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(54) **Title**: COMPOUNDS FOR THE MODULATION OF OSTEOPONTIN EXPRESSION

(57) **Abstract**: The present invention relates to oligomer compounds (oligomers) which target osteopontin mRNA in a cell, leading to reduced expression of osteopontin. Reduction of osteopontin expression is beneficial for the treatment of diseases or disorders associated with overexpression or undesirably high levels of osteopontin, such as ischemia/reperfusion injury, e.g., after organ transplantation, such as renal transplantation.

COMPOUNDS FOR THE MODULATION OF OSTEOPONTIN EXPRESSION

FIELD OF INVENTION

The present invention relates to oligomeric compounds (oligomers) that target osteopontin mRNA in a cell, leading to reduced expression of osteopontin. Reduction of osteopontin expression is believed to be beneficial for a range of medical disorders, such as ischemia/reperfusion injury, such as may occur after renal transplantation.

BACKGROUND

Osteopontin (OPN) is a pro-inflammatory cytokine and integrin-binding ligand that is highly expressed in many inflamed tissues and plays a critical role in wound healing.

Renal transplantation has become the life-saving treatment choice for most patients with end-stage renal disease (ESRD). With the advancement in immunosuppression management, incidents of acute rejection have significantly reduced. However, the surgical procedures involved during a renal transplantation inevitably introduce extensive trauma to the patient, and, of these, the prolonged ischemia followed by reperfusion can lead to a cascade of events which may progress into acute renal failure and increase the mortality rate. Osteopontin (OPN, secreted phosphoprotein 1, bone sialoprotein, urinary stone protein, early t-lymphocyte activation 1) is a proinflammatory cytokine that is known to be upregulated after renal ischemic/reperfusion injury. Osteopontin is a soluble extracellular matrix protein with pleomorphic immunologic activities including activation of macrophage chemotaxis, promotion of Th1 responses, and activation of B cells. Osteopontin has also been implicated in neoplastic transformation, cancer progression, and metastasis. In the kidney, both the degree of osteopontin upregulation in the renal tubules and the sites of its localization have been shown to correlate with the sites and degree of macrophage accumulation and severity of renal impairment. Based on the foregoing, osteopontin is believed to be a therapeutic target for a range of medical disorders, including ischemia/reperfusion injury, such as may occur after organ transplantation, including kidney (renal) transplantation.

Renal proximal tubule epithelial cells are known to highly express osteopontin during various inflammatory states of the kidney. Osteopontin knockout mice have demonstrated reduced post-ischemic macrophage infiltration and less irreversible tubulointerstitial fibrosis. Persy et al. (2003) Kidney Intl. 63:543-553.

Recently, Bertola et al ((2009) Diabetes 58, 125-133) have also shown that liver osteopontin expression directly correlate with severe fatty liver (non-alcoholic steatohepatitis: NASH) fibrosis stage in morbidly obese humans (*36*). OPN is secreted by

cells that mediate fibrogenic repair in NASH (such as NKT cells and fibroblasts). Hedgehog (Hh) signaling has been suggested to regulate OPN transcription. This concept is relevant to related liver fibrosis as Hh pathway activity increases in parallel with fibrosis stage in NASH. Dying hepatocytes produce Hh ligands. Hh ligands, in turn, engage various types of Hh-responsive cells, including HSCs, ductular-type cells, and NKT cells, to trigger fibrogenic responses and this Hh activity correlates with macrophage accumulation and fibrosis stage in fatty liver patients and in rodent models of nonalcoholic fatty liver (Sahai, et al. (2004) Am. J. Physiol Gastrointest. Liver Physiol 287, G264-G273; Syn at al. (2011) Hepatology 53, 106-115).Therefore, OPN induction may represent a conserved pro-fibrogenic mechanism among several distinct types of Hh-responsive liver cells. Such reasoning suggests that inter-individual differences in OPN production may contribute to differences in the outcomes of NASH. Indeed, OPN may also dictate the fibrogenic response in other chronic liver diseases, because it is significantly over-expressed in livers with cirrhosis related to ALD, AIH, PBC, and PSC, and a recent study reported that plasma OPN levels correlate with hepatic inflammation and fibrosis in chronic hepatitis (Syn at al. (2011) Hepatology 53, 106-115*8*). In conclusion, OPN may represent the final common pathway for the fibrotic process for a variety of diseases of the liver, with cirrhosis as a common end stage. An anti-osteopontin LNA oligonucleotide may therefore have the potential to reduce/reverse fibrosis in NASH and other fibrotic liver diseases.Antisense compositions and methods for inhibiting osteopontin expression have been described. WO/1999/007844 and US 6,458,590 disclose antisense sequences and methods, including methods for treatment of restenosis. Antisense compositions are also disclosed in WO/2005/026357, as are methods for treatment of bone cancer and cancer metastasis. Osteopontin-based cancer therapies are disclosed in US Publ. No. 20060252684, which describes antisense targeting of osteopontin-b and osteopontin-c for treatment of tumors and determination of tumor malignancy. Anti-osteopontin agents, including antisense agents, have been described for use in anti-inflammatory, anti-fibrotic and other conditions, and in wound healing and scar prevention (WO/2009/097077). Osteopontin siRNA for therapeutic and screening uses has also been described (WO/2005/100562).

Non-antisense inhibitors of osteopontin have also been described. Polynucleotide aptamer inhibitors of osteopontin have been described in WO/2009/102438. (-)-Agelastatin A, a naturally occurring alkaloid with powerful antitumor effects, has been shown to reduce osteopontin and β-catenin levels within cancer cells. Mason et al. (2008) Mol, Cancer Ther. 7; 548.

SUMMARY OF INVENTION

In accordance with the present invention, a series of oligonucleotides was designed to target different regions of the three alternative transcript variants of human osteopontin (GenBank accession numbers: NM_001040058, NM_000582, NM_001040060).

The invention provides an oligomer of from 10 – 50 nucleotides in length, e.g. 10 - 30 nucleotides in length, which comprises a contiguous nucleotide sequence of a total of from 10 – 30 nucleotides, wherein said contiguous nucleotide sequence is at least 80% (e.g., 85%, 90%, 95%, 98%, or 99%) homologous to a region corresponding toa mammalian osteopontin gene or reverse complement of a mammalian osteopontin mRNA, such as GenBank accession numbers: NM_001040058, NM_000582 or NM_001040060 or naturally occurring variant thereof. Thus, for example, the oligomer hybridizes to a single stranded nucleic acid molecule having the sequence of a portion of NM_001040058, NM_000582 or NM_001040060. Specific oligomer embodiments of the invention are provided.

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 said oligomer. The invention also provides for a pharmaceutical composition comprising the oligomer or 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 disorder or condition associated with overexpression or undesirably high levels of osteopontin, such as ischemia/reperfusion injury, including after renal transplantation.

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 or condition associated with overexpression or undesirably high levels of osteopontin, ischemia/reperfusion injury, including after renal transplantation.

The invention provides for a method of treating a disease or disorder or condition associated with overexpression or undesirably high levels of osteopontin, such as ischemia/reperfusion injury after renal transplantation, said method comprising administering an, e.g. effective dose of, an oligomer, a conjugate or a pharmaceutical composition according to the invention, to a patient suffering from, or likely to suffer from said disease or disorder (such as a patient suffering from or susceptible to the disease or disorder), such as a patient suffering from, or likely to suffer from, ischemia/reperfusion injury, such as may occur after renal transplantation.

The invention provides for a method for the inhibition of osteopontin in a cell which is expressing osteopontin, said method comprising administering an oligomer, or a conjugate according to the invention to said cell so as to affect the inhibition of osteopontin in said cell.

Further provided are methods of down-regulating the expression of osteopontin 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. The invention provides for methods of inhibiting (*e.g.,* by down-regulating) the expression of osteopontin 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 affect down-regulation of expression of osteopontin.

BRIEF DESCRIPTION OF FIGURES

Figure 1 is a bar graph showing the results of evaluation of the oligonucleotides shown in Table 1 in the human A549 cell line for their potential to knock down osteopontin mRNA levels at oligonucleotide concentrations of 1, 5, and 25 nM using lipid transfection.

Figure 2 is a bar graph showing the results of evaluation of the oligonucleotides shown in Table 1 in the human RPTEC cell line for their potential to knock down osteopontin mRNA levels at an oligonucleotide concentration of 20 μM using natural uptake (gymnosis).

Figure 3 is a bar graph showing that SEQ ID NO: 11 and SEQ ID NO: 14 downregulate all three known osteopontin transcript variants. Human RPTEC cells were treated with oligonucleotide at an oligonucleotide concentration of 20 μM using natural uptake (gymnosis).

Figure 4 is a bar graph showing dose-dependent downregulation of osteopontin mRNA in kidneys isolated from mice treated with anti-osteopontin oligonucleotides with SEQ ID NO: 11 and SEQ ID NO: 14.

Figure 5a and 5b are bar graphs showing that liver enzymes are unaffected by treatment of mice with anti-osteopontin oligonucleotides, indicating that the treatment is well tolerated. Figure 5a shows levels of serum alanine-aminotransferase (ALT) and Figure 5b shows levels of serum aspartate-aminotransferase (AST).

Figure 6 is a bar graph showing an induction of osteopontin mRNA after 4 hours of hypoxic incubation of human RPTEC. Osteopontin mRNA levels gradually reverted back to a normal level after 24 hours of reoxygenation in untreated cells, but osteopontin mRNA levels remained low in cells that were pretreated gymnotically with 5 μM of SEQ ID NO: 11.

Figure 7 is a bar graph showing the results of evaluation of SEQ ID NOs: 11, 16-28, 30-41, and 43 in the human RPTEC cell line for their potential to knock down osteopontin mRNA levels at an oligonucleotide concentration of 25 μM using natural uptake (gymnosis).

Figure 8 is a bar graph showing the results of evaluation of SEQ ID NOs: 11, 16-30, 32-35, 37, and 39-41 in the human HRCE cell line for their potential to knock down osteopontin mRNA levels at an oligonucleotide concentration of 25 μM using natural uptake (gymnosis).

DETAILED DESCRIPTION OF THE INVENTION

The Oligomer

The present invention employs oligomeric compounds (referred herein as oligomers), for use in modulating the function of nucleic acid molecules encoding mammalian osteopontin, such as the osteopontin nucleic acid of Genbank Accession No. NM_001040058, NM_000582 and/or NM_001040060, and naturally occurring variants of such nucleic acid molecules encoding mammalian osteopontin. The term "oligomer" in the context of the present invention, refers to a molecule formed by covalent linkage of two or more nucleotides (*i.e.* an oligonucleotide). Herein, a single nucleotide (unit) may also be referred to as a monomer or unit. 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 or monomers, what is referred to is the sequence of bases, such as A, T, G, C or U.

The oligomer consists or comprises of a contiguous nucleotide sequence of from 10 - 50 nucleotides in length, such as 10-30 nucleotides in length.

In various embodiments, the compound of the invention does not comprise RNA (units). It is preferred that the compound according to the invention is a linear molecule or is synthesised as a linear molecule. The oligomer is a single stranded molecule, and preferably does not comprise significant regions of self-complementarity. In some embodiments, the oligomer is essentially not double stranded, such as is not a siRNA. In various embodiments, the oligomer of the invention may consist entirely of the contiguous nucleotide region. Thus, the oligomer is not substantially self-complementary.

The Target

Suitably the oligomer of the invention is capable of down-regulating (e.g. reducing or removing) expression of the osteopontin gene. In this regard, the oligomer of the invention can affect the inhibition of osteopontin, typically in a mammalian cell, such as a human cell, such as a cancer cell, e.g., the human lung carcinoma cell line A549 or a kidney cell, e.g., a human renal proximal tubular epithelial cell (RPTEC) or a human renal cortical epithelial cell (HRCE). In some embodiments, the oligomers of the invention bind to the target nucleic acid and affect inhibition of expression of at least 10% or 20% compared to the normal

expression level, more preferably at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% inhibition compared to the normal expression level (such as the expression level in the absence of the oligomer(s) or conjugate(s)). In some embodiments, such modulation is seen when using from 0.04 to 25nM, such as from 0.8 to 20nM, of the compound of the invention, e.g., 1, 5, 20 nM or 25 nM. In other embodiments, such modulation is seen when using from 5 to 25 μM, such as from 8 to 20μM, of the compound of the invention, e.g., 1, 5, 20 μM or 25 μM. In the same or a different embodiment, the inhibition of expression is less than 100%, such as less than 98% inhibition, less than 95% inhibition, less than 90% inhibition, less than 80% inhibition, such as less than 70% inhibition. Modulation of expression level may be determined by measuring protein levels, *e.g.* by the 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 described above, is, in some embodiments, typically to a level of from 10-20% of the normal levels in the absence of the compound, conjugate or composition of the invention.

As illustrated herein the cell type may, in some embodiments, be a cancer cell, such as an A549 human lung carcinoma cell, or may be a kidney cell, such as a human renal proximal tubular epithelial cell (RPTEC) or a human renal cortical epithelial cell (HRCE). The oligomer concentration used may, in some embodiments, be 5nM. The oligomer concentration used may, in some embodiments, be 25nM. The oligomer concentration used may, in some embodiments be 1nM. It should be noted that this concentration of oligomer used to treat the cell is typically in an *in vitro* cell assay, using transfection (Lipofection), as illustrated in the examples. In the absence of a transfection agent, the oligo concentration required to obtain the down-regulation of the target is typically between 1 and 25μM, such as 5μM.

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

The term “target nucleic acid”, as used herein refers to the DNA or RNA encoding mammalian osteopontin polypeptide, such as human osteopontin, such as NM_001040058,

NM_000582 or NM_001040060 or naturally occurring variants thereof, and RNA nucleic acids derived therefrom, preferably mRNA, such as pre-mRNA, preferably mature mRNA. In some embodiments, for example when used in research or diagnostics the "target nucleic acid" may be a cDNA or a synthetic oligonucleotide derived from the above DNA or RNA nucleic acid targets. The oligomer according to the invention is preferably capable of hybridising to the target nucleic acid. It will be recognised that NM_001040058, NM_000582 and NM_001040060 are cDNA sequences, and as such, correspond to the mature mRNA target sequences, although uracil is replaced with thymidine in the cDNA sequences.

The term "naturally occurring variant thereof" refers to variants of the osteopontin polypeptide of nucleic acid sequence which exist naturally within the defined taxonomic group, such as mammalian, such as mouse, monkey, and preferably human. Typically, when referring to "naturally occurring variants" of a polynucleotide the term also may encompass any allelic variant of the osteopontin-encoding genomic DNA resulting from chromosomal translocation or duplication, and the RNA, such as mRNA derived therefrom. "Naturally occurring variants" may also include variants derived from alternative splicing of the osteopontin 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.

Sequences

The oligomers comprise or consist of a contiguous nucleotide sequence which corresponds to the reverse complement of a nucleotide sequence present in NM_001040058, NM_000582, and/or NM_001040060. Thus, the oligomer can comprise or consist of a sequence selected from the group consisting of SEQ ID NOS: 1-43, wherein said oligomer (or contiguous nucleotide portion thereof) may optionally have one, two, or three mismatches as compared to said selected sequence.

The oligomer may comprise or consist of a contiguous nucleotide sequence which is fully complementary (perfectly complementary) to the equivalent region of a nucleic acid which encodes a mammalian osteopontin (e.g., GenBank accession number NM_001040058, NM_000582 or NM_001040060). Thus, the oligomer can comprise or consist of an antisense nucleotide sequence.

However, in some embodiments, the oligomer may tolerate 1, 2, 3, or 4 (or more) mismatches, when hybridising to the target sequence and still sufficiently bind to the target to show the desired effect, *i.e.* down-regulation of the target. Mismatches may, for example, be compensated by increased length of the oligomer nucleotide sequence and/or an

increased number of nucleotide analogues, such as LNA, present within the nucleotide sequence.

In some embodiments, the contiguous nucleotide sequence comprises no more than 3, such as no more than 2 mismatches when hybridizing to the target sequence, such as to the corresponding region of a nucleic acid which encodes a mammalian osteopontin.

In some embodiments, the contiguous nucleotide sequence comprises no more than a single mismatch when hybridizing to the target sequence, such as the corresponding region of a nucleic acid which encodes a mammalian osteopontin.

The nucleotide sequence of the oligomer of the invention or the contiguous nucleotide sequence is preferably at least 80% homologous to a corresponding sequence selected from the group consisting of SEQ ID NOS: 1-43, 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, at least 98% homologous, or at least 99% homologous, such as 100% homologous (identical).

The nucleotide sequence of the oligomers of the invention or the contiguous nucleotide sequence is preferably at least 80% homologous to the reverse complement of a corresponding sequence present in NM_001040058, NM_000582 or NM_001040060, 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, at least 98% homologous, or at least 99% homologous, such as 100% homologous (identical).

The nucleotide sequence of the oligomers of the invention or the contiguous nucleotide sequence is preferably at least 80% complementary to a sub-sequence present in NM_001040058, NM_000582 or NM_001040060, 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, or at least 99% complementary, such as 100% complementary (perfectly complementary).

In some embodiments the oligomer (or contiguous nucleotide portion thereof) is selected from, or comprises, one of the sequences selected from the group consisting of SEQ ID NOS: 1-43, or a sub-sequence of at least 10 contiguous nucleotides thereof, wherein said oligomer (or contiguous nucleotide portion thereof) may optionally comprise one, two, or three mismatches when compared to the sequence.

In some embodiments the sub-sequence may consist of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 contiguous nucleotides, such as from 12 - 22, such as from 12-18 nucleotides. In some embodiments, the sub-sequence is 16 nucleotides in length and has the base sequence of one of SEQ ID NOS: 3, 5, 9, 11, 16-25, 27-31 or 33-43. In other embodiments, the sub-sequence is 15 nucleotides in length and

has the base sequence of one of SEQ ID NOs: 26 or 32. In some embodiments, the sub-sequence is 14 nucleotides in length and has the base sequence of SEQ ID NOs: 2, 4, 7, 8, 10, 12, 14, or 15. In other embodiments, the sub-sequence is 13 nucleotides in length and has the base sequence of SEQ ID NO: 6 or 13. In still other embodiments, the sub-sequence is 12 nucleotides in length and has the base sequence of SEQ ID NO: 1. Suitably, in some embodiments, the sub-sequence is of the same length as the contiguous nucleotide sequence of the oligomer of the invention.

However, it is recognised that, in some embodiments the nucleotide sequence of the oligomer may comprise additional 5' or 3' nucleotides, such as, independently, 1, 2, 3, 4 or 5 additional nucleotides 5' and/or 3', which are non-complementary to the target sequence. In this respect the oligomer of the invention may, in some embodiments, comprise a contiguous nucleotide sequence which is flanked 5' and or 3' by additional nucleotides. In some embodiments the additional 5' or 3' nucleotides are naturally occurring nucleotides, such as DNA or RNA. In some embodiments, the additional 5' or 3' nucleotides may represent region D as referred to in the context of gapmer oligomers herein.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 1, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 2, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 3, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 4, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 5 or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 6 or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 7 or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 8 or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 9 or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 10, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 11, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 12, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 13, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 14, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 15, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 16, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 17, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 18, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 19, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 20, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 21, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 22, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 23, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 24, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 25, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 26, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 27, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 28, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 29, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 30, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 31, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 32, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 33, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 34, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 35, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 36, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 37, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 38, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 39, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 40, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 41, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 42, or a sub-sequence thereof.

In some embodiments the oligomer according to the invention comprises or consists of a nucleotide sequence according to SEQ ID NO: 43, or a sub-sequence thereof.

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 osteopontin, such as those disclosed herein, the degree of “complementarity” is expressed as the percentage identity (percentage homology) between the sequence of the oligomer (or

region thereof) and the sequence of 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.

Similarly, the degree of “homology” or “identity” is expressed as the percentage identity (percentage homology) between the sequence of the oligomer (or region thereof) and the sequence of the target region that best aligns therewith. As used herein, the terms “homologous” and “homology” are interchangeable with the terms “identical” and “identity”.

The terms "corresponding to" and "corresponds to" refer to the comparison between the nucleotide sequence of the oligomer (*i.e.* the nucleobase or base sequence) or contiguous nucleotide sequence and the equivalent contiguous nucleotide sequence of a further sequence selected from either i) a sub-sequence of the reverse complement of the nucleic acid target, such as the mRNA which encodes the osteopontin protein, such as NM_001040058, NM_000582 and/or NM_001040060 and/or ii) the nucleotide sequences provided herein such as the group consisting of SEQ ID NOS: 1-43, or sub-sequence thereof. Nucleotide analogues are compared directly to their equivalent or corresponding nucleotides. A first sequence which corresponds to a further sequence under i) or ii) typically is identical to that sequence over the length of the first sequence (such as the contiguous nucleotide sequence) or, as described herein may, in some embodiments, is 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%, at least 97%, at least 98%, or at least 99% homologous, such as 100% homologous (identical).

The terms "corresponding nucleotide analogue" and "corresponding nucleotide" are intended to indicate that the nucleobase in the nucleotide analogue and the naturally occurring nucleotide are identical. For example, when the 2-deoxyribose unit of the nucleotide is linked to an adenine, the "corresponding nucleotide analogue" contains a pentose unit (different from 2-deoxyribose) linked to an adenine.

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

Length

The oligomers may comprise or consist of a contiguous nucleotide sequence of a total of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleotides in length.

In some embodiments, the oligomers comprise or consist of a contiguous nucleotide sequence of a total of from 10 to 22 nucleotides, such as 12 -18, 13-17 or 12-16 nucleotides, such as 13, 14, 15, or 16 contiguous nucleotides in length.

In some embodiments, the oligomers comprise or consist of a contiguous nucleotide sequence of a total of 10, 11, 12, 13, or 14 contiguous nucleotides in length.

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

Nucleosides and Nucleoside analogues

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

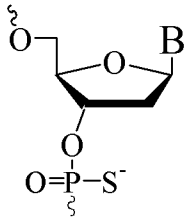
The term "nucleotide" as used herein, refers to a glycoside comprising a sugar moiety, a base moiety and a covalently linked group (linkage group), such as a phosphate or phosphorothioate internucleotide linkage group, and covers both naturally occurring nucleotides, such as DNA or RNA, and non-naturally occurring nucleotides comprising 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 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 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 refer to the nucleobase sequence (*i.e.* the presence of the sugar backbone and internucleoside 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.

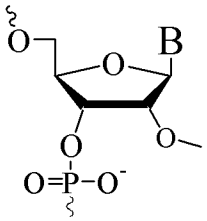
As one of ordinary skill in the art would recognise, the 5' terminal nucleotide of an oligonucleotide does not comprise a 5' internucleotide linkage group, although may or may not comprise a 5' terminal group.

Non-naturally occurring nucleotides include nucleotides which have modified sugar moieties, such as bicyclic nucleotides or 2' modified nucleotides, such as 2' substituted nucleotides.

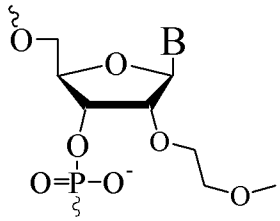
"Nucleotide analogues" are variants of natural nucleotides, such as DNA or RNA nucleotides, by virtue of modifications in the sugar and/or base moieties. Analogues could in principle be merely "silent" or "equivalent" to the natural nucleotides in the context of the oligonucleotide, *i.e.* have no functional effect on the way the oligonucleotide works to inhibit target gene expression. Such "equivalent" analogues may nevertheless be useful if, for example, they are easier or cheaper to manufacture, or are more stable to storage or manufacturing conditions, or represent a tag or label. Preferably, however, the analogues will have a functional effect on the way in which the oligomer works to inhibit expression; for example by producing increased binding affinity to the target and/or increased resistance to intracellular nucleases and/or increased ease of transport into the cell. 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:



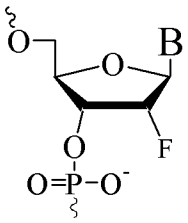
Phosphorthioate



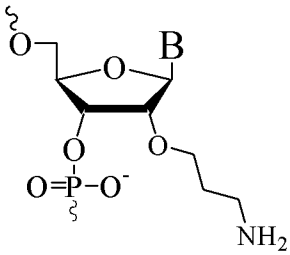
2'-O-Methyl



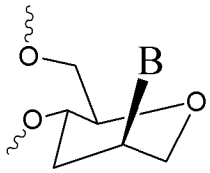
2'-MOE



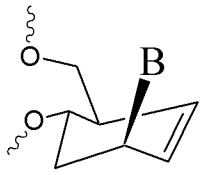
2'-Fluoro



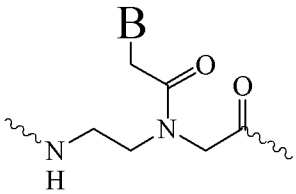
2'-AP



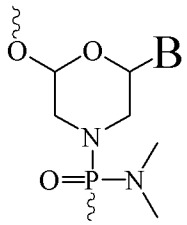
HNA



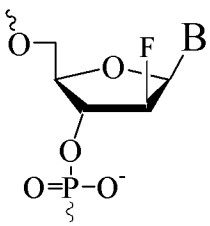
CeNA



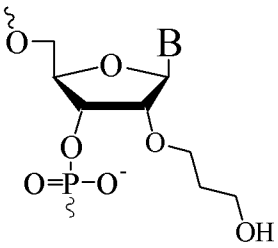
PNA



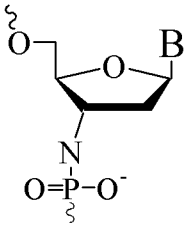
Morpholino



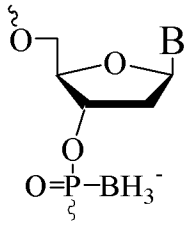
2'-F-ANA



2'-(3-hydroxy)propyl



3'-Phosphoramidate



Boranophosphates

Scheme 1

The oligomer may thus comprise or consist of a simple sequence of natural occurring nucleotides – preferably 2'-deoxynucleotides (referred to here generally as "DNA"), but also possibly ribonucleotides (referred to here generally as "RNA"), or a combination of such naturally occurring nucleotides and one or more non-naturally occurring nucleotides, *i.e.* nucleotide analogues. Such nucleotide analogues may suitably enhance the affinity of the oligomer for the target sequence.

Examples of suitable and preferred nucleotide analogues are provided by WO2007/031091 or are referenced therein.

Incorporation of affinity-enhancing nucleotide analogues in the oligomer, such as LNA or 2'-substituted sugars, can allow the size of the specifically binding oligomer to be reduced, and may also reduce the upper limit to the size of the oligomer before non-specific or aberrant binding takes place.

In some embodiments, the oligomer comprises at least 1 nucleoside analogue. In some embodiments the oligomer comprises at least 2 nucleotide analogues. In some embodiments, the oligomer comprises from 3-8 nucleotide analogues, *e.g.* 6 or 7 nucleotide analogues. In the by far most preferred embodiments, at least one of said nucleotide analogues is a locked nucleic acid (LNA); for example at least 3 or at least 4, or at least 5, or at least 6, or at least 7, or 8, of the nucleotide analogues may be LNA. In some embodiments all the nucleotide analogues may be LNA; in other embodiments approximately half of the nucleotide analogues may be LNA.

It will be recognised that when referring to a preferred nucleotide sequence motif or nucleotide sequence, which consists of only nucleotides, the oligomers of the invention which are defined by that sequence may comprise a corresponding nucleotide analogue (that is, having the same nucleobase) in place of one or more of the nucleotides present in said sequence, such as LNA units or other nucleotide analogues, which raise the duplex stability/T_m of the oligomer/target duplex (*i.e.* affinity enhancing nucleotide analogues).

In some embodiments, any mismatches between the nucleotide sequence of the oligomer and the target sequence are preferably found in regions outside the affinity enhancing nucleotide analogues, such as region B as referred to herein, and/or region D as referred to herein, and/or at the site of nonmodified (such as DNA) nucleotides in the oligonucleotide, and/or in regions which are 5' or 3' to the contiguous nucleotide sequence.

Examples of such modification of the nucleotide include modifying the sugar moiety to provide a 2'-substituent group or to produce a bridged (locked nucleic acid) structure which enhances binding affinity and may also provide increased nuclease resistance.

A preferred nucleotide analogue is LNA, such as 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). Most preferred is beta-D-oxy-LNA.

In some embodiments the nucleotide analogues present within the oligomer of the invention (such as in regions A and C mentioned herein) are independently selected from, for example: 2'-O-alkyl-RNA units, 2'-amino-DNA units, 2'-fluoro-DNA units, LNA units, arabino nucleic acid (ANA) units, 2'-fluoro-ANA units, HNA units, INA (intercalating nucleic acid -Christensen, 2002. Nucl. Acids. Res. 2002 30: 4918-4925, hereby incorporated by reference) units and 2'MOE units. In some embodiments there is only one of the above

types of nucleotide analogues present in the oligomer of the invention, or contiguous nucleotide sequence thereof.

In some embodiments the nucleotide analogues are 2'-O-methoxyethyl-RNA (2'MOE), 2'-fluoro-DNA monomers or LNA nucleotide analogues, and as such the oligonucleotide of the invention may comprise nucleotide analogues which are independently selected from these three types of analogue, or may comprise only one type of analogue selected from the three types. In some embodiments at least one of said nucleotide analogues is 2'-MOE-RNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-MOE-RNA nucleotide units. In some embodiments at least one of said nucleotide analogues is 2'-fluoro DNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-fluoro-DNA nucleotide units.

In some embodiments, the oligomer according to the invention comprises at least one Locked Nucleic Acid (LNA) unit, such as 1, 2, 3, 4, 5, 6, 7, or 8 LNA units, such as from 3 – 7 or 4 to 8 LNA units, or 3, 4, 5, 6 or 7 LNA units. In some embodiments, all the nucleotide analogues are LNA. In some embodiments, the oligomer may comprise both beta-D-oxy-LNA, and one or more of the following LNA units: thio-LNA, amino-LNA, oxy-LNA, and/or ENA in either the beta-D or alpha-L configurations or combinations thereof. In some embodiments all LNA cytosine units are 5'methyl-cytosine. In some embodiments of the invention, the oligomer may comprise both LNA and DNA units. Preferably the combined total of LNA and DNA units is 10-25, such as 10 – 24, preferably 10-20, such as 10 – 18, even more preferably 12-16. In some embodiments of the invention, the nucleotide sequence of the oligomer, such as the contiguous nucleotide sequence consists of at least one LNA and the remaining nucleotide units are DNA units. In some embodiments the oligomer comprises only LNA nucleotide analogues and naturally occurring nucleotides (such as RNA or DNA, most preferably DNA nucleotides), optionally with modified internucleotide linkages such as phosphorothioate.

The term "nucleobase" refers to the base moiety of a nucleotide and covers both naturally occurring as well as non-naturally occurring variants. Thus, "nucleobase" covers not only the known purine and pyrimidine heterocycles but also heterocyclic analogues and tautomers thereof.

Examples of nucleobases include, but are not limited to adenine, guanine, cytosine, thymidine, uracil, xanthine, hypoxanthine, 5-methylcytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

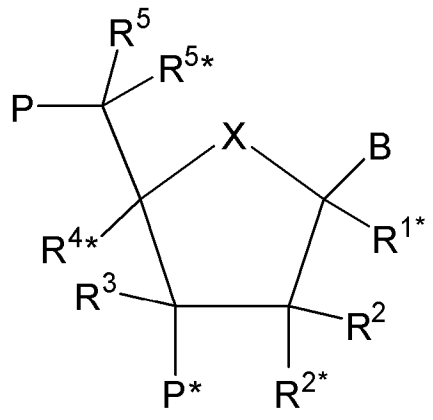
In some embodiments, at least one of the nucleobases present in the oligomer is a modified nucleobase selected from the group consisting of 5-methylcytosine, isocytosine,

pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine.

LNA

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

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



Formula 1

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*})-, -C(R^6R^{6*})-, such as, in some embodiments –O-;

B is selected from hydrogen, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-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 internucleotide linkage to an adjacent monomer, or a 5'-terminal group, such internucleotide linkage or 5'-terminal group optionally including the substituent R^5 or equally applicable the substituent R^{5*};

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

R^{4*} and R^{2*} together designate a bivalent linker group consisting of 1 - 4 groups/atoms selected from -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)$_2$-, -S-, -SO_2-, -

$N(R^a)$-, and >C=Z, wherein Z is selected from -O-, -S-, and -$N(R^a)$-, and R^a and R^b each is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, optionally substituted 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, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene ($=CH_2$), wherein for all chiral centers, asymmetric groups may be found in either *R* or *S* orientation, and;

each of the substituents R^{1*}, R^2, R^3, R^5, R^{5*}, R^6 and R^{6*}, which are present is 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, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where 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 C_{1-4}-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C_{1-4}-alkyl; and basic salts and acid addition salts thereof. 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 biradical consisting of a groups selected from the group consisting of $C(R^aR^b)$-$C(R^aR^b)$-, $C(R^aR^b)$-O-, $C(R^aR^b)$-NR^a-, $C(R^aR^b)$-S-, and $C(R^aR^b)$-$C(R^aR^b)$-O-, wherein each R^a and R^b may optionally be independently selected. In some embodiments, R^a and R^b may be, optionally independently

selected from the group consisting of hydrogen and $_{C1-6}$alkyl, such as methyl, such as hydrogen.

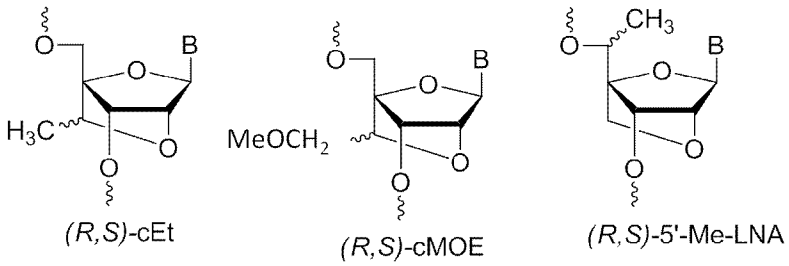
In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-$CH(CH_2OCH_3)$- (2'O-methoxyethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem) – in either the R- or S- configuration.

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-$CH(CH_2CH_3)$- (2'O-ethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem). – in either the R- or S- configuration.

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-$CH(CH_3)$-. – in either the R- or S- configuration.In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-CH_2-O-CH_2- - (Seth at al., 2010, J. Org. Chem).

In some embodiments, R^{4*} and R^{2*} together designate the biradical –O-NR-CH_3- - (Seth at al., 2010, J. Org. Chem) .

In some embodiments, the LNA units have a structure selected from the following group:



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

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

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

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

In some embodiments, R^5 and R^{5*} are each independently selected from the group consisting of H, $-CH_3$, $-CH_2-CH_3$,- CH_2-O-CH_3, and $-CH=CH_2$. Suitably in some embodiments, either R^5 or R^{5*} are hydrogen, where as the other group (R^5 or R^{5*} respectively) is selected from the group consisting of 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 or 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,J_2 or N(H)C(=X)N(H)J_2 wherein X is O or S; and each J_1 and J_2 is, independently, 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 or a protecting group. In some embodiments either R^5 or R^{5*} is substituted C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is substituted methylene wherein preferred substituent groups include one or more groups independently selected from F, NJ_1J_2, N_3, CN, OJ_1, SJ_1, O-C(=O)NJ_1J_2, N(H)C(=NH)NJ, J_2 or N(H)C(O)N(H)J_2. In some embodiments each J_1 and J_2 is, independently H or C_{1-6} alkyl. In some embodiments either R^5 or R^{5*} is methyl, ethyl or methoxymethyl. In some embodiments either R^5 or R^{5*} is methyl. In a further embodiment either R^5 or R^{5*} is ethylenyl. In some embodiments either R^5 or R^{5*} is substituted acyl. In some embodiments either R^5 or R^{5*} is $C(=O)NJ_1J_2$. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. 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, such as a nucleobase referred to herein, such as a nucleobase selected from the group consisting of 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 designate a biradical 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-, and -$C(R^aR^b)$-S-, -$C(R^aR^b)$-$C(R^cR^d)$-S-, wherein R^a, R^b, R^c, R^d, R^e, and R^f each is 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, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2). For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

In a further embodiment R^{4*} and R^{2*} together designate a biradical (bivalent group) selected from -CH_2-O-, -CH_2-S-, -CH_2-NH-, -CH_2-N(CH_3)-, -CH_2-CH_2-O-, -CH_2-CH(CH_3)-, -CH_2-CH_2-S-, -CH_2-CH_2-NH-, -CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-O-, -CH_2-CH_2-CH(CH_3)-, -CH=CH-CH_2-, -CH_2-O-CH_2-O-, -CH_2-NH-O-, -CH_2-N(CH_3)-O-, -CH_2-O-CH_2-, -CH(CH_3)-O-, and –CH(CH_2-O-CH_3)-O-, and/or, -CH_2-CH_2-, 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 designate the biradical C(R^aR^b)-N(R^c)-O-, wherein R^a and R^b are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen, and; wherein R^c is selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen.

In some embodiments, R^{4*} and R^{2*} together designate the biradical C(R^aR^b)-O-C(R^cR^d) -O-, wherein R^a, R^b, R^c, and R^d are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl, such as hydrogen.

In some embodiments, R^{4*} and R^{2*} form the biradical –CH(Z)-O-, wherein Z is selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, substituted C_{1-6} alkyl, substituted C_{2-6} alkenyl, substituted C_{2-6} alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio; and wherein each of the substituted groups, is, independently, mono or poly substituted with optionally protected substituent groups independently selected from halogen, oxo, hydroxyl, OJ_1, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$,

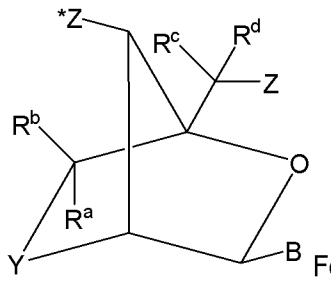
$OC(=X)NJ_1J_2$, $NJ^3C(=X)NJ_1J_2$ and CN, wherein each J_1, J_2 and J_3 is, independently, H or C_{1-6} alkyl, and X is O, S or NJ_1. In some embodiments Z is C_{1-6} alkyl or substituted C_{1-6} alkyl. In some embodiments Z is methyl. In some embodiments Z is substituted C_{1-6} alkyl. In some embodiments said substituent group is C_{1-6} alkoxy. In some embodiments Z is CH_3OCH_2-. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in US 7,399,845 which is hereby incorporated by reference in its entirety. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some some embodiments, R^{1*}, R^2, R^{3*} are hydrogen, and one or both of R^5, 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 designate a biradical which comprise a substituted amino group in the bridge such as consist or comprise of the biradical $–CH_2$-N(R^c)-, wherein R^c is C_{1-12} alkyloxy. In some embodiments R^{4*} and R^{2*} together designate a biradical $–Cq_3q_4$-NOR -, wherein q_3 and q_4 are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or 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, O-C(=O)NJ_1J_2, N(H)C(=NH)N J_1J_2 or N(H)C(=X=N(H)J_2 wherein X is O or S; and each of J_1 and J_2 is, independently, H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl or a protecting group. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. 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*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some embodiments, R^{1*}, R^2, R^3 are hydrogen and one or both of R^5, 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 designate a biradical (bivalent group) C(R^aR^b)-O-, wherein R^a and R^b are each independently halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{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, O-C(=O)NJ_1J_2, N(H)C(=NH)NJ_1J_2, N(H)C(=O)NJ_1J_2 or N(H)C(=S)NJ_1J_2; or R^a and R^b together are =C(q3)(q4); q_3 and q_4 are each, independently, H, halogen, C_1-C_{12}alkyl or substituted C_1-C_{12} alkyl; each substituted group is, independently, mono or poly substituted with substituent groups independently selected from halogen, C_1-

C_6 alkyl, substituted C_1-C_6 alkyl, C_2- C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, OJ_1, SJ_1, NJ_1J_2, N_3, CN, $C(=O)OJ_1$, $C(=O)NJ_1J_2$, $C(=O)J_1$, O-$C(=O)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$. and; each J_1 and J_2 is, independently, H, C1-C_6 alkyl, substituted C1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl, C1-C_6 aminoalkyl, substituted C1-C_6 aminoalkyl or a protecting group. Such compounds are disclosed in WO2009006478A, hereby incorporated in its entirety by reference.

In some embodiments, R^{4*} and R^{2*} form the biradical - Q -, wherein Q is $C(q_1)(q_2)C(q_3)(q_4)$, $C(q_1)=C(q_3)$, $C[=C(q_1)(q_2)]$-$C(q_3)(q_4)$ or $C(q_1)(q_2)$-$C[=C(q_3)(q_4)]$; q_1, q_2, q_3, q_4 are each independently. 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, -$C(=O)NJ_1J_2$, $N(H)C(=NH)NJ_1J_2$, $N(H)C(=O)NJ_1J_2$ or $N(H)C(=S)NJ_1J_2$; each J_1 and J_2 is, independently, H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} aminoalkyl or 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 hydrogen. For all chiral centers, asymmetric groups may be found in either *R* or *S* orientation. Such bicyclic nucleotides are disclosed in WO2008/154401 which is hereby incorporated by reference in its entirity. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are independently selected from the group consisting of hydrogen, halogen, C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{2-6} alkenyl, substituted C_{2-6} alkenyl, C_{2-6} alkynyl or substituted C_{2-6} alkynyl, C_{1-6} alkoxyl, substituted C_{1-6} alkoxyl, acyl, substituted acyl, C_{1-6} aminoalkyl or substituted C_{1-6} aminoalkyl. In some embodiments, R^{1*}, R^2, R^3, R^5, R^{5*} are hydrogen. In some embodiments, R^{1*}, R^2, R^3 are 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).

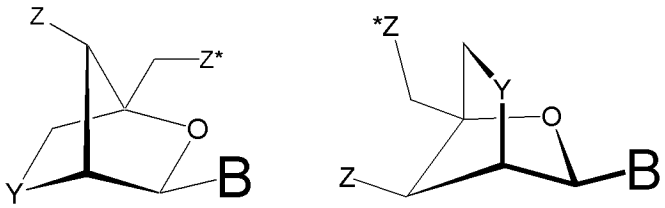
In some embodiments the LNA used in the oligonucleotide compounds of the invention preferably has the structure of the general formula II:



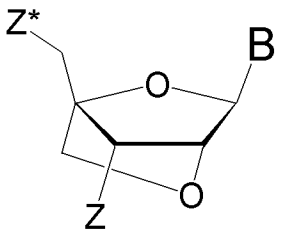
Formula II

wherein Y is selected from the group consisting of -O-, -CH_2O-, -S-, -NH-, $N(R^e)$ and/or –CH_2-; Z and Z* are independently selected among an internucleotide linkage, R^H, a terminal group or a protecting group; B constitutes a natural or non-natural nucleotide base

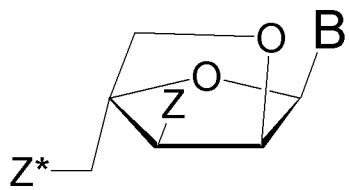
moiety (nucleobase), and R^H is selected from hydrogen and C_{1-4}-alkyl; R^a, R^b R^c, R^d and R^e are, optionally independently, selected from the group consisting of hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, 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, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2); and R^H is selected from hydrogen and C_{1-4}-alkyl. In some embodiments R^a, R^b R^c, R^d and R^e are, optionally independently, selected from the group consisting of hydrogen and C_{1-6} alkyl, such as 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:



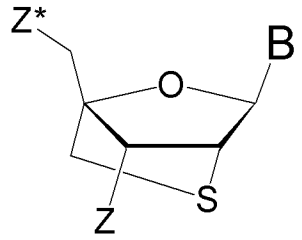
Specific exemplary LNA units are shown below:



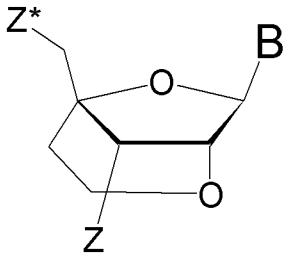
β-D-oxy-LNA



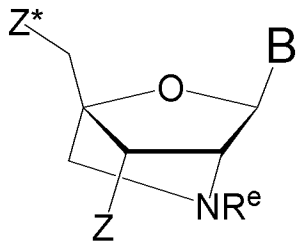
α-L-Oxy-LNA



β-D-thio-LNA



β-D-ENA



β-D-amino-LNA

The term "thio-LNA" comprises a locked nucleotide in which Y in the general formula above is selected from S or -CH_2-S-. Thio-LNA can be in both beta-D and alpha-L-configuration.

The term "amino-LNA" comprises a locked nucleotide in which Y in the general formula above is selected from -N(H)-, N(R)-, CH_2-N(H)-, and -CH_2-N(R)- where R is selected from hydrogen and C_{1-4}-alkyl. Amino-LNA can be in both beta-D and alpha-L-configuration.

The term "oxy-LNA" comprises a locked nucleotide 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 nucleotide in which Y in the general formula above is -CH_2-O- (where the oxygen atom of –CH_2-O- is attached to the 2'-position relative to the base B). R^e is hydrogen or methyl.

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 recruitment

It is recognised that an oligomeric compound may function via non RNase mediated degradation of target mRNA, such as by steric hindrance of translation, or other methods, however, the preferred oligomers of the invention are capable of recruiting an endoribonuclease (RNase), such as RNase H.

It is preferable that the oligomer, or contiguous nucleotide sequence, comprises of a region of at least 6, such as at least 7 consecutive nucleotide units, such as at least 8 or at least 9 consecutive nucleotide units (residues), including 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 consecutive nucleotides, which, when formed in a duplex with the complementary target

RNA is capable of recruiting RNase. The contiguous sequence which 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 size of the contiguous sequence which is capable of recruiting RNAse, such as region B, may be higher, such as 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotide units.

EP 1 222 309 provides *in vitro* methods for determining RNaseH activity, which may be used to determine the ability to recruit RNaseH. A oligomer is deemed capable of recruiting RNase H if, when provided with the complementary 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 of the initial rate determined using DNA only 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 Example 91 - 95 of EP 1 222 309.

In some embodiments, an oligomer is deemed essentially incapable of recruiting RNaseH if, when provided with the complementary 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 the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Example 91 - 95 of EP 1 222 309.

In other embodiments, an oligomer is deemed capable of recruiting RNaseH if, when provided with the complementary 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 the equivalent DNA only oligonucleotide, with no 2' substitutions, with phosphorothioate linkage groups between all nucleotides in the oligonucleotide, using the methodology provided by Example 91 - 95 of EP 1 222 309.

Typically the region of the oligomer which forms the consecutive nucleotide units which, when formed in a duplex with the complementary target RNA is capable of recruiting RNase consists of nucleotide units which form a DNA/RNA like duplex with the RNA target – and include both DNA units and LNA units which are in the alpha-L configuration, particularly preferred being alpha-L-oxy LNA.

The oligomer of the invention may comprise a nucleotide sequence which comprises both nucleotides and nucleotide analogues, and may be 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 X 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.

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 α-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 α-L-LNA monomers consist of fewer monomers recognizable and cleavable by the RNaseH, and more flexibility in the mixmer construction is introduced. In some oligomer embodiments, LNA monomers alternate with other nucleoside analogs such as DNA units.

Gapmer Design

Preferably, the oligomer of the invention is a gapmer. A gapmer oligomer is an oligomer which comprises a contiguous stretch of nucleotides which is capable of recruiting an RNAse, such as RNAseH, such as a region of at least 6 or 7 DNA nucleotides, referred to herein as region B (B), wherein region B is flanked both 5' and 3' by regions of affinity enhancing nucleotide analogues, such as from 1 – 6 nucleotide analogues 5' and 3' to the contiguous stretch of nucleotides which is capable of recruiting RNAse – these regions are referred to as regions A (A) and C (C) respectively.

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 (unlinked nucleic acid) nucleotides (see Fluiter *et al.*, Mol. Biosyst., 2009, 10, 1039 hereby incorporated by reference). UNA is unlocked nucleic acid, typically where the C2 – C3 C-C bond of the ribose has been removed, forming an unlocked "sugar" residue. Preferably the gapmer comprises a (poly)nucleotide sequence of formula (5' to 3'), A-B-C, or optionally A-B-C-D or

D-A-B-C, wherein; region A (A) (5' region) consists or comprises of at least one nucleotide analogue, such as at least one LNA unit, such as from 1-6 nucleotide analogues, such as LNA units, and; region B (B) consists or comprises of at least five consecutive nucleotides which are capable of recruiting RNAse (when formed in a duplex with a complementary RNA molecule, such as the mRNA target), such as DNA nucleotides, and; region C (C) (3'region) consists or comprises of at least one nucleotide analogue, such as at least one LNA unit, such as from 1-6 nucleotide analogues, such as LNA units, and; region D (D), when present consists or comprises of 1, 2 or 3 nucleotide units, such as DNA nucleotides.

In some embodiments, region A consists of 1, 2, 3, 4, 5 or 6 nucleotide analogues, such as LNA units, such as from 2-5 nucleotide analogues, such as 2-5 LNA units, such as 3 or 4 nucleotide analogues, such as 3 or 4 LNA units; and/or region C consists of 1, 2, 3, 4, 5 or 6 nucleotide analogues, such as LNA units, such as from 2-5 nucleotide analogues, such as 2-5 LNA units, such as 3 or 4 nucleotide analogues, such as 3 or 4 LNA units.

In some embodiments B consists or comprises of 5, 6, 7, 8, 9, 10, 11 or 12 consecutive nucleotides which are capable of recruiting RNAse, or from 6-10, or from 7-9, such as 8 consecutive nucleotides which are capable of recruiting RNAse. In some embodiments region B consists or comprises at least one DNA nucleotide unit, such as 1-12 DNA units, preferably from 4-12 DNA units, more preferably from 6-10 DNA units, such as from 7-10 DNA units, most preferably 8, 9 or 10 DNA units.

In some embodiments region A consist of 3 or 4 nucleotide analogues, such as LNA, region B consists of 7, 8, 9 or 10 DNA units, and region C consists of 3 or 4 nucleotide analogues, such as LNA. 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 further include region D, which may have one or 2 nucleotide units, such as DNA units.

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 embodiments, oligomers presented here may be such shortmer gapmers.

In some embodiments the oligomer is consisting of a contiguous nucleotide sequence of a total of 10, 11, 12, 13 or 14 nucleotide units, wherein the contiguous nucleotide sequence is of formula (5' – 3'), A-B-C, or optionally A-B-C-D or D-A-B-C, wherein; A consists of 1, 2 or 3 nucleotide analogue units, such as LNA units; B consists of 7, 8 or 9 contiguous nucleotide units which are capable of recruiting RNAse when formed in a duplex with a complementary RNA molecule (such as a mRNA target); and C consists of

1, 2 or 3 nucleotide analogue units, such as LNA units. When present, D consists of a single DNA unit.

In some embodiments A consists of 1 LNA unit. In some embodiments A consists of 2 LNA units. In some embodiments A consists of 3 LNA units. In some embodiments C consists of 1 LNA unit. In some embodiments C consists of 2 LNA units. In some embodiments C consists of 3 LNA units. In some embodiments B consists of 7 nucleotide units. In some embodiments B consists of 8 nucleotide units. In some embodiments B consists of 9 nucleotide units. In certain embodiments, region B consists of 10 nucleoside monomers. In certain embodiments, region B comprises 1 – 10 DNA monomers. In some embodiments B comprises of from 1 to about 9 DNA units, such as 2, 3, 4, 5, 6, 7 , 8 or 9 DNA units. In some embodiments B consists of DNA units. In some embodiments B comprises of at least one LNA unit which is in the alpha-L configuration, such as 2, 3, 4, 5, 6, 7, 8 or 9 LNA units in the alpha-L-configuration. In some embodiments B comprises of at least one alpha-L-oxy LNA unit or wherein all the LNA units in the alpha-L- configuration are alpha-L-oxy LNA units. In some embodiments the number of nucleotides present in A-B-C are selected from the group consisting of (nucleotide analogue units – region B – nucleotide analogue units): 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, 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. In some embodiments the number of nucleotides in A-B-C are 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 some embodiments both A and C consists of two LNA units each, and B consists of 8 or 9 nucleotide units, preferably DNA units. 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.

Internucleotide 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" or “internucleotide linkage” are intended to mean a group capable of covalently coupling together two nucleotides. Specific and preferred examples include phosphate groups and phosphorothioate groups.

The nucleotides of the oligomer of the invention or contiguous nucleotides sequence thereof are coupled together via linkage groups. Suitably each nucleotide is linked to the 3’ adjacent nucleotide via a linkage group.

Suitable internucleotide linkages include those listed within WO2007/031091, for example the internucleotide linkages listed on the first paragraph of page 34 of WO2007/031091 (hereby incorporated by reference).

It is, in some embodiments, preferred to modify the internucleotide linkage 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, also allow that route of antisense inhibition in reducing the expression of the target gene.

Suitable sulphur (S) containing internucleotide linkages as provided herein may be preferred. Phosphorothioate internucleotide linkages are also preferred, particularly for the gap region (B) of gapmers. Phosphorothioate linkages may also be used for the flanking regions (A and C, and for linking A or C to D, and within region D, as appropriate).

Regions A, B and C, may however comprise internucleotide linkages other than phosphorothioate, such as phosphodiester linkages, particularly, for instance when the use of nucleotide analogues protects the internucleotide linkages within regions A and C from endo-nuclease degradation – such as when regions A and C comprise LNA nucleotides.

The internucleotide linkages in the oligomer may be phosphodiester, phosphorothioate or boranophosphate so as to allow RNase H cleavage of targeted RNA. Phosphorothioate is preferred, for improved nuclease resistance and other reasons, such as ease of manufacture.

In one aspect of the oligomer of the invention, the nucleotides and/or nucleotide analogues 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 otherwise phosphorothioate oligomer, particularly between or adjacent to nucleotide analogue units (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 internucleotide 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 nucleotide analogues, such as LNA, units. Likewise, when referring to specific gapmer oligonucleotide sequences, such as those provided herein, when the C (cytosine) residues are annotated as 5'methyl modified cytosine, in various embodiments, one or more of the Cs present in the oligomer may be unmodified C residues.

Oligomeric Compounds

The oligomers of the invention may, for example, have a sequence selected from the group consisting of SEQ ID NOs 1-15 as shown in Table 1 and SEQ ID NOs 16-43 as shown in Table 4, or a sequence which is a subset of one of the foregoing. In one embodiment, the oligomers are 16mers in which region A consists of three LNA units, preferably β-D-oxy-LNA units, region B consists of 10 units which are DNA units, and region C consists of three LNA units, preferably β-D-oxy-LNA units, in which all LNA cytosines are 5-methylcytosine, and all linkages are phosphorothioate linkages. In another embodiment, the oligomers are 13mers in which region A consists of two LNA units, preferably β-D-oxy-LNA units, region B consists of 8 units which are DNA units, and region C consists of three LNA units, preferably β-D-oxy-LNA units, in which all LNA cytosines are 5-methylcytosine, and all linkages are phosphorothioate linkages. Other embodiments are shown in Tables 1 and 4.

Conjugates

In the context of this disclosure, the term "conjugate" is intended to indicate a heterogeneous molecule formed by the covalent attachment ("conjugation") of the oligomer as described herein to one or more non-nucleotide, or non-polynucleotide moieties. Examples of non-nucleotide or non- polynucleotide moieties include macromolecular agents such as 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.

Therefore, in various embodiments, the oligomer of the invention may comprise both a polynucleotide region which typically consists of a contiguous sequence of nucleotides, and a further non-nucleotide region. When referring to the oligomer of the invention consisting of a contiguous nucleotide sequence, the compound may comprise non-nucleotide components, such as a conjugate component.

In various embodiments of the invention the oligomeric compound is linked to ligands/conjugates, which may be used, e.g. to increase the cellular uptake of oligomeric compounds. WO2007/031091 provides suitable ligands and conjugates, which are hereby incorporated by reference.

The invention also provides for a conjugate comprising the compound according to the invention as herein described, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to said compound. Therefore, in various embodiments where the compound of the invention consists of a specified nucleic acid or nucleotide sequence, as herein disclosed, the compound may also comprise at least one non-nucleotide or non-polynucleotide moiety (e.g. not comprising one or more nucleotides or nucleotide analogues) covalently attached to said compound.

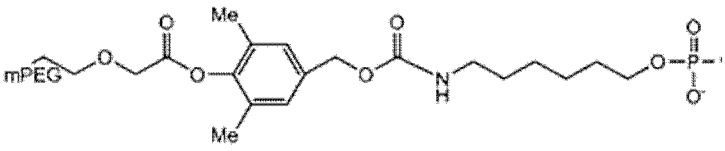
Conjugation (to a conjugate moiety) may enhance the activity, cellular distribution or cellular uptake of the oligomer of the invention. Such moieties include, but are not limited to, antibodies, polypeptides, lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g. hexyl-s-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipids, 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.

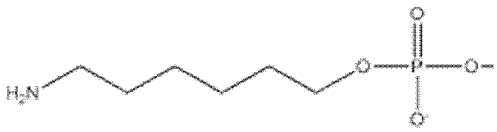
The oligomers of the invention may also be conjugated to active drug substances, for example, aspirin, ibuprofen, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

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

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

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





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. 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 NH_2 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 NH_2 group. In other 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 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 $(CH_2)_w$, 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 (-O-C(O)-$(CH_2)_w$NH).

In other embodiments, the oligomers are functionalized with a hindered ester containing a $(CH_2)_w$-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 (-O-C(O)-$(CH_2)_w$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.

In still other embodiments, the oligomers of the invention are functionalized by 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 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 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.

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 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-diisopropyl-cyanoethoxy phosphoramidite. See, e.g., Manoharan, et al., Tetrahedron Letters, 1991, 34, 7171.

In still further embodiments, the oligomers of the invention may 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, 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 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

The oligomer of the invention may be used in pharmaceutical formulations and compositions. Suitably, such compositions comprise a pharmaceutically acceptable diluent, carrier, salt or adjuvant. WO/2007/031091 provides suitable and preferred pharmaceutically acceptable diluent, carrier and adjuvants - which are hereby incorporated by reference. Suitable dosages, formulations, administration routes, compositions, dosage forms, combinations with other therapeutic agents, pro-drug formulations are also provided in WO/2007/031091- which is hereby incorporated by reference.

Applications

The oligomers of the invention may be utilized as research reagents for, for example, diagnostics, therapeutics and prophylaxis.

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

In diagnostics the oligomers may be used to detect and quantitate osteopontin expression in cell and tissues by northern blotting, *in-situ* hybridisation or similar techniques.

For therapeutics, an animal or a human, suspected of having a disease or disorder, which can be treated by modulating the expression of osteopontin is treated by administering oligomeric compounds 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 osteopontin by administering a therapeutically or prophylactically effective amount of one or more of the oligomers or compositions of the invention. The oligomer, a conjugate or a pharmaceutical composition according to the invention is typically administered in an effective amount.

The invention also provides for the use of the compound or conjugate 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 as a disorder as referred to herein.

The invention also provides for a method for treating a disorder as referred to herein said method comprising administering a compound 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 a patient in need thereof.

Medical Indications

The oligomers and other compositions according to the invention can be used for the treatment of conditions associated with overexpression, undesired or abnormal levels (particularly high levels as might be due to overaccumulation) or expression of a mutated version of the osteopontin.

The invention further provides use of a compound of the invention in the manufacture of a medicament for the treatment of a disease, disorder or condition as referred to herein.

Generally stated, one aspect of the invention is directed to a method of treating a mammal suffering from or susceptible to conditions associated with abnormal levels of osteopontin, comprising administering to the mammal and therapeutically effective amount of an oligomer targeted to osteopontin that comprises one or more LNA units. The oligomer,

a conjugate or a pharmaceutical composition according to the invention is typically administered in an effective amount.

The disease or disorder, as referred to herein, may, in some embodiments, be associated with a mutation in the osteopontin gene or a gene whose protein product is associated with or interacts with osteopontin. Therefore, in some embodiments, the target mRNA is a mutated form of the osteopontin sequence.

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 disease, disorder or condition as referred to herein.

The methods of the invention are preferably employed for treatment or prophylaxis against diseases caused by abnormal or undesired levels of osteopontin.

Alternatively stated, in some embodiments, the invention is furthermore directed to a method for treating abnormal or undesired levels of osteopontin, e.g., higher than desired levels of osteopontin, said method comprising administering a oligomer of the invention, or a conjugate of the invention or a pharmaceutical composition of the invention to a patient in need thereof.

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 a compound, composition, or a conjugate as defined herein for the manufacture of a medicament for the treatment of abnormal or undesired levels of osteopontin or expression of mutant forms of osteopontin (such as allelic variants, such as those associated with one of the diseases referred to herein).

Moreover, the invention relates to a method of treating a subject suffering from a disease or condition such as those referred to herein.

A patient who is in need of treatment is a patient suffering from or likely to suffer from the disease or disorder.

In some embodiments, the term 'treatment' as used herein refers to both treatment of an existing disease (*e.g.* a disease or disorder as herein referred to), or prevention of a disease, *i.e.* prophylaxis. It will therefore be recognised that treatment as referred to herein may, in some embodiments, be prophylactic.

In particular, osteopontin is believed to be a therapeutic target for a range of medical disorders, including ischemia/reperfusion injury, such as may occur after organ transplantation, including kidney (renal) transplantation as well as fibrosis in NASH and other fibrotic liver diseases.

EMBODIMENTS

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

Embodiment 1 - An oligomer of from 10 to 30 nucleotides in length which comprises a contiguous nucleobase sequence of from 10 to 30 nucleobases in length, wherein said contiguous nucleobase sequence is at least 80% homologous to a region corresponding to a mammalian osteopontin gene or the reverse complement of a mammalian osteopontin mRNA, and which inhibits mammalian osteopontin expression.

Embodiment 2 - The oligomer according to embodiment 1, wherein the mammalian osteopontin mRNA is NM_001040058, NM_000582, NM_001040060 or naturally occurring variant thereof.

Embodiment 3 - The oligomer according to embodiment 1 wherein the contiguous nucleobase sequence is at least 80% homologous to a region corresponding to a base sequence of any of SEQ ID NO: 1-43.

Embodiment 4 - The oligomer according to embodiment 1 wherein the contiguous nucleobase sequence comprises zero, one or two mismatches as compared to the corresponding region of a base sequence of any of SEQ ID NO: 1-43.

Embodiment 5 - The oligomer according to embodiment 1 wherein the nucleotide sequence of the oligomer consists of the contiguous nucleotide sequence.

Embodiment 6 - The oligomer according to embodiment 1 wherein the contiguous nucleotide sequence is from 10 to 18 nucleotides in length.

Embodiment 7 - The oligomer according to embodiment 1 wherein the contiguous nucleotide sequence comprises at least one nucleotide analogue.

Embodiment 8 - The oligomer according to embodiment 6 wherein the nucleotide analogue is a sugar-modified nucleotide.

Embodiment 9 - The oligomer according to embodiment 6, wherein each sugar-modified nucleotide is independently 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.

Embodiment 10 - The oligomer according to embodiment 6, wherein the sugar-modified nucleotide is LNA.

Embodiment 11 - The oligomer according to any one of embodiments 6 – 8 which is a gapmer.

Embodiment 12 - The oligomer according to any one of embodiments 1-11, wherein the oligomer is any one of SEQ ID NOs: 1-43.

Embodiment 13 - The oligomer according to any one of embodiments 1 – 12, which inhibits the expression of osteopontin protein or mRNA in a cell which is expressing osteopontin protein or mRNA.

Embodiment 14 - A conjugate comprising the oligomer according to any one of embodiments 1 – 13, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to said oligomer.

Embodiment 15 - A pharmaceutical composition comprising the oligomer according to any one of embodiments 1 – 13, or the conjugate according to embodiment 14, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

Embodiment 16 - The oligomer according to any one of embodiments 1 – 13, or the conjugate according to embodiment 14, for use as a medicament, such as for the treatment of ischemia/reperfusion injury.

Embodiment 17 - The oligomer of embodiment 16 wherein the ischemia/reperfusion injury is ischemia/reperfusion injury to the kidney.

Embodiment 18 - The oligomer according to any one of embodiments 1-13, or a conjugate according to embodiment 14, for use in the treatment of ischemia/reperfusion injury.

Embodiment 19 - The oligomer according to any one of embodiments 1-13, or a conjugate according to embodiment 14, for use in the treatment of fibrotic liver diseases.

Embodiment 20 - A method of treating ischemia/reperfusion injury, said method comprising administering an effective amount of an oligomer according to any one of the embodiments 1-13, or a conjugate according to embodiment 14, or a pharmaceutical composition according to embodiment 15, to a patient suffering from, or likely to suffer from ischemia/reperfusion injury.

Embodiment 21 - A method of treating fibrotic liver diseases, said method comprising administering an effective amount of an oligomer according to any one of the embodiments 1-13, or a conjugate according to embodiment 14, or a pharmaceutical composition according to embodiment 15, to a patient suffering from, or likely to suffer from fibrotic liver diseases.

Embodiment 22 - A method for decreasing osteopontin levels in a cell, said method comprising administering an oligomer according to any one of the embodiments 1-13, or a conjugate according to embodiment 14 to said cell so as to decrease levels of osteopontin in said cell.

EXAMPLES

Example 1: Design of oligonucleotides –first library

Osteopontin is a glycoprotein with a relatively short transcript of around 1.6 kb and is a multifunctional cytokine that serves as a potent chemoattractant for mononuclear cells that are associated with various immunological responses. In the human, three different splice variants have been identified: osteopontin-a (OPN-a) mRNA contains all seven exons, osteopontin-b (OPN-b) mRNA lacks exon 5, and osteopontin-c (OPN-c) mRNA lacks exon 4. Preliminary examination of different transcript variants in human renal proximal tubular epithelial cells (RPTEC) revealed that the predominant form expressed is osteopontin-a, accounting for approximately 82%; whereas osteopontin-b and osteopontin-c are expressed at low level, 16% and 2%, respectively. Furthermore, it is suggested that osteopontin-b and osteopontin-c are commonly expressed in a variety of tumor cells, but not in normal tissues, or in benign tumors. This has an implication for the oligonucleotide design, as it opens up more possibility for designing oligonucleotides targeting only the full length transcript.

In accordance with the present invention, a series of fifteen oligonucleotides were designed to target osteopontin mRNA across different species including mouse, rat, monkey and human (three different transcript variants: GenBank accession numbers NM_001040058, NM_000582, NM_001040060)). GenBank accession No. NM_001040058 (osteopontin variant 1) represents the longest transcript and encodes the longest isoform (osteopontin-a). GenBank accession no. NM000582 (osteopontin variant 2) lacks exon 5 compared to variant 1. The resulting isoform (osteopontin-b) has the same N- and C-termini but is shorter compared to osteopontin-a. GenBank accession no. NM_001040060 (osteopontin variant 3) lacks exon 4 compared to variant 1. The resulting isoform (osteopontin-c) has the same N- and C-termini but is shorter compared to osteopontin-a.

The first library of 15 oligonucleotides was designed as LNA gapmers, with oligonucleotide length from 12 to 16 nucleobases. The oligonucleotides can be mapped into five target regions across the entire transcript. In Table 1, upper case letters indicate LNA, in this case Beta-D-oxy LNA units. Lower case letters represent DNA units. In Table 1, all internucleoside linkages are phosphorothioate linkages and all LNA-cytosines (uppercase) are 5-methylcytosines.

Table 1 Antisense oligonucleotide sequences of the invention

SEQ ID NO	Sequence and modifications (5'-3')	Base sequence	Length (bases)	Target site on NM_001040058
SEQ ID NO: 1	**AC**ctcagtcc**AT**	ACCTCAGTCCAT	12	659-670
SEQ ID NO: 2	**GAC**ctcagtcc**ATA**	GACCTCAGTCCATA	14	658-671
SEQ ID NO: 3	**TGA**cctcagtcca**TAA**	TGACCTCAGTCCATAA	16	657-672
SEQ ID NO: 4	**GA**cctcagtcca**TA**	GACCTCAGTCCATA	14	658-671
SEQ ID NO: 5	**TG**acctcagtccat**AA**	TGACCTCAGTCCATAA	16	657-672

SEQ ID NO: 6	**AA**gcaaatcac**TG**	AAGCAAATCACTG	13	176-188
SEQ ID NO: 7	**AA**gcaaatcact**GC**	AAGCAAATCACTGC	14	175-188
SEQ ID NO: 8	**AAG**caaatcac**TGC**	AAGCAAATCACTGC	14	175-188
SEQ ID NO: 9	**AA**gatttattcaca**CC**	AAGATTTATTCACACC	16	1512-1527
SEQ ID NO: 10	**AA**gatttattca**CA**	AAGATTTATTCACA	14	1514-1527
SEQ ID NO: 11	**AAG**atttattcac**ACC**	AAGATTTATTCACACC	16	1512-1527
SEQ ID NO: 12	**AAG**atttattc**ACA**	AAGATTTATTCACA	14	1514-1527
SEQ ID NO: 13	**CT**aaatgcaaa**GT**	CTAAATGCAAAGT	13	1134-1146
SEQ ID NO: 14	**GA**cctcagaaga**TG**	GACCTCAGAAGATG	14	1091-1104
SEQ ID NO: 15	**GAC**ctcagaag**ATG**	GACCTCAGAAGATG	14	1091-1104

Example 2: 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. 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% CO_2. Cells were routinely passaged 2-3 times weekly.

A549: The human lung carcinoma cell line A549 was cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma) + 10% fetal bovine serum (FBS) + 2 mM Glutamax I + gentamicin (25µg/ml).

Human renal proximal tubular epithelial cell RPTEC: The cell line human RPTEC was cultured in Renal Epithelial Growth Media REGM™ Bullet kit (CC-3190 from Lonza).

Human renal cortical epithelial cells HRCE: The cell line human HRCE was cultured in Renal Epithelial Growth Media REGM™ Bullet kit (CC-3190 from Lonza).

Example 3: In vitro model: Treatment with antisense oligonucleotide using lipid transfection

The cell line, A549, listed in Example 2 was treated with 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 80-90% confluent. Oligo concentrations used ranged from 1 nM to 25 nM final concentration. Formulation of oligo-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 oligo-

containing culture medium. Cells were washed and serum-containing media was added. After oligo treatment cells were allowed to recover for 20 hours before they were harvested for RNA analysis. Results are given in Example 7 below (Table 2).

Example 4: In vitro model: Natural uptake of antisense oligonucleotide

An oligomer delivery method (called natural uptake or 'gymnosis') has been developed that does not require the use of any transfection reagent or any additives to serum whatsoever, but rather takes advantage of the normal growth properties of cells in tissue culture. This method permits the sequence-specific silencing of targets in tissue culture, both at the protein and mRNA level, at oligomer concentrations in the low micromolar range. Optimum results were obtained with locked nucleic acid (LNA) phosphorothioate gapmers. Particularly noteworthy is the observation that the pattern of gene silencing of oligonucleotides delivered in vitro using this gymnosis method correlates particularly well with in vivo silencing. Stein et al. (2010) Nucl. Acids Res. 38: e3, doi:10.1093/nar/gkp841, published online 23 October 2009.

The cell line, human RPTEC, listed in Example 2 was incubated with oligo dissolved in sterile water without any transfection vehicle. Cells were seeded in 6-well cell culture plates (NUNC) and incubated with oligo when 10-30% confluent. Oligo concentrations used ranged from 1µM to 25µM, final concentration. Cells were incubated at 37°C in the oligo containing normal growth serum for 6 days before they were harvested for RNA analysis.

Example 5: In vitro model: Extraction of RNA and cDNA synthesis

Total RNA Isolation and First strand synthesis

Total RNA was extracted from cells transfected as described above and using the Qiagen RNeasy kit (Qiagen cat. no. 74104) according to the manufacturer's instructions. First strand synthesis was performed using Reverse Transcriptase reagents from Ambion according to the manufacturer's instructions.

For each sample 0.25-0.5 µg total RNA was adjusted to (10.8 µl) with RNase free H_2O and mixed with 2 µl random decamers (50 µM) and 4 µl 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 µl 10x Buffer RT, 1 µl MMLV Reverse Transcriptase (100 U/µl) and 0.25 µl RNase inhibitor (10 U/µl) 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 6: In vitro model: Analysis of Oligonucleotide Inhibition of Osteopontin Expression by Real-time PCR

Antisense modulation of osteopontin expression can be assayed in a variety of ways known in the art. For example, osteopontin 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 Osteopontin mRNA Levels

The sample content of human osteopontin mRNA was quantified using the human osteopontin ABI Prism Pre-Developed TaqMan Assay Reagents (Applied Biosystems cat. no. Hs00960942_m1 according to the manufacturer's instructions.

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.

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: The cDNA from the first strand synthesis performed as described in Example 5 was diluted 2-20 times, and analyzed by real time quantitative PCR using Taqman 7500 FAST or 7900 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 μl cDNA to a final volume of 10 μl. Each sample was analysed in duplicate. Assaying 2 fold dilutions of a cDNA that had been prepared on material purified from a cell line expressing the RNA of interest generated standard curves for the assays. Sterile H_2O was used instead of cDNA for the no template control. PCR program: 60° C for 2 minutes, then 95° C for 30 seconds, followed by 40 cycles of 95° C, 3 seconds, 60° C, 20-30 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. or SDS Software Version 2.3.

Example 7: In vitro analysis: Antisense Inhibition of Human Osteopontin Expression by oligonucleotide compounds

Oligonucleotides presented in Table 1 were evaluated in the A549 cell line for their potential to knock down osteopontin expression at concentrations of 1, 5, and 25 nM using lipid transfection (see Figure 1 and Table 2).

Table 2: Antisense Inhibition of Human Osteopontin expression by oligonucleotides.

The data in Table 2 are presented as percentage down-regulation relative to mock transfected cells at 5 nM in the A549 cells. Lower case letters represent DNA units, bold upper case letters represent, LNA preferably β-D-oxy-LNA units. All LNA C are preferably 5'methyl C. Subscript "s" represents phosphorothioate linkage.

SEQ ID NO:	Sequence (5'-3')	Percent inhibition of OPN in A549 cells at 5 nM
1	$\mathbf{A_s}\mathbf{C_s}c_st_sc_sa_sg_st_sc_sc_s\mathbf{A_s}\mathbf{T_s}$	57
2	$\mathbf{G_s}\mathbf{A_s}\mathbf{C_s}c_st_sc_sa_sg_st_sc_sc_s\mathbf{A_s}\mathbf{T_s}\mathbf{A}$	98
3	$\mathbf{T_s}\mathbf{G_s}\mathbf{A_s}c_sc_st_sc_sa_sg_st_sc_sc_sa_s\mathbf{A_s}\mathbf{T_s}\mathbf{A}$	95
4	$\mathbf{G_s}\mathbf{A_s}c_sc_st_sc_sa_sg_st_sc_sc_sa_s\mathbf{T_s}\mathbf{A}$	90
5	$\mathbf{T_s}\mathbf{G_s}a_sc_sc_st_sc_sa_sg_st_sc_sc_sa_st_s\mathbf{A_s}\mathbf{A}$	85
6	$\mathbf{A_s}\mathbf{A_s}g_sc_sa_sa_sa_st_sc_sa_sc_s\mathbf{T_s}\mathbf{G}$	66
7	$\mathbf{A_s}\mathbf{A_s}g_sc_sa_sa_sa_st_sc_sa_sc_st_s\mathbf{G_s}\mathbf{C}$	90
8	$\mathbf{A_s}\mathbf{A_s}\mathbf{G_s}c_sa_sa_sa_st_sc_sa_sc_s\mathbf{T_s}\mathbf{G_s}\mathbf{C}$	94
9	$\mathbf{A_s}\mathbf{A_s}g_sa_st_st_st_sa_st_st_sc_sa_sc_sa_s\mathbf{C_s}\mathbf{C}$	77
10	$\mathbf{A_s}\mathbf{A_s}g_sa_st_st_st_sa_st_st_sc_sa_s\mathbf{C_s}\mathbf{A}$	7
11	$\mathbf{A_s}\mathbf{A_s}\mathbf{G_s}a_st_st_st_sa_st_st_sc_sa_sc_s\mathbf{A_s}\mathbf{C_s}\mathbf{C}$	77
12	$\mathbf{A_s}\mathbf{A_s}\mathbf{G_s}a_st_st_st_sa_st_st_sc_s\mathbf{A_s}\mathbf{C_s}\mathbf{A}$	75
13	$\mathbf{C_s}\mathbf{T_s}a_sa_sa_st_sg_sc_sa_sa_sa_s\mathbf{G_s}\mathbf{T}$	74
14	$\mathbf{G_s}\mathbf{A_s}c_sc_st_sc_sa_sg_sa_sa_sg_sa_s\mathbf{T_s}\mathbf{G}$	76
15	$\mathbf{G_s}\mathbf{A_s}\mathbf{C_s}c_st_sc_sa_sg_sa_sa_sg_s\mathbf{A_s}\mathbf{T_s}\mathbf{G}$	87

As shown in Figure 1 and Table 2, oligonucleotides of SEQ ID NOs: 2, 3, 4, 5, 7, 8, 9, 11, 12, 13, 14 and 15 demonstrated about 70% or greater inhibition of osteopontin expression in these experiments and are therefore preferred.

Also preferred are oligonucleotides based on the illustrated antisense oligo 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 osteopontin expression, preferably at least 90% inhibition.

Example 8: In vitro analysis: Antisense Inhibition of Human Osteopontin Expression by oligonucleotide compounds

Oligonucleotides presented in Table 1 were evaluated in the human RPTEC cell line for their potential to knock down osteopontin at the concentration of 20 μM using natural uptake (gymnosis) without any transfection vehicle (see Figure 2 and Table 3).

The data in Table 3 and Figure 2 are presented as percentage down-regulation relative to mock treated cells at 20μM in the human RPTEC cells. Lower case letters represent DNA units, bold upper case letters represent β-D-oxy-LNA units. All LNA C are preferably 5'methyl C. Subscript "s" represents phosphorothioate linkage.

Table 3: Antisense Inhibition of Human Osteopontin expression by oligonucleotides.

SEQ ID NO:	Sequence (5'-3')	Percent inhibition of OPN in human RPTEC cells at 20 μM
1	$\mathbf{A_s}\mathbf{C_s}c_st_sc_sa_sg_st_sc_sc_s\mathbf{A_s}\mathbf{T_s}$	15
2	$\mathbf{G_s}\mathbf{A_s}\mathbf{C_s}c_st_sc_sa_sg_st_sc_sc_s\mathbf{A_s}\mathbf{T_s}A$	20
3	$\mathbf{T_s}\mathbf{G_s}\mathbf{A_s}c_sc_st_sc_sa_sg_st_sc_sc_sa_s\mathbf{A_s}\mathbf{T_s}A$	86
4	$\mathbf{G_s}\mathbf{A_s}c_sc_st_sc_sa_sg_st_sc_sc_sa_s\mathbf{T_s}A$	87
5	$\mathbf{T_s}\mathbf{G_s}a_sc_sc_st_sc_sa_sg_st_sc_sc_sa_st_s\mathbf{A_s}A$	85
6	$\mathbf{A_s}\mathbf{A_s}g_sc_sa_sa_sa_st_sc_sa_sc_s\mathbf{T_s}G$	51
7	$\mathbf{A_s}\mathbf{A_s}g_sc_sa_sa_sa_st_sc_sa_sc_st_s\mathbf{G_s}C$	-11
8	$\mathbf{A_s}\mathbf{A_s}\mathbf{G_s}c_sa_sa_sa_st_sc_sa_sc_s\mathbf{T_s}\mathbf{G_s}C$	-88
9	$\mathbf{A_s}\mathbf{A_s}g_sa_st_st_st_sa_st_st_sc_sa_sc_sa_s\mathbf{C_s}C$	7
10	$\mathbf{A_s}\mathbf{A_s}g_sa_st_st_st_sa_st_st_sc_sa_s\mathbf{C_s}A$	-68
11	$\mathbf{A_s}\mathbf{A_s}\mathbf{G_s}a_st_st_st_sa_st_st_sc_sa_sc_s\mathbf{A_s}\mathbf{C_s}C$	90
12	$\mathbf{A_s}\mathbf{A_s}\mathbf{G_s}a_st_st_st_sa_st_st_sc_s\mathbf{A_s}\mathbf{C_s}A$	52
13	$\mathbf{C_s}\mathbf{T_s}a_sa_sa_st_sg_sc_sa_sa_sa_s\mathbf{G_s}T$	46
14	$\mathbf{G_s}\mathbf{A_s}c_sc_st_sc_sa_sg_sa_sa_sg_sa_s\mathbf{T_s}G$	77
15	$\mathbf{G_s}\mathbf{A_s}\mathbf{C_s}c_st_sc_sa_sg_sa_sa_sg_s\mathbf{A_s}\mathbf{T_s}G$	-4

As shown in Table 3, oligonucleotides of SEQ ID NOs: 3, 4, 5, 11 and 14 demonstrated about 75% or greater inhibition of osteopontin expression in these experiments and are therefore preferred. Also preferred are oligonucleotides based on the illustrated antisense oligomer 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 comparable inhibition of osteopontin expression.

The ability of compounds SEQ ID NO: 11 and SEQ ID NO: 14 (**AAG**atttattcac**ACC** and **GA**cctcagaaga**TG**, where all linkages are phosphorothioates and uppercase letters indicate LNA) to downregulate the three known osteopontin transcript variants was examined in human RPTEC. As shown in Figure 3, both oligonucleotides target all splice variants of osteopontin. In this experiment, SEQ ID NO: 11 elicited an approximately 80% downregulation of osteopontin mRNA; whereas SEQ ID NO: 14 elicited approximately a 40% downregulation.

Example 9: In vivo screen of antisense oligonucleotides

The antisense oligonucleotides of SEQ ID NOs: 11 and 14 (**AAG**atttattcac**ACC** and **GA**cctcagaaga**TG**, where all linkages are phosphorothioates and uppercase letters indicate LNA) were tested in vivo at a dose of 5 and 25mg/kg every day for a total of 3 doses. Mice were dosed with 10 ml per kg body weight i.v. of the antisense oligonucleotide compounds formulated in the vehicle or vehicle alone. Liver and kidney tissues were harvested 24 hours after the last dose for RNA analysis. The sample content of murine osteopontin mRNA was quantified using the murine osteopontin ABI Prism Pre-Developed TaqMan Assay Reagents (Applied Biosystems cat. no. Mm00436767_m1) according to the manufacturer's instructions. The sample content of murine GAPDH mRNA was quantified using the murine GAPDH ABI Prism Pre-Developed TaqMan Assay Reagents (Applied Biosystems cat. no. 435239E) according to the manufacturer's instructions.

A dose-dependent downregulation of osteopontin mRNA can be observed in kidneys isolated from the treated mice, especially with SEQ ID NO: 11 (Figure 4).

Serum samples from these oligonucleotide-treated mice were assayed for alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) for evidence of oligonucleotide-induced toxicity. The activity of ALT and AST in rat serum were determined using enzymatic assays (Horiba ABX Diagnostics) according to the manufacturer's instructions adjusted to 96-well format. Data were correlated to a 2-fold diluted standard curve generated from an ABX Pentra MultiCal solution. The results were presented as ALT or AST activity in U/L.

The two oligonucleotides appeared to be safe and well tolerated by the animals at the highest dose of 3x25 mg/kg, as can be seen from the results of liver enzyme ALT and AST measurements (Figure 5).

Example 10: *In-vitro biological model mimicking ischemia/reperfusion injury*

An *in-vitro* biological model system was developed using human RPTEC to mimic the clinical ischemia/reperfusion scenario, in which hypoxic conditions would be followed by reoxygenation. This hypoxia/reoxygenation scenario was accomplished by the use of

Anaerocult® P (Merck KgaA, Darmstadt, Germany), which rapidly generates an CO_2 enriched anaerobic environment.

In Figure 6, an induction of osteopontin mRNA can be seen after 4 hours of hypoxia incubation of human RPTEC and this induction gradually reverted back to a normal level after 24 hours of reoxygenation. Interestingly, cells that were pretreated gymnotically with 5 µM of SEQ ID NO: 11 (**AAG**atttattcac**ACC**, where all linkages are phosphorothioates and uppercase letters indicate LNA) remained significantly low in their osteopontin mRNA level. This compound was capable of suppressing the osteopontin level throughout the entire experiment. The scrambled control [**CGT**cagtatgcg**AAT**c (SEQ ID NO: 44),where all linkages are phosphorothioate linkages, lowercase letters indicate DNA, bold uppercase letters indicate oxy-LNA and all LNA-C are 5-methylC] was not capable of suppressing the osteopontin level. This phenomenon was also observed at the protein level, as shown by both Western blotting and ELISA assay.

Example 11: Design of oligonucleotides –second library

A second library of oligonucleotides was designed to target the most abundant form of human osteopontin, osteopontin-a (osteopontin variant 1; GenBank accession No. NM_001040058) which is the longest transcript containing all seven exons. SEQ ID NO: 19, 20, 21 and 34, respectively also target mouse osteopontin. These oligonucleotides are shown in Table 4. In Table 4, upper case letters indicate LNA, in this case Beta-D-oxy LNA. Lower case letters represent DNA units. In Table 4, all internucleoside linkages are phosphorothioate linkages and all LNA-cytosines (uppercase) are 5-methylcytosines.

Table 4- Antisense oligonucleotides targeting osteopontin- second library

SEQ ID NO:	Sequence and modifications (5'-3')	Base sequence (5'-3')	Length (bases)	Target site on NM_001040058
16	**CCA**ccaacacagg**GAG**	CCACCAACACAGGGAG	16	1-16
17	**CTG**ctgctgacaa**CCA**	CTGCTGCTGACAACCA	16	54-69
18	**GCA**actggcctga**GAC**	GCAACTGGCCTGAGAC	16	109-124
19	**GGA**gattctgctt**CTG**	GGAGATTCTGCTTCTG	16	313-328
20	**AGG**acacagcatt**CTG**	AGGACACAGCATTCTG	16	337-352
21	**TCA**gaggacacag**CAT**	TCAGAGGACACAGCAT	16	341-356
22	**TCA**ttggtttctt**CAG**	TCATTGGTTTCTTCAG	16	353-368
23	**AGT**caatggagtc**CTG**	AGTCAATGGAGTCCTG	16	463-478
24	**GAG**actcatcaga**CTG**	GAGACTCATCAGACTG	16	520-535
25	**GAC**cagttcatca**GAT**	GACCAGTTCATCAGAT	16	549-564
26	**ATC**atatgtgtc**TAC**	ATCATATGTGTCTAC	15	622-636
27	**TGT**agcatcaggg**TAC**	TGTAGCATCAGGGTAC	16	705-720
28	**CCT**gtatgcacc**ATT**	CCTTGTATGCACCATT	16	760-775
29	**GGC**tgtcccaatc**AGA**	GGCTGTCCCAATCAGA	16	808-823
30	**TCA**gcactctggt**CAT**	TCAGCACTCTGGTCAT	16	857-872

31	**CTG**actatcaatc**ACA**	CTGACTATCAATCACA	16	942-957
32	**AGC**atatcttca**TGG**	AGCATATCTTCATGG	15	1005-1019
33	**TTC**aggtgtttat**CTT**	TTCAGGTGTTTATCTT	16	1046-1061
34	**TTG**acctcagaag**ATG**	TTGACCTCAGAAGATG	16	1091-1106
35	**AGT**gagaaattgt**ATT**	AGTGAGAAATTGTATT	16	1121-1136
36	**ACA**ttcaaccaat**AAA**	ACATTCAACCAATAAA	16	1206-1221
37	**AGA**ctcaaatага**TAC**	AGACTCAAATAGATAC	16	1222-1237
38	**AGT**ttacagggag**TTT**	AGTTTACAGGGAGTTT	16	1286-1301
39	**GAG**agaataacaa**ATA**	GAGAGAATAACAAATA	16	1367-1382
40	**ATT**tattcacacc**ACA**	ATTTATTCACACCACA	16	1509-1524
41	**TTG**acaccaccaa**ATT**	TTGACACCACCAAATT	16	1547-1562
42	**TAA**ttgctggaca**ACC**	TAATTGCTGGACAACC	16	1583-1598
43	**TTA**attgctggac**AAC**	TTAATTGCTGGACAAC	16	1584-1599

Example 12: In vitro analysis: Antisense Inhibition of Human Osteopontin Expression by oligonucleotide compounds

Oligonucleotides presented in Table 4 were evaluated in the human RPTEC cell line for their potential to knock down osteopontin at the concentration of 25 μM using natural uptake (gymnosis) without any transfection vehicle (see Figure 7).

Oligonucleotides presented in Table 4 were evaluated in the human HRCE cell line for their potential to knock down osteopontin at the concentration of 25 μM using natural uptake (gymnosis) without any transfection vehicle (see Figure 8).

Several of these oligonucleotides, namely SEQ ID NOs 21, 28, 33 and 40, have demonstrated greater than 65% reduction in osteopontin mRNA levels in both cell lines tested. Of these, SEQ ID NO: 40 (**ATT**tattcacacc**ACA**) was the most potent, comparable to SEQ ID NO. 11 (**AAG**atttattcac**ACC**, where all linkages are phosphorothioates and uppercase letters indicate LNA) from the first library.

All of the compositions and methods described and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit and scope of the invention as defined by the appended claims.

* * *

CLAIMS:

1. An oligomer of from 10 to 30 nucleotides in length which comprises a contiguous nucleobase sequence of from 10 to 30 nucleobases in length, wherein said contiguous nucleobase sequence is at least 80% homologous to a region corresponding to a mammalian osteopontin gene or the reverse complement of a mammalian osteopontin mRNA, and which inhibits mammalian osteopontin expression.

2. The oligomer according to claim 1, wherein the mammalian osteopontin mRNA is NM_001040058, NM_000582, NM_001040060 or naturally occurring variant thereof.

3. The oligomer according to claim 1 wherein the contiguous nucleobase sequence is at least 80% homologous to a region corresponding to any of SEQ ID NO: 1-43.

4. The oligomer according to claim 1 wherein the contiguous nucleobase sequence comprises zero, one or two mismatches as compared to the corresponding region of SEQ ID NO: 1-43.

5. The oligomer according to claim 1 wherein the nucleotide sequence of the oligomer consists of the contiguous nucleotide sequence.

6. The oligomer according to claim 1 wherein the contiguous nucleotide sequence is from 10 to 18 nucleotides in length.

7. The oligomer according to claim 1 wherein the contiguous nucleotide sequence comprises at least one nucleotide analogue.

8. The oligomer according to claim 6 wherein the nucleotide analogue is a sugar-modified nucleotide.

9. The oligomer according to claim 6, wherein each sugar-modified nucleotide is independently 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.

10. The oligomer according to claim 6, wherein the sugar-modified nucleotide is LNA.

11. The oligomer according to any one of claims 6 – 8 which is a gapmer.

12. The oligomer according to any one of claims 1-11, wherein the oligomer is any one of SEQ ID NOs: 1-43.

13. The oligomer according to any one of claims 1 – 12, which inhibits the expression of osteopontin protein or mRNA in a cell which is expressing osteopontin protein or mRNA.

14. A conjugate comprising the oligomer according to any one of claims 1 – 13, and at least one non-nucleotide or non-polynucleotide moiety covalently attached to said oligomer.

15. A pharmaceutical composition comprising the oligomer according to any one of claims 1 – 13, or the conjugate according to claim 14, and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

16. The oligomer according to any one of claims 1 – 13, or the conjugate according to claim 14, for use as a medicament, such as for the treatment of ischemia/reperfusion injury.

17. The oligomer of claim 16 wherein the ischemia/reperfusion injury is ischemia/reperfusion injury to the kidney.

18. The use of an oligomer according to any one of claims 1-13, or a conjugate according to claim 14, for the manufacture of a medicament for the treatment of ischemia/reperfusion injury.

19. A method of treating ischemia/reperfusion injury, said method comprising administering an effective amount of an oligomer according to any one of the claims 1-13, or a conjugate according to claim 14, or a pharmaceutical composition according to claim 15, to a patient suffering from, or likely to suffer from ischemia/reperfusion injury.

20. A method for decreasing osteopontin levels in a cell, said method comprising administering an oligomer according to any one of the claims 1-13, or a conjugate according to claim 14 to said cell so as to decrease levels of osteopontin in said cell.

FIGURE 1

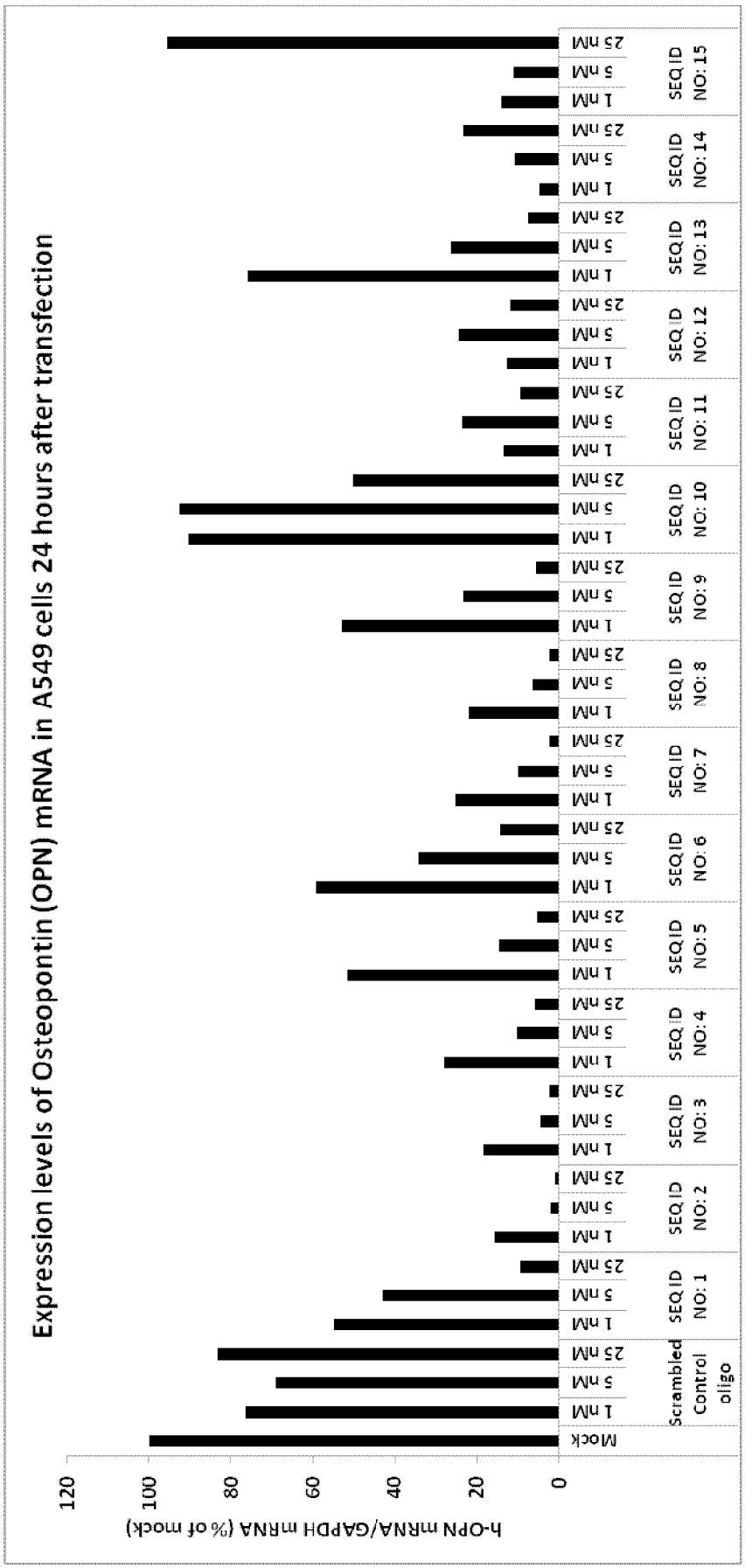

Expression levels of Osteopontin (OPN) mRNA in A549 cells 24 hours after transfection
h-OPN mRNA/GAPDH mRNA (% of mock)
0
20
40
60
80
100
120
Mock
Scrambled Control oligo
1 nM
5 nM
25 nM
SEQ ID NO: 1
SEQ ID NO: 2
SEQ ID NO: 3
SEQ ID NO: 4
SEQ ID NO: 5
SEQ ID NO: 6
SEQ ID NO: 7
SEQ ID NO: 8
SEQ ID NO: 9
SEQ ID NO: 10
SEQ ID NO: 11
SEQ ID NO: 12
SEQ ID NO: 13
SEQ ID NO: 14
SEQ ID NO: 15

FIGURE 2

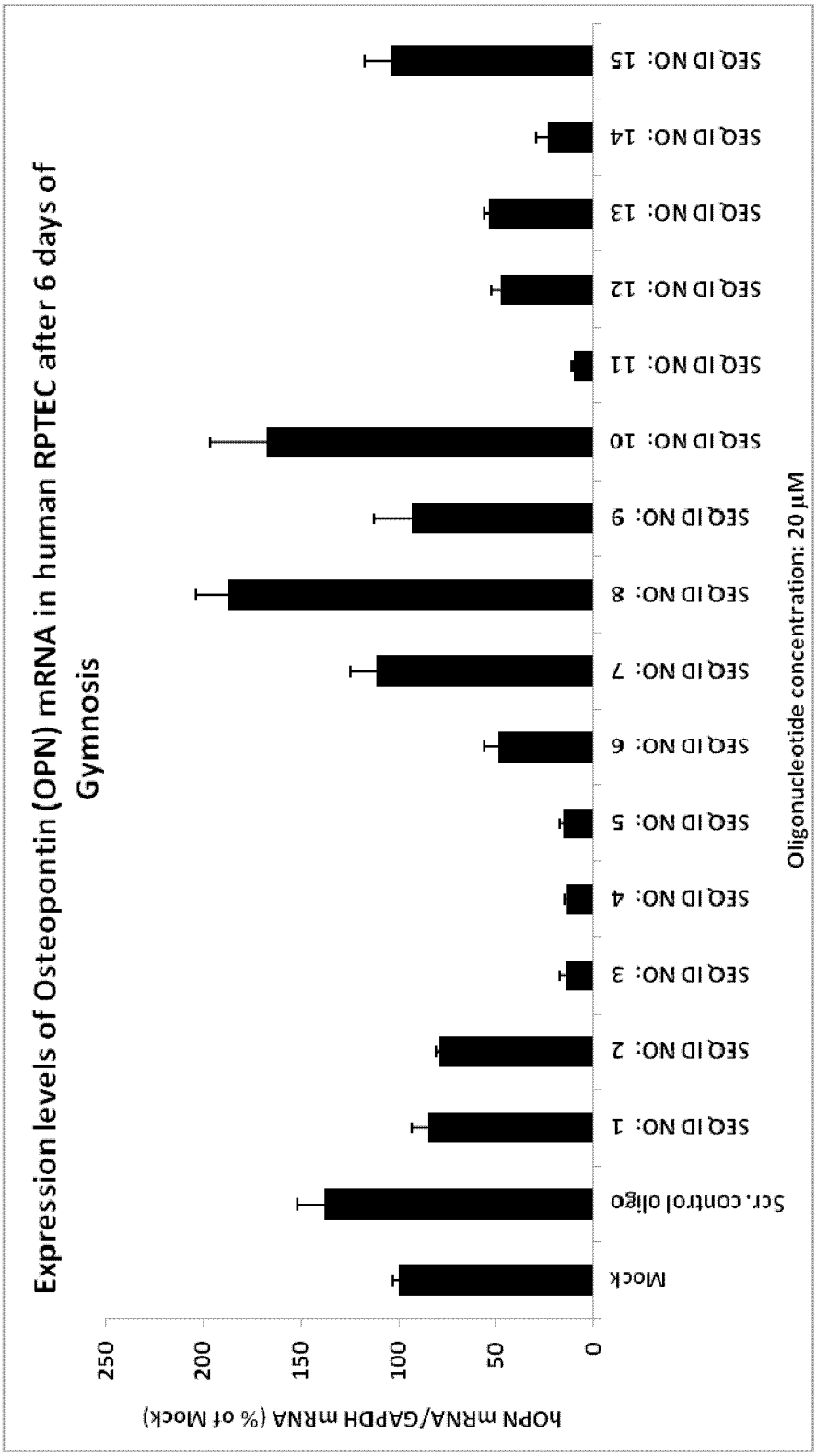

Expression levels of Osteopontin (OPN) mRNA in human RPTEC after 6 days of Gymnosis
hOPN mRNA/GAPDH mRNA (% of Mock)
0
50
100
150
200
250
Mock
Scr. control oligo
SEQ ID NO: 1
SEQ ID NO: 2
SEQ ID NO: 3
SEQ ID NO: 4
SEQ ID NO: 5
SEQ ID NO: 6
SEQ ID NO: 7
SEQ ID NO: 8
SEQ ID NO: 9
SEQ ID NO: 10
SEQ ID NO: 11
SEQ ID NO: 12
SEQ ID NO: 13
SEQ ID NO: 14
SEQ ID NO: 15
Oligonucleotide concentration: 20 µM

FIGURE 3

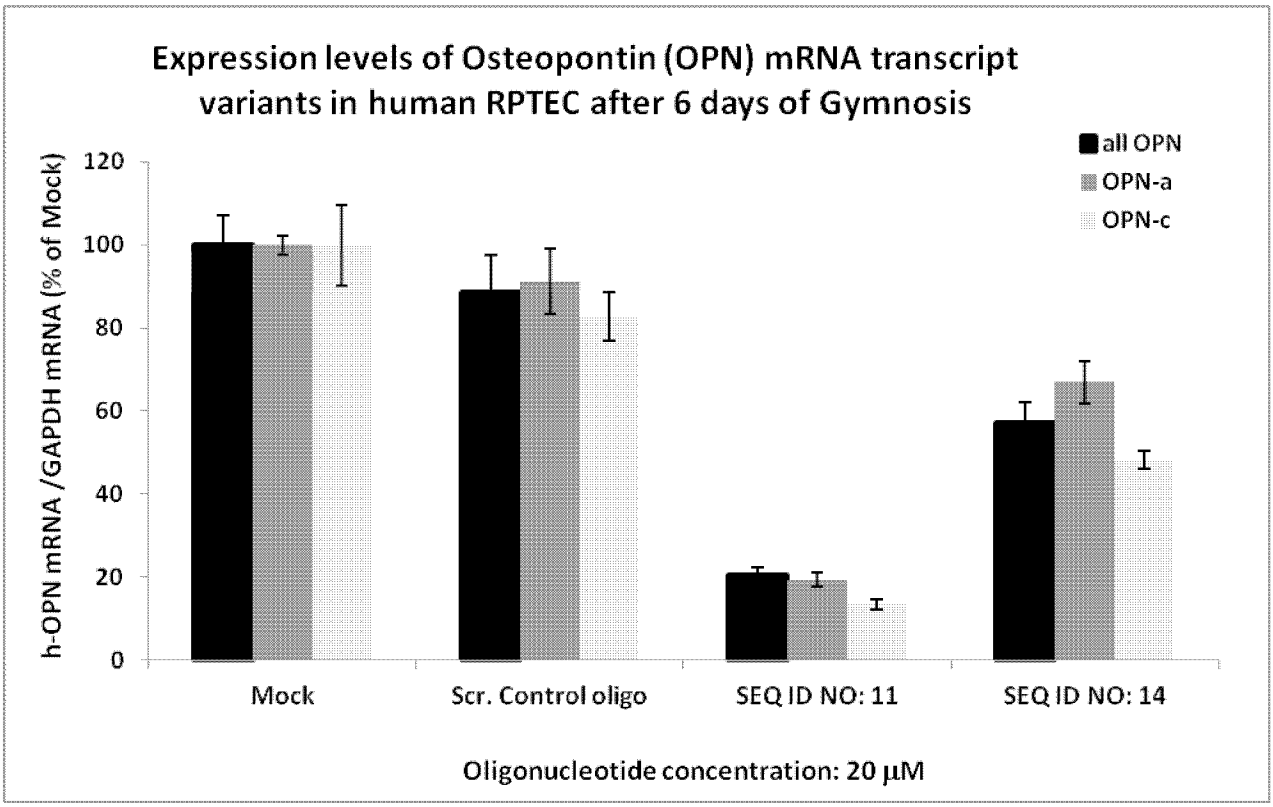

Expression levels of Osteopontin (OPN) mRNA transcript variants in human RPTEC after 6 days of Gymnosis
all OPN
OPN-a
OPN-c
h-OPN mRNA /GAPDH mRNA (% of Mock)
120
100
80
60
40
20
0
Mock
Scr. Control oligo
SEQ ID NO: 11
SEQ ID NO: 14
Oligonucleotide concentration: 20 µM

FIGURE 4

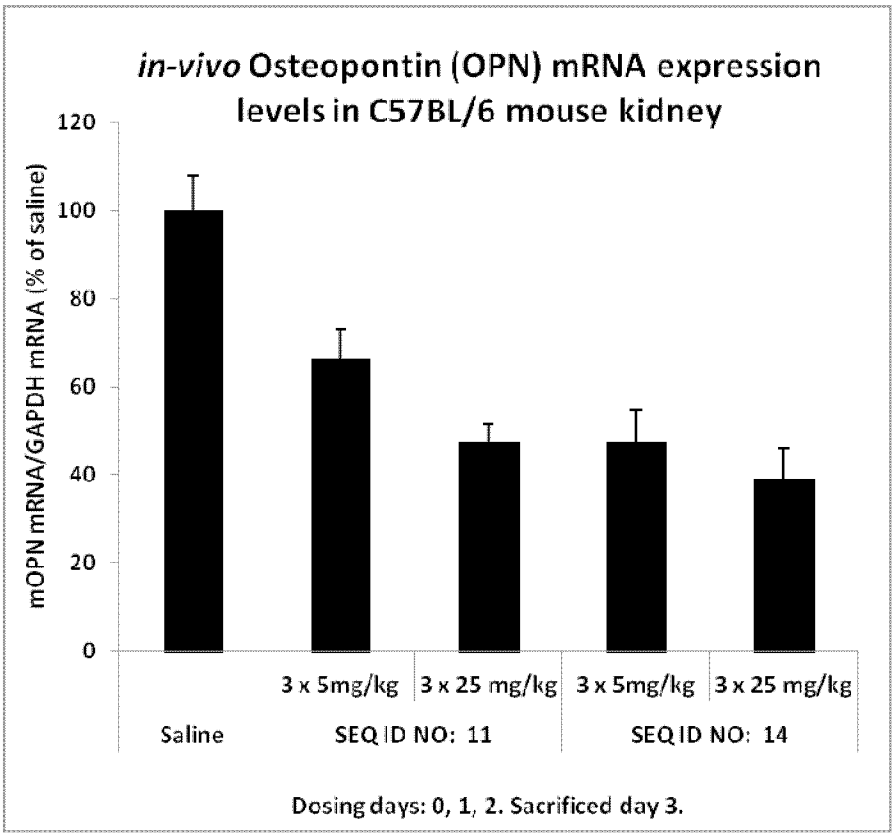

in-vivo Osteopontin (OPN) mRNA expression levels in C57BL/6 mouse kidney
mOPN mRNA/GAPDH mRNA (% of saline)
120
100
80
60
40
20
0
3 x 5mg/kg
3 x 25 mg/kg
3 x 5mg/kg
3 x 25 mg/kg
Saline
SEQ ID NO: 11
SEQ ID NO: 14
Dosing days: 0, 1, 2. Sacrificed day 3.

FIGURE 5a

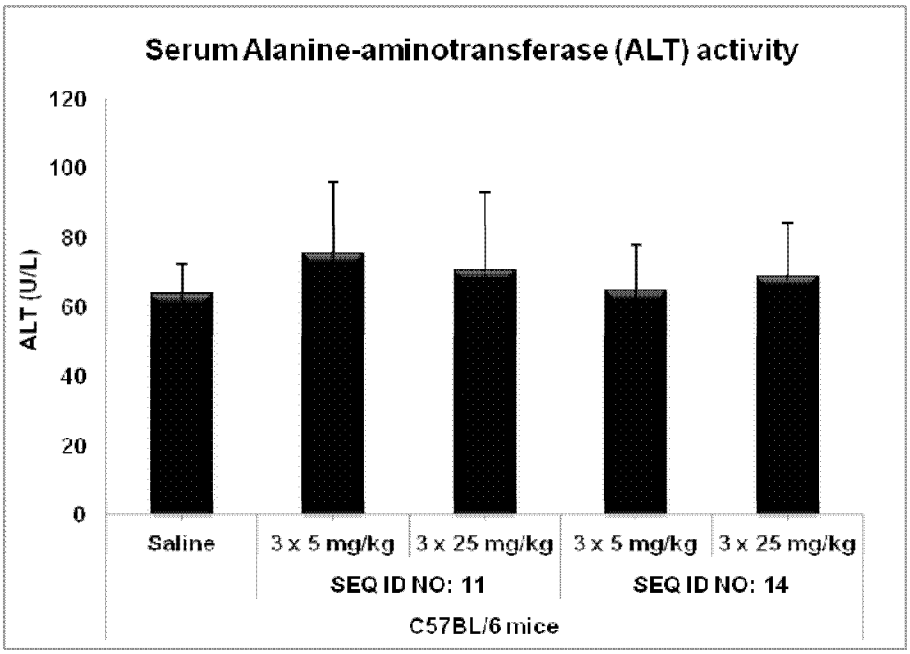


FIGURE 5b

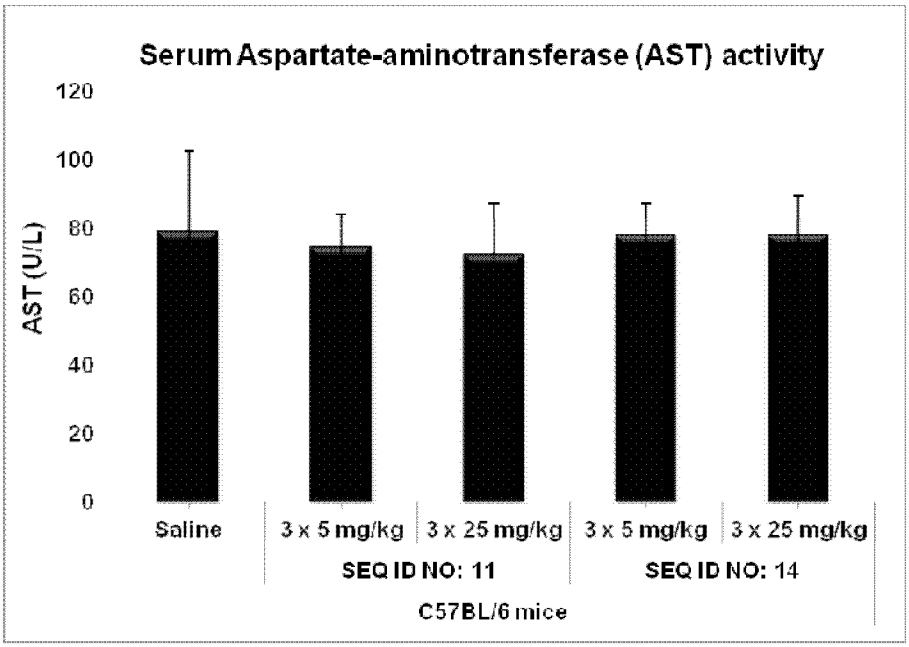


FIGURE 6

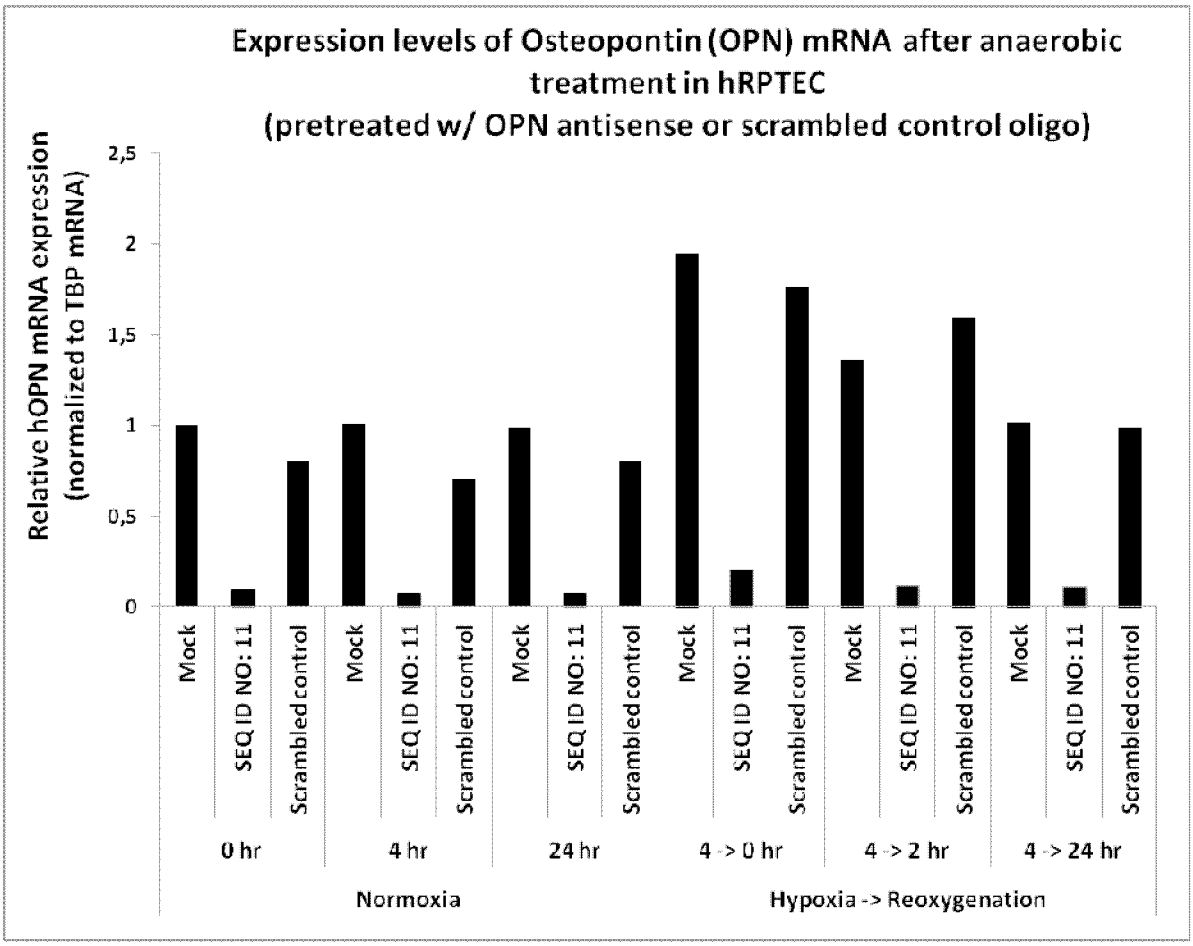

Expression levels of Osteopontin (OPN) mRNA after anaerobic treatment in hRPTEC
(pretreated w/ OPN antisense or scrambled control oligo)
Relative hOPN mRNA expression (normalized to TBP mRNA)
2,5
2
1,5
1
0,5
0
Mock
SEQ ID NO: 11
Scrambled control
0 hr
4 hr
24 hr
4 -> 0 hr
4 -> 2 hr
4 -> 24 hr
Normoxia
Hypoxia -> Reoxygenation

FIGURE 7

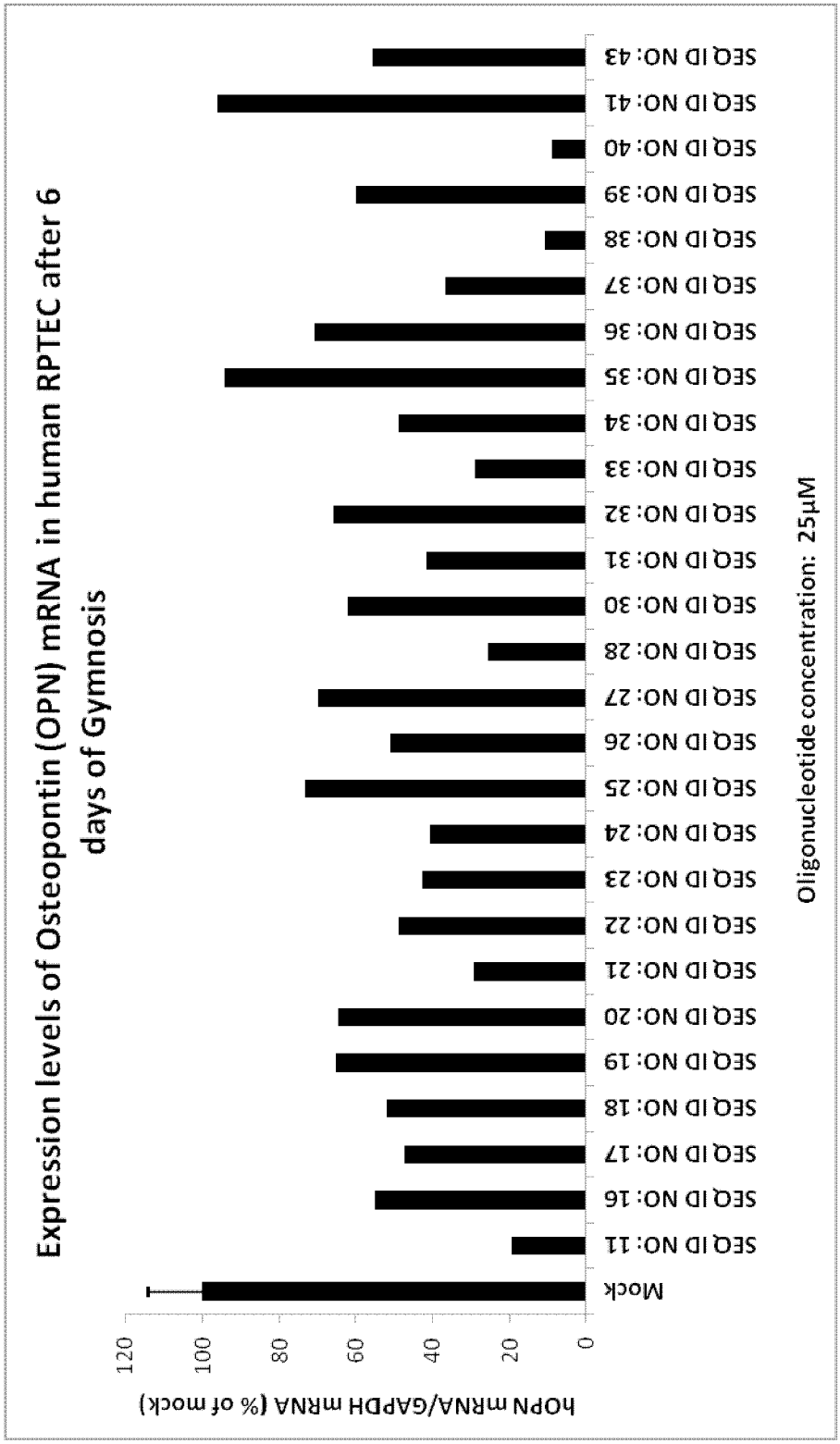
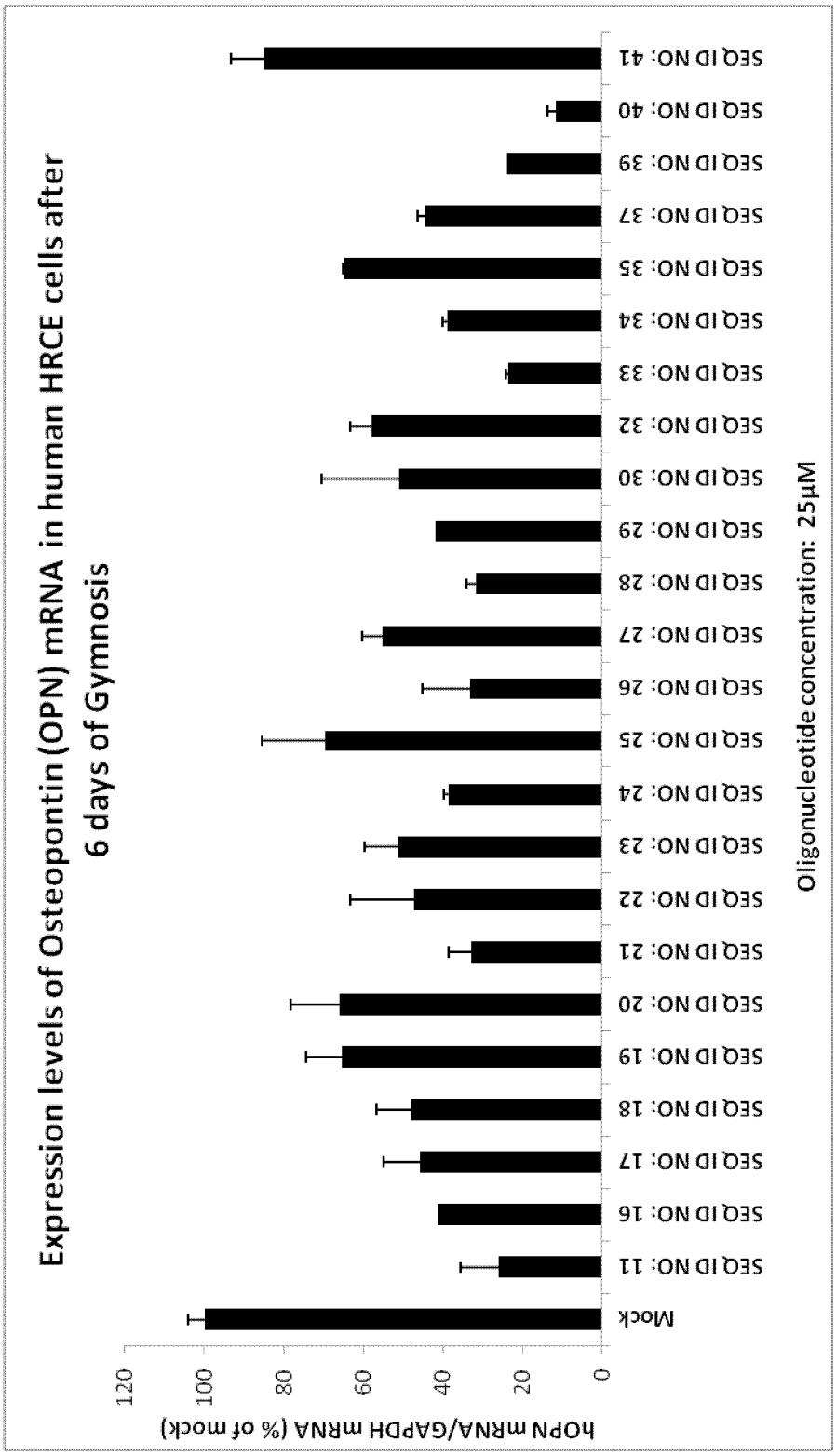

Expression levels of Osteopontin (OPN) mRNA in human RPTEC after 6 days of Gymnosis
hOPN mRNA/GAPDH mRNA (% of mock)
120
100
80
60
40
20
0
Mock
SEQ ID NO: 11
SEQ ID NO: 16
SEQ ID NO: 17
SEQ ID NO: 18
SEQ ID NO: 19
SEQ ID NO: 20
SEQ ID NO: 21
SEQ ID NO: 22
SEQ ID NO: 23
SEQ ID NO: 24
SEQ ID NO: 25
SEQ ID NO: 26
SEQ ID NO: 27
SEQ ID NO: 28
SEQ ID NO: 30
SEQ ID NO: 31
SEQ ID NO: 32
SEQ ID NO: 33
SEQ ID NO: 34
SEQ ID NO: 35
SEQ ID NO: 36
SEQ ID NO: 37
SEQ ID NO: 38
SEQ ID NO: 39
SEQ ID NO: 40
SEQ ID NO: 41
SEQ ID NO: 43
Oligonucleotide concentration: 25µM

FIGURE 8


Expression levels of Osteopontin (OPN) mRNA in human HRCE cells after 6 days of Gymnosis
hOPN mRNA/GAPDH mRNA (% of mock)
0
20
40
60
80
100
120
Oligonucleotide concentration: 25µM
Mock
SEQ ID NO: 11
SEQ ID NO: 16
SEQ ID NO: 17
SEQ ID NO: 18
SEQ ID NO: 19
SEQ ID NO: 20
SEQ ID NO: 21
SEQ ID NO: 22
SEQ ID NO: 23
SEQ ID NO: 24
SEQ ID NO: 25
SEQ ID NO: 26
SEQ ID NO: 27
SEQ ID NO: 28
SEQ ID NO: 29
SEQ ID NO: 30
SEQ ID NO: 32
SEQ ID NO: 33
SEQ ID NO: 34
SEQ ID NO: 35
SEQ ID NO: 37
SEQ ID NO: 39
SEQ ID NO: 40
SEQ ID NO: 41