancer cell line) with 10 micromolar of the oligomer having the sequence of SEQ ID NO: 135 for 5

days followed by 3 days of incubation with a dose range of TNF-alpha resulted in additive, or more than additive, effects on cell proliferation (Figure 12, bottom). Similar results were obtained when cells were treated with TNF• and the oligomer having the sequence of SEQ ID NO: 135 simultaneously for 7 days. HSP27 is reported to participate in the death receptor signaling pathways and these results support the proposed role of HSP27 in modulating this pathway.

Hence, anti-HSP27 oligonucleotides may be used therapeutically in combination with a variety of conventional cytotoxics - examples are TRAIL, 5-fluoruracil, vincristine, doxorubicin, and camptothecin. Other combinations may include use of anti-HSP27 oligonucleotides as a radiation sensitizer, or in combination with various anticancer antibodies or small molecules, particularly where resistance to these agents has occurred. In conclusion, the chaperone functions of HSP27 may play a role in resistance to the cytotoxic activity of many therapies and HSP27 antisense molecules may provide beneficial counteracting activity to enhance the overall therapeutic efficacy of the combination of these agents.

Example 19. Anti-tumor Effects of anti-HSP27 Oligonucleotides in Animal Models

Two human tumor models, PC3 (a human prostate cancer cell line) and DU-145 (a human prostate cancer cell line), were investigated for the capability of anti-HSP27 oligonucleotides to demonstrate tumor growth inhibition (TGI) in xenograft studies in mice. In the first study, subcutaneous tumors of PC3 were established in athymic nude mice and after tumor volume reached approximately 100 mm³, the mice were administered either 3, 10, 30, or 100 mg/kg intravenous doses of one of five oligonucleotides, SEQ ID. NOs: 129, 130, 131, 132, and 135. The dosing regimen was every third day for four total doses. Antitumor effects were observed in SEQ ID NOs: 130, 131, and 135, with tumor growth inhibition at day 10, based on mean tumor volumes (5 mice per group), exhibiting 26% -66% TGI. A second study with PC3 or DU-145 tumor xenografts in mice was conducted using 3 mg/kg of SEQ ID NO: 135 every third day for a total of ten doses. A 30% TGI was observed in both PC3 and DU-145 models at this dose at day 29 (Figure 13). A third study examined the TGI effects of SEQ ID NO: 135 in the PC3 xenograft model at an i.v. dose of 60 mg/kg every third day for ten doses. A 40% TGI was observed at day 29 based on mean tumor volume (6 mice per group) which did not however achieve statistical significance in this study.

Example 20. Target Knockdown in Tumors by anti-HSP27 Oligonucleotides in Animal Models

The human tumor model PC3 (a human prostate cancer cell line) was investigated for the capability of anti-HSP27 oligonucleotides to demonstrate target mRNA and protein knockdown in xenograft studies in mice. In this study, subcutaneous tumors of PC3 were

established in athymic nude mice and after tumor volume reached approximately 100 mm³, the mice were administered either 3, 10, 30, or 100 mg/kg intravenous doses of one of five oligonucleotides having the sequence set forth in SEQ ID NO: 129, 130, 131, 132, and 135 (data not shown for the oligomers having the sequences set forth in SEQ ID NOs: 129, 130 and 132). The dosing regimen was every third day for four total doses. Tumor tissues from these studies were collected either into RNAlater for mRNA analysis or flash-frozen for protein analysis. PC3 tumor sections were processed 24 hr after the final dose of oligonucleotide. Quantitative real-time PCR was conducted as described in Example 14 and protein quantitation by immunoassays was performed as described in Example 17.

HSP27 mRNA in tumor tissue was down-modulated by the oligomers having the sequences set forth in SEQ ID NO: 131 and SEQ ID NO: 135 at the maximum tolerated dose (MTD) for each compound, either 30 mg/kg or 100 mg/kg for the oligomer having the sequences of SEQ ID NO: 131 and SEQ ID NO: 135, respectively (see Figure 14). HSP27 protein down-modulation was also observed for both oligomers (having the sequences set forth in SEQ ID NO: 131 and SEQ ID NO: 135) with the average HSP27 protein knockdown in multiple tumor samples of approximately 3-fold reduction. The mRNA knockdown with the oligomer having the sequence of SEQ ID NO: 135 was approximately 10-fold at the MTD. In conclusion, LNA-based antisense oligonucleotides versus HSP27 demonstrated target-specific mRNA and protein down-modulation in tumor tissues in this dosing regimen.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and molecular biology or related fields are intended to be within the scope of the following claims.

30

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CLAIMS

1. An oligomer of between 10 - 30 monomers in length which comprises a first region of contiguous sequence of a total of between 10 – 30 monomers, wherein said contiguous sequence is at least 80% identical to a region corresponding to a mammalian Hsp27 gene or the reverse complement of a target region of a nucleic acid which encodes a mammalian Hsp27, such as a mammalian Hsp27 gene or mRNA, such as a nucleic acid having the sequence set forth in SEQ ID NO: 137, or a naturally occurring variant thereof.
2. The oligomer according to claim 1, wherein the contiguous sequence is at least 80%, preferably at least 90%, homologous to a region corresponding to any of SEQ ID NO: 91 – 105 and 127, 1-15 and 121, 16-30 and 122, 31-45 and 123, 46-60 and 124, 61-75 and 125, 76-90 and 126, and 106-120 and 128.
3. The oligomer according to claim 1 or 2, wherein the contiguous sequence comprises no mismatches or no more than one or two mismatches with the reverse complement of the corresponding region of 137.
4. The oligomer according to any one of claims 1 to 3, wherein the contiguous sequence is between 10 – 18 monomers in length.
5. The oligomer according to any one of claims 1 to 4, wherein the contiguous sequence comprises nucleoside analogues.
6. The oligomer according to claim 5, wherein the nucleoside analogues are sugar modified nucleosides, such as sugar modified nucleosides selected from the group consisting of: Locked Nucleic Acid (LNA) units; 2'-O-alkyl-RNA units, 2'-OMe-RNA units, 2'-amino-DNA units, and 2'-fluoro-DNA units; preferably the nucleoside analogues are LNA.
7. The oligomer according to claim 5 or 6 which is a gapmer.
8. The oligomer according to any one of claims 1 to 7, which inhibits the expression of Hsp27 gene or mRNA in a cell which is expressing Hsp27 gene or mRNA.

9. The oligomer according to any one of claims 1 to 8 which is selected from the group consisting of SEQ ID NO 129, 130, 131, 132, 133, 134, 135 and 136; preferably said oligomer is SEQ ID NO 131 or SEQ ID NO 135.
10. A conjugate comprising the oligomer according to any one of claims 1 to 9, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to said oligomer.
11. A pharmaceutical composition comprising the oligomer according to any one of claims 1 to 9, or the conjugate according to claim 10, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.
12. The oligomer according to any one of claims 1 to 9, or the conjugate according to claim 10, for use as a medicament, such as for the treatment of cancer, myopathies and asthma.
13. The use of an oligomer according to any one of the claims 1 to 9, or a conjugate as defined in claim 10, for the manufacture of a medicament for the treatment of cancer, myopathies and asthma.
14. A method of treating cancer, myopathies and asthma, said method comprising administering an oligomer according to any one of the claims 1 to 9, or a conjugate according to claim 10, or a pharmaceutical composition according to claim 11, to an animal suffering from, or likely to suffer from cancer, myopathies and asthma.
15. A method for the inhibition of Hsp27 in a cell which is expressing Hsp27, said method comprising administering an oligomer according to any one of the claims 1 to 9, or a conjugate according to claim 10 to said cell so as to inhibit Hsp27 in said cell.

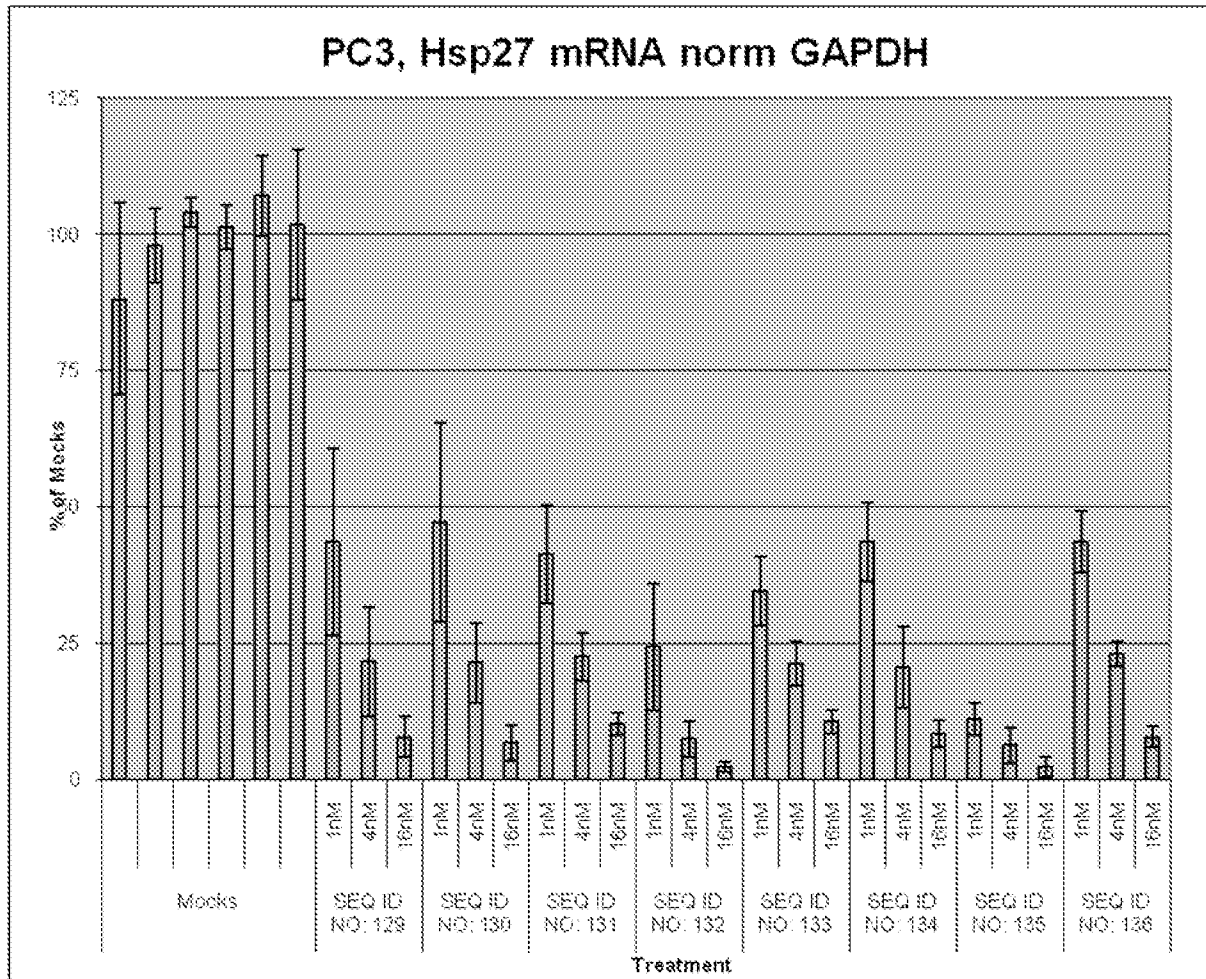


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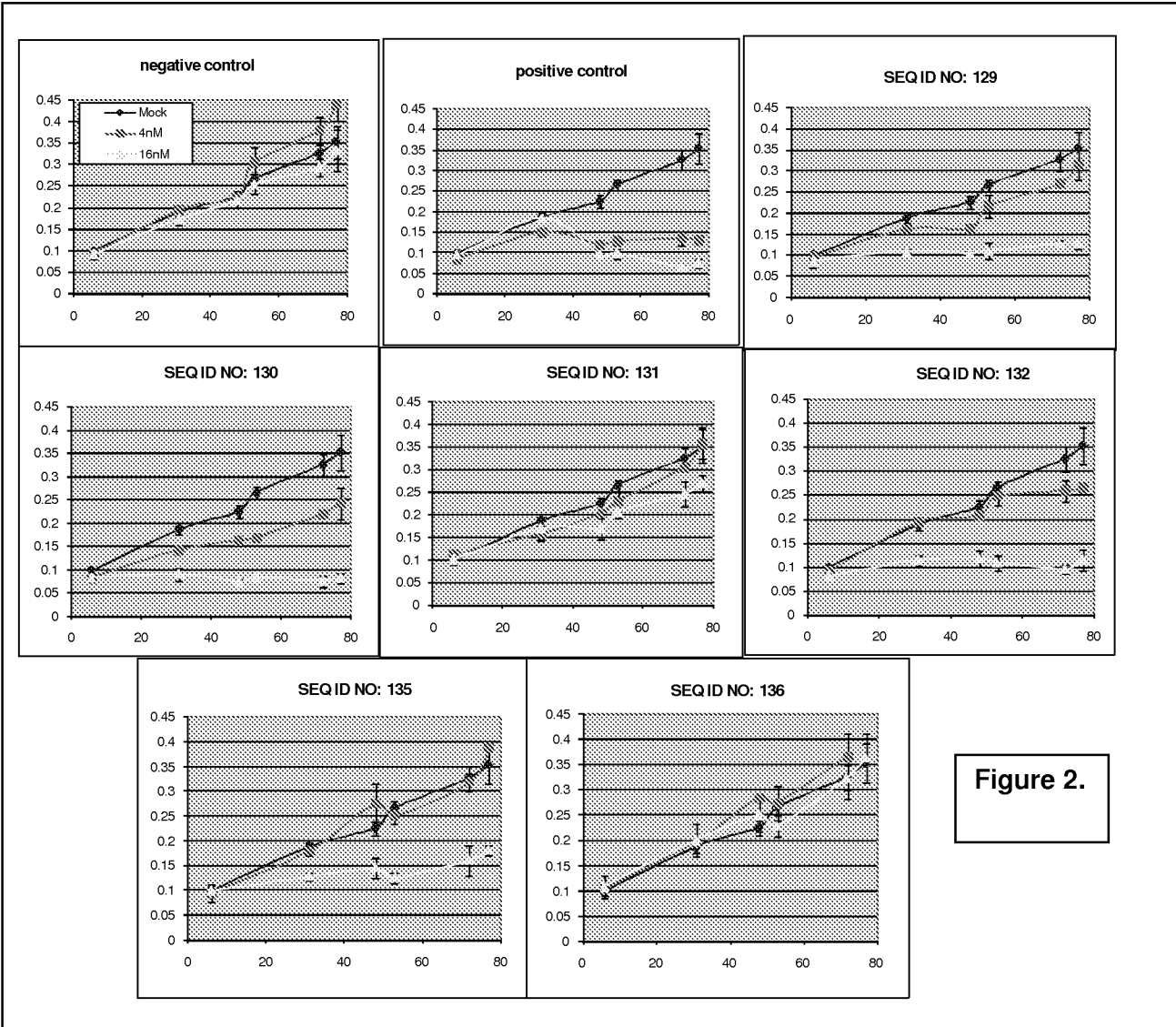


Figure 2.

Figure 3.

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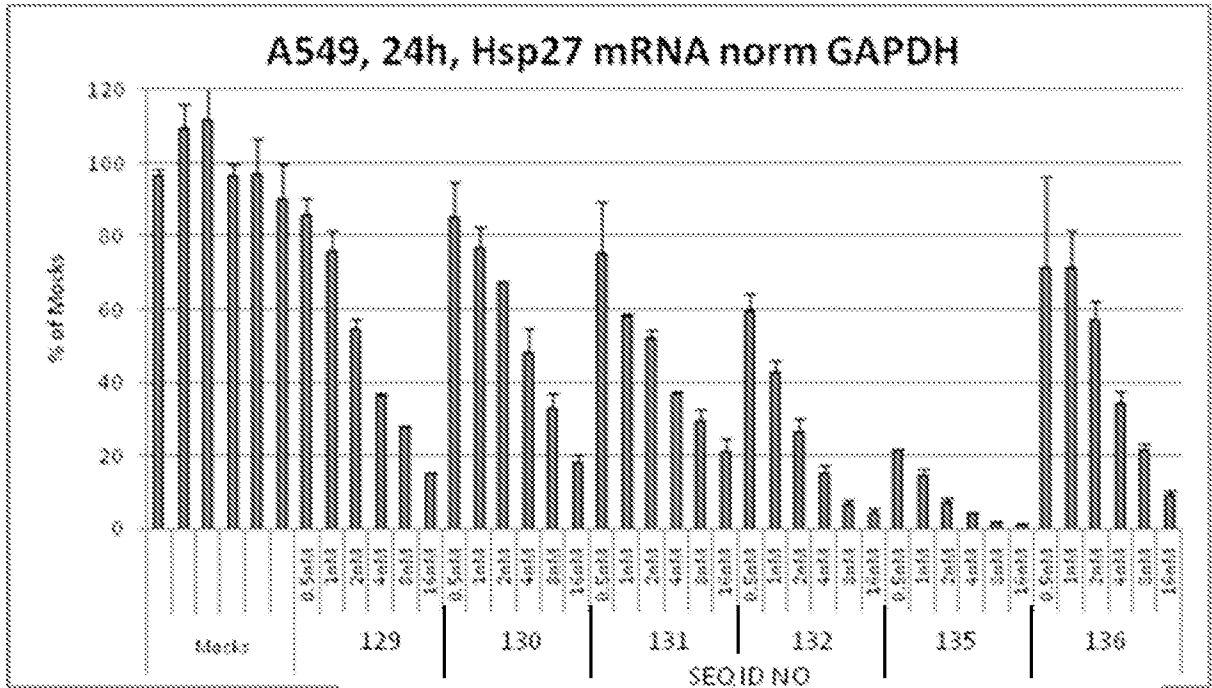


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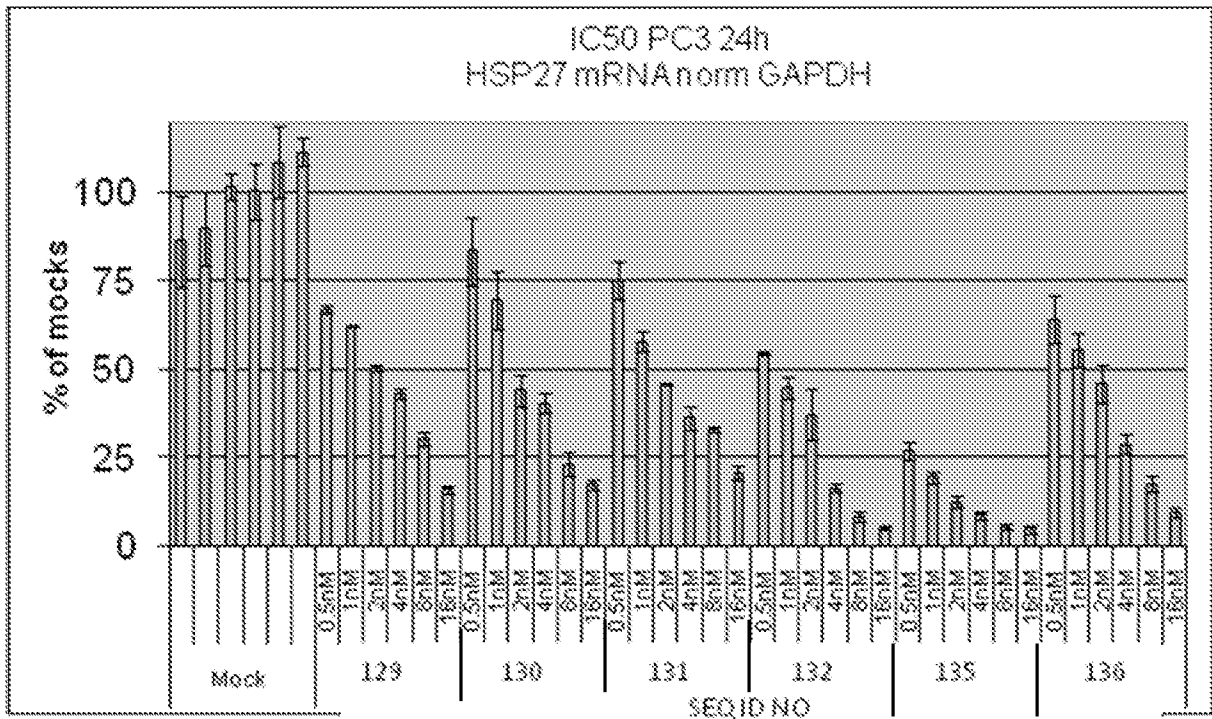


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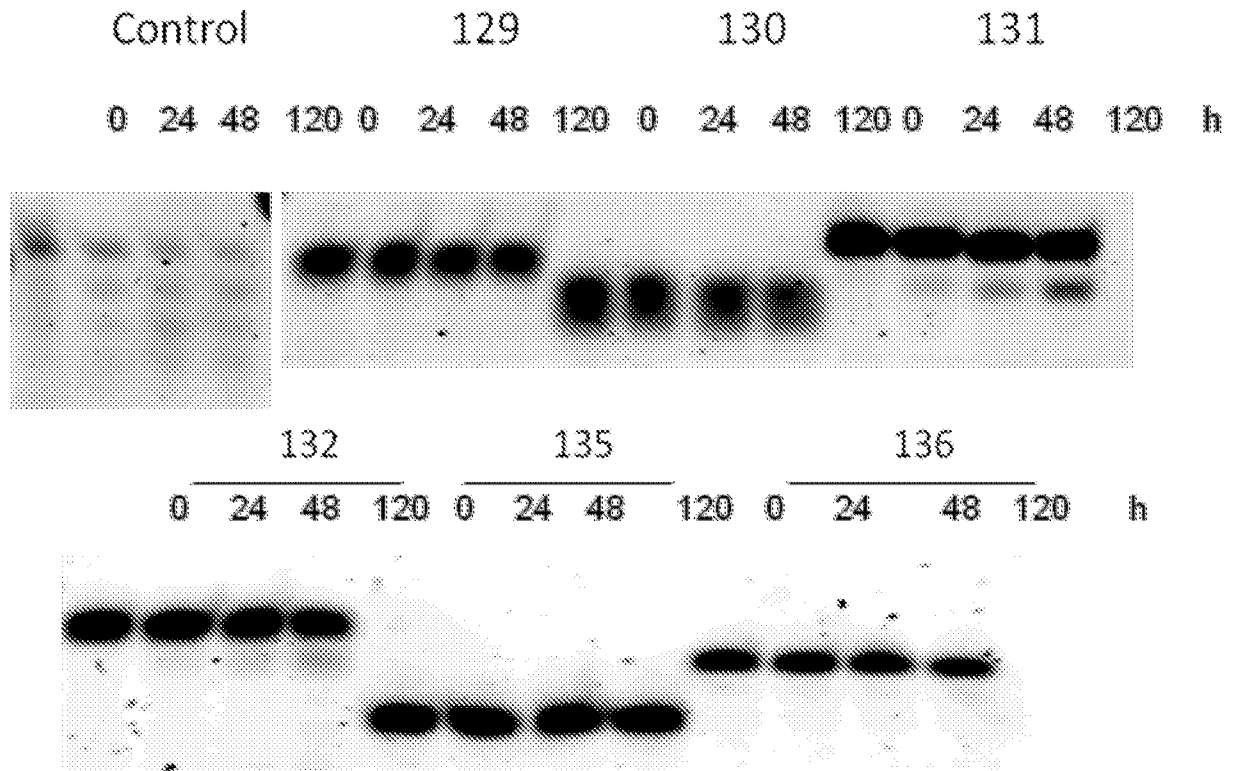


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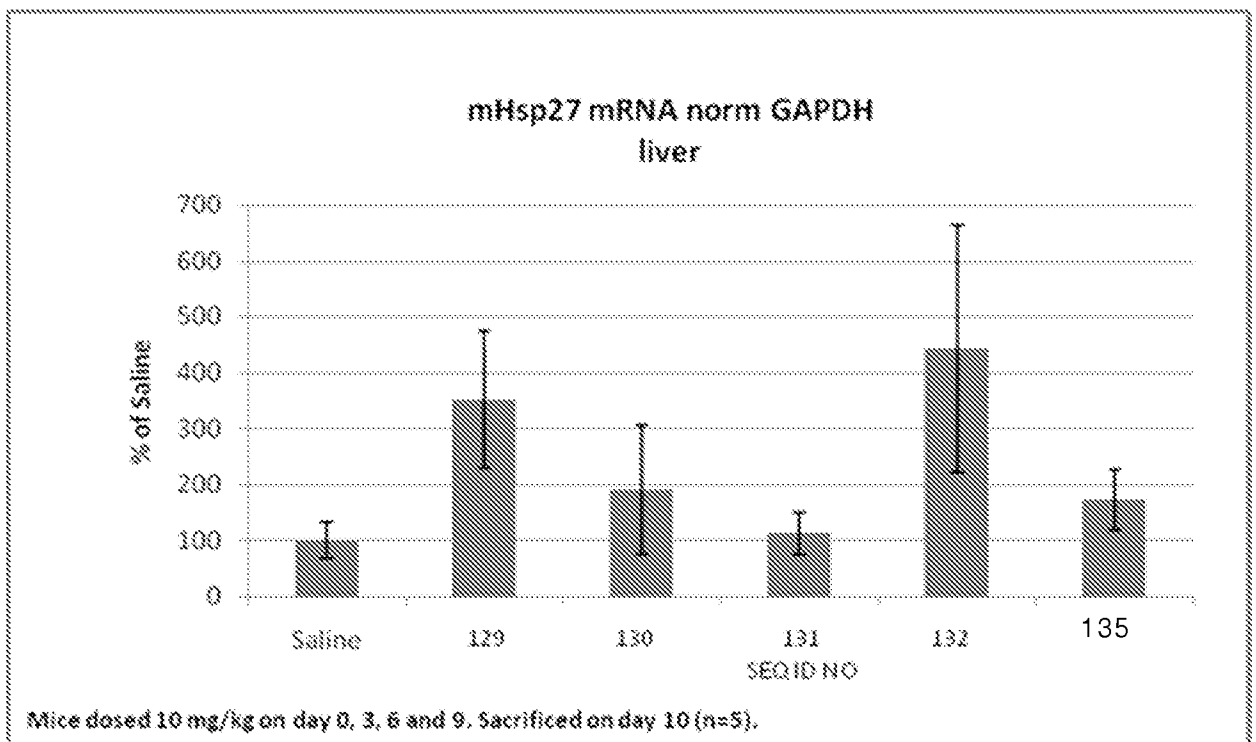


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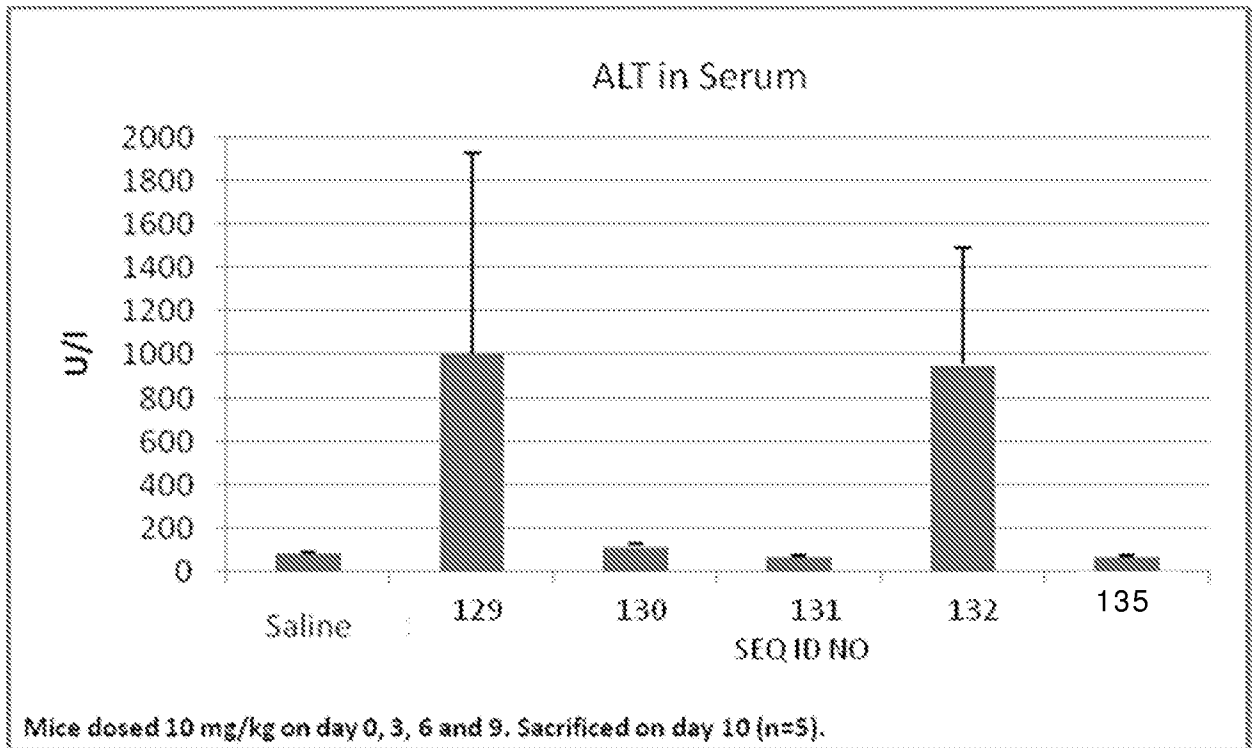


FIGURE 9

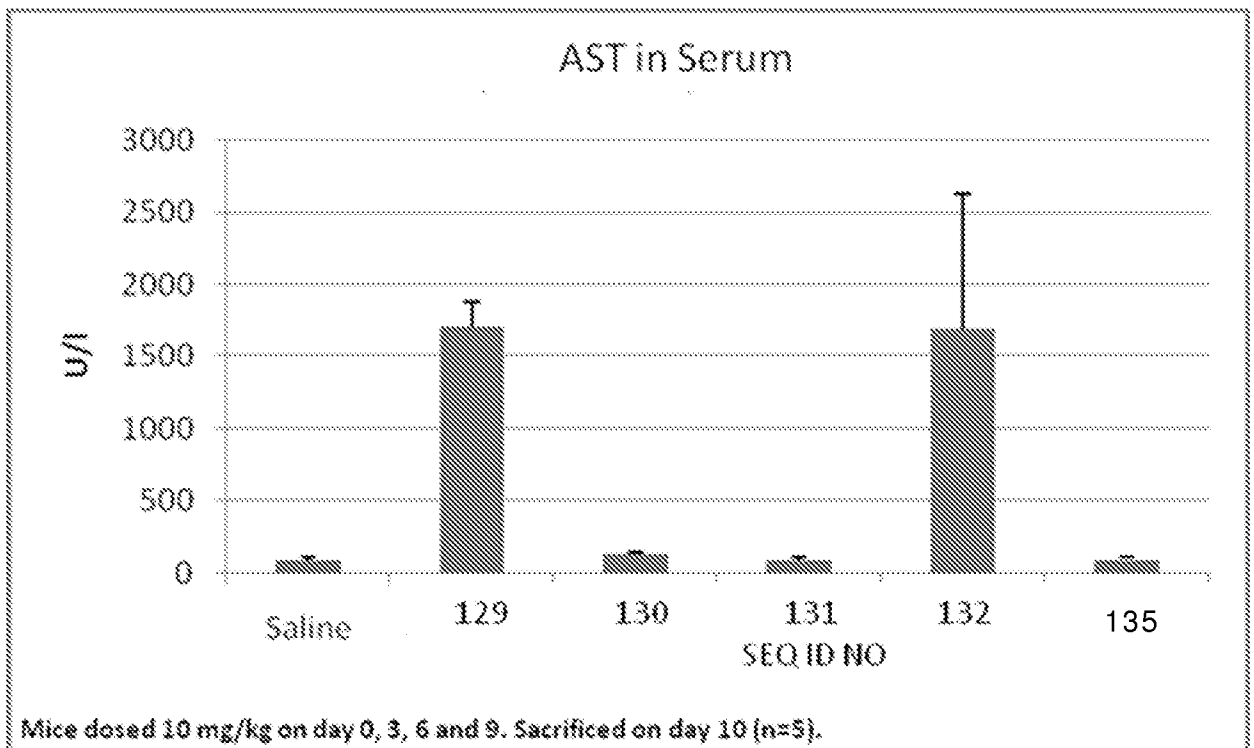
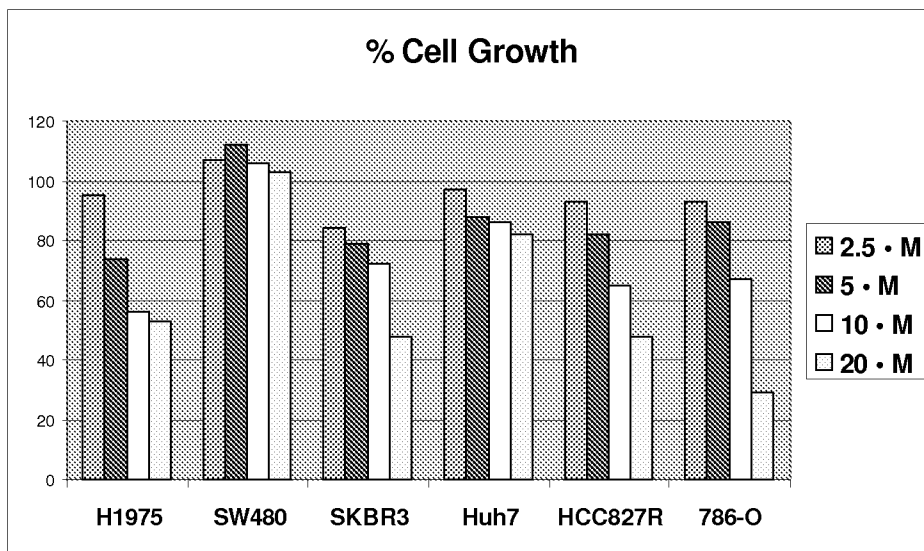
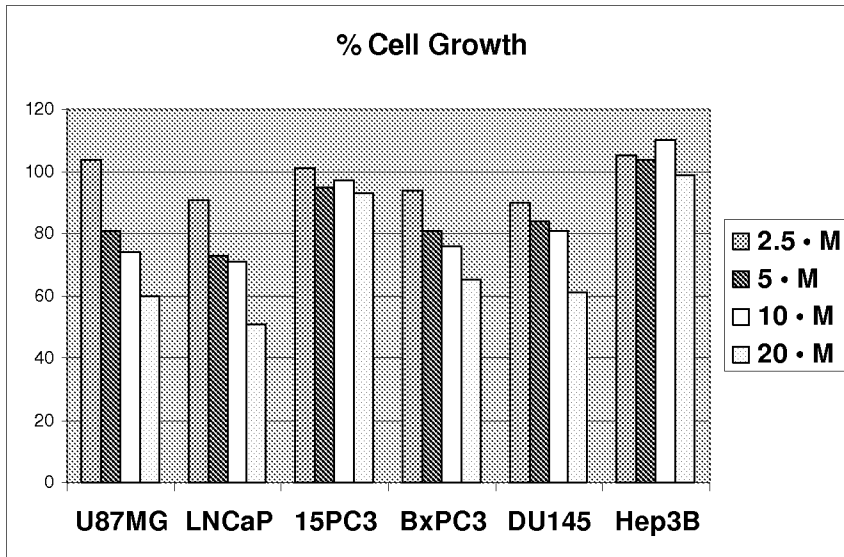


Figure 10



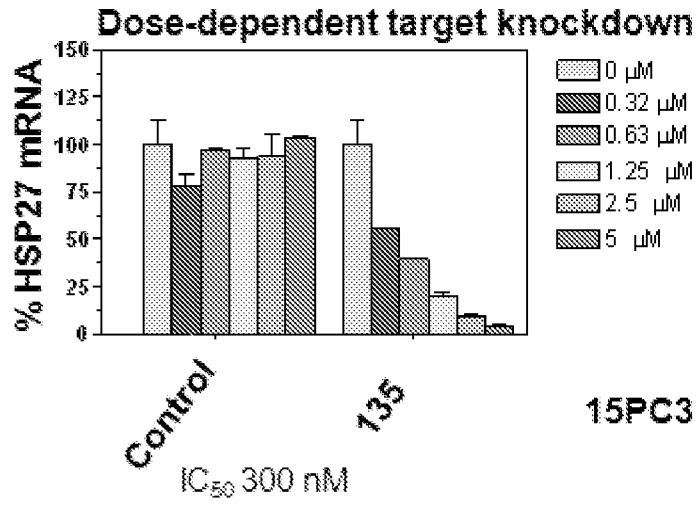


Figure 11A

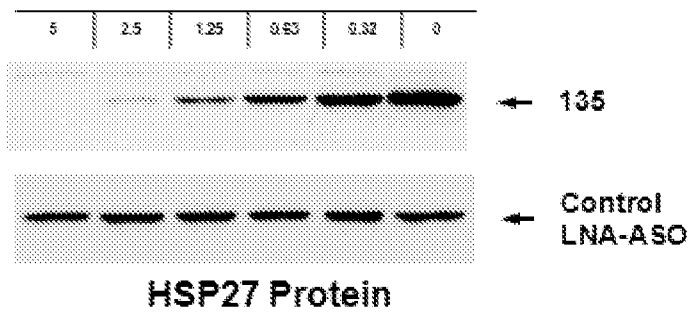
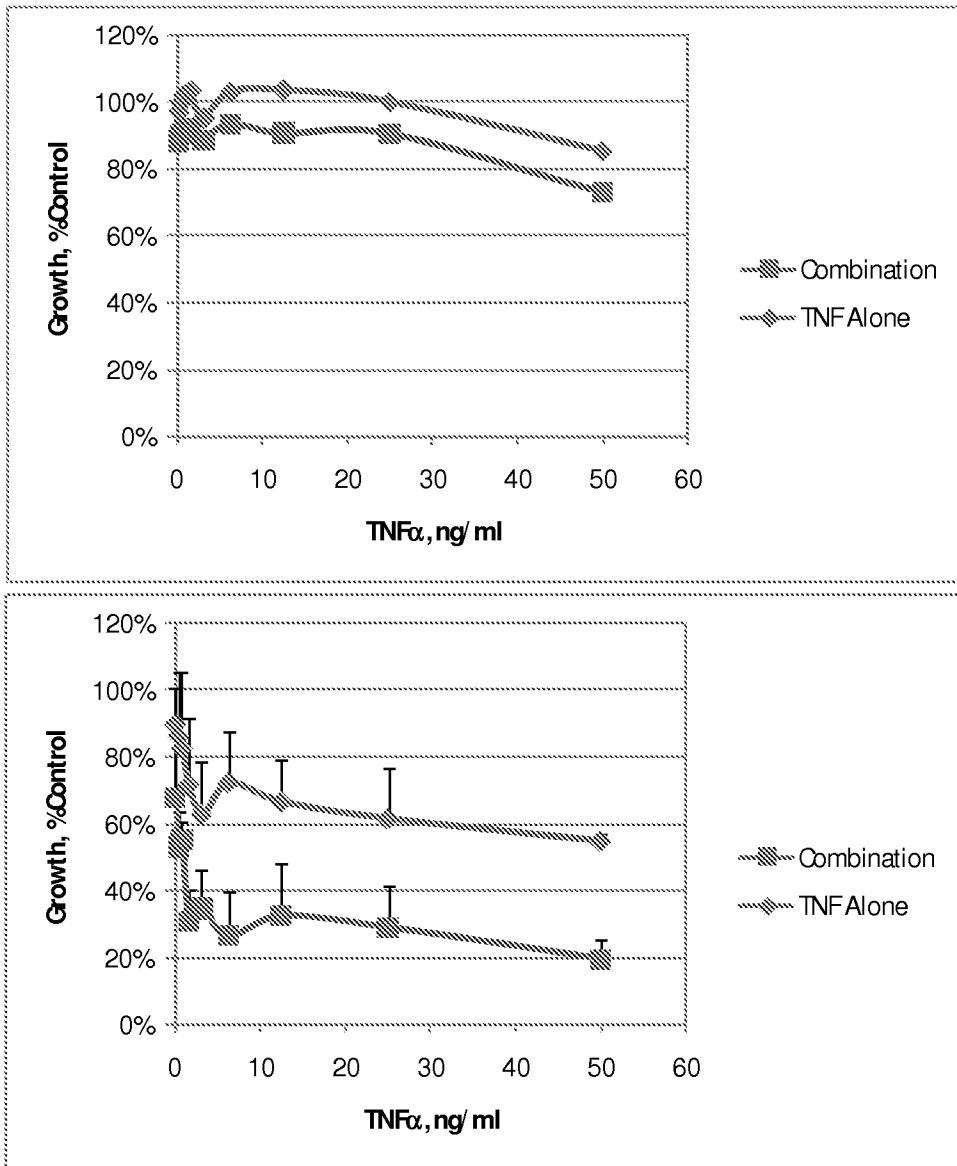


Figure 11B

Figure 12



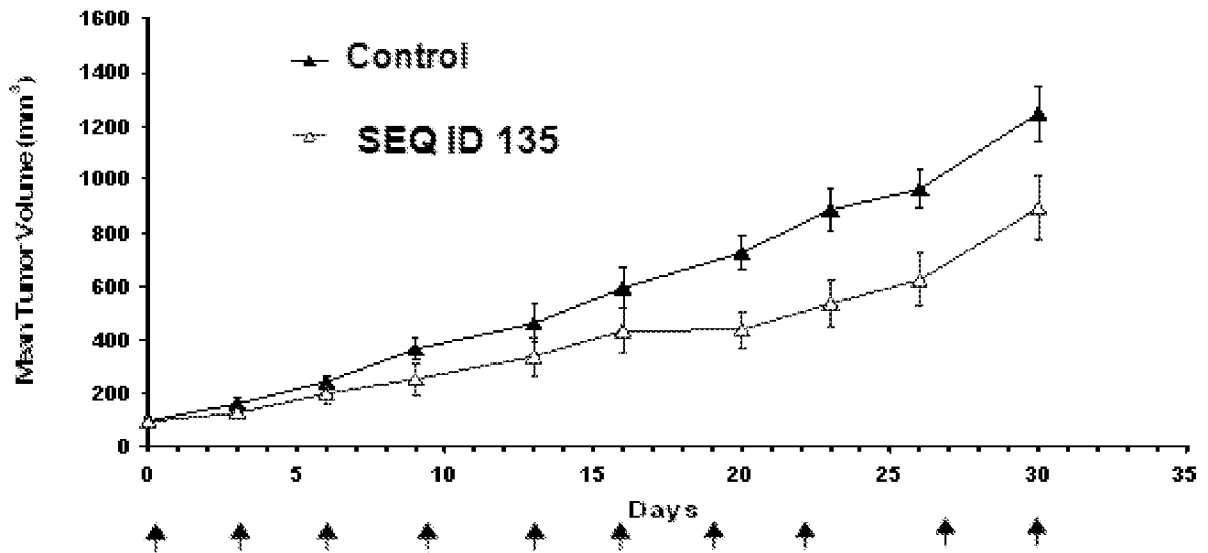
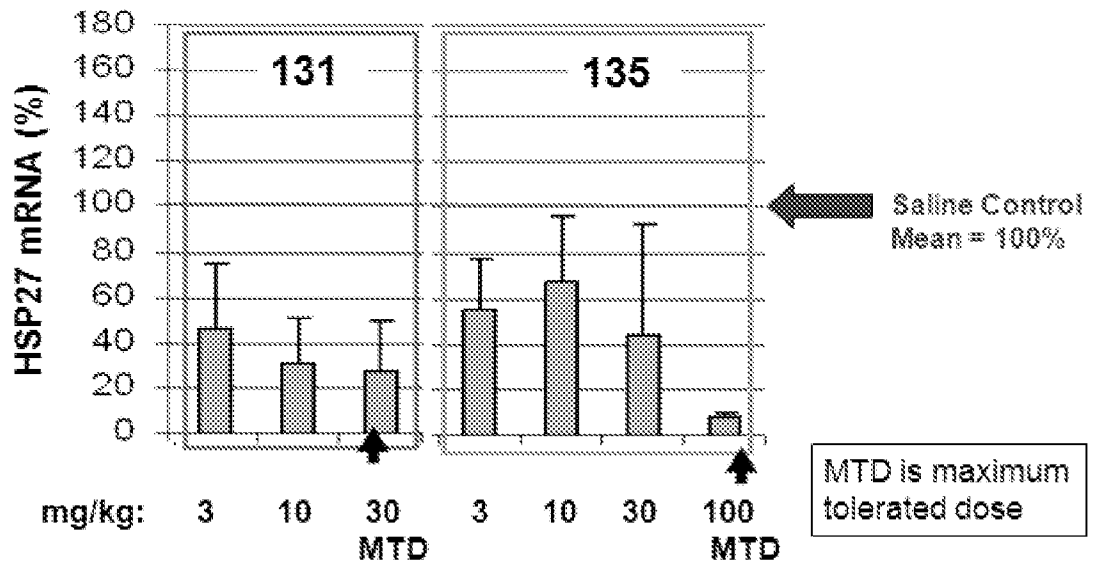


Figure 13

mRNA



Protein

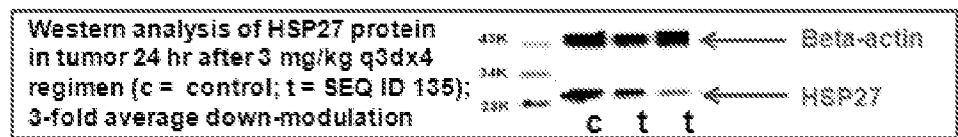


Figure 14

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12N15/11 A61K31/7088

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/025229 A (ISIS PHARMACEUTICALS, INC. [US]; BENNETT C.F.; DEAN N.M.; DOBIE K.W.) 1 March 2007 (2007-03-01) cited in the application page 18 - page 19; table 3 the whole document	1-11, 15
X	WO 2004/030660 A (UNIV BRITISH COLUMBIA [CA]; GLEAVE MARTIN E.; ROCCHI PALMA; SIGNAEVSKY) 15 April 2004 (2004-04-15) the whole document	1-15
P, X	WO 2008/106781 A (UNIV BRITISH COLUMBIA [CA]; RODRIGUEZ-LAFRASSE CLAIRE; HADCHITY ELIE) 12 September 2008 (2008-09-12) the whole document	1

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

28 September 2009

06/10/2009

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Authorized officer

Macchia, Giovanni

WO 2010/001349 INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/IB2009/052860
International application No
PCT/IB2009/052860

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007025229	A	01-03-2007	NONE
WO 2004030660	A	15-04-2004	AU 2003278028 A1 23-04-2004 CA 2498026 A1 15-04-2004 CN 1703229 A 30-11-2005 EP 1545561 A2 29-06-2005 JP 2006503904 T 02-02-2006 KR 20050050111 A 27-05-2005 MX PA05003551 A 19-10-2005 NZ 538841 A 27-10-2006 US 2006040886 A1 23-02-2006 US 2009221678 A1 03-09-2009 US 2006276424 A1 07-12-2006 US 2004127441 A1 01-07-2004 ZA 200502603 A 28-06-2006
WO 2008106781	A	12-09-2008	NONE

INTERNATIONAL SEARCH REPORT

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
- a. type of material
- a sequence listing
- table(s) related to the sequence listing
- b. format of material
- on paper
- in electronic form
- c. time of filing/furnishing
- contained in the international application as filed
- filed together with the international application in electronic form
- furnished subsequently to this Authority for the purpose of search
2. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments: