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(54) Title: ANTISENSE OLIGONUCLEOTIDES TARGETING GBP-1

(57) Abstract: The present invention relates to antisense LNA oligonucleotides (oligomers) complementary to GBP-1 pre-mRNA sequences, which are capable of inhibiting the expression of GBP-1 protein. Inhibition of GBP-1 expression is beneficial for a range of medical disorders including cancer or osteoporosis.

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ANTISENSE OLIGONUCLEOTIDES TARGETING GBP-1

FIELD OF INVENTION

The present invention relates to antisense oligonucleotides (oligomers) complementary to GBP-1 pre-mRNA intron and exon sequences, which are capable of inhibiting the expression of GBP-1. Inhibition of GBP-1 expression is beneficial for a range of medical disorders including cancer or osteoporosis.

BACKGROUND

Guanylate Binding Protein 1 (GBP-1) is a member of the large GTPases, and is a secreted GTPase that is induced by IFNγ and mediate the antibacterial and antiviral activities of IFNγ. GBP-1 is a key mediator of the inhibitory effects of inflammatory cytokines on endothelial cells, regulating the proliferation and invasiveness of endothelial cells, and has been reported as a potential target for modulation of blood vessel growth and tumor angiongenesis (Naschberger et al., Advan. Enzyme Regul. 45 (2005) 215–227).

Mustafa et al., Acta Neuropathologica (2018) 135:581–599 reports that mutations in the GBP-1 is overexpressed in breast cancer and that T lymphocytes facilitate brain metastasis of breast cancer by inducing Guanylate-Binding Protein 1 expression.

Bai et al., Scientifi reports (2018) 8:1048 reports that GBP-1 inhibits osteogenic differentiation of human mesenchymal stromal cells derived from bone marrow, and suggests that GBP1 inhibits osteogenic differentiation of MSCs, and inhibition of GBP1 expression may prevent development of osteoporosis and facilitate MSC-based bone regeneration.

Schnoor et al., Mucosal Immunology 2009 2:1 (33-42) reports that down-regulation of GBP-1 by siRNAs resulted in enhanced permeability that correlated with increased apoptosis in in vitro intestinal epithelia, and suggest that GBP-1 may protect against epithelial apoptosis induced by inflammatory cytokines in inflammatory bowel disease.

Li et al., Mucosal Immunology 2009 2:1 (33-42) reports on siRNA mediated knock-down of GBP-1 in glioma cells, and that dysregulation of EGFR-mediated GBP1 expression may contribute to glioma invasion.

Capaldo *et al.,* Mucosal Immunology (2012) vol 5, pp 681 – 690, reports on siRNAs targeting GBP-1 to show that GBP-1 retards epithelial cell proliferation and TCF signaling through non-canonical inhibition of beta-catenin protein levels.

Pan et al. Virology Journal 2012, 9:292 reports on siRNAs targeting GBP-1 that decrease the levels of selective anti-viral cytokines.

There is therefore a need for GBP-1 inhibitors, for example for use in the treatment of cancer or osteoporosis.

OBJECTIVE OF THE INVENTION

The inventors have identified regions of the GBP-1 pre-mRNA and mRNA for antisense inhibition *in vitro* or *in vivo*. The invention therefore provides for antisense oligonucleotides, including LNA gapmer oligonucleotides, which target these regions of the GBP-1 pre-mRNA or mature mRNA. The present invention provides oligonucleotides which inhibit mammalian, such as human, GBP-1 which are useful in the treatment of a range of medical disorders including cancer or osteoporosis.

STATEMENT OF THE INVENTION

The invention provides for an antisense oligonucleotide, 10-30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary to –SEQ ID NO 11 wherein the antisense oligonucleotide is capable of inhibiting the expression of GBP-1 in a cell which is expressing GBP-1.

The invention provides for an LNA antisense oligonucleotide, 10-30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary to any of SEQ ID NO 11 wherein the antisense oligonucleotide is capable of inhibiting the expression of GBP-1 in a cell which is expressing GBP-1.

The invention provides for an gapmer antisense oligonucleotide, 10-30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary to any of SEQ ID NO 11 wherein the antisense oligonucleotide is capable of inhibiting the expression of GBP-1 in a cell which is expressing GBP-1.

The invention provides for an LNA gapmer antisense oligonucleotide, 10-30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary to any of SEQ ID NO 11 wherein the antisense oligonucleotide is capable of inhibiting the expression of GBP-1 in a cell which is expressing GBP-1.

The oligonucleotides targeting GBP-1 are antisense oligonucleotides, i.e. are complementary to their GBP-1 nucleic acid target.

In an advantageous embodiment, the antisense oligonucleotide of the invention is capable of inhibiting human GBP-1 in a cell which is expressing human GBP-1.

In one aspect, the invention provides for an antisense oligonucleotide, 10-30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary to SEQ ID NO 11.

In suitable embodiments, the antisense oligonucleotide is capable of inhibiting the expression of human GBP-1 transcript in a cell which is expressing human GBP-1 transcript.

The oligonucleotide of the invention as referred to or claimed herein may be in the form of a pharmaceutically acceptable salt, such as a sodium salt or a potassium salt.

In one aspect, the invention provides for a conjugate comprising the oligonucleotide according to the invention, and at least one conjugate moiety covalently attached to said oligonucleotide.

In one aspect, the invention provides for a pharmaceutical composition comprising the oligonucleotide or conjugate of the invention and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

In one aspect, the invention provides for an *in vivo* or *in vitro* method for modulating GBP-1 expression in a target cell which is expressing GBP-1, said method comprising administering an oligonucleotide or conjugate or pharmaceutical composition of the invention in an effective amount to said cell.

In one aspect, the invention provides for a method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide or a conjugate or a pharmaceutical composition of the invention to a subject suffering from or susceptible to the disease.

In some embodiments, the disease is one that is mediated by or associated with GBP-1 expression, or increased GBP-1 expression.

In some embodiments, the disease is selected from the group consisting of: cancer or osteoporosis.

In other aspects, the invention provides for the oligonucleotide, the conjugate or the pharmaceutical composition of the invention for use in medicine.

In other aspects, the invention provides for the oligonucleotide, the conjugate or the pharmaceutical composition of the invention for use in the treatment or prevention of a disease selected from the group consisting of: cancer or osteoporosis.

In other aspects, the invention provides for the use of the oligonucleotide, the conjugate or the pharmaceutical composition of the invention, for the preparation of a medicament for treatment or prevention of a disease selected from the group consisting of: cancer or osteoporosis.

BRIEF DESCRIPTION OF FIGURES

Figure 1: Testing *in vitro* efficacy of various antisense oligonucleotides targeting human and mouse GBP-1 mRNA in A431and MDA-MB-231 cell lines at single concentration.

Figure 2: Comparison of *in vitro* efficacy for antisense oligonucleotides targeting human GBP-1 mRNA in A431and MDA-MB-231 cell lines at single concentration shows good correlation.

Figure 3: Testing selected oligonucleotides targeting human GBP-1 mRNA *in vitro* for concentration dependent potency and efficacy in A231 cell line.

Figure 4: Testing selected oligonucleotides targeting human GBP-1 mRNA *in vitro* for concentration dependent potency and efficacy in MDA-MB-231 cell line.

DEFINITIONS

Oligonucleotide

The term “oligonucleotide” as used herein is defined as it is generally understood by the skilled person as a molecule comprising two or more covalently linked nucleosides. Such covalently bound nucleosides may also be referred to as nucleic acid molecules or oligomers. Oligonucleotides are commonly made in the laboratory by solid-phase chemical synthesis followed by purification. When referring to a sequence of the oligonucleotide, reference is made to the sequence or order of nucleobase moieties, or modifications thereof, of the covalently linked nucleotides or nucleosides. The oligonucleotide of the invention is

man-made, and is chemically synthesized, and is typically purified or isolated. The oligonucleotide of the invention may comprise one or more modified nucleosides or nucleotides.

Antisense oligonucleotides

The term "Antisense oligonucleotide" as used herein is defined as an oligonucleotide capable of modulating expression of a target gene by hybridizing to a target nucleic acid, in particular to a contiguous sequence on a target nucleic acid. The antisense oligonucleotides are not essentially double stranded and are therefore not siRNAs or shRNAs. Preferably, the antisense oligonucleotides of the present invention are single stranded. It is understood that single stranded oligonucleotides of the present invention can form hairpins or intermolecular duplex structures (duplex between two molecules of the same oligonucleotide), as long as the degree of intra or inter self-complementarity is less than 50% across of the full length of the oligonucleotide

Contiguous Nucleotide Sequence

The term "contiguous nucleotide sequence" refers to the region of the oligonucleotide which is complementary to or hybridizes to the target nucleic acid. Although this region of the oligonucleotide is complementary to the target sequence, in some embodiments, not every nucleobase within the contiguous sequence need be complementary provided the the contiguous nucleotide sequence can hybridize to the target sequence and inhibit its expression then a mismatch, or in some embodiments more than 1 mismatch may exist. Adventurously, the contiguous nucleotide sequence is 100% complementary to the target nucleic acid. The term "contiguous nucleotide sequence" is used interchangeably herein with the term "contiguous nucleobase sequence" and the term "oligonucleotide motif sequence". In some embodiments all the nucleotides of the oligonucleotide constitute the contiguous nucleotide sequence. In some embodiments the oligonucleotide comprises the contiguous nucleotide sequence, such as a F-G-F' gapmer region, and may optionally comprise further nucleotide(s), for example a nucleotide linker region which may be used to attach a functional group to the contiguous nucleotide sequence. The nucleotide linker region may or may not be complementary to the target nucleic acid.

Nucleotides

Nucleotides are the building blocks of oligonucleotides and polynucleotides, and for the purposes of the present invention include both naturally occurring and non-naturally occurring nucleotides. In nature, nucleotides, such as DNA and RNA nucleotides comprise a ribose sugar moiety, a nucleobase moiety and one or more phosphate groups (which is

absent in nucleosides). Nucleosides and nucleotides may also interchangeably be referred to as “units” or “monomers”.

Modified nucleoside

The term “modified nucleoside” or “nucleoside modification” as used herein refers to nucleosides modified as compared to the equivalent DNA or RNA nucleoside by the introduction of one or more modifications of the sugar moiety or the (nucleo)base moiety. In a preferred embodiment the modified nucleoside comprise a modified sugar moiety. The term modified nucleoside may also be used herein interchangeably with the term “nucleoside analogue” or modified “units” or modified “monomers”. Nucleosides with an unmodified DNA or RNA sugar moiety are termed DNA or RNA nucleosides herein. Nucleosides with modifications in the base region of the DNA or RNA nucleoside are still generally termed DNA or RNA if they allow Watson Crick base pairing.

Modified internucleoside linkages

The term “modified internucleoside linkage” is defined as generally understood by the skilled person as linkages other than phosphodiester (PO) linkages, that covalently couples two nucleosides together. The oligonucleotides of the invention may therefore comprise modified internucleoside linkages. In some embodiments, the modified internucleoside linkage increases the nuclease resistance of the oligonucleotide compared to a phosphodiester linkage. For naturally occurring oligonucleotides, the internucleoside linkage includes phosphate groups creating a phosphodiester bond between adjacent nucleosides. Modified internucleoside linkages are particularly useful in stabilizing oligonucleotides for *in vivo* use,and may serve to protect against nuclease cleavage at regions of DNA or RNA nucleosides in the oligonucleotide of the invention, for example within the gap region of a gapmer oligonucleotide, as well as in regions of modified nucleosides, such as region F and F’.

In an embodiment, the oligonucleotide comprises one or more internucleoside linkages modified from the natural phosphodiester, such one or more modified internucleoside linkages that is for example more resistant to nuclease attack. Nuclease resistance may be determined by incubating the oligonucleotide in blood serum or by using a nuclease resistance assay (e.g. snake venom phosphodiesterase (SVPD)), both are well known in the art. Internucleoside linkages which are capable of enhancing the nuclease resistance of an oligonucleotide are referred to as nuclease resistant internucleoside linkages. In some embodiments at least 50% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are modified, such as at least 60%, such as at least 70%, such as at least 80 or such as at least 90% of the internucleoside linkages in the

oligonucleotide, or contiguous nucleotide sequence thereof, are nuclease resistant internucleoside linkages. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are nuclease resistant internucleoside linkages. It will be recognized that, in some embodiments the nucleosides which link the oligonucleotide of the invention to a non-nucleotide functional group, such as a conjugate, may be phosphodiester.

A preferred modified internucleoside linkage is phosphorothioate.

Phosphorothioate internucleoside linkages are particularly useful due to nuclease resistance, beneficial pharmacokinetics and ease of manufacture. In some embodiments at least 50% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate, such as at least 60%, such as at least 70%, such as at least 80% or such as at least 90% of the internucleoside linkages in the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are phosphorothioate.

Nuclease resistant linkages, such as phosphorothioate linkages, are particularly useful in oligonucleotide regions capable of recruiting nuclease when forming a duplex with the target nucleic acid, such as region G for gapmers. Phosphorothioate linkages may, however, also be useful in non-nuclease recruiting regions and/or affinity enhancing regions such as regions F and F' for gapmers. Gapmer oligonucleotides may, in some embodiments comprise one or more phosphodiester linkages in region F or F', or both region F and F', which the internucleoside linkage in region G may be fully phosphorothioate. Advantageously, all the internucleoside linkages in the contiguous nucleotide sequence of the oligonucleotide are phosphorothioate linkages.

It is recognized that, as disclosed in EP2 742 135, antisense oligonucleotide may comprise other internucleoside linkages (other than phosphodiester and phosphorothioate), for example alkyl phosphonate / methyl phosphonate internucleosides, which according to EP2 742 135 may for example be tolerated in an otherwise DNA phosphorothioate gap region.

Nucleobase

The term nucleobase includes the purine (e.g. adenine and guanine) and pyrimidine (e.g. uracil, thymine and cytosine) moiety present in nucleosides and nucleotides which form hydrogen bonds in nucleic acid hybridization. In the context of the present invention the term nucleobase also encompasses modified nucleobases which may differ from naturally occurring nucleobases but are functional during nucleic acid hybridization. In this context "nucleobase" refers to both naturally occurring nucleobases such as adenine, guanine,

cytosine, thymidine, uracil, xanthine and hypoxanthine, as well as non-naturally occurring variants. Such variants are for example described in Hirao et al (2012) Accounts of Chemical Research vol 45 page 2055 and Bergstrom (2009) Current Protocols in Nucleic Acid Chemistry Suppl. 37 1.4.1.

In a some embodiments the nucleobase moiety is modified by changing the purine or pyrimidine into a modified purine or pyrimidine, such as substituted purine or substituted pyrimidine, such as a nucleobased selected from isocytosine, pseudoisocytosine, 5-methyl cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, 5-propynyl-uracil, 5-bromouracil 5-thiazolo-uracil, 2-thio-uracil, 2'thio-thymine, inosine, diaminopurine, 6-aminopurine, 2-aminopurine, 2,6-diaminopurine and 2-chloro-6-aminopurine.

The nucleobase moieties may be indicated by the letter code for each corresponding nucleobase, e.g. A, T, G, C or U, wherein each letter may optionally include modified nucleobases of equivalent function. For example, in the exemplified oligonucleotides, the nucleobase moieties are selected from A, T, G, C, and 5-methyl cytosine. Optionally, for LNA gapmers, 5-methyl cytosine LNA nucleosides may be used.

Modified oligonucleotide

The term modified oligonucleotide describes an oligonucleotide comprising one or more sugar-modified nucleosides and/or modified internucleoside linkages. The term chimeric" oligonucleotide is a term that has been used in the literature to describe oligonucleotides with modified nucleosides.

Complementarity

The term "complementarity" describes the capacity for Watson-Crick base-pairing of nucleosides/nucleotides. Watson-Crick base pairs are guanine (G)-cytosine (C) and adenine (A) - thymine (T)/uracil (U). It will be understood that oligonucleotides may comprise nucleosides with modified nucleobases, for example 5-methyl cytosine is often used in place of cytosine, and as such the term complementarity encompasses Watson Crick base-paring between non-modified and modified nucleobases (see for example Hirao et al (2012) Accounts of Chemical Research vol 45 page 2055 and Bergstrom (2009) Current Protocols in Nucleic Acid Chemistry Suppl. 37 1.4.1).

The term "% complementary" as used herein, refers to the number of nucleotides in percent of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which, at a given position, are complementary to (*i.e.* form Watson Crick base pairs with) a contiguous sequence of nucleotides, at a given position of a separate nucleic acid molecule (e.g. the target nucleic acid or target sequence). The percentage is calculated by counting the number of aligned bases that form pairs between the two sequences (when aligned with

the target sequence 5'-3' and the oligonucleotide sequence from 3'-5'), dividing by the total number of nucleotides in the oligonucleotide and multiplying by 100. In such a comparison a nucleobase/nucleotide which does not align (form a base pair) is termed a mismatch. Preferably, insertions and deletions are not allowed in the calculation of % complementarity of a contiguous nucleotide sequence.

The term "fully complementary", refers to 100% complementarity.

Identity

The term "Identity" as used herein, refers to the proportion of nucleotides (expressed in percent) of a contiguous nucleotide sequence in a nucleic acid molecule (e.g. oligonucleotide) which across the contiguous nucleotide sequence, are identical to a reference sequence (e.g. a sequence motif). The percentage of identity is thus calculated by counting the number of aligned bases that are identical (a match) between two sequences (e.g. in the contiguous nucleotide sequence of the compound of the invention and in the reference sequence), dividing that number by the total number of nucleotides in the aligned region and multiplying by 100. Therefore, Percentage of Identity = (Matches x 100)/Length of aligned region (e.g. the contiguous nucleotide sequence). Insertions and deletions are not allowed in the calculation the percentage of identity of a contiguous nucleotide sequence. It will be understood that in determining identity, chemical modifications of the nucleobases are disregarded as long as the functional capacity of the nucleobase to form Watson Crick base pairing is retained (e.g. 5-methyl cytosine is considered identical to a cytosine for the purpose of calculating % identity).

Hybridization

The term "hybridizing" or "hybridizes" as used herein is to be understood as two nucleic acid strands (e.g. an oligonucleotide and a target nucleic acid) forming hydrogen bonds between base pairs on opposite strands thereby forming a duplex. The affinity of the binding between two nucleic acid strands is the strength of the hybridization. It is often described in terms of the melting temperature (T_m) defined as the temperature at which half of the oligonucleotides are duplexed with the target nucleic acid. At physiological conditions T_m is not strictly proportional to the affinity (Mergny and Lacroi. *Oligonucleotides* 13:515–537, 2003). The standard state Gibbs free energy ΔG° is a more accurate representation of binding affinity and is related to the dissociation constant (K_d) of the reaction by $\Delta G^\circ = -RT\ln(K_d)$, where R is the gas constant and T is the absolute temperature. Therefore, a very low ΔG° of the reaction between an oligonucleotide and the target nucleic acid reflects a strong hybridization between the oligonucleotide and target nucleic acid. ΔG° is the energy associated with a reaction where aqueous concentrations are 1M, the pH is 7, and the

temperature is 37°C. The hybridization of oligonucleotides to a target nucleic acid is a spontaneous reaction and for spontaneous reactions ΔG° is less than zero. ΔG° can be measured experimentally, for example, by use of the isothermal titration calorimetry (ITC) method as described in Hansen et al., 1965, *Chem. Comm.* 36–38 and Holdgate et al., 2005, *Drug Discov Today*. The skilled person will know that commercial equipment is available for ΔG° measurements. ΔG° can also be estimated numerically by using the nearest neighbor model as described by SantaLucia, 1998, *Proc Natl Acad Sci USA.* 95: 1460–1465 using appropriately derived thermodynamic parameters described by Sugimoto et al., 1995, *Biochemistry* 34:11211–11216 and McTigue et al., 2004, *Biochemistry* 43:5388–5405. In order to have the possibility of modulating its intended nucleic acid target by hybridization, oligonucleotides of the present invention hybridize to a target nucleic acid with estimated ΔG° values below -10 kcal for oligonucleotides that are 10-30 nucleotides in length. In some embodiments the degree or strength of hybridization is measured by the standard state Gibbs free energy ΔG°. The oligonucleotides may hybridize to a target nucleic acid with estimated ΔG° values below the range of -10 kcal, such as below -15 kcal, such as below -20 kcal and such as below -25 kcal for oligonucleotides that are 8-30 nucleotides in length. In some embodiments the oligonucleotides hybridize to a target nucleic acid with an estimated ΔG° value of -10 to -60 kcal, such as -12 to -40, such as from -15 to -30 kcal or-16 to -27 kcal such as -18 to -25 kcal.

Target nucleic acid

According to the present invention, the target nucleic acid is a nucleic acid which encodes mammalian GBP-1 and may for example be a gene, a GBP-1 RNA, a mRNA, a pre-mRNA, a mature mRNA or a cDNA sequence. The target may therefore be referred to as an GBP-1 target nucleic acid.

Suitably, the target nucleic acid encodes an GBP-1 protein, in particular mammalian GBP-1, such as the human GBP-1 encoding pre-mRNA or mRNA sequences provided herein as SEQ ID NO 11.

In some embodiments, the target nucleic acid is SEQ ID NO: 11 or naturally occurring variants thereof (e.g. GBP-1 sequences encoding a mammalian GBP-1 protein).

If employing the oligonucleotide of the invention in research or diagnostics the target nucleic acid may be a cDNA or a synthetic nucleic acid derived from DNA or RNA.

For *in vivo* or *in vitro* application, the oligonucleotide of the invention is typically capable of inhibiting the expression of the GBP-1 target nucleic acid in a cell which is expressing the GBP-1 target nucleic acid. The contiguous sequence of nucleobases of the oligonucleotide of the invention is typically complementary to the GBP-1 target nucleic acid, as measured

across the length of the oligonucleotide, optionally with no more than one mismatch, excluding the optional nucleotide based linker regions which may link the oligonucleotide to an optional functional group such as a conjugate, or other non-complementary terminal nucleotides (e.g. region D' or D''). The target nucleic acid is a messenger RNA, such as a mature mRNA or a pre-mRNA which encodes mammalian GBP-1 protein, such as human GBP-1, e.g. the human GBP-1 pre-mRNA sequence, such as that disclosed as SEQ ID NO 11. it will be understood that target RNA sequences have uracil (U) bases in place of the thymidine bases (T).

Table 3.

Target Nucleic Acid	Database reference	Sequence ID
GBP-1 *Homo sapiens* pre- mRNA,	Ensembl:ENSG00000117228 MIM:600411; Vega:OTTHUMG00000010614	SEQ ID NO 11

Target Sequence

The term "target sequence" as used herein refers to a sequence of nucleotides present in the target nucleic acid which comprises the nucleobase sequence which is complementary to the oligonucleotide of the invention. The target sequence consists of a region on the target nucleic acid which is complementary to the contiguous nucleotide sequence of the oligonucleotide of the invention. Herein are provided numerous target sequence regions, as defined by regions of the human GBP-1 pre-mRNA (SEQ ID NO 11) which may be targeted by the oligonucleotides of the invention. In some embodiments the target sequence is longer than the complementary sequence of a single oligonucleotide, and may, for example represent a preferred region of the target nucleic acid which may be targeted by several oligonucleotides of the invention.

The oligonucleotide of the invention comprises a contiguous nucleotide sequence which is complementary to or hybridizes to the target nucleic acid, such as a sub-sequence of the target nucleic acid, such as a target sequence described herein.

The oligonucleotide comprises a contiguous nucleotide sequence which are complementary to a target sequence present in the target nucleic acid molecule. The contiguous nucleotide sequence (and therefore the target sequence) comprises of at least 10 contiguous nucleotides, such as 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 contiguous nucleotides, such as from 12-25, such as from 14-18 contiguous nucleotides.

Target Sequence Regions

In some embodiments, the oligonucleotide of the invention, or contiguous nucleotide sequence thereof is complementary, such as fully complementary, to a sequence selected from the group consisting of SEQ ID NO 12, 13, 14, 15, 16, 17, 18 and 19.

In some embodiments, the oligonucleotide of the invention, or contiguous nucleotide sequence thereof is complementary, such as fully complementary, to a region of SEQ ID NO 11, selected from the group consisting of 13 - 65; 114 - 135; 137 - 171; 175 - 213; 230 - 265; 267 - 282; 284 - 314; 325 - 339; 341 - 373; 381 - 422; 424 - 449; 456 - 473; 495 - 524; 544 - 558; 644 - 667; 681 - 706; 736 - 753; 759 - 831; 840 - 872; 874 - 891; 893 - 908; 920 - 955; 957 - 1019; 1021 - 1037; 1039 - 1055; 1086 - 1120; 1120 - 1161; 1182 - 1210; 1212 - 1244; 1246 - 1270; 1272 - 1342; 1358 - 1382; 1413 - 1432; 1450 - 1464; 1482 - 1500; 1534 - 1553; 1555 - 1571; 1573 - 1616; 1621 - 1657; 1665 - 1691; 1693 - 1714; 1741 - 1762; 1777 - 1830; 1832 - 1858; 1869 - 1886; 1888 - 1934; 1955 - 2000; 2004 - 2036; 2061 - 2079; 2098 - 2118; 2120 - 2218; 2220 - 2237; 2277 - 2335; 2337 - 2372; 2387 - 2413; 2415 - 2459; 2481 - 2499; 2499 - 2517; 2519 - 2534; 2562 - 2577; 2589 - 2620; 2622 - 2636; 2646 - 2677; 2732 - 2758; 2789 - 2813; 2815 - 2839; 2848 - 2876; 2878 - 2927; 2929 - 3014; 3017 - 3034; 3025 - 3039; 3036 - 3092; 3133 - 3177; 3203 - 3232; 3234 - 3249; 3250 - 3275; 3311 - 3330; 3332 - 3361; 3396 - 3427; 3486 - 3518; 3520 - 3541; 3552 - 3612; 3639 - 3656; 3660 - 3714; 3722 - 3750; 3779 - 3795; 3797 - 3817; 3848 - 3881; 3887 - 3905; 3907 - 3931; 3933 - 3967; 3997 - 4029; 4031 - 4056; 4066 - 4081; 4090 - 4110; 4112 - 4131; 4140 - 4170; 4183 - 4224; 4237 - 4273; 4275 - 4313; 4324 - 4341; 4343 - 4370; 4372 - 4386; 4438 - 4453; 4450 - 4482; 4550 - 4569; 4588 - 4621; 4647 - 4683; 4726 - 4742; 4792 - 4807; 4824 - 4852; 4885 - 4911; 4940 - 4963; 4970 - 5002; 5014 - 5055; 5057 - 5100; 5102 - 5119; 5121 - 5182; 5203 - 5221; 5226 - 5241; 5252 - 5266; 5271 - 5309; 5331 - 5352; 5366 - 5382; 5384 - 5430; 5452 - 5481; 5483 - 5519; 5542 - 5562; 5564 - 5581; 5602 - 5618; 5627 - 5666; 5668 - 5692; 5693 - 5710; 5712 - 5731; 5745 - 5774; 5778 - 5829; 5841 - 5858; 5860 - 5879; 5894 - 5973; 5975 - 6008; 6010 - 6029; 6052 - 6094; 6127 - 6141; 6143 - 6170; 6179 - 6201; 6215 - 6249; 6266 - 6339; 6341 - 6382; 6384 - 6455; 6457 - 6474; 6482 - 6516; 6527 - 6561; 6563 - 6602; 6611 - 6627; 6629 - 6644; 6669 - 6772; 6774 - 6818; 6831 - 6858; 6875 - 6896; 6907 - 6923; 6960 - 6990; 7001 - 7015; 7017 - 7034; 7051 - 7112; 7163 - 7178; 7195 - 7214; 7230 - 7244; 7258 - 7319; 7321 - 7353; 7355 - 7406; 7408 - 7441; 7461 - 7475; 7477 - 7504; 7526 - 7558; 7647 - 7688; 7730 - 7752; 7762 - 7795; 7824 - 7864; 7899 - 7923; 7949 - 7968; 7972 - 7987; 7989 - 8039; 8047 - 8068; 8070 - 8088; 8114 - 8135; 8137 - 8170; 8172 - 8194; 8196 - 8214; 8218 - 8251; 8253 - 8272; 8283 - 8345; 8358 - 8392; 8394 - 8497; 8499 - 8525; 8607 - 8638; 8664 - 8730; 8748 - 8817; 8847 - 8875; 8877 - 8892; 8894 - 8910; 8927 - 8943; 8945 - 8972; 8974 - 8988; 9018 - 9032; 9061 - 9084; 9092 - 9190; 9192 - 9214; 9262 - 9285; 9287 - 9318; 9331 - 9366; 9378 - 9415;

9432 - 9476; 9501 - 9523; 9531 - 9546; 9548 - 9564; 9566 - 9626; 9636 - 9650; 9660 - 9697; 9699 - 9719; 9785 - 9822; 9824 - 9855; 9857 - 9872; 9874 - 9915; 9921 - 9970; 9980 - 9997; 10049 - 10064; 10065 - 10083; 10085 - 10101; 10103 - 10117; 10119 - 10204; 10206 - 10231; 10233 - 10288; 10290 - 10310; 10342 - 10362; 10376 - 10391; 10402 - 10422; 10424 - 10469; 10483 - 10623; 10634 - 10698; 10700 - 10723; 10725 - 10740; 10742 - 10758; 10780 - 10796; 10798 - 10824; 10831 - 10856; 10863 - 10892; 10943 - 10960; 10982 - 10999; 11014 - 11038; 11044 - 11066; 11075 - 11095; 11108 - 11136; 11142 - 11159; 11177 - 11198; 11208 - 11222; 11217 - 11233; 11245 - 11269; 11281 - 11303; 11320 - 11362; 11368 - 11387; 11389 - 11408; 11410 - 11424; 11444 - 11505; 11515 - 11566; 11570 - 11584; 11605 - 11637; 11665 - 11699; 11701 - 11723; 11725 - 11747; 11792 - 11816; 11822 - 11838; 11840 - 11874; 11876 - 11902; 11908 - 11926; 11945 - 11968; 11990 - 12032; 12034 - 12090; 12092 - 12119; 12143 - 12161; 12164 - 12318; 12317 - 12333; 12345 - 12420; 12440 - 12481; 12496 - 12514; 12516 - 12542; 12544 - 12604; 12632 - 12647; 12649 - 12701; 12745 - 12776; 12787 - 12812; 12876 - 12904; 12906 - 12923; 12925 - 12956; 12980 - 12997; and 13000 - 13042;

In a further aspect, the invention provides for an antisense oligonucleotide, 10-30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary, such as fully complementary to any of target sequence regions R_1 - R_295 (listed in table 1).

Table 1 Further exemplary regions of SEQ ID NO 11 which may be targeted by the oligonucleotides of the invention.

from	to	region
13	65	R_1
114	135	R_2
137	171	R_3
175	213	R_4
230	265	R_5
267	282	R_6
284	314	R_7
325	339	R_8
341	373	R_9
381	422	R_10
424	449	R_11
456	473	R_12

from	to	region
495	524	R_13
544	558	R_14
644	667	R_15
681	706	R_16
736	753	R_17
759	831	R_18
840	872	R_19
874	891	R_20
893	908	R_21
920	955	R_22
957	1019	R_23
1021	1037	R_24

from	to	region
1039	1055	R_25
1086	1120	R_26
1120	1161	R_27
1182	1210	R_28
1212	1244	R_29
1246	1270	R_30
1272	1342	R_31
1358	1382	R_32
1413	1432	R_33
1450	1464	R_34
1482	1500	R_35
1534	1553	R_36

from	to	region
1555	1571	R_37
1573	1616	R_38
1621	1657	R_39
1665	1691	R_40
1693	1714	R_41
1741	1762	R_42
1777	1830	R_43
1832	1858	R_44
1869	1886	R_45
1888	1934	R_46
1955	2000	R_47
2004	2036	R_48
2061	2079	R_49
2098	2118	R_50
2120	2218	R_51
2220	2237	R_52
2277	2335	R_53
2337	2372	R_54
2387	2413	R_55
2415	2459	R_56
2481	2499	R_57
2499	2517	R_58
2519	2534	R_59
2562	2577	R_60
2589	2620	R_61
2622	2636	R_62
2646	2677	R_63
2732	2758	R_64
2789	2813	R_65
2815	2839	R_66
2848	2876	R_67
2878	2927	R_68
2929	3014	R_69
3017	3034	R_70
3025	3039	R_71
3036	3092	R_72
3133	3177	R_73
3203	3232	R_74
3234	3249	R_75
3250	3275	R_76

from	to	region
3311	3330	R_77
3332	3361	R_78
3396	3427	R_79
3486	3518	R_80
3520	3541	R_81
3552	3612	R_82
3639	3656	R_83
3660	3714	R_84
3722	3750	R_85
3779	3795	R_86
3797	3817	R_87
3848	3881	R_88
3887	3905	R_89
3907	3931	R_90
3933	3967	R_91
3997	4029	R_92
4031	4056	R_93
4066	4081	R_94
4090	4110	R_95
4112	4131	R_96
4140	4170	R_97
4183	4224	R_98
4237	4273	R_99
4275	4313	R_100
4324	4341	R_101
4343	4370	R_102
4372	4386	R_103
4438	4453	R_104
4450	4482	R_105
4550	4569	R_106
4588	4621	R_107
4647	4683	R_108
4726	4742	R_109
4792	4807	R_110
4824	4852	R_111
4885	4911	R_112
4940	4963	R_113
4970	5002	R_114
5014	5055	R_115
5057	5100	R_116

from	to	region
5102	5119	R_117
5121	5182	R_118
5203	5221	R_119
5226	5241	R_120
5252	5266	R_121
5271	5309	R_122
5331	5352	R_123
5366	5382	R_124
5384	5430	R_125
5452	5481	R_126
5483	5519	R_127
5542	5562	R_128
5564	5581	R_129
5602	5618	R_130
5627	5666	R_131
5668	5692	R_132
5693	5710	R_133
5712	5731	R_134
5745	5774	R_135
5778	5829	R_136
5841	5858	R_137
5860	5879	R_138
5894	5973	R_139
5975	6008	R_140
6010	6029	R_141
6052	6094	R_142
6127	6141	R_143
6143	6170	R_144
6179	6201	R_145
6215	6249	R_146
6266	6339	R_147
6341	6382	R_148
6384	6455	R_149
6457	6474	R_150
6482	6516	R_151
6527	6561	R_152
6563	6602	R_153
6611	6627	R_154
6629	6644	R_155
6669	6772	R_156

from	to	region
6774	6818	R_157
6831	6858	R_158
6875	6896	R_159
6907	6923	R_160
6960	6990	R_161
7001	7015	R_162
7017	7034	R_163
7051	7112	R_164
7163	7178	R_165
7195	7214	R_166
7230	7244	R_167
7258	7319	R_168
7321	7353	R_169
7355	7406	R_170
7408	7441	R_171
7461	7475	R_172
7477	7504	R_173
7526	7558	R_174
7647	7688	R_175
7730	7752	R_176
7762	7795	R_177
7824	7864	R_178
7899	7923	R_179
7949	7968	R_180
7972	7987	R_181
7989	8039	R_182
8047	8068	R_183
8070	8088	R_184
8114	8135	R_185
8137	8170	R_186
8172	8194	R_187
8196	8214	R_188
8218	8251	R_189
8253	8272	R_190
8283	8345	R_191
8358	8392	R_192
8394	8497	R_193
8499	8525	R_194
8607	8638	R_195
8664	8730	R_196

from	to	region
8748	8817	R_197
8847	8875	R_198
8877	8892	R_199
8894	8910	R_200
8927	8943	R_201
8945	8972	R_202
8974	8988	R_203
9018	9032	R_204
9061	9084	R_205
9092	9190	R_206
9192	9214	R_207
9262	9285	R_208
9287	9318	R_209
9331	9366	R_210
9378	9415	R_211
9432	9476	R_212
9501	9523	R_213
9531	9546	R_214
9548	9564	R_215
9566	9626	R_216
9636	9650	R_217
9660	9697	R_218
9699	9719	R_219
9785	9822	R_220
9824	9855	R_221
9857	9872	R_222
9874	9915	R_223
9921	9970	R_224
9980	9997	R_225
10049	10064	R_226
10065	10083	R_227
10085	10101	R_228
10103	10117	R_229
10119	10204	R_230
10206	10231	R_231
10233	10288	R_232
10290	10310	R_233
10342	10362	R_234
10376	10391	R_235
10402	10422	R_236

from	to	region
10424	10469	R_237
10483	10623	R_238
10634	10698	R_239
10700	10723	R_240
10725	10740	R_241
10742	10758	R_242
10780	10796	R_243
10798	10824	R_244
10831	10856	R_245
10863	10892	R_246
10943	10960	R_247
10982	10999	R_248
11014	11038	R_249
11044	11066	R_250
11075	11095	R_251
11108	11136	R_252
11142	11159	R_253
11177	11198	R_254
11208	11222	R_255
11217	11233	R_256
11245	11269	R_257
11281	11303	R_258
11320	11362	R_259
11368	11387	R_260
11389	11408	R_261
11410	11424	R_262
11444	11505	R_263
11515	11566	R_264
11570	11584	R_265
11605	11637	R_266
11665	11699	R_267
11701	11723	R_268
11725	11747	R_269
11792	11816	R_270
11822	11838	R_271
11840	11874	R_272
11876	11902	R_273
11908	11926	R_274
11945	11968	R_275
11990	12032	R_276

from	to	region
12034	12090	R_277
12092	12119	R_278
12143	12161	R_279
12164	12318	R_280
12317	12333	R_281
12345	12420	R_282
12440	12481	R_283

from	to	region
12496	12514	R_284
12516	12542	R_285
12544	12604	R_286
12632	12647	R_287
12649	12701	R_288
12745	12776	R_289
12787	12812	R_290

from	to	region
12876	12904	R_291
12906	12923	R_292
12925	12956	R_293
12980	12997	R_294
13000	13042	R_295

Target Cell

The term a "target cell" as used herein refers to a cell which is expressing the target nucleic acid. In some embodiments the target cell may be *in vivo* or *in vitro*. In some embodiments the target cell is a mammalian cell such as a rodent cell, such as a mouse cell or a rat cell, or a primate cell such as a monkey cell or a human cell.

In some embodiments the target cell is an in vitro cell line, such as A431 or MDA-MB-231 (available from ATCC).

In preferred embodiments the target cell expresses GBP-1 mRNA, such as the GBP-1 pre-mRNA, e.g. SEQ ID NO 11. The poly A tail of GBP-1 mRNA is typically disregarded for antisense oligonucleotide targeting.

Naturally occurring variant

The term "naturally occurring variant" refers to variants of GBP-1 gene or transcripts which originate from the same genetic loci as the target nucleic acid, but may differ for example, by virtue of degeneracy of the genetic code causing a multiplicity of codons encoding the same amino acid, or due to alternative splicing of pre-mRNA, or the presence of polymorphisms, such as single nucleotide polymorphisms (SNPs), and allelic variants.

Based on the presence of the sufficient complementary sequence to the oligonucleotide, the oligonucleotide of the invention may therefore target the target nucleic acid and naturally occurring variants thereof.

The *homo sapiens* GBP-1 gene is located at chromosome 1: 89,052,319-89,065,360 reverse strand.

The key database entries for GBP-1, including the exon and intron start and end locations, are disclosed in Table 4 below:

Table 4:

Exons	start_SEQIDNO11	end_SEQIDNO11	Intron	start_SEQIDNO11	end_SEQIDNO11
EX_1	1	201	Int_1	201	2108
EX_2	2108	2316	Int_2	2316	5037
EX_3	5037	5164	Int_3	5164	5935
EX_4	5935	6044	Int_4	6044	6318
EX_5	6318	6520	Int_5	6520	7127
EX_6	7127	7369	Int_6	7369	8227
EX_7	8227	8507	Int_7	8507	9133
EX_8	9133	9345	Int_8	9345	10146
EX_9	10146	10248	Int_9	10248	10486
EX_10	10486	10679	Int_10	10679	11893

In some embodiments the contiguous nucleotide sequence of the oligonucleotide of the invention is complementary, such as fully complementary to an exonic region of the human GBP-1 pre-mRNA, selected from the group consisting of EX_1, EX_2, EX_3, EX_4, EX_5, EX_6, EX_7, EX_8, EX_9, EX_10.

In a further aspect, the invention provides for an antisense oligonucleotide, 10-30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary, such as fully complementary to any of target sequence regions selected from the group consisting of 1 - 201; 2108 - 2316; 5037 - 5164; 5935 - 6044; 6318 - 6520; 7127 - 7369; 8227 - 8507; 9133 - 9345; 10146 - 10248; and 10486 – 10679, of SEQ ID NO 11.

In some embodiments the contiguous nucleotide sequence of the oligonucleotide of the invention is complementary, such as fully complementary to an exonic region of the human GBP-1 pre-mRNA, selected from the group consisting of INT_1, INT_2, INT_3, INT_4, INT_5, INT_6, INT_7, INT_8, INT_9, INT_10.

In a further aspect, the invention provides for an antisense oligonucleotide, 10-30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 10 – 30 nucleotides in length, wherein the contiguous nucleotide

sequence is at least 90% complementary, such as fully complementary to any of target sequence regions selected from the group consisting of 201 - 2108; 2316 - 5037; 5164 - 5935; 6044 - 6318; 6520 - 7127; 7369 - 8227; 8507 - 9133; 9345 - 10146; 10248 - 10486; and 10679 - 11893, of SEQ ID NO 11.

In some embodiments, the naturally occurring variants have at least 95% such as at least 98% or at least 99% homology to a mammalian GBP-1 target nucleic acid, such as SEQ ID NO 11.

Modulation of expression

The term "modulation of expression" as used herein is to be understood as an overall term for an oligonucleotide's ability to alter the amount of GBP-1 protein or GBP-1 mRNA when compared to the amount of GBP-1 or GBP-1 mRNA prior to administration of the oligonucleotide. Alternatively, modulation of expression may be determined by reference to a control experiment. It is generally understood that the control is an individual or target cell treated with a saline composition or an individual or target cell treated with a non-targeting oligonucleotide (mock).

One type of modulation is an oligonucleotide's ability to inhibit, down-regulate, reduce, suppress, remove, stop, block, prevent, lessen, lower, avoid or terminate expression of GBP-1, e.g. by degradation of GBP-1 mRNA.

The oligonucelotides of the invention are capable of inhibiting the expression of GBP-1 mRNA in a cell which is expressing GBP-1mRNA.

High affinity modified nucleosides

A high affinity modified nucleoside is a modified nucleotide which, when incorporated into the oligonucleotide enhances the affinity of the oligonucleotide for its complementary target, for example as measured by the melting temperature (T^m). A high affinity modified nucleoside of the present invention preferably result in an increase in melting temperature between +0.5 to +12°C, more preferably between +1.5 to +10°C and most preferably between+3 to +8°C per modified nucleoside. Numerous high affinity modified nucleosides are known in the art and include for example, many 2' substituted nucleosides as well as locked nucleic acids (LNA) (see e.g. Freier & Altmann; Nucl. Acid Res., 1997, 25, 4429-4443 and Uhlmann; Curr. Opinion in Drug Development, 2000, 3(2), 293-213).

Sugar modifications

The oligomer of the invention may comprise one or more nucleosides which have a modified sugar moiety, *i.e.* a modification of the sugar moiety when compared to the ribose sugar moiety found in DNA and RNA.

Numerous nucleosides with modification of the ribose sugar moiety have been made, primarily with the aim of improving certain properties of oligonucleotides, such as affinity and/or nuclease resistance.

Such modifications include those where the ribose ring structure is modified, *e.g.* by replacement with a hexose ring (HNA), or a bicyclic ring, which typically have a biradicle bridge between the C2 and C4 carbons on the ribose ring (LNA), or an unlinked ribose ring which typically lacks a bond between the C2 and C3 carbons (e.g. UNA). Other sugar modified nucleosides include, for example, bicyclohexose nucleic acids (WO2011/017521) or tricyclic nucleic acids (WO2013/154798). Modified nucleosides also include nucleosides where the sugar moiety is replaced with a non-sugar moiety, for example in the case of peptide nucleic acids (PNA), or morpholino nucleic acids.

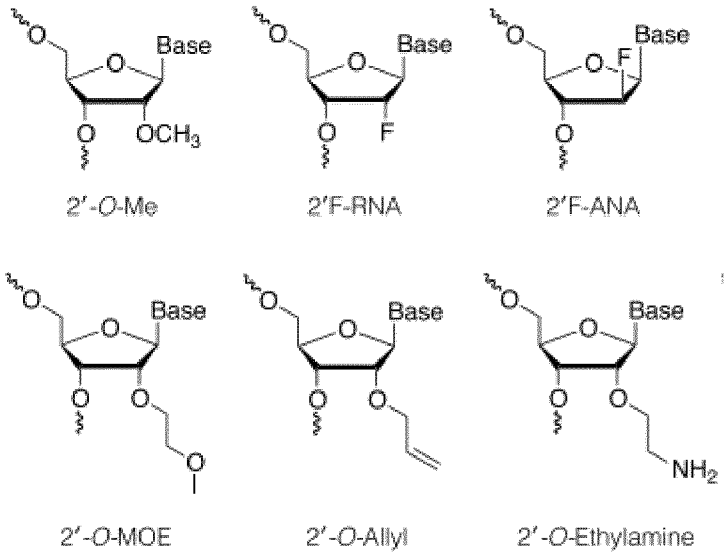
Sugar modifications also include modifications made via altering the substituent groups on the ribose ring to groups other than hydrogen, or the 2'-OH group naturally found in DNA and RNA nucleosides. Substituents may, for example be introduced at the 2', 3', 4' or 5' positions.

2' sugar modified nucleosides.

A 2' sugar modified nucleoside is a nucleoside which has a substituent other than H or –OH at the 2' position (2' substituted nucleoside) or comprises a 2' linked biradicle capable of forming a bridge between the 2' carbon and a second carbon in the ribose ring, such as LNA (2' – 4' biradicle bridged) nucleosides.

Indeed, much focus has been spent on developing 2' substituted nucleosides, and numerous 2' substituted nucleosides have been found to have beneficial properties when incorporated into oligonucleotides. For example, the 2' modified sugar may provide enhanced binding affinity and/or increased nuclease resistance to the oligonucleotide.

Examples of 2' substituted modified nucleosides are 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-Fluoro-RNA, and 2'-F-ANA nucleoside. For further examples, please see e.g. Freier & Altmann; Nucl. Acid Res., 1997, 25, 4429-4443 and Uhlmann; Curr. Opinion in Drug Development, 2000, 3(2), 293-213, and Deleavey and Damha, Chemistry and Biology 2012, 19, 937. Below are illustrations of some 2' substituted modified nucleosides.



In relation to the present invention 2' substituted does not include 2' bridged molecules like LNA.

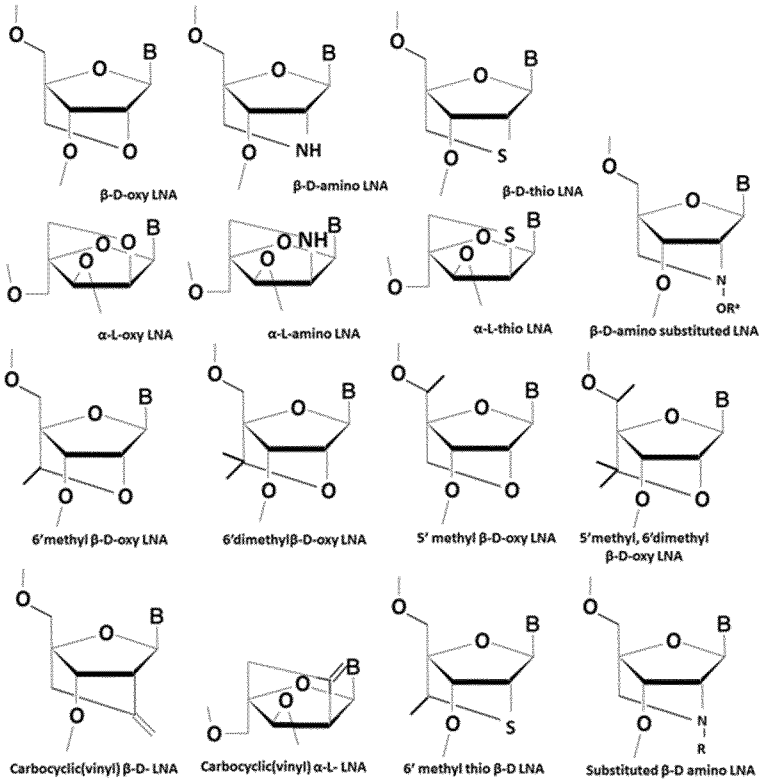
Locked Nucleic Acids (LNA)

A "LNA nucleoside" is a 2'- modified nucleoside which comprises a biradical linking the C2' and C4' of the ribose sugar ring of said nucleoside (also referred to as a "2'- 4' bridge"), which restricts or locks the conformation of the ribose ring. These nucleosides are also termed bridged nucleic acid or bicyclic nucleic acid (BNA) in the literature. The locking of the conformation of the ribose is associated with an enhanced affinity of hybridization (duplex stabilization) when the LNA is incorporated into an oligonucleotide for a complementary RNA or DNA molecule. This can be routinely determined by measuring the melting temperature of the oligonucleotide/complement duplex.

Non limiting, exemplary LNA nucleosides are disclosed in WO 99/014226, WO 00/66604, WO 98/039352, WO 2004/046160, WO 00/047599, WO 2007/134181, WO 2010/077578, WO 2010/036698, WO 2007/090071, WO 2009/006478, WO 2011/156202, WO 2008/154401, WO 2009/067647, WO 2008/150729, Morita et al., Bioorganic & Med.Chem. Lett. 12, 73-76, Seth et al. J. Org. Chem. 2010, Vol 75(5) pp. 1569-81, and Mitsuoka et al., Nucleic Acids Research 2009, 37(4), 1225-1238, and Wan and Seth, J. Medical Chemistry 2016, 59, 9645−9667.

Further non-limiting, exemplary LNA nucleosides are disclosed in Scheme 1.

Scheme 1:



Particular LNA nucleosides are beta-D-oxy-LNA, 6'-methyl-beta-D-oxy LNA such as (S)-6'-methyl-beta-D-oxy-LNA (ScET) and ENA.

A particularly advantageous LNA is beta-D-oxy-LNA.

RNase H Activity and Recruitment

The RNase H activity of an antisense oligonucleotide refers to its ability to recruit RNase H when in a duplex with a complementary RNA molecule. WO01/23613 provides *in vitro* methods for determining RNaseH activity, which may be used to determine the ability to recruit RNaseH. Typically an oligonucleotide is deemed capable of recruiting RNase H if it, when provided with a complementary target nucleic acid sequence, has an initial rate, as measured in pmol/l/min, of at least 5%, such as at least 10% or more than 20% of the of the initial rate determined when using a oligonucleotide having the same base sequence as the modified oligonucleotide being tested, but containing only DNA monomers with phosphorothioate linkages between all monomers in the oligonucleotide, and using the methodology provided by Example 91 - 95 of WO01/23613 (hereby incorporated by reference). For use in determining RHase H activity, recombinant human RNase H1 is available from Lubio Science GmbH, Lucerne, Switzerland.

Gapmer

The antisense oligonucleotide of the invention, or contiguous nucleotide sequence thereof may be a gapmer. Various gapmer designs are described herein.

The antisense gapmers are commonly used to inhibit a target nucleic acid via RNase H mediated degradation. A gapmer oligonucleotide comprises at least three distinct structural regions a 5'-flank, a gap and a 3'-flank, F-G-F' in the '5 -> 3' orientation.. The "gap" region (G) comprises a stretch of contiguous DNA nucleotides which enable the oligonucleotide to recruit RNase H. The gap region is flanked by a 5' flanking region (F) comprising one or more sugar modified nucleosides, advantageously high affinity sugar modified nucleosides, and by a 3' flanking region (F') comprising one or more sugar modified nucleosides, advantageously high affinity sugar modified nucleosides. The one or more sugar modified nucleosides in region F and F' enhance the affinity of the oligonucleotide for the target nucleic acid (*i.e.* are affinity enhancing sugar modified nucleosides). In some embodiments, the one or more sugar modified nucleosides in region F and F' are 2' sugar modified nucleosides, such as high affinity 2' sugar modifications, such as independently selected from LNA and 2'-MOE.

In a gapmer design, the 5' and 3' most nucleosides of the gap region are DNA nucleosides, and are positioned adjacent to a sugar modified nucleoside of the 5' (F) or 3' (F') region respectively. The flanks may be further defined by having at least one sugar modified nucleoside at the end most distant from the gap region, i.e. at the 5' end of the 5' flank and at the 3' end of the 3' flank.

Regions F-G-F' form a contiguous nucleotide sequence. Antisense oligonucleotides of the invention, or the contiguous nucleotide sequence thereof, may comprise a gapmer region of formula F-G-F'.

The overall length of the gapmer design F-G-F' may be, for example 10 to 30 nucleosides, such as 13 to 24, such as 14 to 22 nucleosides, Such as from 14 to 18, such as 15 to17 nucleosides.

By way of example, the gapmer oligonucleotide of the present invention can be represented by the following formulae:

F_{1-8}-G_{5-16}-F'_{1-8}, such as

F_{1-8}-G_{7-16}-F'_{2-8}

with the proviso that the overall length of the gapmer regions F-G-F' is at least 12, such as at least 14 nucleotides in length.

Regions F, G and F' are further defined below and can be incorporated into the F-G-F' formula.

Gapmer - Region G

Region G (gap region) of the gapmer is a region of nucleosides which enables the oligonucleotide to recruit RNaseH, such as human RNase H1, typically DNA nucleosides.

RNaseH is a cellular enzyme which recognizes the duplex between DNA and RNA, and enzymatically cleaves the RNA molecule. Suitably gapmers may have a gap region (G) of at least 5 or 6 contiguous DNA nucleosides, such as 5 – 16 contiguous DNA nucleosides, such as 6 – 15 contiguous DNA nucleosides, such as 7-14 contiguous DNA nucleosides, such as 8 – 12 contiguous DNA nucleotides, such as 8 – 12 contiguous DNA nucleotides in length. The gap region G may, in some embodiments consist of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 contiguous DNA nucleosides. One or more cytosine (C) DNA in the gap region may in some instances be methylated (e.g. when a DNA c is followed by a DNA g) such residues are either annotated as 5-methyl-cytosine (^{me}C). In some embodiments the gap region G may consist of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 contiguous phosphorothioate linked DNA nucleosides. In some embodiments, all internucleoside linkages in the gap are phosphorothioate linkages.

Whilst traditional gapmers have a DNA gap region, there are numerous examples of modified nucleosides which allow for RNaseH recruitment when they are used within the gap region. Modified nucleosides which have been reported as being capable of recruiting RNaseH when included within a gap region include, for example, alpha-L-LNA, C4' alkylated DNA (as described in PCT/EP2009/050349 and Vester *et al.*, Bioorg. Med. Chem. Lett. 18 (2008) 2296 – 2300, both incorporated herein by reference), arabinose derived nucleosides like ANA and 2'F-ANA (Mangos et al. 2003 J. AM. CHEM. SOC. 125, 654-661), UNA (unlocked nucleic acid) (as described in Fluiter *et al.*, Mol. Biosyst., 2009, 10, 1039 incorporated herein by reference). UNA is unlocked nucleic acid, typically where the bond between C2 and C3 of the ribose has been removed, forming an unlocked "sugar" residue. The modified nucleosides used in such gapmers may be nucleosides which adopt a 2' endo (DNA like) structure when introduced into the gap region, *i.e.* modifications which allow for RNaseH recruitment). In some embodiments the DNA Gap region (G) described herein may optionally contain 1 to 3 sugar modified nucleosides which adopt a 2' endo (DNA like) structure when introduced into the gap region.

Region G - "Gap-breaker"

Alternatively, there are numerous reports of the insertion of a modified nucleoside which confers a 3' endo conformation into the gap region of gapmers, whilst retaining some RNaseH activity. Such gapmers with a gap region comprising one or more 3'endo modified nucleosides are referred to as "gap-breaker" or "gap-disrupted" gapmers, see for example WO2013/022984. Gap-breaker oligonucleotides retain sufficient region of DNA nucleosides within the gap region to allow for RNaseH recruitment. The ability of gapbreaker oligonucleotide design to recruit RNaseH is typically sequence or even compound specific –

see Rukov et al. 2015 Nucl. Acids Res. Vol. 43 pp. 8476-8487, which discloses "gapbreaker" oligonucleotides which recruit RNaseH which in some instances provide a more specific cleavage of the target RNA. Modified nucleosides used within the gap region of gap-breaker oligonucleotides may for example be modified nucleosides which confer a 3'endo confirmation, such 2' –O-methyl (OMe) or 2'-O-MOE (MOE) nucleosides, or beta-D LNA nucleosides (the bridge between C2' and C4' of the ribose sugar ring of a nucleoside is in the beta conformation), such as beta-D-oxy LNA or ScET nucleosides.

As with gapmers containing region G described above, the gap region of gap-breaker or gap-disrupted gapmers, have a DNA nucleoside at the 5' end of the gap (adjacent to the 3' nucleoside of region F), and a DNA nucleoside at the 3' end of the gap (adjacent to the 5' nucleoside of region F'). Gapmers which comprise a disrupted gap typically retain a region of at least 3 or 4 contiguous DNA nucleosides at either the 5' end or 3' end of the gap region. Exemplary designs for gap-breaker oligonucleotides include

F_{1-8}-[D_{3-4}-E_1- D_{3-4}].F'_{1-8}

F_{1-8}- [D_{1-4}-E_1- D_{3-4}]-F'_{1-8}

F_{1-8}- [D_{3-4}-E_1- D_{1-4}]-F'_{1-8}

wherein region G is within the brackets [D_n-E_r- D_m], D is a contiguous sequence of DNA nucleosides, E is a modified nucleoside (the gap-breaker or gap-disrupting nucleoside), and F and F' are the flanking regions as defined herein, and with the proviso that the overall length of the gapmer regions F-G-F' is at least 12, such as at least 14 nucleotides in length. In some embodiments, region G of a gap disrupted gapmer comprises at least 6 DNA nucleosides, such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 DNA nucleosides. As described above, the DNA nucleosides may be contiguous or may optionally be interspersed with one or more modified nucleosides, with the proviso that the gap region G is capable of mediating RNaseH recruitment.

Gapmer - flanking regions, F and F'

Region F is positioned immediately adjacent to the 5' DNA nucleoside of region G. The 3' most nucleoside of region F is a sugar modified nucleoside, such as a high affinity sugar modified nucleoside, for example a 2' substituted nucleoside, such as a MOE nucleoside, or an LNA nucleoside.

Region F' is positioned immediately adjacent to the 3' DNA nucleoside of region G. The 5' most nucleoside of region F' is a sugar modified nucleoside, such as a high affinity sugar modified nucleoside, for example a 2' substituted nucleoside, such as a MOE nucleoside, or an LNA nucleoside.

Region F is 1 – 8 contiguous nucleotides in length, such as 2-6, such as 3-4 contiguous nucleotides in length. Advantageously the 5' most nucleoside of region F is a sugar modified nucleoside. In some embodiments the two 5' most nucleoside of region F are sugar modified nucleoside. In some embodiments the 5' most nucleoside of region F is an LNA nucleoside. In some embodiments the two 5' most nucleoside of region F are LNA nucleosides. In some embodiments the two 5' most nucleoside of region F are 2' substituted nucleoside nucleosides, such as two 3' MOE nucleosides. In some embodiments the 5' most nucleoside of region F is a 2' substituted nucleoside, such as a MOE nucleoside.

Region F' is 2 – 8 contiguous nucleotides in length, such as 3-6, such as 4-5 contiguous nucleotides in length. Advantageously, embodiments the 3' most nucleoside of region F' is a sugar modified nucleoside. In some embodiments the two 3' most nucleoside of region F' are sugar modified nucleoside. In some embodiments the two 3' most nucleoside of region F' are LNA nucleosides. In some embodiments the 3' most nucleoside of region F' is an LNA nucleoside. In some embodiments the two 3' most nucleoside of region F' are 2' substituted nucleoside nucleosides, such as two 3' MOE nucleosides. In some embodiments the 3' most nucleoside of region F' is a 2' substituted nucleoside, such as a MOE nucleoside.

It should be noted that when the length of region F or F' is one, it is advantageously an LNA nucleoside.

In some embodiments, region F and F' independently consists of or comprises a contiguous sequence of sugar modified nucleosides. In some embodiments, the sugar modified nucleosides of region F may be independently selected from 2'-O-alkyl-RNA units, 2'-O-methyl-RNA, 2'-amino-DNA units, 2'-fluoro-DNA units, 2'-alkoxy-RNA, MOE units, LNA units, arabino nucleic acid (ANA) units and 2'-fluoro-ANA units.

In some embodiments, region F and F' independently comprises both LNA and a 2' substituted modified nucleosides (mixed wing design).

In some embodiments, region F and F' consists of only one type of sugar modified nucleosides, such as only MOE or only beta-D-oxy LNA or only ScET. Such designs are also termed uniform flanks or uniform gapmer design.

In some embodiments, all the nucleosides of region F or F', or F and F' are LNA nucleosides, such as independently selected from beta-D-oxy LNA, ENA or ScET nucleosides. In some embodiments region F consists of 1-5, such as 2-4, such as 3-4 such as 1, 2, 3, 4 or 5 contiguous LNA nucleosides. In some embodiments, all the nucleosides of region F and F' are beta-D-oxy LNA nucleosides.

In some embodiments, all the nucleosides of region F or F', or F and F' are 2' substituted nucleosides, such as OMe or MOE nucleosides. In some embodiments region F consists of

1, 2, 3, 4, 5, 6, 7, or 8 contiguous OMe or MOE nucleosides. In some embodiments only one of the flanking regions can consist of 2' substituted nucleosides, such as OMe or MOE nucleosides. In some embodiments it is the 5' (F) flanking region that consists 2' substituted nucleosides, such as OMe or MOE nucleosides whereas the 3' (F') flanking region comprises at least one LNA nucleoside, such as beta-D-oxy LNA nucleosides or cET nucleosides. In some embodiments it is the 3' (F') flanking region that consists 2' substituted nucleosides, such as OMe or MOE nucleosides whereas the 5' (F) flanking region comprises at least one LNA nucleoside, such as beta-D-oxy LNA nucleosides or cET nucleosides.

In some embodiments, all the modified nucleosides of region F and F' are LNA nucleosides, such as independently selected from beta-D-oxy LNA, ENA or ScET nucleosides, wherein region F or F', or F and F' may optionally comprise DNA nucleosides (an alternating flank, see definition of these for more details). In some embodiments, all the modified nucleosides of region F and F' are beta-D-oxy LNA nucleosides, wherein region F or F', or F and F' may optionally comprise DNA nucleosides (an alternating flank, see definition of these for more details).

In some embodiments the 5' most and the 3' most nucleosides of region F and F' are LNA nucleosides, such as beta-D-oxy LNA nucleosides or ScET nucleosides.

In some embodiments, the internucleoside linkage between region F and region G is a phosphorothioate internucleoside linkage. In some embodiments, the internucleoside linkage between region F' and region G is a phosphorothioate internucleoside linkage. In some embodiments, the internucleoside linkages between the nucleosides of region F or F', F and F' are phosphorothioate internucleoside linkages.

LNA Gapmer

An LNA gapmer is a gapmer wherein either one or both of region F and F' comprises or consists of LNA nucleosides. A beta-D-oxy gapmer is a gapmer wherein either one or both of region F and F' comprises or consists of beta-D-oxy LNA nucleosides.

In some embodiments the LNA gapmer is of formula: $[LNA]_{1-5}$-[region G] -$[LNA]_{1-5}$, wherein region G is as defined in the Gapmer region G definition.

MOE Gapmers

A MOE gapmers is a gapmer wherein regions F and F' consist of MOE nucleosides. In some embodiments the MOE gapmer is of design $[MOE]_{1-8}$-[Region G]-$[MOE]_{1-8}$, such as $[MOE]_{2-7}$-[Region G]$_{5-16}$-$[MOE]_{2-7}$, such as $[MOE]_{3-6}$-[Region G]-$[MOE]_{3-6}$, wherein region G is as defined in the Gapmer definition. MOE gapmers with a 5-10-5 design (MOE-DNA-MOE) have been widely used in the art.

Mixed Wing Gapmer

A mixed wing gapmer is an LNA gapmer wherein one or both of region F and F' comprise a 2' substituted nucleoside, such as a 2' substituted nucleoside independently selected from the group consisting of 2'-O-alkyl-RNA units, 2'-O-methyl-RNA, 2'-amino-DNA units, 2'-fluoro-DNA units, 2'-alkoxy-RNA, MOE units, arabino nucleic acid (ANA) units and 2'-fluoro-ANA units, such as a MOE nucleosides. In some embodiments wherein at least one of region F and F', or both region F and F' comprise at least one LNA nucleoside, the remaining nucleosides of region F and F' are independently selected from the group consisting of MOE and LNA. In some embodiments wherein at least one of region F and F', or both region F and F' comprise at least two LNA nucleosides, the remaining nucleosides of region F and F' are independently selected from the group consisting of MOE and LNA. In some mixed wing embodiments, one or both of region F and F' may further comprise one or more DNA nucleosides.

Mixed wing gapmer designs are disclosed in WO2008/049085 and WO2012/109395, both of which are hereby incorporated by reference.

Alternating Flank Gapmers

Oligonucleotides with alternating flanks are LNA gapmer oligonucleotides where at least one of the flanks (F or F') comprises DNA in addition to the LNA nucleoside(s). In some embodiments at least one of region F or F', or both region F and F', comprise both LNA nucleosides and DNA nucleosides. In such embodiments, the flanking region F or F', or both F and F' comprise at least three nucleosides, wherein the 5' and 3' most nucleosides of the F and/or F' region are LNA nucleosides.

In some embodiments at least one of region F or F', or both region F and F', comprise both LNA nucleosides and DNA nucleosides. In such embodiments, the flanking region F or F', or both F and F' comprise at least three nucleosides, wherein the 5' and 3' most nucleosides of the F or F' region are LNA nucleosides, and there is at least one DNA nucleoside positioned between the 5' and 3' most LNA nucleosides of region F or F' (or both region F and F').

Region D' or D'' in an oligonucleotide

The oligonucleotide of the invention may in some embodiments comprise or consist of the contiguous nucleotide sequence of the oligonucleotide which is complementary to the target nucleic acid, such as the gapmer F-G-F', and further 5' and/or 3' nucleosides. The further 5' and/or 3' nucleosides may or may not be fully complementary to the target nucleic acid. Such further 5' and/or 3' nucleosides may be referred to as region D' and D'' herein.

The addition of region D' or D'' may be used for the purpose of joining the contiguous nucleotide sequence, such as the gapmer, to a conjugate moiety or another functional group. When used for joining the contiguous nucleotide sequence with a conjugate moiety is can serve as a biocleavable linker. Alternatively, it may be used to provide exonucleoase protection or for ease of synthesis or manufacture.

Region D' and D'' can be attached to the 5' end of region F or the 3' end of region F', respectively to generate designs of the following formulas D'-F-G-F', F-G-F'-D'' or D'-F-G-F'-D''. In this instance the F-G-F' is the gapmer portion of the oligonucleotide and region D' or D'' constitute a separate part of the oligonucleotide.

Region D' or D'' may independently comprise or consist of 1, 2, 3, 4 or 5 additional nucleotides, which may be complementary or non-complementary to the target nucleic acid. The nucleotide adjacent to the F or F' region is not a sugar-modified nucleotide, such as a DNA or RNA or base modified versions of these. The D' or D' region may serve as a nuclease susceptible biocleavable linker (see definition of linkers). In some embodiments the additional 5' and/or 3' end nucleotides are linked with phosphodiester linkages and are DNA or RNA. Nucleotide based biocleavable linkers suitable for use as region D' or D'' are disclosed in WO2014/076195, which include by way of example a phosphodiester linked DNA dinucleotide. The use of biocleavable linkers in poly-oligonucleotide constructs is disclosed in WO2015/113922, where they are used to link multiple antisense constructs (e.g. gapmer regions) within a single oligonucleotide.

In one embodiment the oligonucleotide of the invention comprises a region D' and/or D'' in addition to the contiguous nucleotide sequence which constitutes the gapmer.

In some embodiments, the oligonucleotide of the present invention can be represented by the following formulae:

F-G-F'; in particular F_{1-8}-G_{5-16}-F'_{2-8}

D'-F-G-F', in particular D'_{1-3}-F_{1-8}-G_{5-16}-F'_{2-8}

F-G-F'-D'', in particular F_{1-8}-G_{5-16}-F'_{2-8}-D''_{1-3}

D'-F-G-F'-D'', in particular D'_{1-3}- F_{1-8}-G_{5-16}-F'_{2-8}-D''_{1-3}

In some embodiments the internucleoside linkage positioned between region D' and region F is a phosphodiester linkage. In some embodiments the internucleoside linkage positioned between region F' and region D'' is a phosphodiester linkage.

Conjugate

The term conjugate as used herein refers to an oligonucleotide which is covalently linked to a non-nucleotide moiety (conjugate moiety or region C or third region).

Conjugation of the oligonucleotide of the invention to one or more non-nucleotide moieties may improve the pharmacology of the oligonucleotide, *e.g.* by affecting the activity, cellular distribution, cellular uptake or stability of the oligonucleotide. In some embodiments the conjugate moiety modifies or enhances the pharmacokinetic properties of the oligonucleotide by improving cellular distribution, bioavailability, metabolism, excretion, permeability, and/or cellular uptake of the oligonucleotide. In particular, the conjugate may target the oligonucleotide to a specific organ, tissue or cell type and thereby enhance the effectiveness of the oligonucleotide in that organ, tissue or cell type. The conjugate may serve to reduce activity of the oligonucleotide in non-target cell types, tissues or organs, e.g. off target activity or activity in non-target cell types, tissues or organs.

In an embodiment, the non-nucleotide moiety (conjugate moiety) is selected from the group consisting of carbohydrates, cell surface receptor ligands, drug substances, hormones, lipophilic substances, polymers, proteins, peptides, toxins (e.g. bacterial toxins), vitamins, viral proteins (e.g. capsids) or combinations thereof.

Linkers

A linkage or linker is a connection between two atoms that links one chemical group or segment of interest to another chemical group or segment of interest via one or more covalent bonds. Conjugate moieties can be attached to the oligonucleotide directly or through a linking moiety (e.g. linker or tether). Linkers serve to covalently connect a third region, e.g. a conjugate moiety (Region C), to a first region, e.g. an oligonucleotide or contiguous nucleotide sequence or gapmer region F-G-F' (region A).

In some embodiments of the invention the conjugate or oligonucleotide conjugate of the invention may optionally, comprise a linker region (second region or region B and/or region Y) which is positioned between the oligonucleotide or contiguous nucleotide sequence complementary to the target nucleic acid (region A or first region) and the conjugate moiety (region C or third region).

Region B refers to biocleavable linkers comprising or consisting of a physiologically labile bond that is cleavable under conditions normally encountered or analogous to those encountered within a mammalian body. Conditions under which physiologically labile linkers undergo chemical transformation (e.g., cleavage) include chemical conditions such as pH, temperature, oxidative or reductive conditions or agents, and salt concentration found in or analogous to those encountered in mammalian cells. Mammalian intracellular conditions also include the presence of enzymatic activity normally present in a mammalian cell such as from proteolytic enzymes or hydrolytic enzymes or nucleases. In one embodiment the biocleavable linker is susceptible to S1 nuclease cleavage. DNA phosphodiester containing

biocleavable linkers are described in more detail in WO 2014/076195 (hereby incorporated by reference) – see also region D' or D'' herein.

Region Y refers to linkers that are not necessarily biocleavable but primarily serve to covalently connect a conjugate moiety (region C or third region), to an oligonucleotide (region A or first region). The region Y linkers may comprise a chain structure or an oligomer of repeating units such as ethylene glycol, amino acid units or amino alkyl groups. The oligonucleotide conjugates of the present invention can be constructed of the following regional elements A-C, A-B-C, A-B-Y-C, A-Y-B-C or A-Y-C. In some embodiments the linker (region Y) is an amino alkyl, such as a C2 – C36 amino alkyl group, including, for example C6 to C12 amino alkyl groups. In a preferred embodiment the linker (region Y) is a C6 amino alkyl group.

Treatment

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 recognized that treatment as referred to herein may, in some embodiments, be prophylactic.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to oligonucleotides, such as antisense oligonucleotides, capable of inhibiting the expression of GBP-1.

The oligonucleotides of the invention targeting GBP-1 are capable of hybridizing to and inhibiting the expression of a GBP-1 target nucleic acid in a cell which is expressing the GBP-1 target nucleic acid.

The GBP-1 target nucleic acid may be a mammalian GBP-1 mRNA or pre-mRNA, such as a human GBP-1 mRNA or pre-mRNA, for example a pre-mRNA or mRNA originating from the *Homo sapiens* GBP-1 gene (Homo sapiens Chromosome 1: 89,052,319-89,065,360 reverse strand) – such as SEQ ID NO 11.

The oligonucleotides of the invention are capable of inhibiting the expression of GBP-1 target nucleic acid, such as the GBP-1 mRNA, in a cell which is expressing the target nucleic acid, such as the GBP-1 mRNA.

In some embodiments, oligonucleotides of the invention are capable of inhibiting the expression of GBP-1 target nucleic acid in a cell which is expressing the target nucleic acid, so to reduce the level of GBP-1 target nucleic acid (e.g. the mRNA) by at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% inhibition compared to the expression level

of the GBP-1 target nucleic acid (e.g. the mRNA) in the cell. Suitably the cell is selected from the group consisting of A431 and MDA-MB-231 cells. Example 1 provides a suitable assay for evaluating the ability of the oligonucleotides of the invention to inhibit the expression of the target nucleic acid. Suitably the evaluation of a compounds ability to inhibit the expression of the target nucleic acid is performed in vitro, such a gymnotic *in vitro* assay, for example as according to Example 1.

An aspect of the present invention relates to an antisense oligonucelotide, such as an LNA antisense oligonucleotide gapmer which comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementarity, such as is fully complementary to a sequence selected from the group consisting of SEQ ID NO 12 – 19.

An aspect of the present invention relates to an antisense oligonucleotide, such as an LNA antisense oligonucleotide gapmer which comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementarity, such as is fully complementary to any of SEQ ID Nos: 12 – 19.

In some embodiments, the oligonucleotide comprises a contiguous sequence of 10 – 30 nucleotides, which is at least 90% complementary, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, or 100% complementary with a region of the target nucleic acid or a target sequence, such as one selected from SEQ ID Nos 12 – 19.

The inventors have identified particularly effective sequences of the GBP-1 target nucleic acid which may be targeted by the oligonucleotide of the invention.

In some embodiments the target sequence is SEQ ID NO 12.

In some embodiments the target sequence is SEQ ID NO 13.

In some embodiments the target sequence is SEQ ID NO 14.

In some embodiments the target sequence is SEQ ID NO 15.

In some embodiments the target sequence is SEQ ID NO 16.

In some embodiments the target sequence is SEQ ID NO 17.

In some embodiments the target sequence is SEQ ID NO 18.

In some embodiments the target sequence is SEQ ID NO 19.

In some embodiments, the oligonucleotide of the invention comprises a contiguous nucleotides sequence of 12 – 16, such as 13, 14, 15, contiguous nucleotides in length, wherein the contiguous nucleotide sequence is fully complementary to SEQ ID NO 12

In some embodiments, the oligonucleotide of the invention comprises a contiguous nucleotides sequence of 12 – 16, such as 13, 14, 15, contiguous nucleotides in length, wherein the contiguous nucleotide sequence is fully complementary to SEQ ID NO 13.

In some embodiments, the oligonucleotide of the invention comprises a contiguous nucleotides sequence of 12 – 16, such as 13, 14, 15, contiguous nucleotides in length, wherein the contiguous nucleotide sequence is fully complementary to SEQ ID NO 14.

In some embodiments, the oligonucleotide of the invention comprises a contiguous nucleotides sequence of 12 – 16, such as 13, 14, 15, contiguous nucleotides in length, wherein the contiguous nucleotide sequence is fully complementary to SEQ ID NO 15.

In some embodiments, the oligonucleotide of the invention comprises a contiguous nucleotides sequence of 12 – 16, such as 13, 14, 15, contiguous nucleotides in length, wherein the contiguous nucleotide sequence is fully complementary to SEQ ID NO 16.

In some embodiments, the oligonucleotide of the invention comprises a contiguous nucleotides sequence of 12 – 16, such as 13, 14, or 15 contiguous nucleotides in length, wherein the contiguous nucleotide sequence is fully complementary to SEQ ID NO 17.

In some embodiments, the oligonucleotide of the invention comprises a contiguous nucleotides sequence of 12 – 16, such as 13, 14, or 15, contiguous nucleotides in length, wherein the contiguous nucleotide sequence is fully complementary to SEQ ID NO 18.

In some embodiments, the oligonucleotide of the invention comprises a contiguous nucleotides sequence of 12 – 19, such as 13, 14, 15, 16, 17 or 18 contiguous nucleotides in length, wherein the contiguous nucleotide sequence is fully complementary to SEQ ID NO 19.

In some embodiments, the antisense oligonucleotide of the invention or the contiguous nucleotide sequence thereof is a gapmer, such as an LNA gapmer, a mixed wing gapmer, or an alternating flank gapmer.

In some embodiments, the antisense oligonucleotide according to the invention, comprises a contiguous nucleotide sequence of at least 10 contiguous nucleotides, such as at least 12 contiguous nucleotides, such as at least 13 contiguous nucleotides, such as at least 14 contiguous nucleotides, such as at least 15 contiguous nucleotides, such as at least 16 contiguous nucleotides, such as at least 17 contiguous nucleotides, which is fully complementary to SEQ ID NO 11.

In some embodiments, the antisense oligonucleotide according to the invention, comprises a contiguous nucleotide sequence of at least 10 contiguous nucleotides, such as at least 12 contiguous nucleotides, such as at least 13 contiguous nucleotides, such as at least 14

contiguous nucleotides, such as at least 15 contiguous nucleotides, such as at least 16 contiguous nucleotides, which is fully complementary to SEQ ID NO 12.

In some embodiments, the antisense oligonucleotide according to the invention, comprises a contiguous nucleotide sequence of at least 10 contiguous nucleotides, such as at least 12 contiguous nucleotides, such as at least 13 contiguous nucleotides, such as at least 14 contiguous nucleotides, such as at least 15 contiguous nucleotides, such as at least 16 contiguous nucleotides, which is fully complementary to SEQ ID NO 13.

In some embodiments, the antisense oligonucleotide according to the invention, comprises a contiguous nucleotide sequence of at least 10 contiguous nucleotides, such as at least 12 contiguous nucleotides, such as at least 13 contiguous nucleotides, such as at least 14 contiguous nucleotides, such as at least 15 contiguous nucleotides, such as at least 16 contiguous nucleotides, which is fully complementary to SEQ ID NO 14.

In some embodiments, the antisense oligonucleotide according to the invention, comprises a contiguous nucleotide sequence of at least 10 contiguous nucleotides, such as at least 12 contiguous nucleotides, such as at least 13 contiguous nucleotides, such as at least 14 contiguous nucleotides, such as at least 15 contiguous nucleotides, such as at least 16 contiguous nucleotides, which is fully complementary to SEQ ID NO 15.

In some embodiments, the antisense oligonucleotide according to the invention, comprises a contiguous nucleotide sequence of at least 10 contiguous nucleotides, such as at least 12 contiguous nucleotides, such as at least 13 contiguous nucleotides, such as at least 14 contiguous nucleotides, such as at least 15 contiguous nucleotides, such as at least 16 contiguous nucleotides, which is fully complementary to SEQ ID NO 16.

In some embodiments, the antisense oligonucleotide according to the invention, comprises a contiguous nucleotide sequence of at least 10 contiguous nucleotides, such as at least 12 contiguous nucleotides, such as at least 13 contiguous nucleotides, such as at least 14 contiguous nucleotides, such as at least 15 contiguous nucleotides, such as at least 16 contiguous nucleotides, which is fully complementary to SEQ ID NO 17.

In some embodiments, the antisense oligonucleotide according to the invention, comprises a contiguous nucleotide sequence of at least 10 contiguous nucleotides, such as at least 12 contiguous nucleotides, such as at least 13 contiguous nucleotides, such as at least 14 contiguous nucleotides, such as at least 15 contiguous nucleotides, such as at least 16 contiguous nucleotides, which is fully complementary to SEQ ID NO 18.

In some embodiments, the antisense oligonucleotide according to the invention, comprises a contiguous nucleotide sequence of at least 10 contiguous nucleotides, such as at least 12 contiguous nucleotides, such as at least 13 contiguous nucleotides, such as at least 14

contiguous nucleotides, such as at least 15 contiguous nucleotides, such as at least 16 contiguous nucleotides, such as at least 17 contiguous nucleotides, which is fully complementary to SEQ ID NO 19.

In some embodiments the contiguous nucleotide sequence of the antisense oligonucleotide according to the invention is less than 20 nucleotides in length. In some embodiments the contiguous nucleotide sequence of the antisense oligonucleotide according to the invention is 12 - 24 nucleotides in length. In some embodiments the contiguous nucleotide sequence of the antisense oligonucleotide according to the invention is 12 - 22 nucleotides in length. In some embodiments the contiguous nucleotide sequence of the antisense oligonucleotide according to the invention is 12 - 20 nucleotides in length. In some embodiments the contiguous nucleotide sequence of the antisense oligonucleotide according to the invention is 12 - 18 nucleotides in length. In some embodiments the contiguous nucleotide sequence of the antisense oligonucleotide according to the invention is 14 - 17 nucleotides in length.

Advantageously, in some embodiments all of the internucleoside linkages between the nucleosides of the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.

In some embodiments, the contiguous nucleotide sequence is fully complementary to SEQ ID NO 12.

In some embodiments, the contiguous nucleotide sequence is fully complementary to SEQ ID NO 13.

In some embodiments, the contiguous nucleotide sequence is fully complementary to SEQ ID NO 14.

In some embodiments, the contiguous nucleotide sequence is fully complementary to SEQ ID NO 15.

In some embodiments, the contiguous nucleotide sequence is fully complementary to SEQ ID NO 16.

In some embodiments, the contiguous nucleotide sequence is fully complementary to SEQ ID NO 17.

In some embodiments, the contiguous nucleotide sequence is fully complementary to SEQ ID NO 18.

In some embodiments, the contiguous nucleotide sequence is fully complementary to SEQ ID NO 19.

In some embodiments, the antisense oligonucleotide is a gapmer oligonucleotide comprising a contiguous nucleotide sequence of formula 5'-F-G-F'-3', where region F and F'

independently comprise 1 - 8 sugar modified nucleosides, and G is a region between 5 and 16 nucleosides which are capable of recruiting RNaseH.

In some embodiments, the sugar modified nucleosides of region F and F' are independently selected from the group consisting of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA, 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA and LNA nucleosides.

In some embodiments, region G comprises 5 – 16 contiguous DNA nucleosides.

In some embodiments, wherein the antisense oligonucleotide is a gapmer oligonucleotide, such as an LNA gapmer oligonucleotide.

In some embodiments, the LNA nucleosides are beta-D-oxy LNA nucleosides.

In some embodiments, the internucleoside linkages between the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.

Table 5

SEQ ID NO	Motif	CMP ID NO	Compound	Target region – SEQ ID	Target region Sequence
1	tcaaatgtagtgacgc	1_1	TCAAatgtagtga^{m}cGC	12	gcgtcactacatttga
2	caaatgtagtgacgc	2_1	CAaatgtagtgACGC	12	gcgtcactacatttga
3	tgaggattatacatgg	3_1	TGAggattatacaTGG	13	ccatgtataatcctca
4	gtttggtaagatcctc	4_1	GTTtggtaagatcCTC	14	gaggatcttaccaaac
5	cccattgaagttataa	5_1	CCCattgaagttaTAA	15	ttataacttcaatggg
6	cctaatcacagcatac	6_1	CCtaatcacagcATAC	16	gtatgctgtgattagg
7	tttagcttatggtaca	7_1	TTTAgcttatggtaCA	17	tgtaccataagctaaa
8	ctgtataaggtggttt	8_1	CTGtataaggtggTTT	18	aaaccaccttatacag
9	taagaagctagggtgg	9_1	TAAgaagctagggtGG	19	ccaccctagcttcttagtg
10	cactaagaagctaggg	10_1	CACtaagaagctagGG	19	ccaccctagcttcttagtg

In the compound column, capital letters are beta-D-oxy LNA nucleosides, and LNA C are all 5-methyl C, lower case letters are DNA nucleosides, and a superscript m before a lower

case c represent a 5-methyl cytosine DNA nucleoside, and all internucleoside linkages are phosphorothioate internucleoside linkages.

The invention provides antisense oligonucleotides according to the invention, such as antisense oligonucleotides 12 – 24 nucleosides in length, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence comprising at least 12 contiguous nucleotides present in SEQ ID NOs – 1 - 10.

The invention provides antisense oligonucleotides according to the invention, such as antisense oligonucleotides 14 – 24 nucleosides in length, wherein the antisense oligonucleotide comprises a contiguous nucleotide sequence comprising at least 14 contiguous nucleotides present in SEQ ID NOs – 1 – 10.

The invention provides LNA gapmers according to the invention comprising or consisting of a contiguous nucleotide sequence selected from SEQ ID NO 1 – 10.
The invention provides LNA gapmers as depicted in Table 5 (Compound). The invention provides LNA gapmers as depicted in Table 6 (see Examples).

The invention provides antisense oligonucleotides selected from the group consisting of: TCAAatgtagtgacGC; CAaatgtagtgACGC; TGAggattatacaTGG; GTTtggtaagatcCTC; CCCattgaagttaTAA; CCtaatcacagcATAC; TTTAgcttatggtaCA; CTGtataaggtggTTT; TAAgaagctagggtGG; and CACtaagaagctagGG
wherein a capital letter is a LNA nucleoside, and a lower case letter is a DNA nucleoside. In some embodiments all internucleoside linkages in contiguous nucleoside sequence are phosphorothioate internucleoside linkages. Optionally LNA cytosine may be 5-methyl cytosine. Optionally DNA cytosine may be 5-methyl cytosine.
The invention provides antisense oligonucleotides selected from the group consisting of: TCAAatgtagtgacGC; CAaatgtagtgACGC; TGAggattatacaTGG; GTTtggtaagatcCTC; CCCattgaagttaTAA; CCtaatcacagcATAC; TTTAgcttatggtaCA; CTGtataaggtggTTT; TAAgaagctagggtGG; and CACtaagaagctagGG
; wherein a capital letter is a beta-D-oxy-LNA nucleoside, and a lower case letter is a DNA nucleoside. In some embodiments all internucleoside linkages in contiguous nucleoside sequence are phosphorothioate internucleoside linkages. Optionally LNA cytosine may be 5-methyl cytosine. Optionally DNA cytosine may be 5-methyl cytosine.

The invention provides antisense oligonucleotides selected from the group consisting of: TCAAatgtagtgacGC; CAaatgtagtgACGC; TGAggattatacaTGG; GTTtggtaagatcCTC; CCCattgaagttaTAA; CCtaatcacagcATAC; TTTAgcttatggtaCA; CTGtataaggtggTTT; TAAgaagctagggtGG; and CACtaagaagctagGG ; wherein a capital letter is a beta-D-oxy-LNA nucleoside, wherein all LNA cytosines are 5-methyl cytosine, and a lower case letter is a DNA nucleoside, wherein all internucleoside linkages in contiguous nucleoside sequence are phosphorothioate internucleoside linkages, and optionally DNA cytosine may be 5-methyl cytosine.

Method of manufacture

In a further aspect, the invention provides methods for manufacturing the oligonucleotides of the invention comprising reacting nucleotide units and thereby forming covalently linked contiguous nucleotide units comprised in the oligonucleotide. Preferably, the method uses phophoramidite chemistry (see for example Caruthers et al, 1987, Methods in Enzymology vol. 154, pages 287-313). In a further embodiment the method further comprises reacting the contiguous nucleotide sequence with a conjugating moiety (ligand) to covalently attach the conjugate moiety to the oligonucleotide. In a further aspect a method is provided for manufacturing the composition of the invention, comprising mixing the oligonucleotide or conjugated oligonucleotide of the invention with a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

Pharmaceutical salts

The compounds according to the present invention may exist in the form of their pharmaceutically acceptable salts. The term “pharmaceutically acceptable salt” refers to conventional acid-addition salts or base-addition salts that retain the biological effectiveness and properties of the compounds of the present invention and are formed from suitable non-toxic organic or inorganic acids or organic or inorganic bases. Acid-addition salts include for example those derived from inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, sulfamic acid, phosphoric acid and nitric acid, and those derived from organic acids such as *p*-toluenesulfonic acid, salicylic acid, methanesulfonic acid, oxalic acid, succinic acid, citric acid, malic acid, lactic acid, fumaric acid, and the like. Base-addition salts include those derived from ammonium, potassium, sodium and, quaternary ammonium hydroxides, such as for example, tetramethyl ammonium hydroxide. The chemical modification of a pharmaceutical compound into a salt is a technique well known to pharmaceutical chemists in order to obtain improved physical and chemical stability, hygroscopicity, flowability and solubility of compounds. It is for example described in Bastin, Organic Process Research & Development 2000, 4, 427-435 or in Ansel, In:

Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th ed. (1995), pp. 196 and 1456-1457. For example, the pharmaceutically acceptable salt of the compounds provided herein may be a sodium salt.

Pharmaceutical Composition

In a further aspect, the invention provides pharmaceutical compositions comprising any of the aforementioned oligonucleotides and/or oligonucleotide conjugates or salts thereof and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS) and pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts. In some embodiments the pharmaceutically acceptable diluent is sterile phosphate buffered saline. In some embodiments the oligonucleotide is used in the pharmaceutically acceptable diluent at a concentration of 50 - 300μM solution. In some embodiments, the oligonucleotide of the invention is administered at a dose of 10 - 1000μg.

Suitable formulations for use in the present invention are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed., 1985. For a brief review of methods for drug delivery, see, e.g., Langer (Science 249:1527-1533, 1990).

WO 2007/031091 provides further suitable and preferred examples of pharmaceutically acceptable diluents, carriers and adjuvants (hereby incorporated by reference). Suitable dosages, formulations, administration routes, compositions, dosage forms, combinations with other therapeutic agents, pro-drug formulations are also provided in WO2007/031091.

Oligonucleotides or oligonucleotide conjugates of the invention may be mixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

These compositions may be sterilized by conventional sterilization techniques or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents, such as in a sealed package of tablets or capsules. The composition in solid form can also be

packaged in a container for a flexible quantity, such as in a squeezable tube designed for a topically applicable cream or ointment.

In some embodiments, the oligonucleotide or oligonucleotide conjugate of the invention is a prodrug. In particular with respect to oligonucleotide conjugates the conjugate moiety is cleaved of the oligonucleotide once the prodrug is delivered to the site of action, e.g. the target cell.

Applications

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

In research, such oligonucleotides may be used to specifically modulate the synthesis of GBP-1 protein in cells (e.g. *in vitro* cell cultures) and experimental animals thereby facilitating functional analysis of the target or an appraisal of its usefulness as a target for therapeutic intervention. Typically, the target modulation is achieved by degrading or inhibiting the mRNA producing the protein, thereby prevent protein formation or by degrading or inhibiting a modulator of the gene or mRNA producing the protein.

If employing the oligonucleotide of the invention in research or diagnostics the target nucleic acid may be a cDNA or a synthetic nucleic acid derived from DNA or RNA.

The present invention provides an *in vivo* or *in vitro* method for modulating GBP-1 expression in a target cell which is expressing GBP-1, said method comprising administering an oligonucleotide of the invention in an effective amount to said cell.

In some embodiments, the target cell, is a mammalian cell such as a human, cynomolgus monkey or murine cell. The target cell may be an *in vitro* cell culture or an *in vivo* cell forming part of a tissue in a mammal.

In some embodiments the cell is a cancer cell.

In diagnostics the oligonucleotides may be used to detect and quantitate GBP-1 expression in cell and tissues by northern blotting, *in-situ* hybridisation or similar techniques.

For therapeutics, the oligonucleotides of the invention can be used to modulate the expression of GBP-1 in an animal (e.g. a human) suspected of having a disease or disorder mediated by or associated with aberrant GBP-1 expression.

In a particular embodiment, an oligonucleotide of the invention is used to inhibit the expression of GBP-1 in an animal suspected of having a disease or disorder mediated by or associated with aberrant GBP-1 expression. In an embodiment, the disease or disorder is one mediated by or associated with elevated expression of GBP-1 in the affected cells. For example, the affected cells could be tumour/cancer cells that may express higher than normal amounts of GBP-1.

The invention provides methods for treating or preventing a disease, comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide, an oligonucleotide conjugate or a pharmaceutical composition of the invention to a subject suffering from or susceptible to the disease.

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

The oligonucleotide, oligonucleotide 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 oligonucleotide or oligonucleotide conjugate or pharmaceutical composition as defined or described herein for the manufacture of a medicament for the treatment of a disease or disorder as referred to herein, or for a method of the treatment of a disease or disorder as referred to herein.

The disease or disorder, as referred to herein, is associated with expression of GBP-1. In some embodiments the disease or disorder may be associated with a mutation in the GBP-1 gene. Therefore, in some embodiments, the target nucleic acid is a mutated form of the GBP-1 sequence.

The methods of the invention may be employed for treatment or prophylaxis against diseases caused by abnormal levels and/or activity of GBP-1.

The methods of the invention may be employed for treatment or prophylaxis against diseases caused by elevated levels and/or activity of GBP-1. By elevated we mean greater than the level typically found in normal tissues. The degree of elevated expression indicative of a diseased cell/tissue can be determined by a clinician. However, the amount of increase in expression relative to normal cells/tissues could by an increase of 5%, 10%, 15%,.20%, 25%, 30%, 50%, 75%, 90%, 100%, 150%, 175%, 200%, 250% or more above normal levels. Such typical “normal” levels can be determined by measurement of levels in normal (non-diseased) cells or from a reference data set. A reference normal level, be it from direct measurements or from a reference data set, is usually one that is an average from multiple (e.g. >5) measurements.

In a particular embodiment, the patient is identified as having a disease or condition characterised by elevated GBP-1 expression prior to administration of the oligonucleotide, conjugate or pharmaceutical composition according to the invention. Such identification can be carried out according to a variety of methods as described herein. In certain embodiments, the level of GBP-1 expression is determined from a biological sample

previously isolated from the patient/subject .Such sample, could be a biopsy (such as tumour tissue) or fluid (such as blood) sample.

There are various well-known methods for determining the amount of protein or mRNA in a cell. Immunohistochemistry, ELISA, or mass spectroscopy methods, such as liquid-chromatography mass spectroscopy (LC-MS) are particularly suitable methods.

For mRNA determination, methods involving hybridisation to the target mRNA using a complementary nucleic acid can be employed. Various adaptations of reverse transcription polymerase chain reaction (RT-PCR), such as quantitative PCR or competitive RT-PCR, are suitable quantitative methods for determining the relative amount of a mRNA species in a normal cell versus an aberrant cell. The person skilled in the art is able to employ a suitable method for detection of the amount or protein or mRNA in the cell or cells.

The invention further relates to use of an oligonucleotide, oligonucleotide conjugate or a pharmaceutical composition as defined herein for the manufacture of a medicament for the treatment of abnormal levels and/or activity of GBP-1. As noted above, such abnormal levels may be elevated levels.

In one embodiment, the invention relates to oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions for use in the treatment of diseases or disorders selected from cancer (such as breast cancer or metastases breast cancer) and osteoporosis.

Administration

The oligonucleotides or pharmaceutical compositions of the present invention may be administered topical or enteral or parenteral (such as, intravenous, subcutaneous, intra-muscular, intracerebral, intracerebroventricular or intrathecal).

In a preferred embodiment the oligonucleotide or pharmaceutical compositions of the present invention are administered by a parenteral route including intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion, intrathecal or intracranial, e.g. intracerebral or intraventricular, intravitreal administration. In one embodiment the active oligonucleotide or oligonucleotide conjugate is administered intravenously. In another embodiment the active oligonucleotide or oligonucleotide conjugate is administered subcutaneously.

In some embodiments, the oligonucleotide, oligonucleotide conjugate or pharmaceutical composition of the invention is administered at a dose of 0.1 – 15 mg/kg, such as from 0.2 –

10 mg/kg, such as from 0.25 – 5 mg/kg. The administration can be once a week, every 2nd week, every third week or even once a month.

Combination therapies

In some embodiments the oligonucleotide, oligonucleotide conjugate or pharmaceutical composition of the invention is for use in a combination treatment with another therapeutic agent. The therapeutic agent can for example be the standard of care for the diseases or disorders described above.

The work leading to this invention has received funding from the European Union Seventh Framework Programme [FP7-2007-2013] under grant agreement "HEALTH-F2-2013-602114 " (Athero-B-Cell).

EXAMPLES

Example 1: Testing in vitro efficacy of antisense oligonucleotides targeting human GBP1 mRNA in A431 and MDA-MB-231 cells at single concentration.

All oligonucleotides were made by standard automated phosphoramidite oligonucleotide synthesis.

A431 and MDA-MB-231 cell lines were purchased from ATCC and maintained as recommended by the supplier in a humidified incubator at 37°C with 5% CO_2. For assays, 3000 cells/well were seeded in a 96 multi well plate in culture media. Cells were incubated for 24 hours before addition of oligonucleotides dissolved in PBS. Final concentration of oligonucleotides: 25 µM. 3 days after addition of oligonucleotides, the cells were harvested. RNA was extracted using the PureLink Pro 96 RNA Purification kit (Thermo Fisher Scientific) according to the manufacturer's instructions and eluated in 50µl water. The RNA was subsequently diluted 10 times with DNase/RNase free Water (Gibco) and heated to 90°C for one minute.

For gene expressions analysis, One Step RT-qPCR was performed using qScript™ XLT One-Step RT-qPCR ToughMix®, Low ROX™ (Quantabio) in a duplex set up. The following TaqMan primer assays were used for qPCR: GBP1 Hs00977005_m1 (FAM-MGB) and endogenous control GAPDH, Hs99999905_m1 (VIC-MGB). All primer sets were purchased from Thermo Fisher Scientific. The relative GBP1 mRNA expression level in the table is shown as percent of control (PBS-treated cells).

Table 6 - Selected oligonucleotides used:

SEQ ID NO	CMP ID NO	Compound	Rel. mRNA level A431 at 25µM	SD	Rel. mRNA level MDA-MB-231 at 25µM	SD
1	1_1	TCAAatgtagtga^{m}cGC	3	1	4	1
2	2_1	CAaatgtagtgACGC	3	1	3	0
3	3_1	TGAggattatacaTGG	3	0	4	1
4	4_1	GTTtggtaagatcCTC	7	0	11	1
5	5_1	CCCattgaagttaTAA	8	0	7	0
6	6_1	CCtaatcacagcATAC	9	2	11	2
7	7_1	TTTAgcttatggtaCA	11	1	19	0
8	8_1	CTGtataaggtggTTT	12	2	27	4
9	9_1	TAAgaagctagggtGG	12	0	19	0
10	10_1	CACtaagaagctagGG	14	1	22	1

For compounds: Capital letters represent LNA nucleosides (beta-D-oxy LNA nucleosides were used), all LNA cytosines are 5-methyl cytosine, lower case letters represent DNA nucleosides, DNA cytosines preceded with a superscript ^{m} represents a 5-methyl C-DNA nucleoside. All internucleoside linkages are phosphorothioate internucleoside linkages. See also figures 1 and 2.

Example 2: Testing *in vitro* potency and efficacy of selected oligonucleotides targeting human GBP1 mRNA in A431 and MDA-MB-231 cells at different concentrations for a dose response curve.

Human A431 and MDA-MB-231 cell line was described in Example 1. The assay was performed as described in Example 1. Concentration of oligonucleotides: from 50 µM, 1:1 dilutions in 8 steps. 3 days after addition of oligonucleotides, the cells were harvested. RNA extraction and duplex One Step RT-qPCR were performed as described in Example 1. n=2 biological replicates. IC_{50} determinations were performed in GraphPad Prism6. The relative

GBP1 mRNA level at treatment with 50 µM oligonucleotide is shown in the table as percent of control (PBS).

CMP ID NO	IC_{50} in A431 [µM]	SD	Rel. mRNA level A431 at Max KD	SD	IC_{50} in MDA-MB-231 [µM]	SD	Rel. mRNA level MDA-MB-231 at Max KD	SD
1_1	0,12	0,03	2,2	1,6	0,3	0,1	3,1	2,1
2_1	0,13	0,01	5,1	3,2	0,3	0,1	4,9	1,9
3_1	0,08	0,03	2,8	1,4	0,2	0,1	3,4	1,9
4_1	0,17	0,04	7,9	2,1	0,3	0,3	11,9	4,0
5_1	0,16	0,00	8,0	1,5	0,5	0,1	8,7	2,1
6_1	0,36	0,02	7,9	0,2	0,9	0,3	11,6	5,6
7_1	0,18	0,01	7,9	2,3	0,6	0,2	12,6	2,4
8_1	0,22	0,03	10,6	3,0	0,8	0,2	23,7	4,3
9_1	0,88	0,97	11,8	5,8	1,0	0,4	25,7	8,4
10_1	0,35	0,03	12,6	2,7	1,5	0,5	26,6	4,4

See also figures 3 and 4.

CLAIMS

1. An antisense oligonucleotide, 10-30 nucleotides in length, wherein said antisense oligonucleotide comprises a contiguous nucleotide sequence 10 – 30 nucleotides in length, wherein the contiguous nucleotide sequence is at least 90% complementary to any of SEQ ID NO: 11, wherein the antisense oligonucleotide is capable of inhibiting the expression of human GBP-1 in a cell which is expressing human GBP-1; or a pharmaceutically acceptable salt thereof.
2. The antisense oligonucleotide according to claim 1, wherein the contiguous nucleotide sequence is fully complementary to any of SEQ ID NO 12 - 19
3. The antisense oligonucleotide according to claim 1, wherein the contiguous nucleotide sequence is fully complementary to a region in SEQ ID NO 11 selected from the group consisting of 13 - 65; 114 - 135; 137 - 171; 175 - 213; 230 - 265; 267 - 282; 284 - 314; 325 - 339; 341 - 373; 381 - 422; 424 - 449; 456 - 473; 495 - 524; 544 - 558; 644 - 667; 681 - 706; 736 - 753; 759 - 831; 840 - 872; 874 - 891; 893 - 908; 920 - 955; 957 - 1019; 1021 - 1037; 1039 - 1055; 1086 - 1120; 1120 - 1161; 1182 - 1210; 1212 - 1244; 1246 - 1270; 1272 - 1342; 1358 - 1382; 1413 - 1432; 1450 - 1464; 1482 - 1500; 1534 - 1553; 1555 - 1571; 1573 - 1616; 1621 - 1657; 1665 - 1691; 1693 - 1714; 1741 - 1762; 1777 - 1830; 1832 - 1858; 1869 - 1886; 1888 - 1934; 1955 - 2000; 2004 - 2036; 2061 - 2079; 2098 - 2118; 2120 - 2218; 2220 - 2237; 2277 - 2335; 2337 - 2372; 2387 - 2413; 2415 - 2459; 2481 - 2499; 2499 - 2517; 2519 - 2534; 2562 - 2577; 2589 - 2620; 2622 - 2636; 2646 - 2677; 2732 - 2758; 2789 - 2813; 2815 - 2839; 2848 - 2876; 2878 - 2927; 2929 - 3014; 3017 - 3034; 3025 - 3039; 3036 - 3092; 3133 - 3177; 3203 - 3232; 3234 - 3249; 3250 - 3275; 3311 - 3330; 3332 - 3361; 3396 - 3427; 3486 - 3518; 3520 - 3541; 3552 - 3612; 3639 - 3656; 3660 - 3714; 3722 - 3750; 3779 - 3795; 3797 - 3817; 3848 - 3881; 3887 - 3905; 3907 - 3931; 3933 - 3967; 3997 - 4029; 4031 - 4056; 4066 - 4081; 4090 - 4110; 4112 - 4131; 4140 - 4170; 4183 - 4224; 4237 - 4273; 4275 - 4313; 4324 - 4341; 4343 - 4370; 4372 - 4386; 4438 - 4453; 4450 - 4482; 4550 - 4569; 4588 - 4621; 4647 - 4683; 4726 - 4742; 4792 - 4807; 4824 - 4852; 4885 - 4911; 4940 - 4963; 4970 - 5002; 5014 - 5055; 5057 - 5100; 5102 - 5119; 5121 - 5182; 5203 - 5221; 5226 - 5241; 5252 - 5266; 5271 - 5309; 5331 - 5352; 5366 - 5382; 5384 - 5430; 5452 - 5481; 5483 - 5519; 5542 - 5562; 5564 - 5581; 5602 - 5618; 5627 - 5666; 5668 - 5692; 5693 - 5710; 5712 - 5731; 5745 - 5774; 5778 - 5829; 5841 - 5858; 5860 - 5879; 5894 - 5973; 5975 - 6008; 6010 - 6029; 6052 - 6094; 6127 - 6141; 6143 - 6170; 6179 - 6201; 6215 - 6249; 6266 - 6339; 6341 - 6382; 6384 - 6455; 6457 - 6474; 6482 - 6516; 6527 - 6561; 6563 - 6602;

6611 - 6627; 6629 - 6644; 6669 - 6772; 6774 - 6818; 6831 - 6858; 6875 - 6896; 6907 - 6923; 6960 - 6990; 7001 - 7015; 7017 - 7034; 7051 - 7112; 7163 - 7178; 7195 - 7214; 7230 - 7244; 7258 - 7319; 7321 - 7353; 7355 - 7406; 7408 - 7441; 7461 - 7475; 7477 - 7504; 7526 - 7558; 7647 - 7688; 7730 - 7752; 7762 - 7795; 7824 - 7864; 7899 - 7923; 7949 - 7968; 7972 - 7987; 7989 - 8039; 8047 - 8068; 8070 - 8088; 8114 - 8135; 8137 - 8170; 8172 - 8194; 8196 - 8214; 8218 - 8251; 8253 - 8272; 8283 - 8345; 8358 - 8392; 8394 - 8497; 8499 - 8525; 8607 - 8638; 8664 - 8730; 8748 - 8817; 8847 - 8875; 8877 - 8892; 8894 - 8910; 8927 - 8943; 8945 - 8972; 8974 - 8988; 9018 - 9032; 9061 - 9084; 9092 - 9190; 9192 - 9214; 9262 - 9285; 9287 - 9318; 9331 - 9366; 9378 - 9415; 9432 - 9476; 9501 - 9523; 9531 - 9546; 9548 - 9564; 9566 - 9626; 9636 - 9650; 9660 - 9697; 9699 - 9719; 9785 - 9822; 9824 - 9855; 9857 - 9872; 9874 - 9915; 9921 - 9970; 9980 - 9997; 10049 - 10064; 10065 - 10083; 10085 - 10101; 10103 - 10117; 10119 - 10204; 10206 - 10231; 10233 - 10288; 10290 - 10310; 10342 - 10362; 10376 - 10391; 10402 - 10422; 10424 - 10469; 10483 - 10623; 10634 - 10698; 10700 - 10723; 10725 - 10740; 10742 - 10758; 10780 - 10796; 10798 - 10824; 10831 - 10856; 10863 - 10892; 10943 - 10960; 10982 - 10999; 11014 - 11038; 11044 - 11066; 11075 - 11095; 11108 - 11136; 11142 - 11159; 11177 - 11198; 11208 - 11222; 11217 - 11233; 11245 - 11269; 11281 - 11303; 11320 - 11362; 11368 - 11387; 11389 - 11408; 11410 - 11424; 11444 - 11505; 11515 - 11566; 11570 - 11584; 11605 - 11637; 11665 - 11699; 11701 - 11723; 11725 - 11747; 11792 - 11816; 11822 - 11838; 11840 - 11874; 11876 - 11902; 11908 - 11926; 11945 - 11968; 11990 - 12032; 12034 - 12090; 12092 - 12119; 12143 - 12161; 12164 - 12318; 12317 - 12333; 12345 - 12420; 12440 - 12481; 12496 - 12514; 12516 - 12542; 12544 - 12604; 12632 - 12647; 12649 - 12701; 12745 - 12776; 12787 - 12812; 12876 - 12904; 12906 - 12923; 12925 - 12956; 12980 - 12997; and 13000 - 13042;

4. The antisense oligonucleotide according to any one of claims 1 – 3, wherein the antisense oligonucleotide is a gapmer oligonucleotide comprising a contiguous nucleotide sequence of formula 5'-F-G-F'-3', where region F and F' independently comprise 1 - 8 sugar modified nucleosides, and G is a region between 5 and 16 nucleosides which are capable of recruiting RNaseH.
5. The antisense oligonucleotide according to claim 4, wherein the sugar modified nucleosides of region F and F' are independently selected from the group consisting of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA, 2'-amino-DNA, 2'-fluoro-DNA, arabino nucleic acid (ANA), 2'-fluoro-ANA and LNA nucleosides.
6. The antisense oligonucleotide according to claim 4 or 5, wherein region G comprises 5 – 16 contiguous DNA nucleosides.

7. The antisense oligonucleotide according to any one of claims 1 – 6, wherein the antisense oligonucleotide is a LNA gapmer oligonucleotide.
8. The antisense oligonucleotide according to any one of claims 4 – 7, wherein the LNA nucleosides are beta-D-oxy LNA nucleosides.
9. The antisense oligonucleotide according to any one of claims 1 – 8, wherein the internucleoside linkages between the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.
10. The antisense oligonucleotide according to any one of claims 1 – 9, wherein the oligonucleotide comprises a contiguous nucleotide sequence selected from the group consisting of: 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 and SEQ ID NO:10.
11. The antisense oligonucleotide according to any one of claims 1 – 10, wherein the oligonucleotide comprises or consists of a contiguous nucleotide sequence:
    TCAAatgtagtgacGC (SEQ ID NO 1);
    CAaatgtagtgACGC (SEQ ID NO 2);
    TGAggattatacaTGG (SEQ ID NO 3);
    GTTtggtaagatcCTC (SEQ ID NO 4);
    CCCattgaagttaTAA (SEQ ID NO 5);
    CCtaatcacagcATAC (SEQ ID NO 6);
    TTTAgcttatggtaCA (SEQ ID NO 7);
    CTGtataaggtggTTT (SEQ ID NO 8);
    TAAgaagctagggtGG (SEQ ID NO 9); and
    CACtaagaagctagGG (SEQ ID NO 10);
    wherein a capital letter represents a LNA nucleoside, a lower case letter represents a DNA nucleoside.
12. The antisense oligonucleotide according to any one of claims 1 – 11, wherein the oligonucleotide comprises or consists of a contiguous nucleotide sequence:
    TCAAatgtagtga^{m}cGC (SEQ ID NO 1);
    CAaatgtagtgACGC (SEQ ID NO 2);
    TGAggattatacaTGG (SEQ ID NO 3);
    GTTtggtaagatcCTC (SEQ ID NO 4);
    CCCattgaagttaTAA (SEQ ID NO 5);
    CCtaatcacagcATAC (SEQ ID NO 6);
    TTTAgcttatggtaCA (SEQ ID NO 7);

CTGtataaggtggTTT (SEQ ID NO 8);
TAAgaagctagggtGG (SEQ ID NO 9); and
CACtaagaagctagGG (SEQ ID NO 10);wherein a capital letter represents a beta-D-oxy LNA nucleoside, a lower case letter represents a DNA nucleoside, wherein each LNA cytosine is 5-methyl cytosine, and ^{m}c is 5-methyl cytosine DNA, and wherein the internucleoside linkages between the nucleosides are phosphorothioate internucleoside linkages.

13. A conjugate comprising the oligonucleotide according to any one of claims 1 – 12, and at least one conjugate moiety covalently attached to said oligonucleotide.
14. A pharmaceutical composition comprising the oligonucleotide of claim 1-12 or the conjugate of claim 13 and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.
15. An *in vivo* or *in vitro* method for modulating GBP-1 expression in a target cell which is expressing GBP-1, said method comprising administering an oligonucleotide of any one of claims 1-12, the conjugate according to claim 13, or the pharmaceutical composition of claim 14 in an effective amount to said cell.
16. A method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide of any one of claims 1 - 12 or the conjugate according to claim 13 or the pharmaceutical composition of claim 14 to a subject suffering from or susceptible to the disease.
17. The method of claim 16, wherein the disease is cancer or osteoporosis.
18. The oligonucleotide of any one of claims 1 - 12 or the conjugate according to claim 13 or the pharmaceutical composition of claim 16 for use in medicine.
19. The oligonucleotide of any one of claims 1 - 12 or the conjugate according to claim 13 or the pharmaceutical composition of claim 14 for use in the treatment or prevention of cancer or osteoporosis.
20. Use of the oligonucleotide of claim 1 - 12 or the conjugate according to claim 13 or the pharmaceutical composition of claim 14, for the preparation of a medicament for treatment or prevention of a disease selected from the group consisting of cancer or osteoporosis.

FIGURES

Figure 1

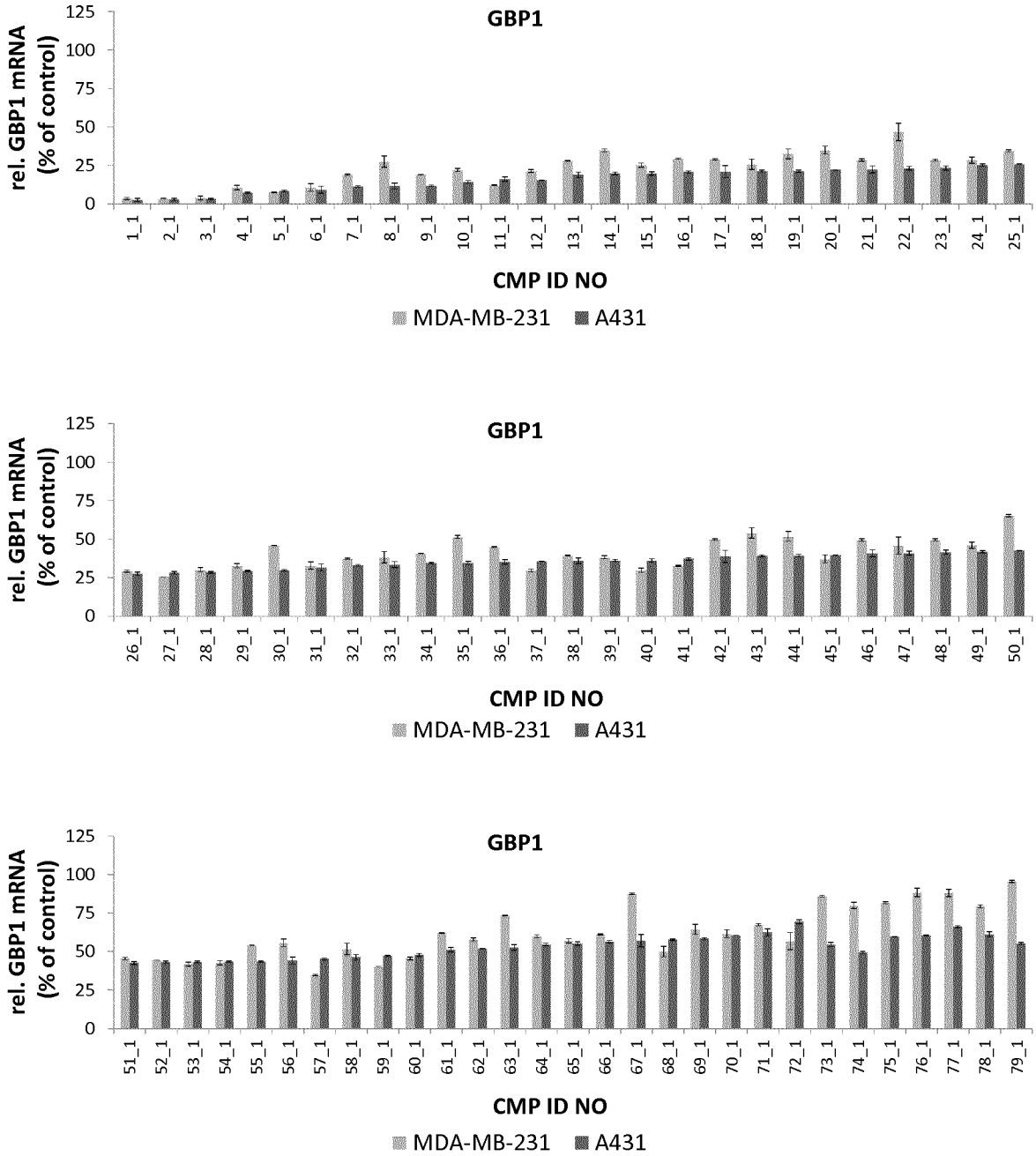


Figure 2

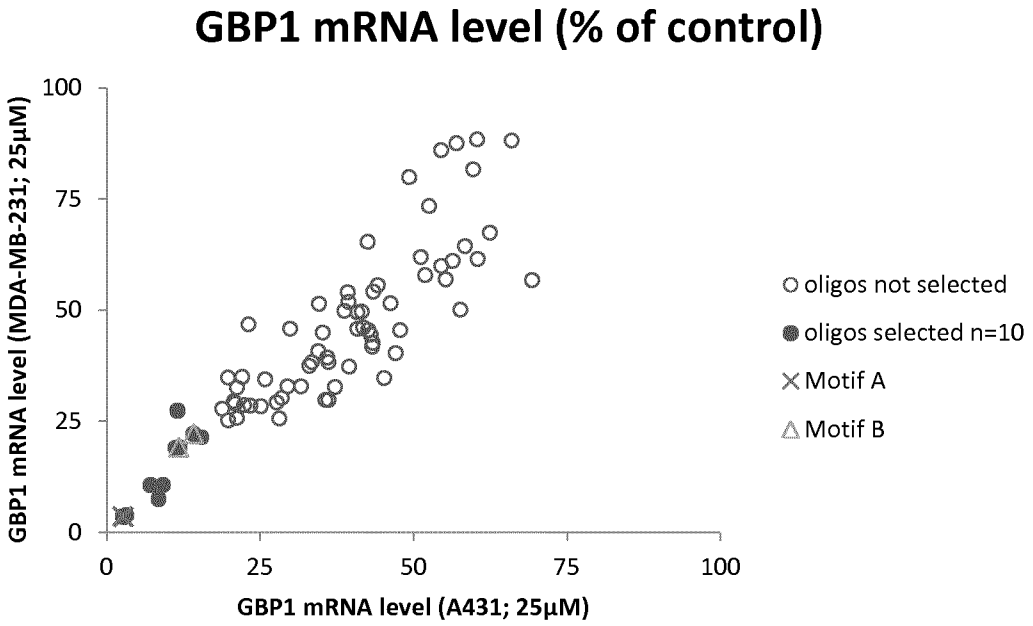


Figure 3

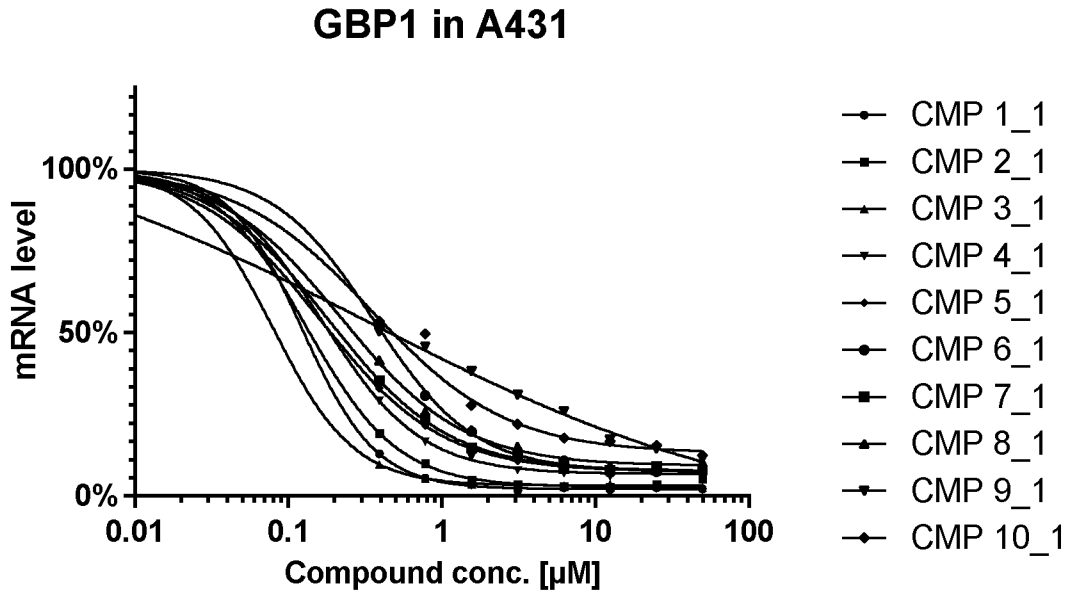
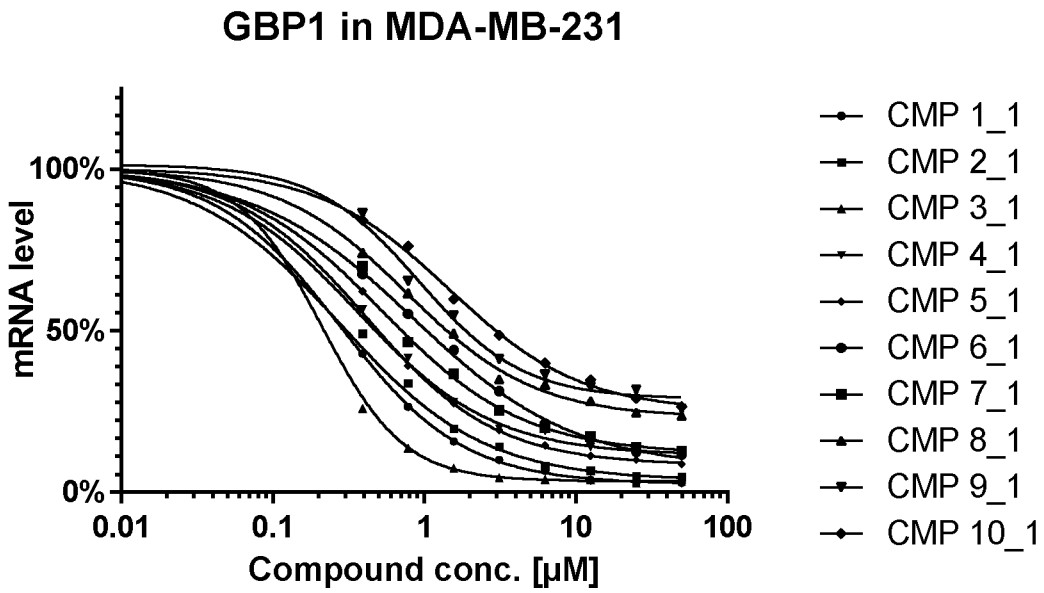


Figure 4


GBP1 in MDA-MB-231
mRNA level
100%
50%
0%
0.01
0.1
1
10
100
Compound conc. [µM]
CMP 1_1
CMP 2_1
CMP 3_1
CMP 4_1
CMP 5_1
CMP 6_1
CMP 7_1
CMP 8_1
CMP 9_1
CMP 10_1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/067537

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/712 A61K31/7125
ADD. C07H21/00 A61P35/00 A61P19/10

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K C07H

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MING LI ET AL: "Guanylate binding protein 1 is a novel effector of EGFR-driven invasion in glioblastoma", THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 208, no. 13, 12 December 2011 (2011-12-12), pages 2657-2673, XP055621088, US ISSN: 0022-1007, DOI: 10.1084/jem.20111102 the whole document -----	1,3-9, 13-20
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 2019	25/11/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Andres, Serge

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] 7 June 2015 (2015-06-07), "Sequence 664929 from Patent EP2850184.", XP002794230, retrieved from EBI accession no. EM_PAT:JE353062 Database accession no. JE353062 sequence -& EP 2 850 184 A1 (RANA THERAPEUTICS INC [US]; GEN HOSPITAL CORP [US]) 25 March 2015 (2015-03-25) -----	1-9,13, 14,16,18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2019/067537

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-20(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-20(partially)

An antisense oligonucleotide complementary to hGBP-1 in the region defined by nucleotides 12034-12090 (including SEQ ID 12). Modified forms thereof, compositions comprising it and medicinal uses.

2-5. claims: 1-20(partially)

As for subject 1., but concerning the hGBP-1 regions defined respectively by nucleotides 12649-12701 (including SEQ ID 15), 6669-6772 (including SEQ ID 16), 11990-1123032 (including SEQ ID 17) or 12345-12420 (including SEQ ID 19).

6-8. claims: 1, 2, 4-20(all partially)

As for subject 1, but concerning the hGBP-1 regions defined respectively by SEQ IDs 13, 14 or 18.

9-298. claims: 1, 3-9, 13-20(all partially)

As for subject 1., but concerning respectively all other regions of hGBP-1 as listed in claim 3 (or Table 1).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/067537

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/067537

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