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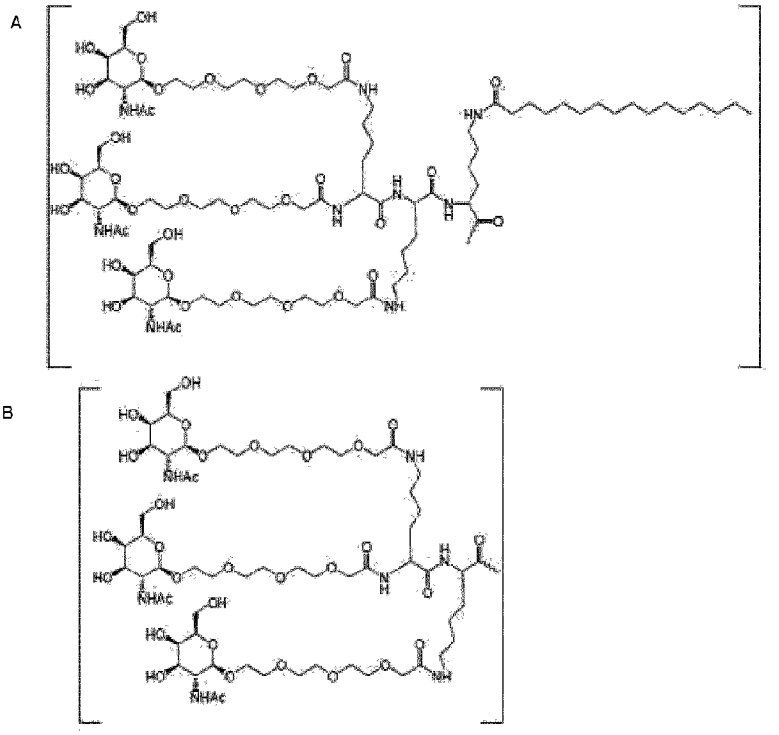

(57) Abrégé/Abstract:
The present invention relates to antisense oligonucleotides that are capable of reducing expression of PD-L1 in a target cell. The oligonucleotides hybridize to PD-L1 mRNA. The present invention further relates to conjugates of the oligonucleotide and pharmaceutical compositions and methods for treatment of viral liver infections such as HBV, HCV and HDV; parasite infections such as malaria, toxoplasmosis, leishmaniasis and trypanosomiasis or liver cancer or metastases in the liver using the oligonucleotide.

Abstract

The present invention relates to antisense oligonucleotides that are capable of reducing expression of PD-L1 in a target cell. The oligonucleotides hybridize to PD-L1 mRNA. The present invention further relates to conjugates of the oligonucleotide and pharmaceutical compositions and methods for treatment of viral liver infections such as HBV, HCV and HDV; parasite infections such as malaria, toxoplasmosis, leishmaniasis and trypanosomiasis or liver cancer or metastases in the liver using the oligonucleotide.

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OLIGONUCLEOTIDES FOR REDUCTION OF PD-L1 EXPRESSION

FIELD OF INVENTION

The present invention relates to oligonucleotides (oligomers) that are complementary to programmed death ligand-1 (PD-L1), leading to reduction of the expression of PD-L1 the liver. The present invention also relates to a method of alleviating the T cell exhaustion caused by an infection of the liver or cancer in the liver. Relevant infections are chronic HBV, HCV and HDV and parasite infections like malaria and toxoplasmosis (e.g. caused by protozoa of the *Plasmodium,* in particular of the species *P. vivax, P. malariae* and *P. falciparum).*

BACKGROUND

The costimulatory pathway consisting of the programmed death-1 (PD-1) receptor and its ligand, PD-L1 (or B7-H1 or CD274) is known to contribute directly to T cell exhaustion resulting in lack of viral control during chronic infections of the liver. The PD-1 pathway also plays a role in autoimmunity as mice disrupted in this pathway develop autoimmune diseases.

It has been shown that antibodies that block the interaction between PD-1 and PD-L1 enhance T cell responses, in particular the response of CD8+ cytotoxic T cells (see Barber et al 2006 Nature Vol 439 p682 and Maier et al 2007 J. Immunol. Vol 178 p 2714).

WO 2006/042237 describes a method of diagnosing cancer by assessing PD-L1 (B7-H1) expression in tumors and suggests delivering an agent, which interferes with the PD-1/PD-L1 interaction, to a patient. Interfering agents can be antibodies, antibody fragments, siRNA or antisense oligonucleotides. There are no specific examples of such interfering agents nor is there any mentioning of chronic liver infections.

RNA interference mediated inhibition of PD-L1 using double stranded RNA (dsRNA, RNAi or siRNA) molecules have also been disclosed in for example WO 2005/007855, WO 2007/084865 and US 8,507,663. None of these describes targeted delivery to the liver.

Dolina et al. 2013 Molecular Therapy-Nucleic Acids, 2 e72 describes in vivo delivery of PD-L1 targeting siRNA molecules to Kupffer cells thereby enhancing NK and CD8+ T cell clearance in MCMV infected mice. This paper concludes that PD-L1 targeting siRNA molecules delivered to hepatocytes are not effective in relation to enhancing CD8+ T cell effector function.

The siRNA approach is significantly different from the single stranded antisense oligonucleotide approach since the biodistribution and the mode of actions is quite different. As described in Xu et al 2003 Biochem. Biophys. Res .Comm. Vol 306 page 712-717, antisense oligonucleotides and siRNAs have different preferences for target sites in the mRNA.

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WO2016/138278 describes inhibition of immune checkpoints including PD-L1, using two or more single stranded antisense oligonucleotides that are linked at their 5' ends. The application does not mention HBV or targeted delivery to the liver.

Objective of the invention

The present invention identifies novel oligonucleotides and oligonucleotide conjugates which reduce PD-L1 mRNA very efficiently in liver cells, both in parenchymal cells (e.g. hepatocytes) and in non-parenchymal cells such as Kupffer cells and liver sinusoidal endothelial cells (LSECs). By reducing or silencing PD-L1, the oligonucleotides and oligonucleotide conjugates decrease PD-1-mediated inhibition and thereby promote immunostimulation of exhausted T cells. Alleviation of the T cell exhaustion in a chronic pathogenic infection of the liver will result in regained immune control and reduced levels of viral antigens in the blood during a chronic pathogenic infection of the liver. Natural killer (NK) cells and natural killer T (NKT) cells may also be activated by the oligonucleotides and oligonucleotide conjugates of the present invention.

The oligonucleotide conjugates secures local reduction of PD-L1 in liver cells and therefore reduces the risk of autoimmune side effects, such as pneumonitis, non-viral hepatitis and colitis associated with systemic depletion of PD-L1.

SUMMARY OF INVENTION

The present invention relates to oligonucleotides or conjugates thereof targeting a nucleic acid capable of modulating the expression of PD-L1 and to treat or prevent diseases related to the functioning of the PD-L1. The oligonucleotides or oligonucleotide conjugates may in particular be used to treat diseases where the immune response against an infectious agent has been exhausted.

Disclosed herein is an antisense oligonucleotide which comprises or consists of a contiguous nucleotide sequence of 10 to 30 nucleotides in length capable of reducing the expression of PD-L1, and having at least 90% identity to a sequence selected from the group consisting of SEQ ID NO: 6, 8, 9, 13, 41, 42, 58, 77, 92, 111, 128, 151, 164, 166, 169, 171, 222, 233, 245, 246, 250, 251, 252, 256, 272, 273, 287, 292, 303, 314, 318, 320, 324, 336, 342, 343, 344, 345, 346, 349, 359, 360, 374, 408, 409, 415, 417, 424, 429, 430, 458, 464, 466, 474, 490, 493, 512, 519, 529, 533, 534, 547, 566, 567, 578, 582, 601, 619, 620, 636, 637, 638, 640, 645, 650, 651, 652, 653, 658, 659, 660, 665, 678, 679, 680, 682, 683, 684, 687, 694, 706, 716, 728, 733, 734, and 735.

In one aspect, the present invention provides an antisense oligonucleotide of formula CCtatttaacatcAGAC, wherein capital letters represent beta-D-oxy LNA nucleosides, lowercase

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letters represent DNA nucleosides, all LNA C are 5-methyl cytosine and all internucleoside linkages are phosphorothioate internucleoside linkages.

The invention also provides an antisense oligonucleotide conjugate comprising the antisense oligonucleotide and a conjugate moiety covalently attached to the antisense oligonucleotide.

The invention also provides an antisense oligonucleotide conjugate selected from the group consisting of the following CMP ID NO: 766_2, 767 2, 768_2, 769_2 and 770_2.

The invention also provides an antisense oligonucleotide conjugate comprising an antisense oligonucleotide of the sequence SEQ ID NO: 466.

The invention further provides a pharmaceutically acceptable salt of the antisense oligonucleotide or the antisense oligonucleotide conjugate, including sodium salt and potassium salt.

The invention further provides a pharmaceutical composition comprising the antisense oligonucleotide, conjugate, or salt thereof and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

The invention further provides an *in vitro* method for modulating PD-L1 expression in a target cell which is expressing PD-L1, said method comprising administering the antisense oligonucleotide, conjugate, salt thereof or pharmaceutical composition in an effective amount to the target cell.

In a further aspect the invention provides methods for treating or preventing a disease, disorder or dysfunction by administering a therapeutically or prophylactically effective amount of the oligonucleotide of the invention to a subject suffering from or susceptible to the disease, disorder or dysfunction, in particular diseases selected from viral liver infections or parasite infections.

In a further aspect the oligonucleotide, oligonucleotide conjugates or pharmaceutical composition of the invention is used in the treatment or prevention of viral liver infections such as HBV, HCV and HDV or a parasite infections such as malaria, toxoplasmosis, leishmaniasis and trypanosomiasis or liver cancer or metastases in the liver.

In one aspect, the invention provides the antisense oligonucleotide, conjugate, salt thereof, or pharmaceutical composition for use in restoration of immune response against a virus or parasite.

The invention also provides the antisense oligonucleotide, conjugate, salt thereof or pharmaceutical composition, for use as a medicament, including in treatment of HBV infection.

The invention also provides use of the antisense oligonucleotide, conjugate, or salt thereof, for

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the preparation of a medicament for treatment of HBV infection.

The invention also provides use of the antisense oligonucleotide, conjugate, salt thereof, or pharmaceutical composition, for treatment of HBV infection.

BRIEF DESCRIPTION OF FIGURES

Figure 1: Illustrates exemplary antisense oligonucleotide conjugates, where the oligonucleotide either is represented as a wavy line (A-D) or as "oligonucleotide" (E-H) or as T_2 (I) and the asialoglycoprotein receptor targeting conjugate moieties are trivalent N-acetylgalactosamine moieties. Compounds A to D comprise a di-lysine brancher molecule a PEG3 spacer and three terminal GalNAc carbohydrate moieties. In compound A and B the oligonucleotide is attached directly to the asialoglycoprotein receptor targeting conjugate moiety without a linker. In compound C and D the oligonucleotide is attached directly to the asialoglycoprotein receptor targeting conjugate moiety via a C6 linker. Compounds E-I comprise a trebler brancher molecule and spacers of varying length and structure and three terminal GalNAc carbohydrate moieties.

Figure 2: Graph showing EC50 (A) and PD-L1 knock down as % of saline (B) for the compounds tested in Example 2, in relation to their position on the target nucleic acid. The cell line in which the compound were tested are THP1(•) and Karpas (✳).

Figure 3: Structural formula of the trivalent GalNAc cluster (GN2). GN2 is useful as conjugation moiety in the present invention. The wavy line illustrates the site of conjugation of the cluster to e.g. a C6 amino linker or directly to the oligonucleotide.

Figure 4: Structural formula of CMP ID NO 766_2.

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Figure 5: Structural formula of CMP ID NO 767_2.

Figure 6: Structural formula of CMP ID NO 768_2.

Figure 7: Structural formula of CMP ID NO 769_2.

Figure 8: Structural formula of CMP ID NO 770_2.

Figure 9: Western blot detecting PD-L1 protein expression in liver from poly(IC) induced animals following treatment with saline and the indicated CMP ID NO's. Each blot shows a naked oligonucleotide versus a GalNAc conjugated version of the same oligonucleotide, blot A) CMP ID NO 744_1 and 755_2, B) CMP ID NO 747_1 and 758_2, C) CMP ID NO 748_1 and 759_2, D) CMP ID NO 752_1 and 763_2 and E) CMP ID NO 753_1 and 764_2. The upper band is the vinculin loading control, the lower band is the PD-L1 protein. The first lane in each blot show saline treated mice without Poly(IC) induction. These mice express very little PD-L protein.

Figure 10: Population of mononuclear cells in the liver after treatment with ● vehicle (group 10 and 1), ◆ DNA vaccine (group 11 and 2), ○ anti-PD-L1 antibody (group 12), ▲naked PD-L1 ASO + DNA vaccine (group 7) or △ GalNAc conjugated PD-L1 ASO + DNA vaccine (group 8), for each group the individual animals are represented and the average is indicated by the vertical line for each group (see table 18). Statistical significance between the DNA vaccine group and the three treatment groups has been assessed and if present it is indicated by * between the groups (* = P< 0.05, *** = P< 0.001 and **** = P< 0.0001). A) represents the number of T cells in the liver following treatment. B) represents the fraction of CD4+ T cells and C) represents the fraction of CD8+ T cells.

Figure 11: Modulation of PD-L1 positive cells in the liver after treatment with ● vehicle (group 10 and 1), ◆ DNA vaccine (group 11 and 2), ○ anti-PD-L1 antibody (group 12), ▲naked PD-L1 ASO + DNA vaccine (group 7) or △ GalNAc conjugated PD-L1 ASO + DNA vaccine (group 8), for each group the individual animals are represented and the average is indicated by the vertical line for each group (see table 19). Statistical significance between the DNA vaccine group and the three treatment groups has been assessed and if present it is indicated by * between the groups (* = P< 0.05 and **** = P< 0.0001). A) represents the pertentage of CD8+ T cells which express PD-L1 in the liver following treatment. B) represents the pertentage of CD4+ T cells which express PD-L1 in the liver following treatment and C) represents the pertentage of B cells which express PD-L1 in the liver following treatment.

Figure 12: HBV antigen specific CD8+ cytokine secreting cells in the liver after treatment with ● vehicle (group 10 and 1), ◆ DNA vaccine (group 11 and 2), ○ anti-PD-L1 antibody (group 12), ▲naked PD-L1 ASO + DNA vaccine (group 7) or △ GalNAc conjugated PD-L1 ASO + DNA vaccine (group 8), for each group the individual animals are represented and the average is indicated by the vertical line for each group (see table 20). Statistical significance between the

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DNA vaccine group and the three treatment groups has been assessed and if present it is indicated by * between the groups (* = P< 0.05). A) represents the pertentage of IFN-γ secreting CD8+ T cells in the liver which are specific towards HBV PreS2+S antigen following treatment. B) represents the pertentage of IFN-γ secreting CD8+ T cells in the liver which are specific towards HBV core antigen following treatment and C) represents the pertentage of IFN-γ and TNF-α secreting CD8+ T cells in the liver which are specific towards HBV PreS2+S antigen following treatment.

Figure 13: HBV-DNA, HBsAg and HBeAg in AAV/HBV mice following treatment with GalNAc conjugated PD-L1 antisense CMP NO: 759_2 (▼) compared to vehicle (■). The vertical line indicates the end of treatment.

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 oligonucleotides 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. Preferably, the antisense oligonucleotides of the present invention are single stranded.

Contiguous Nucleotide Sequence

The term "contiguous nucleotide sequence" refers to the region of the oligonucleotide which is complementary to the target nucleic acid. The term 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 and may optionally comprise further nucleotide(s), for example a nucleotide linker

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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".

Modified internucleoside linkage

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. Nucleotides with modified internucleoside linkage are also termed "modified nucleotides". 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.

In an embodiment, the oligonucleotide comprises one or more internucleoside linkages modified from the natural phosphodiester to a linkage 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%,

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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 modified. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are modified. 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. In some embodiments all of the internucleoside linkages of the oligonucleotide, or contiguous nucleotide sequence thereof, are nuclease resistant internucleoside linkages.

Modified internucleoside linkages may be selected from the group comprising phosphorothioate, diphosphorothioate and boranophosphate. In some embodiments, the modified internucleoside linkages are compatible with the RNaseH recruitment of the oligonucleotide of the invention, for example phosphorothioate, diphosphorothioate or boranophosphate.

In some embodiments the internucleoside linkage comprises sulphur (S), such as a phosphorothioate internucleoside linkage.

A phosphorothioate internucleoside linkage is particularly useful due to nuclease resistance, beneficial pharmakokinetics 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.

In some embodiments, the oligonucleotide comprises one or more neutral internucleoside linkage, particularly a internucleoside linkage selected from phosphotriester, methylphosphonate, MMI, amide-3, formacetal or thioformacetal.

Further internucleoside linkages are disclosed in WO2009/124238. In an embodiment the internucleoside linkage is selected from linkers disclosed in WO2007/031091. Particularly, the internucleoside linkage may be selected from -O-P(O)$_2$-O-, -O-P(O,S)-O-, -O-P(S)$_2$-O-, -S-P(O)$_2$-O-, -S-P(O,S)-O-, -S-P(S)$_2$-O-, -O-P(O)$_2$-S-, -O-P(O,S)-S-, -S-P(O)$_2$-S-, -O-PO(R^H)-O-, 0-PO(OCH$_3$)-0-, -O-PO(NR^H)-O-, -O-PO(OCH$_2$CH$_2$S-R)-O-, -O-PO(BH$_3$)-O-, -O-PO(NHR^H)-O-, -O-P(O)$_2$-NR^H-, -NR^H-P(O)$_2$-O-, -NR^H-CO-O-, -NR^H-CO-NR^H-, and/or the internucleoside linker may be selected form the group consisting of: -O-CO-O-, -O-CO-NR^H-, -NR^H-CO-CH$_2$-, -O-CH$_2$-CO-NR^H-, -O-CH$_2$-CH$_2$-NR^H-, -CO-NR^H-CH$_2$-, -CH$_2$-NR^{H}CO-, -O-CH$_2$-CH$_2$-S-, -S-CH$_2$-CH$_2$-O-, -S-CH$_2$-CH$_2$-S-, -CH$_2$-SO$_2$-CH$_2$-, -CH$_2$-CO-NR^H-, -O-CH$_2$-CH$_2$-NR^H-CO -, -CH$_2$-NCH$_3$-O-CH$_2$-, where R^H is selected from hydrogen and C1 -4-alkyl.

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Nuclease resistant linkages, such as phosphothioate 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, or the non-modified nucleoside region of headmers and tailmers. 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, or the modified nucleoside region of headmers and tailmers.

Each of the design regions may however comprise internucleoside linkages other than phosphorothioate, such as phosphodiester linkages, in particularly in regions where modified nucleosides, such as LNA, protect the linkage against nuclease degradation. Inclusion of phosphodiester linkages, such as one or two linkages, particularly between or adjacent to modified nucleoside units (typically in the non-nuclease recruiting regions) can modify the bioavailability and/or bio-distribution of an oligonucleotide – see WO2008/113832.

In an embodiment all the internucleoside linkages in the oligonucleotide are phosphorothioate and/or boranophosphate linkages. Preferably, all the internucleoside linkages in the oligonucleotide are phosphorothioate linkages.

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

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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 nucleotide sequence, at a given position of a separate nucleic acid molecule (e.g. the target nucleic acid). 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.

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

The following is an example of an oligonucleotide (SEQ ID NO: 5) that is fully complementary to the target nucleic acid (SEQ ID NO: 772).

```
5'gcagtagagccaatta3'  (SEQ ID NO:772)
3'cgtcatctcggttaat5'  (SEQ ID NO: 5)
```

Identity

The term "Identity" 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 identical to (*i.e.* in their ability to form Watson Crick base pairs with the complementary nucleoside) a contiguous nucleotide sequence, at a given position of a separate nucleic acid molecule (e.g. the target nucleic acid). The percentage is calculated by counting

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the number of aligned bases that are identical between the two sequences, including gaps, dividing by the total number of nucleotides in the oligonucleotide and multiplying by 100. Percent Identity = (Matches x 100)/Length of aligned region (with gaps).

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 Lacroix, 2003, *Oligonucleotides* 13:515–537). The standard state Gibbs free energy $\Delta G°$ is a more accurate representation of binding affinity and is related to the dissociation constant (K_d) of the reaction by $\Delta G° = -RT\ln(K_d)$, where R is the gas constant and T is the absolute temperature. Therefore, a very low $\Delta G°$ of the reaction between an oligonucleotide and the target nucleic acid reflects a strong hybridization between the oligonucleotide and target nucleic acid. $\Delta G°$ 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 $\Delta G°$ is less than zero. $\Delta G°$ 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 $\Delta G°$ measurements. $\Delta G°$ 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 $\Delta G°$ 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 $\Delta G°$. The oligonucleotides may hybridize to a target nucleic acid with estimated $\Delta G°$ 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 $\Delta G°$ 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.

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Target nucleic acid

According to the present invention, the target nucleic acid is a nucleic acid which encodes mammalian PD-L1 and may for example be a gene, a RNA, a mRNA, and pre-mRNA, a mature mRNA or a cDNA sequence. The target may therefore be referred to as a PD-L1 target nucleic acid. The oligonucleotide of the invention may for example target exon regions of a mammalian PD-L1, or may for example target intron region in the PD-L1 pre-mRNA (see Table 1).

Table 1: human PD-L1 Exons and Introns

Exonic regions in the human PD-L1 premRNA (SEQ ID NO 1)			Intronic regions in the human PD-L1 premRNA (SEQ ID NO 1)		
ID	**start**	**end**	**ID**	**start**	**end**
e1	1	94	i1	95	5597
e2	5598	5663	i2	5664	6576
e3	6577	6918	i3	6919	12331
e4	12332	12736	i4	12737	14996
e5	14997	15410	i5	15411	16267
e6	16268	16327	i6	16328	17337
e7	17338	20064			

Suitably, the target nucleic acid encodes a PD-L1 protein, in particular mammalian PD-L1, such as human PD-L1 (See for example tables 2 and 3, which provide reference to the mRNA and pre-mRNA sequences for human, monkey, and mouse PD-L1). In the context of the present invention pre-mRNA is also considered as a nucleic acid that encodes a protein.

In some embodiments, the target nucleic acid is selected from the group consisting of SEQ ID NO: 1, 2 and 3 or naturally occurring variants thereof (e.g. sequences encoding a mammalian PD-L1 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 PD-L1 target nucleic acid in a cell which is expressing the PD-L1 target nucleic acid. The contiguous sequence of nucleobases of the oligonucleotide of the invention is typically complementary to the PD-L1 target nucleic acid, as measured across the length of the oligonucleotide, optionally with the exception of one or two mismatches, and optionally excluding 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 may, in some embodiments, be a RNA or DNA, such as a messenger RNA, such as a mature mRNA or a pre-mRNA. In some embodiments the target nucleic acid is a RNA or DNA which encodes mammalian PD-L1 protein, such as

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human PD-L1, e.g. the human PD-L1 premRNA sequence, such as that disclosed as SEQ ID NO 1 or the human mRNA sequence with NCBI reference number NM_014143. Further information on exemplary target nucleic acids is provided in tables 2 and 3.

Table 2: Genome and assembly information for PD-L1 across species.

Species	Chr.	Strand	Genomic coordinates Start	End	Assembly	NCBI reference sequence* accession number for mRNA
Human	9	fwd	5450503	5470566	GRCh38:CM000671.2	NM_014143
Cynomolgus monkey	15		73560846	73581371	GCF_000364345.1	XM_005581779
Mouse	19	fwd	29367455	29388095	GRCm38:CM001012.2	NM_021893

Fwd = forward strand. The genome coordinates provide the pre-mRNA sequence (genomic sequence). The NCBI reference provides the mRNA sequence (cDNA sequence).

*The National Center for Biotechnology Information reference sequence database is a comprehensive, integrated, non-redundant, well-annotated set of reference sequences including genomic, transcript, and protein. It is hosted at www.ncbi.nlm.nih.gov/refseq.

Table 3: Sequence details for PD-L1 across species.

Species	RNA type	Length (nt)	SEQ ID NO
Human	premRNA	20064	1
Monkey Cyno	premRNA GCF ref	20261	2
Monkey Cyno	premRNA Internal	20340	3
Mouse	premRNA	20641	4

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. In some embodiments, 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. 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 target sequence may be a sub-sequence of the target nucleic acid.

In some embodiments the sub-sequence is a sequence selected from the group consisting of a1-a149 (see tables 4). In some embodiments the sub-sequence is a sequence selected from the group consisting of a human PD-L1 mRNA exon, such as a PD-L1 human mRNA exon selected from the group consisting of e1, e2, e3, e4, e5, e6, and e7 (see table 1 above).

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In some embodiments the sub-sequence is a sequence selected from the group consisting of a human PD-L1 mRNA intron, such as a PD-L1 human mRNA intron selected from the group consisting of i1, i2, i3, i4, i5 and i6 (see table 1 above).

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 of at least 8 nucleotides which is complementary to or hybridizes to a target sequence present in the target nucleic acid molecule. The contiguous nucleotide sequence (and therefore the target sequence) comprises of at least 8 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 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 preferred embodiments the target cell expresses PD-L1 mRNA, such as the PD-L1 pre-mRNA or PD-L1 mature mRNA. The poly A tail of PD-L1 mRNA is typically disregarded for antisense oligonucleotide targeting.

Naturally occurring variant

The term "naturally occurring variant" refers to variants of PD-L1 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, 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.

In some embodiments, the naturally occurring variants have at least 95% such as at least 98% or at least 99% homology to a mammalian PD-L1 target nucleic acid, such as a target nucleic acid selected form the group consisting of SEQ ID NO 1, 2 and 3.

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Numerous single nucleotide polymorphisms are known in the PD-L1 gene, for example those disclosed in the following table (human premRNA start/reference sequence is SEQ ID NO 2)

Variant name	Variant alleles	minor allele	Minor allele frequency	Start on SEQ ID NO: 1
rs73397192	G/A	A	0,10	2591
rs12342381	A/G	G	0,12	308
rs16923173	G/A	A	0,13	14760
rs2890658	C/A	A	0,16	14628
rs2890657	G/C	C	0,21	2058
rs3780395	A/G	A	0,21	14050
rs147367592	AG/-	-	0,21	13425
rs7023227	T/C	T	0,22	6048
rs2297137	G/A	A	0,23	15230
rs1329946	G/A	A	0,23	2910
rs5896124	-/G	G	0,23	2420
rs61061063	T/C	C	0,23	11709
rs1411263	T/C	C	0,23	8601
rs59906468	A/G	G	0,23	15583
rs6476976	T/C	T	0,24	21012
rs35744625	C/A	A	0,24	3557
rs17804441	T/C	C	0,24	7231
rs148602745	C/T	T	0,25	22548
rs4742099	G/A	A	0,25	20311
rs10815228	T/C	C	0,25	21877
rs58817806	A/G	G	0,26	20769
rs822342	T/C	T	0,27	3471
rs10481593	G/A	A	0,27	7593
rs822339	A/G	A	0,28	2670
rs860290	A/C	A	0,28	2696
rs822340	A/G	A	0,28	2758
rs822341	T/C	T	0.28	2894
rs12002985	C/G	C	0.28	6085
rs822338	C/T	C	0.28	1055
rs866066	C/T	T	0.28	451
rs6651524	A/T	T	0.28	8073
rs6415794	A/T	A	0.28	8200
rs4143815	G/C	C	0.28	17755
rs111423622	G/A	A	0.28	24096
rs6651525	C/A	A	0.29	8345
rs4742098	A/G	G	0.29	19995
rs10975123	C/T	T	0.30	10877
rs2282055	T/G	G	0.30	5230
rs4742100	A/C	C	0.30	20452
rs60520638	-/TC	TC	0.30	9502
rs17742278	T/C	C	0.30	6021
rs7048841	T/C	T	0.30	10299

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Variant name	Variant alleles	minor allele	Minor allele frequency	Start on SEQ ID NO: 1
rs10815229	T/G	G	0.31	22143
rs10122089	C/T	C	0.32	13278
rs1970000	C/A	C	0.32	14534
rs112071324	AGAGAG/-	AGAGAG	0.33	16701
rs2297136	G/A	G	0.33	17453
rs10815226	A/T	T	0.33	9203
rs10123377	A/G	A	0.36	10892
rs10123444	A/G	A	0.36	11139
rs7042084	G/T	G	0.36	7533
rs10114060	G/A	A	0.36	11227
rs7028894	G/A	G	0.36	10408
rs4742097	C/T	C	0.37	5130
rs1536926	G/T	G	0.37	13486
rs1411262	C/T	T	0.39	8917
rs7041009	G/A	A	0.45	12741

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 PD-L1 when compared to the amount of PD-L1 before 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). It may however also be an individual treated with the standard of care.

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 PD-L1, e.g. by degradation of mRNA or blockage of transcription. Another type of modulation is an oligonucleotide's ability to restore, increase or enhance expression of PD-L1, e.g. by repair of splice sites or prevention of splicing or removal or blockage of inhibitory mechanisms such as microRNA repression.

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

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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. Nucleosides with modified sugar moieties also include 2' modified nucleosides, such as 2' substituted 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, such as enhanced nucleoside resistance and enhanced affinity.

2' 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, and includes 2' substituted nucleosides and LNA (2' – 4' biradicle bridged) nucleosides. 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.

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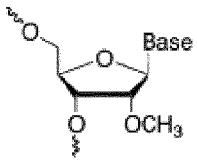
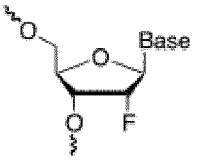
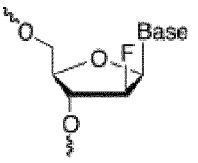


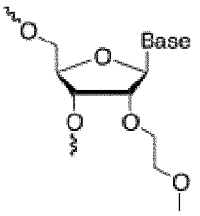
2′-*O*-Me



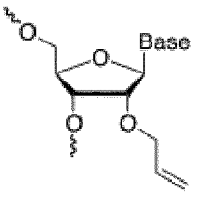
2′F-RNA



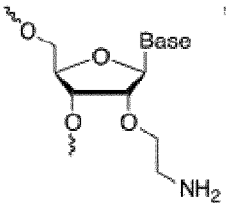
2′F-ANA



2′-*O*-MOE



2′-*O*-Allyl

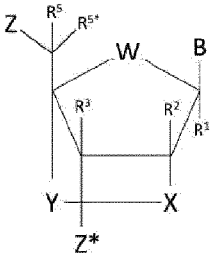


2′-*O*-Ethylamine

Locked Nucleic Acid Nucleosides (LNA).

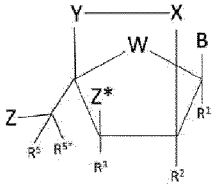
LNA nucleosides are modified nucleosides which comprise a linker group (referred to as a biradicle or a bridge) between C2' and C4' of the ribose sugar ring of a nucleotide. These nucleosides are also termed bridged nucleic acid or bicyclic nucleic acid (BNA) in the literature.

In some embodiments, the modified nucleoside or the LNA nucleosides of the oligomer of the invention has a general structure of the formula I or II:



β-D

or



α-L

Formula I Formula II

wherein W is selected from -O-, -S-, -N(R^a)-, -C(R^aR^b)-, such as, in some embodiments –O-;

B designates a nucleobase or modified nucleobase moiety;

Z designates an internucleoside linkage to an adjacent nucleoside, or a 5'-terminal group;

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

X designates a group selected from the list consisting of -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)$_2$-, -S-, -SO_2-, -N(R^a)-, and >C=Z

In some embodiments, X is selected from the group consisting of: –O-, -S-, NH-, NR^aR^b, -CH_2-, CR^aR^b, -C(=CH_2)-, and -C(=CR^aR^b)-

In some embodiments, X is -O-

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Y designates a group selected from the group consisting of -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)$_2$-, -S-, -SO_2-, -N(R^a)-, and >C=Z

In some embodiments, Y is selected from the group consisting of: –CH_2-, -C(R^aR^b)-, –CH_2CH_2-, -C(R^aR^b)-C(R^aR^b)-, –$CH_2CH_2CH_2$-, -C(R^aR^b)C(R^aR^b)C(R^aR^b)-, -C(R^a)=C(R^b)-, and -C(R^a)=N-

In some embodiments, Y is selected from the group consisting of: -CH_2-, -CHR^a-, -$CHCH_3$-, CR^aR^b-

or -X-Y- together designate a bivalent linker group (also referred to as a radicle) together designate a bivalent linker group consisting of 1, 2, 3 or 4 groups/atoms selected from the group consisting of -C(R^aR^b)-, -C(R^a)=C(R^b)-, -C(R^a)=N-, -O-, -Si(R^a)$_2$-, -S-, -SO_2-, -N(R^a)-, and >C=Z,

In some embodiments, -X-Y- designates a biradicle selected from the groups consisting of: -X-CH_2-, -X-CR^aR^b-, -X-CHR^a-, -X-C(HCH_3)-, -O-Y-, -O-CH_2-, -S-CH_2-, -NH-CH_2-, -O-$CHCH_3$-, -CH_2-O-CH_2, -O-CH(CH_3CH_3)-, -O-CH_2-CH_2-, OCH_2-CH_2-CH_2-,-O-CH_2OCH_2-, -O-NCH_2-, -C(=CH_2)-CH_2-, -NR^a-CH_2-, N-O-CH_2, -S-CR^aR^b- and -S-CHR^a-.

In some embodiments –X-Y- designates –O-CH_2- or –O-CH(CH_3)-.

wherein Z is selected from -O-, -S-, and -N(R^a)-,

and R^a and, when present R^b, each is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, hydroxy, optionally substituted C_{1-6}-alkoxy, C_{2-6}-alkoxyalkyl, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, where aryl and heteroaryl may be optionally substituted and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH_2), wherein for all chiral centers, asymmetric groups may be found in either *R* or *S* orientation.

wherein R^1, R^2, R^3, R^5 and R^{5*} are independently selected from the group consisting of: hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkynyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkoxyalkyl, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-

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carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene.

In some embodiments R^1, R^2, R^3, R^5 and R^{5*} are independently selected from C_{1-6} alkyl, such as methyl, and hydrogen.

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

In some embodiments R^1, R^2, R^3, are all hydrogen, and either R^5 and R^{5*} is also hydrogen and the other of R^5 and $R^{5*}$is other than hydrogen, such as C_{1-6} alkyl such as methyl.

In some embodiments, R^a is either hydrogen or methyl. In some embodiments, when present, R^b is either hydrogen or methyl.

In some embodiments, one or both of R^a and R^b is hydrogen

In some embodiments, one of R^a and R^b is hydrogen and the other is other than hydrogen

In some embodiments, one of R^a and R^b is methyl and the other is hydrogen

In some embodiments, both of R^a and R^b are methyl.

In some embodiments, the biradicle –X-Y- is –O-CH_2-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such LNA nucleosides are disclosed in WO99/014226, WO00/66604, WO98/039352 and WO2004/046160, and include what are commonly known as **beta-D-oxy LNA** and **alpha-L-oxy LNA** nucleosides.

In some embodiments, the biradicle –X-Y- is –S-CH_2-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such **thio LNA** nucleosides are disclosed in WO99/014226 and WO2004/046160.

In some embodiments, the biradicle –X-Y- is –NH-CH_2-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such **amino LNA** nucleosides are disclosed in WO99/014226 and WO2004/046160.

In some embodiments, the biradicle –X-Y- is –O-CH_2-CH_2- or –O-CH_2-CH_2- CH_2-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such LNA nucleosides are disclosed in WO00/047599 and Morita et al, Bioorganic & Med.Chem. Lett. 12 73-76, and include what are commonly known as 2'-O-4'C-ethylene bridged nucleic acids (ENA).

In some embodiments, the biradicle –X-Y- is –O-CH_2-, W is O, and all of R^1, R^2, R^3, and one of R^5 and R^{5*} are hydrogen, and the other of R^5 and R^{5*} is other than hydrogen such as C_{1-6} alkyl,

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such as methyl. Such **5' substituted** LNA nucleosides are disclosed in WO2007/134181.

In some embodiments, the biradicle –X-Y- is –O-CR^aR^b-, wherein one or both of R^a and R^b are other than hydrogen, such as methyl, W is O, and all of R^1, R^2, R^3, and one of R^5 and R^{5*} are hydrogen, and the other of R^5 and R^{5*} is other than hydrogen such as C_{1-6} alkyl, such as methyl. Such **bis modified LNA nucleosides** are disclosed in WO2010/077578.

In some embodiments, the biradicle –X-Y- designate the bivalent linker group –O-$CH(CH_2OCH_3)$- (2' O-methoxyethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem. Vol 75(5) pp. 1569-81). In some embodiments, the biradicle –X-Y- designate the bivalent linker group –O-$CH(CH_2CH_3)$- (2'O-ethyl bicyclic nucleic acid - Seth at al., 2010, J. Org. Chem. Vol 75(5) pp. 1569-81). In some embodiments, the biradicle –X-Y- is –O-CHR^a-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such **6' substituted** LNA nucleosides are disclosed in WO10036698 and WO07090071.

In some embodiments, the biradicle –X-Y- is –O-$CH(CH_2OCH_3)$-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such LNA nucleosides are also known as **cyclic MOEs** in the art (cMOE) and are disclosed in WO07090071.

In some embodiments, the biradicle –X-Y- designate the bivalent linker group –O-$CH(CH_3)$-. – in either the R- or S- configuration. In some embodiments, the biradicle –X-Y- together designate the bivalent linker group –O-CH_2-O-CH_2- (Seth at al., 2010, J. Org. Chem). In some embodiments, the biradicle –X-Y- is –O-$CH(CH_3)$-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such 6' methyl LNA nucleosides are also known as **cET nucleosides** in the art, and may be either (S)cET or (R)cET stereoisomers, as disclosed in WO07090071 (beta-D) and WO2010/036698 (alpha-L)).

In some embodiments, the biradicle –X-Y- is –O-CR^aR^b-, wherein in neither R^a or R^b is hydrogen, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. In some embodiments, R^a and R^b are both methyl. Such **6' di-substituted** LNA nucleosides are disclosed in WO 2009006478.

In some embodiments, the biradicle –X-Y- is –S-CHR^a-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such **6' substituted thio** LNA nucleosides are disclosed in WO11156202. In some 6' substituted thio LNA embodiments R^a is methyl.

In some embodiments, the biradicle –X-Y- is –C(=CH2)-$C(R^aR^b)$-, such as –$C(=CH_2)$-CH_2- , or –$C(=CH_2)$-$CH(CH_3)$-W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. Such **vinyl carbo** LNA nucleosides are disclosed in WO08154401 and WO09067647.

In some embodiments the biradicle –X-Y- is –N(-OR^a)-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. In some embodiments R^a is C_{1-6} alkyl such as methyl. Such LNA nucleosides

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are also known as N substituted LNAs and are disclosed in WO2008/150729. In some embodiments, the biradicle –X-Y- together designate the bivalent linker group –O-NR^a-CH_3- (Seth at al., 2010, J. Org. Chem). In some embodiments the biradicle –X-Y- is –N(R^a)-, W is O, and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. In some embodiments R^a is C_{1-6} alkyl such as methyl.

In some embodiments, one or both of R^5 and R^{5*} is hydrogen and, when substituted the other of R^5 and R^{5*} is C_{1-6} alkyl such as methyl. In such an embodiment, R^1, R^2, R^3, may all be hydrogen, and the biradicle –X-Y- may be selected from –O-CH2- or –O-C(HCR^a)-, such as –O-C(HCH3)-.

In some embodiments, the biradicle is –CR^aR^b-O-CR^aR^b-, such as CH_2-O-CH_2-, W is O and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. In some embodiments R^a is C_{1-6} alkyl such as methyl. Such LNA nucleosides are also known as conformationally restricted nucleotides (CRNs) and are disclosed in WO2013036868.

In some embodiments, the biradicle is –O-CR^aR^b-O-CR^aR^b-, such as O-CH_2-O-CH_2-, W is O and all of R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen. In some embodiments R^a is C_{1-6} alkyl such as methyl. Such LNA nucleosides are also known as COC nucleotides and are disclosed in Mitsuoka et al., Nucleic Acids Research 2009 37(4), 1225-1238.

It will be recognized than, unless specified, the LNA nucleosides may be in the beta-D or alpha-L stereoisoform.

Certain examples of LNA nucleosides are presented in Scheme 1.

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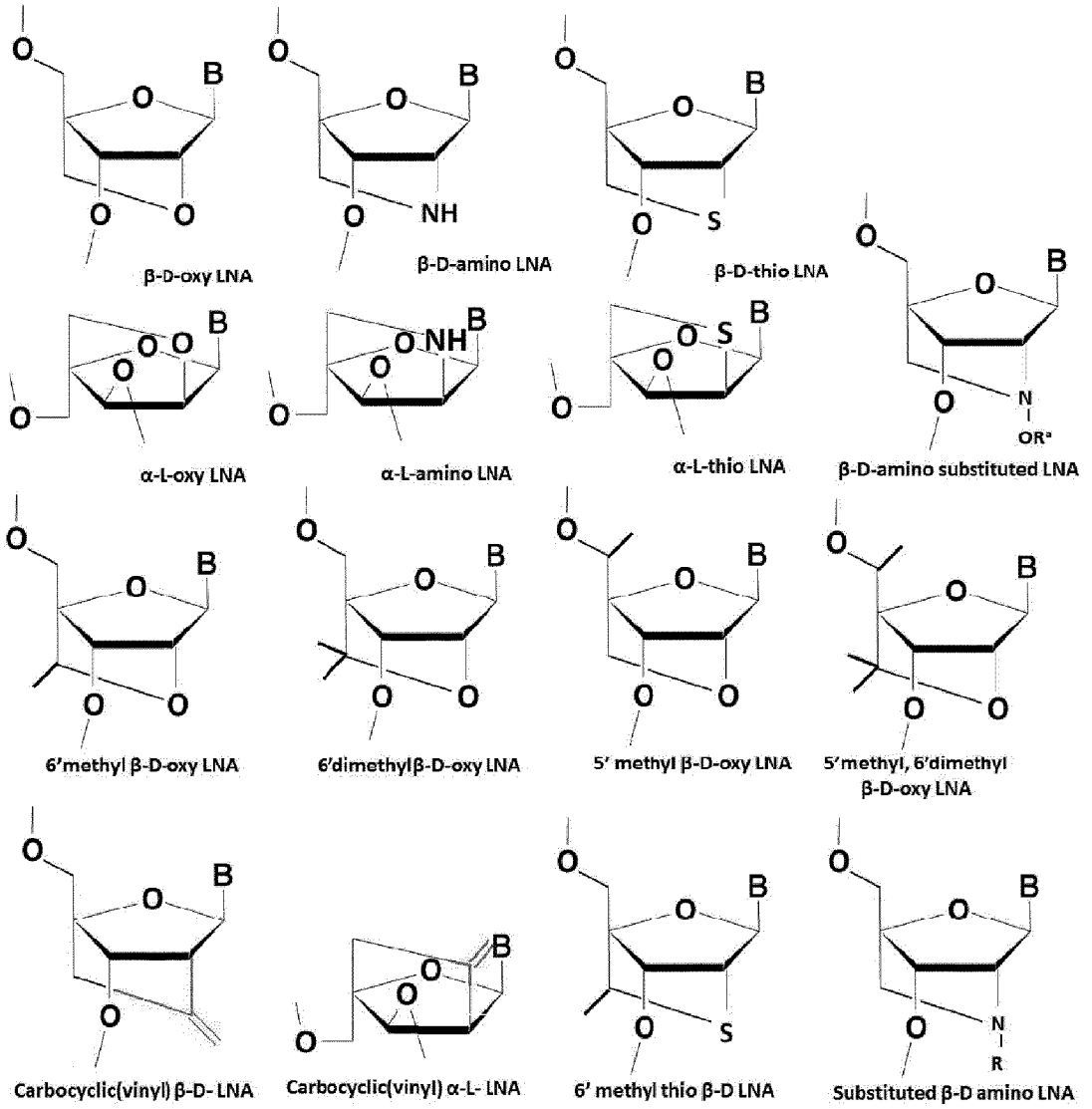
Scheme 1



As illustrated in the examples, in some embodiments of the invention the LNA nucleosides in the oligonucleotides are beta-D-oxy-LNA nucleosides.

Nuclease mediated degradation

Nuclease mediated degradation refers to an oligonucleotide capable of mediating degradation of a complementary nucleotide sequence when forming a duplex with such a sequence.

In some embodiments, the oligonucleotide may function via nuclease mediated degradation of the target nucleic acid, where the oligonucleotides of the invention are capable of recruiting a nuclease, particularly and endonuclease, preferably endoribonuclease (RNase), such as RNase H. Examples of oligonucleotide designs which operate via nuclease mediated mechanisms are oligonucleotides which typically comprise a region of at least 5 or 6 DNA nucleosides and are

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flanked on one side or both sides by affinity enhancing nucleosides, for example gapmers, headmers and tailmers.

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.

Gapmer

The term gapmer as used herein refers to an antisense oligonucleotide which comprises a region of RNase H recruiting oligonucleotides (gap) which is flanked 5' and 3' by regions which comprise one or more affinity enhancing modified nucleosides (flanks or wings). Various gapmer designs are described herein and a characterized by their ability to recruit RNaseH. Headmers and tailmers are oligonucleotides capable of recruiting RNase H where one of the flanks is missing, i.e. only one of the ends of the oligonucleotide comprises affinity enhancing modified nucleosides. For headmers the 3' flank is missing (i.e. the 5' flank comprises affinity enhancing modified nucleosides) and for tailmers the 5' flank is missing (i.e. the 3' flank comprises affinity enhancing modified nucleosides).

LNA Gapmer

The term LNA gapmer is a gapmer oligonucleotide wherein at least one of the affinity enhancing modified nucleosides is an LNA nucleoside.

Mixed Wing Gapmer

The term mixed wing gapmer or mixed flank gapmer refers to a LNA gapmer wherein at least one of the flank regions comprise at least one LNA nucleoside and at least one non-LNA modified nucleoside, such as at least one 2' substituted modified nucleoside, such as, for example, 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(s). In some embodiments the mixed wing gapmer has one flank which comprises only LNA nucleosides (e.g. 5' or 3') and the other flank

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(3' or 5' respectfully) comprises 2' substituted modified nucleoside(s) and optionally LNA nucleosides.

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Gapbreaker

The term "gapbreaker oligonucleotide" is used in relation to a gapmer capable of maintaining RNAseH recruitment even though the gap region is disrupted by a non-RNaseH recruiting nucleoside (a gap-breaker nucleoside, E) such that the gap region comprise less than 5 consecutive DNA nucleosides. Non-RNaseH recruiting nucleosides are for example nucleosides in the 3' endo conformation, such as LNA's where 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 nucleoside. The ability of gapbreaker oligonucleotide 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.

In some embodiments, the oligonucleotide of the invention is a gapbreaker oligonucleotide. In some embodiments the gapbreaker oligonucleotide comprise a 5'-flank (F), a gap (G) and a 3'-flank (F'), wherein the gap is disrupted by a non-RNaseH recruiting nucleoside (a gap-breaker nucleoside, E) such that the gap contain at least 3 or 4 consecutive DNA nucleosides. In some embodiments the gapbreaker nucleoside (E) is an LNA nucleoside where the bridge between C2' and C4' of the ribose sugar ring of a nucleoside is in the beta conformation and is placed within the gap region such that the gap-breaker LNA nucleoside is flanked 5' and 3' by at least 3 (5') and 3 (3') or at least 3 (5') and 4 (3') or at least 4(5') and 3(3') DNA nucleosides, and wherein the oligonucleotide is capable of recruiting RNaseH.

The gapbreaker oligonucleotide can be represented by the following formulae:

F-G-E-G-F'; in particular F_{1-7}-G_{3-4}-E_1-G_{3-4}-F'_{1-7}

D'-F-G-F', in particular D'_{1-3}-F_{1-7}- G_{3-4}-E_1-G_{3-4}-F'_{1-7}

F-G-F'-D'', in particular F_{1-7}- G_{3-4}-E_1-G_{3-4}-F'_{1-7}-D''_{1-3}

D'-F-G-F'-D'', in particular D'_{1-3}-F_{1-7}- G_{3-4}-E_1-G_{3-4}-F'_{1-7}-D''_{1-3}

Where region D' and D'' are as described in the section "Gapmer design".

In some embodiments the gapbreaker nucleoside (E) is a beta-D-oxy LNA or ScET or another beta-LNA nucleosides shown in Scheme 1).

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), also termed a oligonucleotide conjugate.

Conjugation of the oligonucleotides 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

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conjugate moiety targets the oligonucleotide to the liver. A the same time the conjugate 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 one embodiment of the invention the oligonucleotide conjugate of the invention display improved inhibition of PD-L1 in the target cell when compared to an unconjugated oligonucleotide. In another embodiment the oligonucleotide conjugate of the invention has improved cellular distribution between liver and other organs, such as spleen or kidney (i.e. more conjugated oligonucleotide goes to the liver than the spleen or kidney) when compared to an unconjugated oligonucleotide. In another embodiment the oligonucleotide conjugate of the invention show improved cellular uptake into the liver of the conjugate oligonucleotide when compared to an unconjugated oligonucleotide.

WO 93/07883 and WO2013/033230 provides suitable conjugate moieties. Further suitable conjugate moieties are those capable of binding to the asialoglycoprotein receptor (ASGPr). In particular tri-valent N-acetylgalactosamine conjugate moieties are suitable for binding to the the ASGPr, see for example WO 2014/076196, WO 2014/207232 and WO 2014/179620. The conjugate moiety is essentially the part of the antisense oligonucleotides conjugates which is not composed of nucleic acids.

Oligonucleotide conjugates and their synthesis has also been reported in comprehensive reviews by Manoharan in Antisense Drug Technology, Principles, Strategies, and Applications, S.T. Crooke, ed., Ch. 16, Marcel Dekker, Inc., 2001 and Manoharan, Antisense and Nucleic Acid Drug Development, 2002, 12, 103.

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 complementary to the target nucleic acid (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

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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. In a preferred embodiment the nuclease susceptible linker comprises between 1 and 10 nucleosides, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleosides, more preferably between 2 and 6 nucleosides and most preferably between 2 and 4 linked nucleosides comprising at least two consecutive phosphodiester linkages, such as at least 3 or 4 or 5 consecutive phosphodiester linkages. Preferably the nucleosides are DNA or RNA. Phosphodiester containing biocleavable linkers are described in more detail in WO 2014/076195.

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 or contiguous nucleotide sequence complementary to the target nucleic acid (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.

Restoration of immune response against pathogens

The immune response is divided into the innate and adaptive immune response. The innate immune system provides an immediate, but non-specific response. The adaptive immune

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response is activated by innate immune response and is highly specific to a particular pathogen. Upon presentation of a pathogen-derived antigen on the surface of antigen-presenting cells,

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immune cells of the adaptive immune response (i.e. T and B lymphocytes) are activated through their antigen-specific receptors leading to a pathogenic-specifc immune response and development of immunological memory. Chronic viral infections, such as HBV and HCV, are associated with T cell exhaustion characterized by unresponsiveness of the viral-specific T cells. T cell exhaustion is well studied, for a review see for example Yi et al 2010 Immunology129, 474-481. Chronic viral infections are also associated with reduced function of NK cells that are innate immune cells. Enhancing viral immune response is important for clearance of chronic infection. Restoration of immune response against pathogens, mediated by T cells and NK cells, can be assessed by measurement of proliferation, cytokine secretion and cytolytic function (Dolina et al. 2013 Molecular Therapy-Nucleic Acids, 2 e72 and Example 6 herein).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of antisense oligonucleotides and conjugates thereof and pharmaceutical compositions comprising these to restore immune response against pathogens that have infected an animal, in particular a human. The antisense oligonucleotide conjugates of the present invention are particular useful against pathogens that have infected the liver, in particular chronic liver infections like HBV. The conjugates allow targeted distribution of the oligonucleotides and prevents systemic knockdown of the target nucleic acid.

The Oligonucleotides of the Invention

The invention relates to oligonucleotides capable of modulating expression of PD-L1. The modulation is may achieved by hybridizing to a target nucleic acid encoding PD-L1 or which is involved in the regulation of PD-L1. The target nucleic acid may be a mammalian PD-L1 sequence, such as a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and/or SEQ ID NO: 3. The target nucleic acid may be a pre-mRNA, an mRNA or any RNA sequence expressed from a mammalian cell that supports the expression or regulation of PD-L1.

The oligonucleotide of the invention is an antisense oligonucleotide which targets PD-L1.

In one aspect of the invention the oligonucleotides of the invention are conjugated to a conjugate moiety, in particular an asialoglycoprotein receptor targeting conjugate moiety.

In some embodiments the antisense oligonucleotide of the invention is capable of modulating the expression of the target by inhibiting or down-regulating it. Preferably, such modulation produces an inhibition of expression of at least 20% compared to the normal expression level of the target, more preferably at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% inhibition compared to the normal expression level of the target. Preferably, such modulation produces an inhibition of expression of at least 20% compared to the expression level when the cell or

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organism is challenged by an infectious agent, or treated with an agent simulating the challenge by an infectious agent (eg poly I:C or LPS), more preferably at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% inhibition compared to the expression level when the cell or organism is challenged by an infectious agent, or treated with an agent simulating the challenge by an infectious agent (eg poly I:C or LPS). In some embodiments oligonucleotides of the invention may be capable of inhibiting expression levels of PD-L1 mRNA by at least 60% or 70% *in vitro* using KARPAS-299 or THP1 cells. In some embodiments compounds of the invention may be capable of inhibiting expression levels of PD-L1 protein by at least 50% *in vitro* using KARPAS-299 or THP1 cells. Suitably, the examples provide assays which may be used to measure PD-L1 RNA (e.g. example 1). The target modulation is triggered by the hybridization between a contiguous nucleotide sequence of the oligonucleotide and the target nucleic acid. In some embodiments the oligonucleotide of the invention comprises mismatches between the oligonucleotide and the target nucleic acid. Despite mismatches, hybridization to the target nucleic acid may still be sufficient to show a desired modulation of PD-L1 expression. Reduced binding affinity resulting from mismatches may advantageously be compensated by increased number of nucleotides in the oligonucleotide and/or an increased number of modified nucleosides capable of increasing the binding affinity to the target, such as 2' modified nucleosides, including LNA, present within the oligonucleotide sequence.

In some embodiments the antisense oligonucleotide of the invention is capable of restoring pathogen-specific T cells. In some embodiments, oligonucleotides of the invention are capable of increasing the pathogen-specific T cells by at least 40%, 50%, 60% or 70% when compared to untreated controls or controls treated with standard of care. In one embodiment the antisense oligonucleotide or conjugate of the invention is capable increasing HBV-specific T cells when compared to untreated controls or controls treated with standard of care. Suitably, the examples provide assays which may be used to measure the HBV-specific T cells (e.g. T cell proliferation, cytokine secretion and cytolytic activity). In another embodiment the the antisense oligonucleotide or conjugate of the invention is capable increasing HCV-specific T cells when compared to untreated controls or controls treated with standard of care. In another embodiment the the antisense oligonucleotide or conjugate of the invention is capable increasing HDV-specific T cells when compared to untreated controls or controls treated with standard of care.

In some embodiments the antisense oligonucleotide of the invention is capable reducing HBsAg levels in an animal or human. In some embodiments, oligonucleotides of the invention are capable of reducing the HBsAg levels by at least 40%, 50%, 60% or 70%, more preferably by at least 80%, 90% or 95% when compared to the level prior to treatment. Most preferably oligonucleotides of the invention are capable of achieving seroconversion of HBsAg in an animal or human infected with HBV.

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An aspect of the present invention relates to an antisense oligonucleotide which comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementarity to a PD-L1 target nucleic acid.

In some embodiments, the oligonucleotide comprises a contiguous sequence 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.

In a preferred embodiment the oligonucleotide of the invention, or contiguous nucleotide sequence thereof is fully complementary (100% complementary) to a region of the target nucleic acid, or in some embodiments may comprise one or two mismatches between the oligonucleotide and the target nucleic acid.

In some embodiments the oligonucleotide comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementary, such as fully (or 100%) complementary, to a region target nucleic acid region present in SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments the oligonucleotide sequence is 100% complementary to a corresponding target nucleic acid region present SEQ ID NO: 1 and SEQ ID NO: 2. In some embodiments the oligonucleotide sequence is 100% complementary to a corresponding target nucleic acid region present SEQ ID NO: 1 and SEQ ID NO: 3.

In some embodiments, the oligonucleotide or oligonucleotide conjugate comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementary, such as 100% complementarity, to a corresponding target nucleic acid region wherein the contiguous nucleotide sequence is complementary to a sub-sequence of the target nucleic acid selected from the group consisting of position 371-3068, 5467-12107 and 15317-19511 on SEQ ID NO: 1. In a further embodiment the sub-sequence of the target nucleic acid is selected from the group consisting of position 371-510, 822-1090, 1992-3068, 5467-5606, 6470-12107, 15317-15720, 15317-18083, 18881-19494 and 1881-19494 on SEQ ID NO: 1. In a preferred embodiment the sub-sequence of the target nucleic acid is selected from the group consisting of position 7300-7333, 8028-8072, 9812-9859, 11787-11873 and 15690-15735 on SEQ ID NO: 1.

In some embodiments, the oligonucleotide or oligonucleotide conjugate comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementary, such as 100% complementarity, to a corresponding target nucleic acid region present in SEQ ID NO: 1, wherein the target nucleic acid region is selected from the group consisting of region a1 to a449 in table 4.

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Table 4: Regions of SEQ ID NO 1 which may be targeted using oligonucleotide of the invention

Reg. a	Position in SEQ ID NO 1		Len-gth	Reg. a	Position in SEQ ID NO 1		Len-gth	Reg. a	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
a1	51	82	32	a151	6994	7020	27	a301	13092	13115	24
a2	87	116	30	a152	7033	7048	16	a302	13117	13134	18
a3	118	133	16	a153	7050	7066	17	a303	13136	13169	34
a4	173	206	34	a154	7078	7094	17	a304	13229	13249	21
a5	221	287	67	a155	7106	7122	17	a305	13295	13328	34
a6	304	350	47	a156	7123	7144	22	a306	13330	13372	43
a7	354	387	34	a157	7146	7166	21	a307	13388	13406	19
a8	389	423	35	a158	7173	7193	21	a308	13408	13426	19
a9	425	440	16	a159	7233	7291	59	a309	13437	13453	17
a10	452	468	17	a160	7300	7333	34	a310	13455	13471	17
a11	470	484	15	a161	7336	7351	16	a311	13518	13547	30
a12	486	500	15	a162	7353	7373	21	a312	13565	13597	33
a13	503	529	27	a163	7375	7412	38	a313	13603	13620	18
a14	540	574	35	a164	7414	7429	16	a314	13630	13663	34
a15	576	649	74	a165	7431	7451	21	a315	13665	13679	15
a16	652	698	47	a166	7453	7472	20	a316	13706	13725	20
a17	700	750	51	a167	7474	7497	24	a317	13727	13774	48
a18	744	758	15	a168	7517	7532	16	a318	13784	13821	38
a19	774	801	28	a169	7547	7601	55	a319	13831	13878	48
a20	805	820	16	a170	7603	7617	15	a320	13881	13940	60
a21	827	891	65	a171	7632	7647	16	a321	13959	14013	55
a22	915	943	29	a172	7649	7666	18	a322	14015	14031	17
a23	950	982	33	a173	7668	7729	62	a323	14034	14049	16
a24	984	1000	17	a174	7731	7764	34	a324	14064	14114	51
a25	1002	1054	53	a175	7767	7817	51	a325	14116	14226	111
a26	1060	1118	59	a176	7838	7860	23	a326	14229	14276	48
a27	1124	1205	82	a177	7862	7876	15	a327	14292	14306	15
a28	1207	1255	49	a178	7880	7944	65	a328	14313	14384	72
a29	1334	1349	16	a179	7964	8012	49	a329	14386	14408	23
a30	1399	1425	27	a180	8028	8072	45	a330	14462	14481	20
a31	1437	1458	22	a181	8086	8100	15	a331	14494	14519	26
a32	1460	1504	45	a182	8102	8123	22	a332	14557	14577	21
a33	1548	1567	20	a183	8125	8149	25	a333	14608	14628	21
a34	1569	1586	18	a184	8151	8199	49	a334	14646	14668	23
a35	1608	1662	55	a185	8218	8235	18	a335	14680	14767	88
a36	1677	1700	24	a186	8237	8276	40	a336	14765	14779	15
a37	1702	1721	20	a187	8299	8344	46	a337	14815	14844	30

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Reg. a	Position in SEQ ID NO 1		Len-gth	Reg. a	Position in SEQ ID NO 1		Len-gth	Reg. a	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
a38	1723	1745	23	a188	8346	8436	91	a338	14848	14925	78
a39	1768	1794	27	a189	8438	8470	33	a339	14934	14976	43
a40	1820	1835	16	a190	8472	8499	28	a340	14978	15009	32
a41	1842	1874	33	a191	8505	8529	25	a341	15013	15057	45
a42	1889	1979	91	a192	8538	8559	22	a342	15064	15091	28
a43	1991	2011	21	a193	8562	8579	18	a343	15094	15140	47
a44	2013	2038	26	a194	8581	8685	105	a344	15149	15165	17
a45	2044	2073	30	a195	8688	8729	42	a345	15162	15182	21
a46	2075	2155	81	a196	8730	8751	22	a346	15184	15198	15
a47	2205	2228	24	a197	8777	8800	24	a347	15200	15221	22
a48	2253	2273	21	a198	8825	8865	41	a348	15232	15247	16
a49	2275	2303	29	a199	8862	8894	33	a349	15250	15271	22
a50	2302	2333	32	a200	8896	8911	16	a350	15290	15334	45
a51	2335	2366	32	a201	8938	8982	45	a351	15336	15369	34
a52	2368	2392	25	a202	8996	9045	50	a352	15394	15416	23
a53	2394	2431	38	a203	9048	9070	23	a353	15433	15451	19
a54	2441	2455	15	a204	9072	9139	68	a354	15453	15491	39
a55	2457	2494	38	a205	9150	9168	19	a355	15496	15511	16
a56	2531	2579	49	a206	9170	9186	17	a356	15520	15553	34
a57	2711	2732	22	a207	9188	9202	15	a357	15555	15626	72
a58	2734	2757	24	a208	9204	9236	33	a358	15634	15652	19
a59	2772	2786	15	a209	9252	9283	32	a359	15655	15688	34
a60	2788	2819	32	a210	9300	9331	32	a360	15690	15735	46
a61	2835	2851	17	a211	9339	9354	16	a361	15734	15764	31
a62	2851	2879	29	a212	9370	9398	29	a362	15766	15787	22
a63	2896	2912	17	a213	9400	9488	89	a363	15803	15819	17
a64	2915	2940	26	a214	9490	9537	48	a364	15846	15899	54
a65	2944	2973	30	a215	9611	9695	85	a365	15901	15934	34
a66	2973	2992	20	a216	9706	9721	16	a366	15936	15962	27
a67	2998	3016	19	a217	9723	9746	24	a367	15964	15985	22
a68	3018	3033	16	a218	9748	9765	18	a368	15987	16023	37
a69	3036	3051	16	a219	9767	9788	22	a369	16025	16061	37
a70	3114	3139	26	a220	9794	9808	15	a370	16102	16122	21
a71	3152	3173	22	a221	9812	9859	48	a371	16134	16183	50
a72	3181	3203	23	a222	9880	9913	34	a372	16185	16281	97
a73	3250	3271	22	a223	9923	9955	33	a373	16283	16298	16
a74	3305	3335	31	a224	9966	10007	42	a374	16305	16323	19
a75	3346	3363	18	a225	10009	10051	43	a375	16325	16356	32
a76	3391	3446	56	a226	10053	10088	36	a376	16362	16404	43

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Reg. a	Position in SEQ ID NO 1		Len-gth	Reg. a	Position in SEQ ID NO 1		Len-gth	Reg. a	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
a77	3448	3470	23	a227	10098	10119	22	a377	16406	16456	51
a78	3479	3497	19	a228	10133	10163	31	a378	16494	16523	30
a79	3538	3554	17	a229	10214	10240	27	a379	16536	16562	27
a80	3576	3597	22	a230	10257	10272	16	a380	16564	16580	17
a81	3603	3639	37	a231	10281	10298	18	a381	16582	16637	56
a82	3663	3679	17	a232	10300	10318	19	a382	16631	16649	19
a83	3727	3812	86	a233	10339	10363	25	a383	16655	16701	47
a84	3843	3869	27	a234	10409	10426	18	a384	16737	16781	45
a85	3874	3904	31	a235	10447	10497	51	a385	16783	16804	22
a86	3926	3955	30	a236	10499	10529	31	a386	16832	16907	76
a87	3974	3993	20	a237	10531	10546	16	a387	16934	16965	32
a88	3995	4042	48	a238	10560	10580	21	a388	16972	17035	64
a89	4053	4073	21	a239	10582	10596	15	a389	17039	17069	31
a90	4075	4123	49	a240	10600	10621	22	a390	17072	17109	38
a91	4133	4157	25	a241	10623	10664	42	a391	17135	17150	16
a92	4158	4188	31	a242	10666	10685	20	a392	17167	17209	43
a93	4218	4250	33	a243	10717	10773	57	a393	17211	17242	32
a94	4277	4336	60	a244	10775	10792	18	a394	17244	17299	56
a95	4353	4375	23	a245	10794	10858	65	a395	17304	17344	41
a96	4383	4398	16	a246	10874	10888	15	a396	17346	17400	55
a97	4405	4446	42	a247	10893	10972	80	a397	17447	17466	20
a98	4448	4464	17	a248	10974	10994	21	a398	17474	17539	66
a99	4466	4493	28	a249	10996	11012	17	a399	17561	17604	44
a100	4495	4558	64	a250	11075	11097	23	a400	17610	17663	54
a101	4571	4613	43	a251	11099	11124	26	a401	17681	17763	83
a102	4624	4683	60	a252	11140	11157	18	a402	17793	17810	18
a103	4743	4759	17	a253	11159	11192	34	a403	17812	17852	41
a104	4761	4785	25	a254	11195	11226	32	a404	17854	17928	75
a105	4811	4858	48	a255	11235	11261	27	a405	17941	18005	65
a106	4873	4932	60	a256	11279	11337	59	a406	18007	18035	29
a107	4934	4948	15	a257	11344	11381	38	a407	18041	18077	37
a108	4955	4974	20	a258	11387	11411	25	a408	18085	18146	62
a109	4979	5010	32	a259	11427	11494	68	a409	18163	18177	15
a110	5012	5052	41	a260	11496	11510	15	a410	18179	18207	29
a111	5055	5115	61	a261	11512	11526	15	a411	18209	18228	20
a112	5138	5166	29	a262	11528	11551	24	a412	18230	18266	37
a113	5168	5198	31	a263	11570	11592	23	a413	18268	18285	18
a114	5200	5222	23	a264	11594	11634	41	a414	18287	18351	65
a115	5224	5284	61	a265	11664	11684	21	a415	18365	18395	31

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Reg. a	Position in SEQ ID NO 1		Len-gth	Reg. a	Position in SEQ ID NO 1		Len-gth	Reg. a	Position in SEQ ID NO 1		Len-gth
	from	to			from	to			from	to	
a116	5286	5302	17	a266	11699	11719	21	a416	18402	18432	31
a117	5317	5332	16	a267	11721	11746	26	a417	18434	18456	23
a118	5349	5436	88	a268	11753	11771	19	a418	18502	18530	29
a119	5460	5512	53	a269	11787	11873	87	a419	18545	18590	46
a120	5514	5534	21	a270	11873	11905	33	a420	18603	18621	19
a121	5548	5563	16	a271	11927	11942	16	a421	18623	18645	23
a122	5565	5579	15	a272	11946	11973	28	a422	18651	18708	58
a123	5581	5597	17	a273	11975	11993	19	a423	18710	18729	20
a124	5600	5639	40	a274	12019	12114	96	a424	18731	18758	28
a125	5644	5661	18	a275	12116	12135	20	a425	18760	18788	29
a126	5663	5735	73	a276	12137	12158	22	a426	18799	18859	61
a127	5737	5770	34	a277	12165	12192	28	a427	18861	18926	66
a128	5778	5801	24	a278	12194	12216	23	a428	18928	18980	53
a129	5852	5958	107	a279	12218	12246	29	a429	19001	19018	18
a130	6007	6041	35	a280	12262	12277	16	a430	19034	19054	21
a131	6049	6063	15	a281	12283	12319	37	a431	19070	19092	23
a132	6065	6084	20	a282	12334	12368	35	a432	19111	19154	44
a133	6086	6101	16	a283	12370	12395	26	a433	19191	19213	23
a134	6119	6186	68	a284	12397	12434	38	a434	19215	19240	26
a135	6189	6234	46	a285	12436	12509	74	a435	19255	19356	102
a136	6236	6278	43	a286	12511	12543	33	a436	19358	19446	89
a137	6291	6312	22	a287	12545	12565	21	a437	19450	19468	19
a138	6314	6373	60	a288	12567	12675	109	a438	19470	19512	43
a139	6404	6447	44	a289	12677	12706	30	a439	19514	19541	28
a140	6449	6482	34	a290	12708	12724	17	a440	19543	19568	26
a141	6533	6555	23	a291	12753	12768	16	a441	19570	19586	17
a142	6562	6622	61	a292	12785	12809	25	a442	19588	19619	32
a143	6624	6674	51	a293	12830	12859	30	a443	19683	19739	57
a144	6679	6762	84	a294	12864	12885	22	a444	19741	19777	37
a145	6764	6780	17	a295	12886	12916	31	a445	19779	19820	42
a146	6782	6822	41	a296	12922	12946	25	a446	19822	19836	15
a147	6824	6856	33	a297	12948	12970	23	a447	19838	19911	74
a148	6858	6898	41	a298	12983	13003	21	a448	19913	19966	54
a149	6906	6954	49	a299	13018	13051	34	a449	19968	20026	59
a150	6969	6992	24	a300	13070	13090	21				

In some embodiment the oligonucleotide or contiguous nucleotide sequence is complementary to a region of the target nucleic acid, wherein the target nucleic acid region is selected from the group consisting of a7, a26, a43, a119, a142, a159, a160, a163, a169, a178, a179, a180, a189,

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a201, a202, a204, a214, a221, a224, a226, a243, a254, a258, 269, a274, a350, a360, a364, a365, a370, a372, a381, a383, a386, a389, a400, a427, a435 and a438.

In a preferred embodiment the oligonucleotide or contiguous nucleotide sequence is complementary to a region of the target nucleic acid, wherein the target nucleic acid region is selected from the group consisting of a160, a180, a221, a269 and a360.

In some embodiments, the oligonucleotide of the invention comprises or consists of 8 to 35 nucleotides in length, such as from 9 to 30, such as 10 to 22, such as from 11 to 20, such as from 12 to 18, such as from 13 to 17 or 14 to 16 contiguous nucleotides in length. In a preferred embodiment, the oligonucleotide comprises or consists of 16 to 20 nucleotides in length. It is to be understood that any range given herein includes the range endpoints. Accordingly, if an oligonucleotide is said to include from 10 to 30 nucleotides, both 10 and 30 nucleotides are included.

In some embodiments, the contiguous nucleotide sequence comprises or consists of 8, 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 in length. In a preferred embodiment, the oligonucleotide comprises or consists of 16, 17, 18, 19 or 20 nucleotides in length.

In some embodiments, the oligonucleotide or contiguous nucleotide sequence comprises or consists of a sequence selected from the group consisting of sequences listed in table 5.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 5 to 743 (see motif sequences listed in table 5).

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 5 to 743 and 771.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to a sequence selected from the group consisting of SEQ ID NO: 6, 8, 9, 13, 41, 42, 58, 77, 92, 111, 128, 151, 164, 166, 169, 171, 222, 233, 245, 246, 250, 251, 252, 256, 272, 273, 287, 292, 303, 314, 318, 320, 324, 336, 342, 343, 344, 345, 346, 349, 359, 360, 374, 408, 409, 415, 417, 424, 429, 430, 458, 464, 466, 474, 490, 493, 512, 519, 519, 529, 533, 534, 547, 566, 567, 578, 582, 601, 619, 620, 636, 637, 638, 640, 645, 650, 651, 652, 653, 658, 659, 660, 665, 678, 679, 680, 682, 683, 684, 687, 694, 706, 716, 728, 733, 734, and 735.

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In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to SEQ ID NO: 287.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to SEQ ID NO: 342.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to SEQ ID NO: 640.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to SEQ ID NO: 466.

In some embodiments, the antisense oligonucleotide or contiguous nucleotide sequence comprises or consists of 10 to 30 nucleotides in length with at least 90% identity, preferably 100% identity, to SEQ ID NO: 566.

In embodiments where the oligonucleotide is longer than the contigious nucleotide sequence (which is complementary to the target nucleic acid), the motif sequences in table 5 form the contigious nucleotide sequence part of the antisense oligonucleotides of the invention. In some embodiments the sequence of the oligonucleotide is equivalent to the contigious nucleotide sequence (e.g. if no biocleavable linkers are added).

It is understood that the contiguous nucleobase sequences (motif sequence) can be modified to for example increase nuclease resistance and/or binding affinity to the target nucleic acid. Modifications are described in the definitions and in the "Oligonucleotide design" section. Table 5 lists preferred designs of each motif sequence.

Oligonucleotide design

Oligonucleotide design refers to the pattern of nucleoside sugar modifications in the oligonucleotide sequence. The oligonucleotides of the invention comprise sugar-modified nucleosides and may also comprise DNA or RNA nucleosides. In some embodiments, the oligonucleotide comprises sugar-modified nucleosides and DNA nucleosides. Incorporation of modified nucleosides into the oligonucleotide of the invention may enhance the affinity of the oligonucleotide for the target nucleic acid. In that case, the modified nucleosides can be referred to as affinity enhancing modified nucleotides, the modified nucleosides may also be termed units.

In an embodiment, the oligonucleotide comprises at least 1 modified nucleoside, such as at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at

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least 11, at least 12, at least 13, at least 14, at least 15 or at least 16 modified nucleosides. In an embodiment the oligonucleotide comprises from 1 to 10 modified nucleosides, such as from 2 to 8 modified nucleosides, such as from 3 to 7 modified nucleosides, such as from 4 to 6 modified nucleosides, such as 3, 4, 5, 6 or 7 modified nucleosides.

In an embodiment, the oligonucleotide comprises one or more sugar modified nucleosides, such as 2' sugar modified nucleosides. Preferably the oligonucleotide of the invention comprise the one or more 2' sugar modified nucleoside 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. Even more preferably the one or more modified nucleoside is a locked nucleic acid (LNA).

In a further embodiment the oligonucleotide comprises at least one modified internucleoside linkage. In a preferred embodiment all the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate or boranophosphate internucleoside linkages. In some embodiments all the internucleotide linkages in the contiguous sequence of the oligonucleotide are phosphorothioate linkages.

In some embodiments, the oligonucleotide of the invention comprises at least one LNA nucleoside, such as 1, 2, 3, 4, 5, 6, 7, or 8 LNA nucleosides, such as from 2 to 6 LNA nucleosides, such as from 3 to 7 LNA nucleosides, 4 to 6 LNA nucleosides or 3, 4, 5, 6 or 7 LNA nucleosides. In some embodiments, at least 75% of the modified nucleosides in the oligonucleotide are LNA nucleosides, such as 80%, such as 85%, such as 90% of the modified nucleosides are LNA nucleosides. In a still further embodiment all the modified nucleosides in the oligonucleotide are LNA nucleosides. In a further embodiment, the oligonucleotide may comprise both beta-D-oxy-LNA, and one or more of the following LNA nucleosides: thio-LNA, amino-LNA, oxy-LNA, and/or ENA in either the beta-D or alpha-L configurations or combinations thereof. In a further embodiment, all LNA cytosine units are 5-methyl-cytosine. In a preferred embodiment the oligonucleotide or contiguous nucleotide sequence has at least 1 LNA nucleoside at the 5' end and at least 2 LNA nucleosides at the 3' end of the nucleotide sequence.

In some embodiments, the oligonucleotide of the invention comprises at least one modified nucleoside which is a 2'-MOE-RNA nucleoside, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-MOE-RNA nucleosides. In some embodiments, at least one of said modified nucleoside is 2'-fluoro DNA, such as 2, 3, 4, 5, 6, 7, 8, 9 or 10 2'-fluoro-DNA nucleosides.

In some embodiments, the oligonucleotide of the invention comprises at least one LNA nucleoside and at least one 2' substituted modified nucleoside.

In some embodiments of the invention, the oligonucleotide comprise both 2' sugar modified nucleosides and DNA units. Preferably the oligonucleotide comprises both LNA and DNA

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nucleosides (units). Preferably, the combined total of LNA and DNA units is 8-30, such as 10 – 25, preferably 12-22, such as 12 – 18, even more preferably 11-16. In some embodiments of the invention, the nucleotide sequence of the oligonucleotide, such as the contiguous nucleotide sequence consists of at least one or two LNA nucleosides and the remaining nucleosides are DNA units. In some embodiments the oligonucleotide comprises only LNA nucleosides and naturally occurring nucleosides (such as RNA or DNA, most preferably DNA nucleosides), optionally with modified internucleoside linkages such as phosphorothioate.

In an embodiment of the invention the oligonucleotide of the invention is capable of recruiting RNase H.

The structural design of the oligonucleotide of the invention may be selected from gapmers, gapbreakers, headmers and tailmers.

Gapmer design

In a preferred embodiment the oligonucleotide of the invention has a gapmer design or structure also referred herein merely as "Gapmer". In a gapmer structure the oligonucleotide comprises at least three distinct structural regions a 5'-flank, a gap and a 3'-flank, F-G-F' in '5 -> 3' orientation. In this design, flanking regions F and F' (also termed wing regions) comprise a contiguous stretch of modified nucleosides, which are complementary to the PD-L1 target nucleic acid, while the gap region, G, comprises a contiguous stretch of nucleotides which are capable of recruiting a nuclease, preferably an endonuclease such as RNase, for example RNase H, when the oligonucleotide is in duplex with the target nucleic acid. Nucleosides which are capable of recruiting a nuclease, in particular RNase H, can be selected from the group consisting of DNA, alpha-L-oxy-LNA, 2'-Flouro-ANA and UNA. Regions F and F', flanking the 5' and 3' ends of region G, preferably comprise non-nuclease recruiting nucleosides (nucleosides with a 3' endo structure), more preferably one or more affinity enhancing modified nucleosides. In some embodiments, the 3' flank comprises at least one LNA nucleoside, preferably at least 2 LNA nucleosides. In some embodiments, the 5' flank comprises at least one LNA nucleoside. In some embodiments both the 5' and 3' flanking regions comprise a LNA nucleoside. In some embodiments all the nucleosides in the flanking regions are LNA nucleosides. In other embodiments, the flanking regions may comprise both LNA nucleosides and other nucleosides (mixed flanks), such as DNA nucleosides and/or non-LNA modified nucleosides, such as 2' substituted nucleosides. In this case the gap is defined as a contiguous sequence of at least 5 RNase H recruiting nucleosides (nucleosides with a 2' endo structure, preferably DNA) flanked at the 5' and 3' end by an affinity enhancing modified nucleoside, preferably LNA, such as beta-D-oxy-LNA. Consequently, the nucleosides of the 5' flanking region and the 3' flanking region which are adjacent to the gap region are modified nucleosides, preferably non-nuclease recruiting nucleosides.

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Region F

Region F (5' flank or 5' wing) attached to the '5 end of region G comprises, contains or consists of at least one modified nucleoside such as at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 modified nucleosides. In an embodiment region F comprises or consists of from 1 to 7 modified nucleosides, such as from 2 to 6 modified nucleosides, such as from 2 to 5 modified nucleosides, such as from 2 to 4 modified nucleosides, such as from 1 to 3 modified nucleosides, such as 1, 2, 3 or 4 modified nucleosides. The F region is defined by having at least on modified nucleoside at the 5' end and at the 3' end of the region.

In some embodiments, the modified nucleosides in region F have a 3' endo structure.

In an embodiment, one or more of the modified nucleosides in region F are 2' modified nucleosides. In one embodiment all the nucleosides in Region F are 2' modified nucleosides.

In another embodiment region F comprises DNA and/or RNA in addition to the 2' modified nucleosides. Flanks comprising DNA and/or RNA are characterized by having a 2' modified nucleoside in the 5' end and the 3' end (adjacent to the G region) of the F region. In one embodiment the region F comprise DNA nucleosides, such as from 1 to 3 contiguous DNA nucleosides, such as 1 to 3 or 1 to 2 contiguous DNA nucleosides. The DNA nucleosides in the flanks should preferably not be able to recruit RNase H. In some embodiments the 2' modified nucleosides and DNA and/or RNA nucleosides in the F region alternate with 1 to 3 2' modified nucleosides and 1 to 3 DNA and/or RNA nucleosides. Such flanks can also be termed alternating flanks. The length of the 5' flank (region F) in oligonucleotides with alternating flanks may be 4 to 10 nucleosides, such as 4 to 8, such as 4 to 6 nucleosides, such as 4, 5, 6 or 7 modified nucleosides. In some embodiments only the 5' flank of the oligonucleotide is alternating. Specific examples of region F with alternating nucleosides are

$2'_{1-3}$-N'_{1-4}-$2'_{1-3}$

$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$- N'_{1-2}-$2'_{1-2}$

Where 2' indicates a modified nucleoside and N' is a RNA or DNA. In some embodiments all the modified nucleosides in the alternating flanks are LNA and the N' is DNA.In a further embodiment one or more of the 2' modified nucleosides in region F are 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 the F region comprises both LNA and a 2' substituted modified nucleoside. These are often termed mixed wing or mixed flank oligonucleotides.

In one embodiment of the invention all the modified nucleosides in region F are LNA nucleosides. In a further embodiment all the nucleosides in Region F are LNA nucleosides. In a further embodiment the LNA nucleosides in region F are independently selected from the group

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consisting of oxy-LNA, thio-LNA, amino-LNA, cET, and/or ENA, in either the beta-D or alpha-L configurations or combinations thereof. In a preferred embodiment region F comprise at least 1 beta-D-oxy LNA unit, at the 5' end of the contiguous sequence.

Region G

Region G (gap region) preferably comprise, contain or consist of at least 4, such as at least 5, such as at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15 or at least 16 consecutive nucleosides capable of recruiting the aforementioned nuclease, in particular RNaseH. In a further embodiment region G comprise, contain or consist of from 5 to 12, or from 6 to 10 or from 7 to 9, such as 8 consecutive nucleotide units capable of recruiting aforementioned nuclease.

The nucleoside units in region G, which are capable of recruiting nuclease are in an embodiment selected from the group consisting of DNA, alpha-L-LNA, C4' alkylated DNA (as described in PCT/EP2009/050349 and Vester *et al.*, Bioorg. Med. Chem. Lett. 18 (2008) 2296 – 2300), 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). UNA is unlocked nucleic acid, typically where the bond between C2 and C3 of the ribose has been removed, forming an unlocked "sugar" residue.

In a still further embodiment at least one nucleoside unit in region G is a DNA nucleoside unit, such as from 1 to 18 DNA units, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,12, 13, 14, 15, 16 or 17 DNA units, preferably from 2 to 17 DNA units, such as from 3 to 16 DNA units, such as from 4 to 15 DNA units. such as from 5 to 14 DNA units, such as from 6 to 13 DNA units, such as from 7 to 12 DNA units, such as from 8 to 11 DNA units, more preferably from units 8 to 17 DNA units, or from 9 to 16 DNA units, 10 to 15 DNA units or 11 to 13 DNA units, such as 8, 9, 10, 11, 12, 13, 14, 154, 16, 17 DNA units. In some embodiments, region G consists of 100% DNA units.

In further embodiments the region G may consist of a mixture of DNA and other nucleosides capable of mediating RNase H cleavage. Region G may consist of at least 50% DNA, more preferably 60 %, 70% or 80 % DNA, and even more preferred 90% or 95% DNA.

In a still further embodiment at least one nucleoside unit in region G is an alpha-L-LNA nucleoside unit, such as at least one alpha-L-LNA, such as 2, 3, 4, 5, 6, 7, 8 or 9 alpha-L-LNA. In a further embodiment, region G comprises the least one alpha-L-LNA is alpha-L-oxy-LNA. In a further embodiment region G comprises a combination of DNA and alpha-L-LNA nucleoside units.

In some embodiments, nucleosides in region G have a 2' endo structure.

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In some embodiments region G may comprise a gapbreaker nucleoside, leading to a gapbreaker oligonucleotide, which is capable of recruiting RNase H.

Region F'

Region F' (3' flank or 3' wing) attached to the '3 end of region G comprises, contains or consists of at least one modified nucleoside such as at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 modified nucleosides. In an embodiment region F' comprise or consist of from 1 to 7 modified nucleosides, such as from 2 to 6 modified nucleoside, such as from 2 to 4 modified nucleosides, such as from 1 to 3 modified nucleosides, such as 1, 2, 3 or 4 modified nucleosides. The F' region is defined by having at least on modified nucleoside at the 5' end and at the 3' end of the region.

In some embodiments, the modified nucleosides in region F' have a 3' endo structure.

In an embodiment, one or more of the modified nucleosides in region F' are 2' modified nucleosides. In one embodiment all the nucleosides in Region F' are 2' modified nucleosides.

In an embodiment, one or more of the modified nucleosides in region F' are 2' modified nucleosides.

In one embodiment all the nucleosides in Region F' are 2' modified nucleosides. In another embodiment region F' comprises DNA or RNA in addition to the 2' modified nucleosides. Flanks comprising DNA or RNA are characterized by having a 2' modified nucleoside in the 5' end (adjacent to the G region) and the 3' end of the F' region. In one embodiment the region F' comprises DNA nucleosides, such as from 1 to 4 contiguous DNA nucleosides, such as 1 to 3 or 1 to 2 contiguous DNA nucleosides. The DNA nucleosides in the flanks should preferably not be able to recruit RNase H. In some embodiments the 2' modified nucleosides and DNA and/or RNA nucleosides in the F' region alternate with 1 to 3 2' modified nucleosides and 1 to 3 DNA and/or RNA nucleosides, such flanks can also be termed alternating flanks. The length of the 3' flank (region F') in oligonucleotides with alternating flanks may be 4 to 10 nucleosides, such as 4 to 8, such as 4 to 6 nucleosides, such as 4, 5, 6 or 7 modified nucleosides. In some embodiments only the 3' flank of the oligonucleotide is alternating. Specific examples of region F' with alternating nucleosides are

$2'_{1-2}$-N'_{1-4}-$2'_{1-4}$

$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$- N'_{1-2}-$2'_{1-2}$

Where 2' indicates a modified nucleoside and N' is a RNA or DNA. In some embodiments all the modified nucleosides in the alternating flanks are LNA and the N' is DNA.In a further embodiment modified nucleosides in region F' are 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.

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In some embodiments the F' region comprises both LNA and a 2' substituted modified nucleoside. These are often termed mixed wing or mixed flank oligonucleotides.

In one embodiment of the invention all the modified nucleosides in region F' are LNA nucleosides. In a further embodiment all the nucleosides in Region F' are LNA nucleosides. In a further embodiment the LNA nucleosides in region F' are independently selected from the group consisting of oxy-LNA, thio-LNA, amino-LNA, cET and/or ENA, in either the beta-D or alpha-L configurations or combinations thereof. In a preferred embodiment region F' has at least 2 beta-D-oxy LNA unit, at the 3' end of the contiguous sequence.

Region D' and D"

Region D' and D" can be attached to the 5' end of region F or the 3' end of region F', respectively. Region D' or D" are optional.

Region D' or D" may independently comprise 0 to 5, such as 1 to 5, such as 2 to 4, such as 0, 1, 2, 3, 4 or 5 additional nucleotides, which may be complementary or non-complementary to the target nucleic acid. In this respect the oligonucleotide of the invention, may in some embodiments comprise a contiguous nucleotide sequence capable of modulating the target which is flanked at the 5' and/or 3' end by additional nucleotides. Such additional nucleotides may serve as a nuclease susceptible biocleavable linker (see definition of linkers). In some embodiments the additional 5' and/or 3' end nucleosides are linked with phosphodiester linkages, and may be DNA or RNA. In another embodiment, the additional 5' and/or 3' end nucleosides are modified nucleosides which may for example be included to enhance nuclease stability or for ease of synthesis. In one embodiment, the oligonucleotide of the invention, comprises a region D' and/or D" at the 5' or 3' end of the contiguous nucleotide sequence. In a further embodiment the D' and/or D" region is composed of 1 to 5 phosphodiester linked DNA or RNA nucleosides which are not complementary to the target nucleic acid.

The gapmer oligonucleotide of the present invention can be represented by the following formulae:

5'-F-G-F'-3'; in particular F_{1-7}-G_{4-12}-F'_{1-7}

5'-D'-F-G-F'-3', in particular D'_{1-3}-F_{1-7}-G_{4-12}-F'_{1-7}

5'-F-G-F'-D"-3', in particular F_{1-7}-G_{4-12}-F'_{1-7}-$D"_{1-3}$

5'-D'-F-G-F'-D'-3", in particular D'_{1-3}-F_{1-7}-G_{4-12}-F'_{1-7}-$D"_{1-3}$

The preferred number and types of nucleosides in regions F, G and F', D' and D" have been described above. The oligonucleotide conjugates of the present invention have a region C covalently attached to either the 5' or 3' end of the oligonucleotide, in particular the gapmer oligonucleotides presented above.

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In one embodiment the oligonucleotide conjugate of the invention comprises a oligonucleotide with the formula 5'-D'-F-G-F'-3' or 5'-F-G-F'-D''-3', where region F and F' independently comprise 1 - 7 modified nucleosides, G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH and region D' or D'' comprise 1 - 5 phosphodiester linked nucleosides. Preferably region D' or D'' is present in the end of the oligonucleotide where conjugation to a conjugate moiety is contemplated.

Examples of oligonucleotides with alternating flanks can be represented by the following formulae:

$2'_{1-3}$-N'_{1-4}-$2'_{1-3}$-G_{6-12}-$2'_{1-2}$-N'_{1-4}-$2'_{1-4}$

$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$-G_{6-12}-$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$- N'_{1-2}-$2'_{1-2}$

F-G_{6-12}-$2'_{1-2}$-N'_{1-4}-$2'_{1-4}$

F-G_{6-12}-$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$

$2'_{1-3}$-N'_{1-4}-$2'_{1-3}$-G_{6-12}-F'

$2'_{1-2}$-N'_{1-2}-$2'_{1-2}$-N_{1-2}-$2'_{1-2}$-G_{6-12}-F'

Where a flank is indicated by F or F' it only contains 2' modified nucleosides, such as LNA nucleosides. The preferred number and types of nucleosides in the alternating regions, and region F, G and F', D' and D'' have been described above.

In some embodiments the oligonucleotide is a gapmer consisting of 16, 17, 18, 19, 20, 21, 22 nucleotides in length, wherein each of regions F and F' independently consists of 1, 2, 3 or 4 modified nucleoside units complementary to the PD-L1 target nucleic acid and region G consists of 8, 9, 10, 11,12,13,14,15,16,17 nucleoside units, capable of recruiting nuclease when in duplex with the PD-L1 target nucleic acid and region D' consists of 2 phosphodiester linked DNAs.

In a further embodiments, the oligonucleotide is a gapmer wherein each of regions F and F' independently consists of 3, 4, 5 or 6 modified nucleoside units, such as nucleoside units containing a 2'-O-methoxyethyl-ribose sugar (2'-MOE) or nucleoside units containing a 2'-fluoro-deoxyribose sugar and/or LNA units, and region G consists of 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 nucleoside units, such as DNA units or other nuclease recruiting nucleosides such as alpha-L-LNA or a mixture of DNA and nuclease recruiting nucleosides.

In a further specific embodiment, the oligonucleotide is a gapmer wherein each of regions F and F' region consists of two LNA units each, and region G consists of 12, 13, 14 nucleoside units, preferably DNA units. Specific gapmer designs of this nature include 2-12-2, 2-13-2 and 2-14-2.

In a further specific embodiment, the oligonucleotide is a gapmer wherein each of regions F and F' independently consists of three LNA units, and region G consists of 8, 9, 10, 11, 12, 13 or 14 nucleoside units, preferably DNA units. Specific gapmer designs of this nature include 3-8-3, 3-9-3 3-10-3, 3-11-3, 3-12-3, 3-13-3 and 3-14-3.

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In a further specific embodiment, the oligonucleotide is a gapmer wherein each of regions F and F' consists of four LNA units each, and region G consists of 8 or 9, 10, 11 or 12 nucleoside units, preferably DNA units. Specific gapmer designs of this nature include 4-8-4, 4-9-4, 4-10-4, 4-11-4 and 4-12-4.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 6 nucleosides and independently 1 to 4 modified nucleosides in the wings including 1-6-1, 1-6-2, 2-6-1, 1-6-3, 3-6-1, 1-6-4, 4-6-1, 2-6-2, 2-6-3, 3-6-2 2-6-4, 4-6-2, 3-6-3, 3-6-4 and 4-6-3 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 7 nucleosides and independently 1 to 4 modified nucleosides in the wings including 1-7-1, 2-7-1, 1-7-2, 1-7-3, 3-7-1, 1-7-4, 4-7-1, 2-7-2, 2-7-3, 3-7-2, 2-7-4, 4-7-2, 3-7-3, 3-7-4, 4-7-3 and 4-7-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 8 nucleosides and independently 1 to 4 modified nucleosides in the wings including 1-8-1, 1-8-2, 1-8-3, 3-8-1, 1-8-4, 4-8-1,2-8-1, 2-8-2, 2-8-3, 3-8-2, 2-8-4, , 4-8-2, 3-8-3, 3-8-4, 4-8-3 and 4-8-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 9 nucleosides and independently 1 to 4 modified nucleosides in the wings including, 1-9-1, 2-9-1, 1-9-2, 1-9-3, 3-9-1, 1-9-4, 4-9-1, 2-9-2, 2-9-3, 3-9-2, 2-9-4, 4-9-2, 3-9-3, 3-9-4, 4-9-3 and 4-9-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 10 nucleosides including, 1-10-1, 2-10-1, 1-10-2, 1-10-3, 3-10-1, 1-10-4, 4-10-1, 2-10-2, 2-10-3, 3-10-2, 2-10-4, 4-10-2, 3-10-3, 3-10-4, 4-10-3 and 4-10-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 11 nucleosides including, 1-11-1, 2-11-1, 1-11-2, 1-11-3, 3-11-1, 1-11-4, 4-11-1, 2-11-2, 2-11-3, 3-11-2, 2-11-4, 4-11-2, 3-11-3, 3-11-4, 4-11-3 and 4-11-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 12 nucleosides including, 1-12-1, 2-12-1, 1-12-2, 1-12-3, 3-12-1, 1-12-4, 4-12-1, 2-12-2, 2-12-3, 3-12-2, 2-12-4, 4-12-2, 3-12-3, 3-12-4, 4-12-3 and 4-12-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 13 nucleosides including, 1-13-1, 2-13-1, 1-13-2, 1-13-3, 3-13-1, 1-13-4, 4-13-1, 2-13-2, 2-13-3, 3-13-2, 2-13-4, 4-13-2, 3-13-3, 3-13-4, 4-13-3 and 4-13-4 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 14 nucleosides including, 1-14-1, 2-14-1, 1-14-2, 1-14-3, 3-14-1, 1-14-4, 4-14-1, 2-14-2, 2-14-3, 3-14-2, 2-14-4, 4-14-2, 3-14-3, 3-14-4, 4-14-3 and 4-14-4 gapmers.

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Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 15 nucleosides including, 1-15-1, 2-15-1, 1-15-2, 1-15-3, 3-15-1, 1-15-4, 4-15-1, 2-15-2, 2-15-3, 3-15-2, 2-15-4, 4-15-2 and 3-15-3 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 16 nucleosides including, 1-16-1, 2-16-1, 1-16-2, 1-16-3, 3-16-1, 1-16-4, 4-16-1, 2-16-2, 2-16-3, 3-16-2, 2-16-4, 4-16-2 and 3-16-3 gapmers.

Specific gapmer designs of this nature include F-G-F' designs selected from a group consisting of a gap with 17 nucleosides including, 1-17-1, 2-17-1, 1-17-2, 1-17-3, 3-17-1, 1-17-4, 4-17-1, 2-17-2, 2-17-3 and 3-17-2 gapmers.

In all instances the F-G-F' design may further include region D' and/or D", which may have 1, 2 or 3 nucleoside units, such as DNA units, such as 2 phosphodiester linked DNA units. Preferably, the nucleosides in region F and F' are modified nucleosides, while nucleotides in region G are preferably unmodified nucleosides.

In each design, the preferred modified nucleoside is LNA.

In another embodiment all the internucleoside linkages in the gap in a gapmer are phosphorothioate and/or boranophosphate linkages. In another embodiment all the internucleoside linkages in the flanks (F and F' region) in a gapmer are phosphorothioate and/or boranophosphate linkages. In another preferred embodiment all the internucleoside linkages in the D' and D" region in a gapmer are phosphodiester linkages.

For specific gapmers as disclosed herein, when the cytosine (C) residues are annotated as 5-methyl-cytosine, in various embodiments, one or more of the Cs present in the oligonucleotide may be unmodified C residues.

In a particular embodiment, the gapmer is a so-called shortmer as described in WO2008/113832.

Further gapmer designs are disclosed in WO2004/046160 and WO2007/146511.

For certain embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO: 5_1 to 743_1 and 771_1.

For certain embodiments of the invention, the oligonucleotide is selected from the group of oligonucleotide compounds with CMP-ID-NO 6_1, 8_1, 9_1, 13_1, 41_1, 42_1, 58_1, 77_1, 92_1, 111_1, 128_1, 151_1, 164_1, 166_1, 169_1, 171_1, 222_1, 233_1, 245_1, 246_1, 250_1, 251_1, 252_1, 256_1, 272_1, 273_1, 287_1, 292_1, 303_1, 314_1, 318_1, 320_1, 324_1, 336_1, 342_1, 343_1, 344_1, 345_1, 346_1, 349_1, 359_1, 360_1, 374_1, 408_1, 409_1, 415_1, 417_1, 424_1, 429_1, 430_1, 458_1, 464_1, 466_1, 474_1, 490_1, 493_1,

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512_1, 519_1, 519_1, 529_1, 533_1, 534_1, 547_1, 566_1, 567_1, 578_1, 582_1, 601_1, 619_1, 620_1, 636_1, 637_1, 638_1, 640_1, 645_1, 650_1, 651_1, 652_1, 653_1, 658_1, 659_1, 660_1, 665_1, 678_1, 679_1, 680_1, 682_1, 683_1, 684_1, 687_1, 694_1, 706_1, 716_1, 728_1, 733_1, 734_1, and 735_1.

In one preferred embodiment of the invention, the oligonucleotide is CMP-ID-NO: 287_1.

In another preferred embodiment of the invention, the oligonucleotide is CMP-ID-NO: 342_1.

In another preferred embodiment of the invention, the oligonucleotide is CMP-ID-NO: 640_1.

In another preferred embodiment of the invention, the oligonucleotide is CMP-ID-NO: 466_1.

In another preferred embodiment of the invention, the oligonucleotide is CMP-ID-NO: 566_1.

In a further embodiment of the invention the contiguous nucleotide sequence of the oligonucleotide motifs and oligonucleotide compounds of the invention comprise two to four additional phosphodiester linked nucleosides at the 5' end of the contiguous nucleotide sequence (e.g. region D'). In one embodiment the nucleosides serve as a biocleavable linker (see sectionon biocleavable linkers). In a preferred embodiment a ca (cytidine-adenosine) dinucleotide is linked to the 5' end of contiguous nucleotide sequence (i.e. any one of the motif sequences or oligonucleotide compounds listed in table 5) via a phosphodiester linkage. In a preferred emboduiment the ca di nucleotide is not complementary to the target sequence at the position where the reminder of the contigious nucleotide is complementary.

In some embodiments of the invention the oligonucleotide or contiguous nucleotide sequence is selected from the group consisting of the nucleotide motif sequences with SEQ ID NO: 766, 767, 768, 769 and 770.

In some embodiments of the invention the oligonucleotide is selected from the group consisting of the oligonucleotide compounds with CMP-ID-NO 766_1, 767_1, 768_1, 769_1 and 770_1.

Carbohydrate conjugate moieties

Carbohydrate conjugate moieties include but are not limited to galactose, lactose, n-acetylgalactosamine, mannose and mannose-6-phosphate. Carbohydrate conjugates may be used to enhance delivery or activity in a range of tissues, such as liver and/or muscle. See, for example, EP1495769, WO99/65925, Yang et al., Bioconjug Chem (2009) 20(2): 213-21. Zatsepin & Oretskaya Chem Biodivers. (2004) 1(10): 1401-17.

In some embodiments the carbohydrate conjugate moiety is multivalent, such as, for example 2, 3 or 4 identical or non-identical carbohydrate moieties may be covalently joined to the oligonucleotide, optionally via a linker or linkers. In some embodiments the invention provides a conjugate comprising the oligonucleotide of the invention and a carbohydrate conjugate moiety.

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In some embodiments, the conjugate moiety is or may comprise mannose or mannose-6-phosphate. This is particular useful for targeting muscle cells, see for example US 2012/122801.

Conjugate moieties capable of binding to the asialoglycoprotein receptor (ASGPr) are particular useful for targeting hepatocytes in liver. In some embodiments the invention provides a oligonucleotide conjugate comprising the oligonucleotide of the invention and an asialoglycoprotein receptor targeting conjugate moiety. The asialoglycoprotein receptor targeting conjugate moiety comprises one or more carbohydrate moieties capable of binding to the asialoglycoprotein receptor (ASPGr binding carbohydrate moieties) with affinity equal to or greater than that of galactose. The affinities of numerous galactose derivatives for the asialoglycoprotein receptor have been studied (see for example: Jobst, S.T. and Drickamer, K. JB.C. 1996, 271, 6686) or are readily determined using methods typical in the art.

One aspect of the present invention is an antisense oligonucleotide conjugate comprising a) an oligonucleotide (Region A) comprising a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementarity to a PD-L1 target nucleic acid; and b) at least one asialoglycoprotein receptor targeting conjugate moiety (Region C) covalently attached to the oligonucleotide in a). The oligonucleotide or a contiguous nucleotide sequence can be as described in any of the sections “oligonucleotides of the invention”, “oligonucleotide design and “gapmer design”.

In some embodiments asialoglycoprotein receptor targeting conjugate moiety comprises at least one ASPGr binding carbohydrate moiety selected from the group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine and N-isobutanoylgalactosamine. In some embodiments, the asialoglycoprotein receptor targeting conjugate moiety is mono-valent, di-valent, tri-valent or tetra-valent (i.e. containing 1, 2, 3 or 4 terminal carbohydrate moieties capable of binding to the asialoglycoprotein receptor). Preferably, the asialoglycoprotein receptor targeting conjugate moiety is di-valent, even more preferred it is trivalent. In a preferred embodiment the asialoglycoprotein receptor targeting conjugate moiety comprises 1 to 3 N-acetylgalactosamine (GalNAc) moieties (also termed a GalNAc conjugate). In some embodiments the oligonucleotide conjugate comprises a asialoglycoprotein receptor targeting conjugate moiety that is a tri-valent N-acetylgalactosamine (GalNAc) moiety. GalNAc conjugates have been used with phosphodiester, methylphosphonate and PNA antisense oligonucleotides (e.g. US 5,994517 and Hangeland *et al.*, Bioconjug Chem. 1995 Nov-Dec;6(6):695-701, Biessen et al 1999 Biochem J. 340, 783-792 and Maier et al 2003 Bioconjug Chem 14, 18-29) and siRNAs (e.g. WO 2009/126933, WO 2012/089352 & WO 2012/083046) and with LNA and 2'-MOE modified nucleosides WO 2014/076196 WO 2014/207232 and WO 2014/179620.

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To generate the asialoglycoprotein receptor targeting conjugate moiety the ASPGr binding carbohydrate moieties (preferably GalNAc) are attached to a brancher molecule through the C-I carbons of the saccharides. The ASPGr binding carbohydrate moieties are preferably linked to the brancher molecule via spacers. A preferred spacer is a flexible hydrophilic spacer (U.S. Patent 5885968; Biessen et al. J. Med. Chern. 1995 Vol. 39 p. 1538-1546). A preferred flexible hydrophilic spacer is a PEG spacer. A preferred PEG spacer is a PEG3 spacer (three ethylene units). The brancher molecule can be any small molecule which permits attachment of two or three terminal ASPGr binding carbohydrate moieties and further permits attachment of the branch point to the oligonucleotide. An exemplary brancher molecule is a di-lysine. A di-lysine molecule contains three amine groups through which three ASPGr binding carbohydrate moieties may be attached and a carboxyl reactive group through which the di-lysine may be attached to the oligonucleotide. Alternative brancher molecules may be a doubler or trebler such as those supplied by Glen Research. In some embodiments the brancher may be selected from the from the group consisting of 1,3-bis-[5-(4,4'-dimethoxytrityloxy)pentylamido]propyl-2-[(2-cyanoethyl)-(N,N-diisopropyl)] phosphoramidite (Glen Research Catalogue Number: 10-1920-xx), tris-2,2,2-[3-(4,4'-dimethoxytrityloxy)propyloxymethyl]ethyl-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research Catalogue Number: 10-1922-xx), tris-2,2,2-[3-(4,4'-dimethoxytrityloxy)propyloxymethyl]methyleneoxypropyl-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite and 1-[5-(4,4'-dimethoxy-trityloxy)pentylamido]-3-[5-fluorenomethoxy-carbonyl-oxy-pentylamido]-propyl-2-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (Glen Research Catalogue Number: 10-1925-xx). WO 2014/179620 and PCT application No. PCT/EP2015/073331 describes the generation of various GalNAc conjugate moieties. One or more linkers may be inserted between the brancher molecule and the oligonucleotide. In a preferred embodiment the linker is a biocleavable linker. The linker may be selected from the linkers described in the section “Linkers” and its subsections.

The asialoglycoprotein receptor targeting conjugate moiety, in particular the GalNAc conjugate moiety, may be attached to the 3'- or 5'-end of the oligonucleotide using methods known in the art. In preferred embodiments the asialoglycoprotein receptor targeting conjugate moiety is linked to the 5’-end of the oligonucleotide.

Pharmacokinetic modulators in relation to siRNAs delivery has been described in WO 2012/083046. In some embodiments the carbohydrate conjugate moiety comprises a pharmacokinetic modulator selected from the group consisting of a hydrophobic group having 16 or more carbon atoms, hydrophobic group having 16-20 carbon atoms, palmitoyl, hexadec-8-enoyl, oleyl, (9E,12E)-octadeca-9,12dienoyl, dioctanoyl, and C16-C20 acyl, and cholesterol. In a

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preferred embodiment the pharmacokinetic modulator containing carbohydrate conjugate moiety is a GalNAc conjugate.

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Preferred carbohydrate conjugate moieties comprises one to three terminal ASPGr binding carbohydrate moieties, preferably N-acetylgalactosamine moiety(s). In some embodiments the carbohydrate conjugate moiety comprises three ASPGr binding carbohydrate moieties, preferably N-acetylgalactosamine moieties, linked via a spacer to a brancher molecule. The spacer molecule can be between 8 and 30 atoms long. A preferred carbohydrate conjugate moiety comprises three terminal GalNAc moieties linked via a PEG spacer to a di-lysine brancher molecule. Preferably the PEG spacer is a 3PEG spacer. Suitable asialoglycoprotein receptor targeting conjugate moieties are shown in Figure 1. A preferred asialoglycoprotein receptor targeting conjugate moiety is shown in figure 3.

Other GalNAc conjugate moieties can include, for example, small peptides with GalNAc moieties attached such as Tyr-Glu-Glu-(aminohexyl GalNAc)3 (YEE(ahGalNAc)3; a glycotripeptide that binds to asialoglycoprotein receptor on hepatocytes, see, e.g., Duff, et al., Methods Enzymol, 2000, 313, 297); lysine-based galactose clusters (e.g., L3G4; Biessen, et al., Cardovasc. Med., 1999, 214); and cholane-based galactose clusters (e.g., carbohydrate recognition motif for asialoglycoprotein receptor).

In some embodiments of the invention the antisense oligonucleotide conjugate is selected from the group consisting of the following CPM ID NO: 766_2, 767_2, 768_2, 769_2 and 770_2.

In a preferred embodiment the antisense oligonucleotide conjugate corresponds to the compound represented in figure 4.

In another preferred embodiment the antisense oligonucleotide conjugate corresponds to the compound represented in figure 5.

In another preferred embodiment the antisense oligonucleotide conjugate corresponds to the compound represented in figure 6.

In another preferred embodiment the antisense oligonucleotide conjugate corresponds to the compound represented in figure 7.

In another preferred embodiment the antisense oligonucleotide conjugate corresponds to the compound represented in figure 8.

Linkers

Biocleavable linkers (Region B)

The use of a conjugate is often associated with enhanced pharmacokinetic or pharmeodynamic dynamic properties. However, the presence of a conjugate moiety may interfere with the activity of the oligonucleotide against its intended target, for example via steric hindrance preventing hybridization or nuclease recruitment (e.g. RNAseH). The use of a physiologically labile bond (biocleavable linker) between the oligonucleotide (region A or first region) and the conjugate moiety (region C or third region), allows for the improved properties due to the presence of the

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conjugate moiety, whilst ensuring that once at the target tissue, the conjugate group does not prevent effective activity of the oligonucleotide.

Cleavage of the physiologically labile bond occurs spontaneously when a molecule containing the labile bond reaches an appropriate intra-and/or extra-cellular environment. For example, a pH labile bond may be cleaved when the molecule enters an acidified endosome. Thus, a pH labile bond may be considered to be an endosomal cleavable bond. Enzyme cleavable bonds may be cleaved when exposed to enzymes such as those present in an endosome or lysosome or in the cytoplasm. A disulfide bond may be cleaved when the molecule enters the more reducing environment of the cell cytoplasm. Thus, a disulfide may be considered to be a cytoplasmic cleavable bond. As used herein, a pH-labile bond is a labile bond that is selectively broken under acidic conditions (pH<7). Such bonds may also be termed endosomally labile bonds, since cell endosomes and lysosomes have a pH less than 7.

For biocleavable linkers associated with a conjugate moiety for targeted delivery it is preferred that, the cleavage rate seen in the target tissue (for example muscle, liver, kidney or a tumor) is greater than that found in blood serum. Suitable methods for determining the level (%) of cleavage in target tissue versus serum or cleavage by S1 nuclease are described in the "Materials and methods" section. In some embodiments, the biocleavable linker (also referred to as the physiologically labile linker, or nuclease susceptible linker or region B), in a conjugate of the invention, is at least about 20% cleaved, such as at least about 30% cleaved, such as at least about 40% cleaved, such as at least about 50% cleaved, such as at least about 60% cleaved, such as at least about 70% cleaved, such as at least about 75% cleaved when compared against a standard.

In some embodiments, the oligonucleotide conjugate of the invention comprises three regions: i) a first region (region A), which comprises 10 – 25 contiguous nucleotides complementary to the target nucleic acid; ii) a second region (region B) which comprises a biocleavable linker and iii) a third region (region C) which comprises a conjugate moiety, such as an asialoglycoprotein receptor targeting conjugate moiety, wherein the third region is covalent linked to the second region which is covalently linked to the first region.

In one embodiment of the present invention the oligonucleotide conjugate comprises a biocleavable linker (Region B) between the contiguous nucleotide sequence (region A) and the asialoglycoprotein receptor targeting conjugate moiety (region C).

In some embodiments, the biocleavable linker may be situated either at the 5' end and/or the 3'-end of the contiguous nucleotides complementary to the target nucleic acid (region A). In a preferred embodiment the biocleavable linker is at the 5'-end.

In some embodiments, the cleavable linker is susceptible to nuclease(s) which may for example, be expressed in the target cell. In some embodiments the biocleavable linker is

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composed of 2 to 5 consecutive phosphodiester linkages. The linker may be a short region (e.g. 1 – 10 as detailed in the definition of linkers) phosphodiester linked nucleosides. In some embodiments, the nucleosides in the biocleavable linker region B is (optionally independently) selected from the group consisting of DNA and RNA or modifications thereof which do not interfere with nuclease cleavage. Modifications of DNA and RNA nucleosides which do not interfere with nuclease cleavage may be non-naturally occurring nucleobases. Certain sugar-modified nucleosides may also allow nuclease cleavage such as an alpha-L-oxy-LNA. In some embodiments, all the nucleosides of region B comprise (optionally independently) either a 2'-OH ribose sugar (RNA) or a 2'-H sugar - i.e. RNA or DNA. In a preferred embodiment, at least two consecutive nucleosides of region B are DNA or RNA nucleosides (such as at least 3 or 4 or 5 consecutive DNA or RNA nucleosides). In an even more preferred embodiment, the nucleosides of region B are DNA nucleosides Preferably region B consists of between 1 to 5, or 1 to 4, such as 2, 3, 4 consecutive phosphodiester linked DNA nucleosides. In preferred embodiments region B is so short that it does not recruit RNAseH. In some embodiments, region B comprises no more than 3 or no more than 4 consecutive phospodiester linked DNA and/or RNA nucleosides (such as DNA nucleosides).

Where region B is composed of phosphodiester linked nucleosides, region A and B may together form the oligonucleotide that is linked to region C. In this context region A can be differentiated from region B in that Region A starts with at least one, preferably at least two, modified nucleosides with increased binding affinity to the target nucleic acid (e.g. LNA or nucleosides with a 2' substituted sugar moiety) and region A on its own is capable of modulation of the expression the target nucleic acid in a relevant cell line. Furthermore, if region A comprises DNA or RNA nucleosides these are linked with nuclease resistant internucleoside linkage, such phosphorothioate or boranophosphate. Region B on the other hand comprises phophodiester linkages between DNA or RNA nucleosides. In some embodiments region B is not complementary to or comprises at least 50% mismatches to the target nucleic acid.

In some embodiments, region B is not complementary to the target nucleic acid sequence or to the contiguous nucleotides complementary to the target nucleic acid in region A.

In some embodiments, region B is complementary with the target nucleic acid sequence. In this respect region A and B together may form a single contiguous sequence which is complementary to the target sequence.

In some aspects of the invention the internucleoside linkage between the first (region A) and the second region (region B) may be considered part of the second region.

In some embodiments, the sequence of bases in region B is selected to provide an optimal endonuclease cleavage site, based upon the predominant endonuclease cleavage enzymes present in the target tissue or cell or sub-cellular compartment. In this respect, by isolating cell

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extracts from target tissues and non-target tissues, endonuclease cleavage sequences for use in region B may be selected based upon a preferential cleavage activity in the desired target cell (e.g. liver/hepatocytes) as compared to a non-target cell (e.g. kidney). In this respect, the potency of the compound for target down-regulation may be optimized for the desired tissue/cell.

In some embodiments region B comprises a dinucleotide of sequence AA, AT, AC, AG, TA, TT, TC, TG, CA, CT, CC, CG, GA, GT, GC, or GG, wherein C may be 5-methylcytosine, and/or T may be replaced with U. Preferably, the internucleoside linkage is a phosphodiester linkage. In some embodiments region B comprises a trinucleotide of sequence AAA, AAT, AAC, AAG, ATA, ATT, ATC, ATG, ACA, ACT, ACC, ACG, AGA, AGT, AGC, AGG, TAA, TAT, TAC, TAG, TTA, TTT, TTC, TAG, TCA, TCT, TCC, TCG, TGA, TGT, TGC, TGG, CAA, CAT, CAC, CAG, CTA, CTG, CTC, CTT, CCA, CCT, CCC, CCG, CGA, CGT, CGC, CGG, GAA, GAT, GAC, CAG, GTA, GTT, GTC, GTG, GCA, GCT, GCC, GCG, GGA, GGT, GGC, and GGG wherein C may be 5-methylcytosine and/or T may be replaced with U. Preferably, the internucleoside linkages are phosphodiester linkages. In some embodiments region B comprises a trinucleotide of sequence AAAX, AATX, AACX, AAGX, ATAX, ATTX, ATCX, ATGX, ACAX, ACTX, ACCX, ACGX, AGAX, AGTX, AGCX, AGGX, TAAX, TATX, TACX, TAGX, TTAX, TTTX, TTCX, TAGX, TCAX, TCTX, TCCX, TCGX, TGAX, TGTX, TGCX, TGGX, CAAX, CATX, CACX, CAGX, CTAX, CTGX, CTCX, CTTX, CCAX, CCTX, CCCX, CCGX, CGAX, CGTX, CGCX, CGGX, GAAX, GATX, GACX, CAGX, GTAX, GTTX, GTCX, GTGX, GCAX, GCTX, GCCX, GCGX, GGAX, GGTX, GGCX, and GGGX, wherein X may be selected from the group consisting of A, T, U, G, C and analogues thereof, wherein C may be 5-methylcytosine and/or T may be replaced with U. Preferably, the internucleoside linkages are phosphodiester linkages. It will be recognized that when referring to (naturally occurring) nucleobases A, T, U, G, C, these may be substituted with nucleobase analogues which function as the equivalent natural nucleobase (e.g. base pair with the complementary nucleoside).

Other linkers (Region Y)

The linker can have at least two functionalities, one for attaching to the oligonucleotide and the other for attaching to the conjugate moiety. Example linker functionalities can be electrophilic for reacting with nucleophilic groups on the oligonucleotide or conjugate moiety, or nucleophilic for reacting with electrophilic groups. In some embodiments, linker functionalities include amino, hydroxyl, carboxylic acid, thiol, phosphoramidate, phosphorothioate, phosphate, phosphite, unsaturations (e.g., double or triple bonds), and the like. Some example linkers (region Y) include 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl)cyclohexane-l-carboxylate (SMCC), 6- aminohexanoic acid (AHEX or AHA), 6-aminohexyloxy, 4-aminobutyric acid, 4- aminocyclohexylcarboxylic acid, succinimidyl 4-(N-maleimidomethyl)cyclohexane- l-carboxy-(6-amido-caproate) (LCSMCC), succinimidyl m-

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maleimido-benzoylate (MBS), succinimidyl N-e-maleimido-caproylate (EMCS), succinimidyl 6-(beta - maleimido-propionamido) hexanoate (SMPH), succinimidyl N-(a-maleimido acetate) (AMAS), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), beta -alanine (beta -ALA), phenylglycine (PHG), 4-aminocyclohexanoic acid (ACHC), beta -(cyclopropyl) alanine (beta -CYPR), amino dodecanoic acid (ADC), alylene diols, polyethylene glycols, amino acids, and the like. 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. The amino alkyl group may be added to the oligonucleotide (region A or region A-B) as part of standard oligonucleotide synthesis, for example using a (e.g. protected) amino alkyl phosphoramidite. The linkage group between the amino alkyl and the oligonucleotide may for example be a phosphorothioate or a phosphodiester, or one of the other nucleoside linkage groups referred to herein. The amino alkyl group is covalently linked to the 5' or 3'-end of the oligonucleotide. Commercially available amino alkyl linkers are for example 3'-Amino-Modifier reagent for linkage at the 3'-end of the oligonucleotide and for linkage at the 5 '-end of an oligonucleotide 5'- Amino-Modifier C6 is available. These reagents are available from Glen Research Corporation (Sterling, Va.). These compounds or similar ones were utilized by Krieg, et al, Antisense Research and Development 1991, 1, 161 to link fluorescein to the 5'- terminus of an oligonucleotide. A wide variety of further linker groups are known in the art and can be useful in the attachment of conjugate moieties to oligonucleotides. A review of many of the useful linker groups can be found in, for example, Antisense Research and Applications, S. T. Crooke and B. Lebleu, Eds., CRC Press, Boca Raton, Fla., 1993, p. 303-350. Other compounds such as acridine have been attached to the 3 '-terminal phosphate group of an oligonucleotide via a polymethylene linkage (Asseline, et al., Proc. Natl. Acad. Sci. USA 1984, 81, 3297). Any of the above groups can be used as a single linker (region Y) or in combination with one or more further linkers (region Y-Y'or region Y-B or B-Y).

Linkers and their use in preparation of conjugates of oligonucleotides are provided throughout the art such as in WO 96/11205 and WO 98/52614 and U.S. Pat. Nos. 4,948,882; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,580,731; 5,486,603; 5,608,046; 4,587,044; 4,667,025; 5,254,469; 5,245,022; 5,112,963; 5,391,723; 5,510475; 5,512,667; 5,574,142; 5,684,142; 5,770,716; 6,096,875; 6,335,432; and 6,335,437,WO 2012/083046.

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

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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). 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 Composition

In a further aspect, the invention provides pharmaceutical compositions comprising any of the aforementioned oligonucleotides and/or oligonucleotide conjugates and a pharmaceutically acceptable diluent, solvent, 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.

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. 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

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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.

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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 or oligonucleotide conjugates of the present invention may be utilized as research reagents for, for example, diagnostics, therapeutics and prophylaxis.

In research, such oligonucleotides or oligonucleotide conjugates may be used to specifically modulate the synthesis of PD-L1 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 PD-L1 expression in a target cell which is expressing PD-L1, said method comprising administering an oligonucleotide or oligonucleotide conjugate of the invention in an effective amount to said cell.

In some embodiments, the target cell, is a mammalian cell in particular a human 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 preferred embodiments the target cell is present in the liver. Liver target cell can be selected from parenchymal cells (e.g. hepatocytes) and non-parenchymal cells such as Kupffer cells, LSECs, stellate cells (or Ito cells), cholangiocytes and liver-associated leukocytes (including T cells and NK cells). In some embodiments the target cell is an antigen-presenting cell. Antigen-presenting cells displays foreign antigens complexed with major histocompatibility complex (MHC) class I or class II on their surfaces. In some embodiments the antigen-presenting cell expresses MHC class II (i.e. professional antigen-presenting cells such as dendritic cells, macrophages and B cells).

In diagnostics the oligonucleotides may be used to detect and quantitate PD-L1 expression in cell and tissues by northern blotting, *in-situ* hybridisation or similar techniques.

For therapeutics oligonucleotides or oligonucleotide conjugates of the present invention or pharmaceutical compositions thereof may be administered to an animal or a human, suspected of having a disease or disorder, which can be alleviated or treated by reduction of the expression of PD-L1, in particular by reduction of the expression of PD-L1 in liver target cells.

The invention provides methods for treating or preventing a disease, comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide, an oligonucleotide

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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, oligonucleotide conjugate or a pharmaceutical composition according to the invention 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 of the invention as described for the manufacture of a medicament for the treatment of a disease or disorder as referred to herein. In one embodiment the disease is selected from a) viral liver infections such as HBV, HCV and HDV; b) parasite infections such as malaria, toxoplasmosis, leishmaniasis and trypanosomiasis and c) liver cancer or metastases in the liver.

In one embodiment, the invention relates to oligonucleotides, oligonucleotide conjugates or pharmaceutical compositions for use in the treatment of diseases or disorders selected from viral or parasitic infections. In a further embodiment the disease is selected from a) viral liver infections such as HBV, HCV and HDV; b) parasite infections such as malaria, toxoplasmosis, leishmaniasis and trypanosomiasis and c) liver cancer or metastases in the liver.

The disease or disorder, as referred to herein, is associated with immune exhaustion. In particular the disease or disorder is associated with exhaustion of virus-specific T-cell responses. In some embodiments disease or disorder may be alleviated or treated by reduction of PD-L1 expression.

The methods of the invention are preferably employed for treatment or prophylaxis against diseases associated with immune exhaustion.

In one embodiment of the invention the oligonucleotide, oligonucleotide conjugate or pharmaceutical compositions of the invention are used in restoration of immune response against a liver cancer or metastases in the liver.

In one embodiment of the invention the oligonucleotide, oligonucleotide conjugate or pharmaceutical compositions of the invention are used in restoration of immune response against a pathogen. In some embodiments the pathogen can be found in the liver. The pathogens can be a virus or a parasite, in particular those described herein. In a preferred embodiment the pathogen is HBV.

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 restoration of immunity against a viral or parasite infection as mentioned herein.

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Oligonucleotides or oligonucleotide conjugates or pharmaceutical compositions of the present invention can be used in the treatment of viral infections, in particular viral infections in the liver where the PD-1 patheway is affected (see for example Kapoor and Kottilil 2014 Future Virol Vol. 9 pp. 565-585 and Salem and El-Badawy 2015 World J Hepatol Vol. 7 pp. 2449-2458). Viral liver infections can be selected from the group consisting of hepatitis viruses, in particular HBV, HCV and HDV, in particular chronic forms of these infections. In one embodiment the oligonucleotides or oligonucleotide conjugates or pharmaceutical compositions of the present invention are used to treat HBV, in particular chronic HBV. Indicators of chronic HBV infections are high levels of viral load (HBV DNA) and even higher levels of empty HBsAg particles (>100-fold in excess of virions) in the circulation.

Oligonucleotides or oligonucleotide conjugates of the present invention can also be used to treat viral liver infections that occur as co-infections with HIV. Other viral infections which can be treated with the oligonucleotides or oligonucleotide conjugates or pharmaceutical compositions of the present invention are lcmv (Lymphocytic Choriomeningitis Virus), and HIV as a mono infection, HSV-1 and -2, and other herpesviruses. These viruses are not hepatotrophic, however they may be sensitive to PDL1 down regulation.

In some embodiments the restoration of immunity or immune response involves improvement of the T-cell and/or NK cell response and/or alleviation of the T-cell exhaustion, in particular the HBV-specific T-cell response, the HCV-specific T-cell response and or the HDV-specific T-cell response is restored. An improvement of the T cell response can for example be assessed as an increase in T cells in the liver, in particular an increase in CD8+ and/or CD4+ T cells when compared to a control (e.g. the level prior to treatment or the level in a vehicle treated subject) In a further embodiment it is the virus specific CD8+ T cells that are restored or increased when compared to cotrol), in particular HBV specific CD8+ T cells or HCV specific CD8+ T cells or HDV specific CD8+ T cells are restored or increased when compared to control. In a preferred embodiment CD8+ T cells specific for HBV s antigen (HBsAg) and/or CD8+ T cells specific for HBV e antigen (HBeAg) and/or CD8+ T cells specific for HBV core antigen (HBcAg) are increased in subjects treated with an oligonucleotide, oligonucleotide conjugate or pharmaceutical composition of the present invention compared to control. Preferably the HBV antigen specific CD8+ T cells produce one or more cytokines, such as interferon-gamma (IFN-γ) or tumor necrosis factor alpha (TNF-α). The increase in CD8+ T cells described above is in particular observed in the liver. The increase described herein should be statistically significant when compared to a control. Preferably the increase is at least 20%, such as 25%, such as 50% such as 75% when compared to control. In another embodiment natural killer (NK) cells and/or natural killer T (NKT) cells are activated by the oligonucleotides or oligonucleotide conjugates of the present invention.

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Oligonucleotides or oligonucleotide conjugates or pharmaceutical compositions of the present invention can be used in the treatment parasite infections, in particular parasite infections where the PD-1 pathway is affected (see for example Bhadra et al. 2012 J Infect Dis vol 206 pp. 125-134; Bhadra et al. 2011 Proc Natl Acad Sci U S A Vol. 108 pp. 9196-9201; Esch et al. J Immunol vol 191 pp 5542-5550; Freeman and Sharpe 2012 Nat Immunol Vol 13 pp. 113-115; Gutierrez et al. 2011 Infect Immun Vol 79 pp. 1873-1881; Joshi et al. 2009 PLoS Pathog Vol 5 e1000431; Liang et al. 2006 Eur J Immunol Vol. 36 pp 58-64; Wykes et al. 2014 Front Microbiol Vol 5 pp 249). Parasite infections can be selected from the group consisting of malaria, toxoplasmosis, leishmaniasis and trypanosomiasis. Malaria infection is caused by protozoa of the genus *Plasmodium,* in particular of the species *P. vivax, P. malariae* and *P. falciparum.* Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii.* Leishmaniasis is a disease caused by protozoan parasites of the genus *Leishmania.* Trypanosomiasis is caused by the protozoan of the genus *Trypanosoma.* Chaga disease which is the tropical form caused by the species *Trypanosoma cruzi*, and sleeping disease is caused by the species *Trypanosoma brucei.*

In some embodiments the restoration of immunity involves restoration of a parasite-specific T cell and NK cell response, in particular a *Plasmodium*-specific T-cell response, a *Toxoplasma gondii*-specific T-cell and NK cell response, a *Leishmania*-specific T-cell and NK cell response, a *Trypanosoma cruzi*-specific T-cell and NK cell response or *a Trypanosoma brucei*-specific T-cell and NK cell response. In a further embodiment it is the parasite-specific CD8+ T cell and NK cell response that is restored.

Administration

The oligonucleotides or pharmaceutical compositions of the present invention may be administered topical (such as, to the skin, inhalation, ophthalmic or otic) or enteral (such as, orally or through the gastrointestinal tract) 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 0.1 – 10 mg/kg, such as from 0.2 – 10 mg/kg, such as from 0.25 – 10 mg/kg, such as from 0.1 – 5

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mg/kg, such as from 0.2 – 5 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.

For the treatment of chronic HBV infections a combination of antiviral drugs and immune system modulators is recommended as standard of care. The antiviral drugs effective against HBV are for example nucleos(t)ide analogs. There are five nucleos(t)ide analogs licensed for therapy of HBV namely lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telbivudine (Tyzeka), entecavir (Baraclude) these are effective in suppressing viral replication (HBV DNA) but have no effect on HBsAg levels. Other antiviral drugs include ribavirin and an HBV antibody therapy (monoclonal or polyclonal). The immune system modulators can for example be interferon alpha-2a and PEGylated interferon alpha-2a (Pegasys) or TLR7 agonists (e.g. GS-9620) or therapeutic vaccines. IFN-α treatment show only very modest effect in reducing viral load, but result in some HBsAg decline, albeit very inefficiently (<10% after 48 week therapy).

The oligonucleotide or oligonucleotide conjugates of the present invention may also be combined with other antiviral drugs effective against HBV such as the antisense oligonucleotides described in WO2012/145697 and WO 2014/179629 or the siRNA molecules described in WO 2005/014806, WO 2012/024170, WO 2012/2055362, WO 2013/003520 and WO 2013/159109.

When the oligonucleotides or oligonucleotide conjugates of this invention are administered in combination therapies with other agents, they may be administered sequentially or concurrently to an individual. Alternatively, pharmaceutical compositions according to the present invention may be comprised of a combination of an oligonucleotide or oligonucleotide conjugate of the present invention in association with a pharmaceutically acceptable excipient, as described herein, and another therapeutic or prophylactic agent known in the art.

EMBODIMENTS

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

1. An antisense oligonucleotide which comprises or consists of a contiguous nucleotide sequence of 10 to 30 nucleotides in length capable of reducing the expression of PD-L1.

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2. The oligonucleotide of embodiment 1, wherein the contiguous nucleotide sequence is at least 90% complementarity to a PD-L1 target nucleic acid.

3. The oligonucleotide of embodiment 1 or 2, wherein the contiguous nucleotide sequence is complementary to a target nucleic acid selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and/or SEQ ID NO: 3.

4. The oligonucleotide of embodiment 1 to 3, wherein the contiguous nucleotide sequence is complementary to a region within position 1 and 15720 on SEQ ID NO: 1.

5. The oligonucleotide of embodiment 1 to 4, wherein the oligonucleotide is capable of hybridizing to a target nucleic acid of selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2 and/or SEQ ID NO: 3 with a ΔG° below -10 kcal.

6. The oligonucleotide of embodiment 1 to 5, wherein the contiguous nucleotide sequence is complementary to a sub-sequence of the target nucleic acid, wherein the sub-sequence is selected from the group consisting of position 371-3068, 5467-12107, 15317-15720, 15317-18083, 15317-19511 and 18881-19494 on SEQ ID NO: 1.

7. The oligonucleotide of embodiment 6, wherein the sub-sequence is selected from the group consisting of position 7300-7333, 8028-8072, 9812-9859, 11787-11873 and 15690-15735 on SEQ ID NO: 1.

8. The oligonucleotide of embodiment 2 to 7, wherein the target nucleic acid is RNA.

9. The oligonucleotide of embodiment 8, wherein the RNA is mRNA.

10. The oligonucleotide of embodiment 9, wherein the mRNA is pre-mRNA or mature mRNA.

11. The oligonucleotide of embodiment 1-10, wherein the contiguous nucleotide sequence comprises or consists of at least 14 contiguous nucleotides, particularly 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 contiguous nucleotides.

12. The oligonucleotide of embodiment 1-10, wherein the contiguous nucleotide sequence comprises or consists of from 16 to 20 nucleotides.

13. The oligonucleotide of embodiment 1-10, wherein the oligonucleotide comprises or consists of 14 to 35 nucleotides in length.

14. The oligonucleotide of embodiment 13, wherein the oligonucleotide comprises or consists of 18 to 22 nucleotides in length.

15. The oligonucleotide of embodiment 1-14, wherein the oligonucleotide or contiguous nucleotide sequence is single stranded.

16. The oligonucleotide of embodiment 1-15, wherein the contiguous nucleotide sequence is complementary to a sub-sequence of the target nucleic acid, wherein the subsequence is

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selected from the group consisting of A7, A26, A43, A119, A142, A159, A160, A163, A169, A178, A179, A180, A189, A201, A202, A204, A214, A221, A224, A226, A243, A254, A258, 269, A274, A350, A360, A364, A365, A370, A372, A381, A383, A386, A389, A400, A427, A435 and A438.

17. The oligonucleotide of embodiment 16, wherein the subsequence is selected from the group consisting of A221, A360, A180, A160 and A269.

18. The oligonucleotide of embodiment 1-17, wherein the oligonucleotide is not siRNA and is not self-complementary.

19. The oligonucleotide of embodiment 1-18, wherein the contiguous nucleotide sequence comprises or consists of a sequence selected from SEQ ID NO: 5 to 743 or 771.

20. The oligonucleotide of embodiment 1-19, wherein the contiguous nucleotide sequence comprises or consists of a sequence selected from SEQ ID NO: 6, 8, 9, 13, 41, 42, 58, 77, 92, 111, 128, 151, 164, 166, 169, 171, 222, 233, 245, 246, 250, 251, 252, 256, 272, 273, 287, 292, 303, 314, 318, 320, 324, 336, 342, 343, 344, 345, 346, 349, 359, 360, 374, 408, 409, 415, 417, 424, 429, 430, 458, 464, 466, 474, 490, 493, 512, 519, 519, 529, 533, 534, 547, 566, 567, 578, 582, 601, 619, 620, 636, 637, 638, 640, 645, 650, 651, 652, 653, 658, 659, 660, 665, 678, 679, 680, 682, 683, 684, 687, 694, 706, 716, 728, 733, 734, and 735.

21. The oligonucleotide of embodiment 1-20, wherein the contiguous nucleotide sequence comprises or consists of a sequence selected from SEQ ID NO: 466, 640, 342, 287 and 566.

22. The oligonucleotide of embodiment 1-21 wherein the contiguous nucleotide sequence has zero to three mismatches compared to the target nucleic acid it is complementary to.

23. The oligonucleotide of embodiment 22, wherein the contiguous nucleotide sequence has one mismatch compared to the target nucleic acid.

24. The oligonucleotide of embodiment 22, wherein the contiguous nucleotide sequence has two mismatches compared to the target nucleic acid.

25. The oligonucleotide of embodiment 22, wherein the contiguous nucleotide sequence is fully complementary to the target nucleic acid sequence.

26. The oligonucleotide of embodiment 1 -25, comprising one or more modified nucleosides.

27. The oligonucleotide of embodiment 26, wherein the one or more modified nucleoside is a high-affinity modified nucleosides.

28. The oligonucleotide of embodiment 26 or 27, wherein the one or more modified nucleoside is a 2' sugar modified nucleoside.

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29. The oligonucleotide of embodiment 28, wherein the one or more 2' sugar modified nucleoside is 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, 2'-fluoro-ANA and LNA nucleosides.

30. The oligonucleotide of embodiment 28, wherein the one or more modified nucleoside is a LNA nucleoside.

31. The oligonucleotide of embodiment 30, wherein the modified LNA nucleoside is oxy-LNA.

32. The oligonucleotide of embodiment 31, wherein the modified nucleoside is beta-D-oxy-LNA.

33. The oligonucleotide of embodiment 30, wherein the modified nucleoside is thio-LNA.

34. The oligonucleotide of embodiment 30, wherein the modified nucleoside is amino-LNA.

35. The oligonucleotide of embodiment 30, wherein the modified nucleoside is cET.

36. The oligonucleotide of embodiment 30, wherein the modified nucleoside is ENA.

37. The oligonucleotide of embodiment 30, wherein the modified LNA nucleoside is selected from beta-D-oxy-LNA, alpha-L-oxy-LNA, beta-D-amino-LNA, alpha-L-amino-LNA, beta-D-thio-LNA, alpha-L-thio-LNA, (S)cET, (R)cET beta-D-ENA and alpha-L-ENA.

38. The oligonucleotide of embodiment 30-37, wherein there in addition to the modified LNA nucleoside is at least one 2' substituted modified nucleoside.

39. The oligonucleotide of embodiment 38, wherein the 2' substituted modified nucleoside is selected from the group consisting of 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-fluoro-DNA, 2'-fluoro-ANA.

40. The oligonucleotide of any one of embodiments 1-39, wherein the oligonucleotide comprises at least one modified internucleoside linkage.

41. The oligonucleotide of embodiment 40, wherein the modified internucleoside linkage is nuclease resistant.

42. The oligonucleotide of embodiment 40 or 41, wherein at least 50% of the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages or boranophosphate internucleoside linkages.

43. The oligonucleotide of embodiment 40 or 41, wherein all the internucleoside linkages within the contiguous nucleotide sequence are phosphorothioate internucleoside linkages.

44. The oligonucleotide of embodiment 1-43, wherein the oligonucleotide is capable of recruiting RNase H.

45. The oligonucleotide of embodiment 44, wherein the oligonucleotide is a gapmer.

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46. The oligonucleotide of embodiment 44 or 45, wherein the oligonucleotide is a gapmer of formula 5'-F-G-F'-3', where region F and F' independently comprise or consist of 1 - 7 modified nucleosides and G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH.

47. The oligonucleotide of embodiment 44 or 45, wherein the gapmer has formula 5'-D'-F-G-F'-3' or 5'-F-G-F'-D''-3', where region F and F' independently comprise 1 - 7 modified nucleosides, G is a region between 6 and 16 nucleosides which are capable of recruiting RNaseH and region D' or D'' comprise 1 - 5 phosphodiester linked nucleosides.

48. The oligonucleotide of embodiment 47, wherein D' or D'' are optional.

49. The oligonucleotide of embodiment 47, wherein region D' consist of two phosphodiester linked nucleosides.

50. The oligonucleotide of embodiment 49, wherein the phosphodiester linked nucleosides are ca (cytidine-adenosine).

51. The oligonucleotide of embodiment 46 or 47, wherein the modified nucleoside is a 2' sugar modified nucleoside 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.

52. The oligonucleotide of embodiments 46 to 51, wherein one or more of the modified nucleosides in region F and F' is a LNA nucleoside.

53. The oligonucleotide of embodiment 52, wherein all the modified nucleosides in region F and F' are LNA nucleosides.

54. The oligonucleotide of embodiment 53, wherein region F and F' consist of LNA nucleosides.

55. The oligonucleotide of embodiment 52-54, wherein all the modified nucleosides in region F and F' are oxy-LNA nucleosides.

56. The oligonucleotide of embodiment 52, wherein at least one of region F or F' further comprises at least one 2' substituted modified nucleoside 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 and 2'-fluoro-DNA.

57. The oligonucleotide of embodiment 46-56, wherein the RNaseH recruiting nucleosides in region G are independently selected from DNA, alpha-L-LNA, C4' alkylated DNA, ANA and 2'F-ANA and UNA.

58. The oligonucleotide of embodiment 57, wherein the nucleosides in region G is DNA and/or alpha-L-LNA nucleosides.

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59. The oligonucleotide of embodiment 57 or 58, wherein region G consists of at least 75% DNA nucleosides.

60. The oligonucleotide of embodiment 1-59, wherein the oligonucleotide is selected from any one of the CMP ID NO: 5_1 to 743_1 and 771_1 (table 5).

61. The oligonucleotide of embodiment 1-60, wherein the oligonucleotide is selected from the group consisting of CMP ID NO: 6_1, 8_1, 9_1, 13_1, 41_1, 42_1, 58_1, 77_1, 92_1, 111_1, 128_1, 151_1, 164_1, 166_1, 169_1, 171_1, 222_1, 233_1, 245_1, 246_1, 250_1, 251_1, 252_1, 256_1, 272_1, 273_1, 287_1, 292_1, 303_1, 314_1, 318_1, 320_1, 324_1, 336_1, 342_1, 343_1, 344_1, 345_1, 346_1, 349_1, 359_1, 360_1, 374_1, 408_1, 409_1, 415_1, 417_1, 424_1, 429_1, 430_1, 458_1, 464_1, 466_1, 474_1, 490_1, 493_1, 512_1, 519_1, 519_1, 529_1, 533_1, 534_1, 547_1, 566_1, 567_1, 578_1, 582_1, 601_1, 619_1, 620_1, 636_1, 637_1, 638_1, 640_1, 645_1, 650_1, 651_1, 652_1, 653_1, 658_1, 659_1, 660_1, 665_1, 678_1, 679_1, 680_1, 682_1, 683_1, 684_1, 687_1, 694_1, 706_1, 716_1, 728_1, 733_1, 734_1, and 735_1.

62. The oligonucleotide of embodiment 1-61, wherein the oligonucleotide is selected from the group consisting of CMP ID NO: 287_1, 342_1, 466_1, 640_1, 566_1, 766_1, 767_1, 768_1, 769_1 and 770_1.

63. An antisense oligonucleotide conjugate comprising

a. an oligonucleotide according to any one of claims 1-62 (Region A); and

b. at least one at least one conjugate moiety (Region C) covalently attached to said oligonucleotide.

64. The oligonucleotide conjugate of embodiment 63, wherein the conjugate moiety is selected from carbohydrates, cell surface receptor ligands, drug substances, hormones, lipophilic substances, polymers, proteins, peptides, toxins, vitamins, viral proteins or combinations thereof.

65. The oligonucleotide conjugate of embodiment 63 or 64, wherein the conjugate moiety is a carbohydrate containing moiety.

66. The oligonucleotide conjugate of embodiment 65, wherein the carbohydrate conjugate moiety comprises at least one asialoglycoprotein receptor targeting moiety covalently attached to an oligonucleotide according to any one of claims 1-62.

67. The oligonucleotide conjugate of embodiment 66, wherein the asialoglycoprotein receptor targeting conjugate moiety comprises at least one carbohydrate moiety selected from group consisting of galactose, galactosamine, N-formyl-galactosamine, N-acetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine and N-isobutanoylgalactosamine.

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68. The oligonucleotide conjugate of embodiment 66 or 67, wherein the asialoglycoprotein receptor targeting conjugate moiety is mono-valent, di-valent, tri-valent or tetra-valent.

69. The oligomer conjugate of embodiment 68, wherein the asialoglycoprotein receptor targeting conjugate moiety consists of two to four terminal GalNAc moieties, a PEG spacer linking each GalNAc moiety to a brancher molecule.

70. The oligonucleotide conjugate of embodiment 66 to 69, wherein the asialoglycoprotein receptor targeting conjugate moiety is a tri-valent N-acetylgalactosamine (GalNAc) moiety.

71. The oligonucleotide conjugate of embodiment 66 to 70, wherein the conjugate moiety is selected from one of the trivalent GalNAc moieties in figure 1.

72. The oligonucleotide conjugate of embodiment 71, wherein the conjugate moiety is the trivalent GalNAc moiety in figure 3.

73. The oligonucleotide conjugate of embodiment 63-72, where a linker is present between the oligonucleotide or contiguous oligonucleotide sequence and the conjugate moiety.

74. The oligonucleotide conjugate of embodiment 73, wherein the linker is a physiologically labile linker (region B).

75. The oligonucleotide conjugate of embodiment 74, wherein the physiologically labile linker is nuclease susceptible linker.

76. The oligonucleotide conjugate of embodiment 74 or 75, wherein the physiologically labile linker is composed of 2 to 5 consecutive phosphodiester linkages.

77. The oligonucleotide conjugate of embodiment 76, wherein the physiologically labile linker is equivalent to region D' or D'' presented in embodiment 47 to 50.

78. The oligonucleotide conjugate of any one of embodiments 63-77, wherein the oligonucleotide conjugate is selected from CMP ID NO: 766_2, 767_2,768_2, 769_2 and 770_2.

79. The oligonucleotide conjugate of embodiment 78, wherein the oligonucleotide conjugate is selected from the oligonucleotide conjugated represented in figure 4, 5, 6, 7 and 8.

80. The oligonucleotide conjugate of embodiment 63-76, which display improved inhibition of PD-L1 in the target cell, or improved cellular distribution between liver and the spleen or improved cellular uptake into the liver of the conjugate oligonucleotide as compared to an unconjugated oligonucleotide.

81. A pharmaceutical composition comprising the oligonucleotide of embodiment 1-62 or a conjugate of embodiment 63-80 and a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant.

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82. A method for manufacturing the oligonucleotide of embodiment 1-62, comprising reacting nucleotide units thereby forming covalently linked contiguous nucleotide units comprised in the oligonucleotide.

83. The method of embodiment 82, further comprising reacting the contiguous nucleotide sequence with a non-nucleotide conjugation moiety.

84. A method for manufacturing the composition of embodiment 81, comprising mixing the oligonucleotide with a pharmaceutically acceptable diluent, carrier, salt and/or adjuvant.

85. An *in vivo* or *in vitro* method for modulating PD-L1 expression in a target cell which is expressing PD-L1, said method comprising administering an oligonucleotide of embodiment 1-62 or a conjugate of embodiment 63-80 or the pharmaceutical composition of embodiment 81 in an effective amount to said cell.

86. A method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide of embodiment 1-62 or a conjugate of embodiment 63-80 or the pharmaceutical composition of embodiment 81 to a subject suffering from or susceptible to the disease.

87. A method for restoration of immunity against a virus or parasite comprising administering a therapeutically or prophylactically effective amount of an oligonucleotide conjugate of embodiment 63-80 or the oligonucleotide of embodiment 1-62 or the pharmaceutical composition of embodiment 81 to a subject infected with a virus or parasite.

88. The method of embodiment 87, the restoration of immunity is an increase in the liver of CD8+ T cells specific to one or more HBV antigens when compared to a control.

89. The oligonucleotide of embodiment 1-62 or a conjugate of embodiment 63-80 or the pharmaceutical composition of embodiment 81, for use as a medicament for treatment or prevention of a disease in a subject.

90. Use of the oligonucleotide of oligonucleotide of embodiment 1-62 or a conjugate of embodiment 63-80 for the preparation of a medicament for treatment or prevention of a disease in a subject.

91. The oligonucleotide of embodiment 1-62 or a conjugate of embodiment 63-80 or the pharmaceutical composition of embodiment 81, for use in restoration of immunity against a virus or parasite.

92. The use of embodiment 91, wherein the restoration of immunity is an increase in the liver of CD8+ T cells specific to one or more HBV antigens when compared to a control.

93. The use of embodiment 92, wherein the HBV antigen is the HBsAg.

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94. The method, the oligonucleotide or the use of embodiments 86 - 93, wherein the disease is associated with in vivo activity of PD-L1.

95. The method, the oligonucleotide or the use of embodiments 86 - 94, wherein the disease is associated with increased expression of PD-L1 in an antigen presenting cell.

96. The method, the oligonucleotide or the use of embodiments 95, wherein the PD-L1 is reduced by at least 30%, or at least or at least 40%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 95% compared to the expression without or before treatment with the oligonucleotide of embodiment 1-62 or a conjugate of embodiment 63-80 or the pharmaceutical composition of embodiment 81.

97. The method, the oligonucleotide or the use of embodiments 86 - 95, wherein the disease is selected from a viral liver infection or a parasite infections.

98. The method, the oligonucleotide or the use of embodiment 98, wherein the viral infection is HBV, HCV or HDV.

99. The method, the oligonucleotide or the use of embodiment 86 - 95, wherein the disease is chronic HBV.

100. The method, the oligonucleotide or the use of embodiment 98, wherein the parasite infection is malaria, toxoplasmosis, leishmaniasis or trypanosomiasis.

101. The method, the oligonucleotide or the use of embodiments 86 - 100, wherein the subject is a mammal.

102. The method, the oligonucleotide or the use of embodiment 101, wherein the mammal is human.

EXAMPLES

Materials and methods

Motif sequences and oligonucleotide compounds

Table 5: list of oligonucleotide motif sequences (indicated by SEQ ID NO) targeting the human PD-L1 transcript (SEQ ID NO: 1), designs of these, as well as specific antisense oligonucleotide compounds (indicated by CMP ID NO) designed based on the motif sequence.

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 1	dG
5	taattggctctactgc	2-11-3	TAattggctctacTGC	5_1	236	-20
6	tcgcataagaatgact	4-10-2	TCGCataagaatgaCT	6_1	371	-19
7	tgaacacacagtcgca	2-12-2	TGaacacacagtcgCA	7_1	382	-19
8	ctgaacacacagtcgc	3-10-3	CTGaacacacagtCGC	8_1	383	-22
9	tctgaacacacagtcg	3-11-2	TCTgaacacacagtCG	9_1	384	-19
10	ttctgaacacacagtc	3-11-2	TTCtgaacacacagTC	10_1	385	-17

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SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 1	dG
11	acaagtcatgttacta	2-11-3	ACaagtcatgttaCTA	11_1	463	-16
12	acacaagtcatgttac	2-12-2	ACacaagtcatgttAC	12_1	465	-14
13	cttacttagatgctgc	2-11-3	CTtacttagatgcTGC	13_1	495	-20
14	acttacttagatgctg	2-11-3	ACttacttagatgCTG	14_1	496	-18
15	gacttacttagatgct	3-11-2	GACttacttagatgCT	15_1	497	-19
16	agacttacttagatgc	2-11-3	AGacttacttagaTGC	16_1	498	-18
17	gcaggaagagacttac	3-10-3	GCAggaagagactTAC	17_1	506	-20
18	aataaattccgttcagg	4-9-4	AATAaattccgttCAGG	18_1	541	-22
19	gcaaataaattccgtt	3-10-3	GCAaataaattccGTT	19_2	545	-18
19	gcaaataaattccgtt	4-8-4	GCAAataaattcCGTT	19_1	545	-20
20	agcaaataaattccgt	4-9-3	AGCAaataaattcCGT	20_1	546	-20
21	cagagcaaataaattcc	4-10-3	CAGAgcaaataaatTCC	21_1	548	-21
22	tggacagagcaaataaat	4-11-3	TGGAcagagcaaataAAT	22_1	551	-19
23	atggacagagcaaata	4-8-4	ATGGacagagcaAATA	23_1	554	-20
24	cagaatggacagagca	2-11-3	CAgaatggacagaGCA	24_1	558	-21
25	ttctcagaatggacag	3-11-2	TTCtcagaatggacAG	25_1	562	-17
26	ctgaactttgacatag	4-8-4	CTGAactttgacATAG	26_1	663	-20
27	aagacaaacccagactga	2-13-3	AAgacaaacccagacTGA	27_1	675	-21
28	tataagacaaacccagac	4-10-4	TATAagacaaacccAGAC	28_1	678	-22
29	ttataagacaaacccaga	4-10-4	TTATaagacaaaccCAGA	29_1	679	-23
30	tgttataagacaaaccc	4-10-3	TGTTataagacaaaCCC	30_1	682	-22
31	tagaacaatggtacttt	4-9-4	TAGAacaatggtaCTTT	31_1	708	-20
32	gtagaacaatggtact	4-10-2	GTAGaacaatggtaCT	32_1	710	-19
33	aggtagaacaatggta	3-10-3	AGGtagaacaatgGTA	33_1	712	-19
34	aagaggtagaacaatgg	4-9-4	AAGAggtagaacaATGG	34_1	714	-21
35	gcatccacagtaaatt	2-12-2	GCatccacagtaaaTT	35_1	749	-17
36	gaaggttatttaattc	2-11-3	GAaggttatttaaTTC	36_1	773	-13
37	ctaatcgaatgcagca	4-9-3	CTAAtcgaatgcaGCA	37_1	805	-22
38	tacccaatctaatcga	3-10-3	TACccaatctaatCGA	38_1	813	-20
39	tagttacccaatctaa	3-10-3	TAGttacccaatcTAA	39_1	817	-19
40	catttagttacccaat	3-10-3	CATttagttacccAAT	40_1	821	-18
41	tcatttagttacccaa	3-10-3	TCAtttagttacccAA	41_1	822	-19
42	ttcatttagttaccca	2-10-4	TTcatttagttaCCCA	42_1	823	-22
43	gaattaatttcatttagt	4-10-4	GAATtaatttcattTAGT	43_1	829	-19
44	cagtgaggaattaattt	4-9-4	CAGTgaggaattaATTT	44_1	837	-20
45	ccaacagtgaggaatt	4-8-4	CCAAcagtgaggAATT	45_1	842	-21
46	cccaacagtgaggaat	3-10-3	CCCaacagtgaggAAT	46_1	843	-22
47	tatacccaacagtgagg	2-12-3	TAtacccaacagtgAGG	47_1	846	-21
48	ttatacccaacagtgag	2-11-4	TTatacccaacagTGAG	48_1	847	-21
49	tttatacccaacagtga	3-11-3	TTTatacccaacagTGA	49_1	848	-21
50	cctttatacccaacag	3-10-3	CCTttatacccaaCAG	50_1	851	-23
51	taacctttatacccaa	4-8-4	TAACctttatacCCAA	51_1	854	-22

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SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 1	dG
52	aataacctttataccca	3-10-4	AATaacctttataCCCA	52_1	855	-23
53	gtaaataacctttata	3-11-2	GTAaataacctttaTA	53_1	859	-14
54	actgtaaataacctttat	4-10-4	ACTGtaaataacctTTAT	54_1	860	-20
55	atatatatgcaatgag	3-11-2	ATAtatatgcaatgAG	55_1	903	-14
56	agatatatatgcaatg	2-12-2	AGatatatatgcaaTG	56_1	905	-12
57	gagatatatatgcaat	3-10-3	GAGatatatatgcAAT	57_1	906	-15
58	ccagagatatatatgc	2-11-3	CCagagatatataTGC	58_1	909	-19
59	caatattccagagatat	4-9-4	CAATattccagagATAT	59_1	915	-20
60	gcaatattccagagata	4-10-3	GCAAtattccagagATA	60_1	916	-22
61	agcaatattccagagat	3-11-3	AGCaatattccagaGAT	61_1	917	-22
62	cagcaatattccagag	3-9-4	CAGcaatattccAGAG	62_1	919	-22
63	aatcagcaatattccag	4-9-4	AATCagcaatattCCAG	63_1	921	-23
64	acaatcagcaatattcc	4-9-4	ACAAtcagcaataTTCC	64_1	923	-21
65	actaagtagttacacttct	2-14-3	ACtaagtagttacactTCT	65_1	957	-20
66	ctaagtagttacacttc	4-11-2	CTAAgtagttacactTC	66_1	958	-18
67	gactaagtagttacactt	3-12-3	GACtaagtagttacaCTT	67_1	959	-20
68	tgactaagtagttaca	3-9-4	TGActaagtagtTACA	68_1	962	-19
69	ctttgactaagtagtta	4-10-3	CTTTgactaagtagTTA	69_1	964	-19
70	ctctttgactaagtag	3-10-3	CTCtttgactaagTAG	70_1	967	-19
71	gctctttgactaagta	4-10-2	GCTCtttgactaagTA	71_1	968	-21
72	ccttaaatactgttgac	2-11-4	CCttaaatactgtTGAC	72_1	1060	-20
73	cttaaatactgttgac	2-12-2	CTtaaatactgttgAC	73_1	1060	-13
74	tccttaaatactgttg	3-10-3	TCCttaaatactgTTG	74_1	1062	-18
75	tctccttaaatactgtt	4-11-2	TCTCcttaaatactgTT	75_1	1063	-19
76	tatcatagttctcctt	2-10-4	TAtcatagttctCCTT	76_1	1073	-21
77	agtatcatagttctcc	3-10-3	AGTatcatagttcTCC	77_1	1075	-22
78	gagtatcatagttctc	2-11-3	GAgtatcatagttCTC	78_1	1076	-18
79	agagtatcatagttct	2-10-4	AGagtatcatagTTCT	79_1	1077	-18
79	agagtatcatagttct	3-10-3	AGAgtatcatagtTCT	79_2	1077	-19
80	cagagtatcatagttc	3-10-3	CAGagtatcatagTTC	80_1	1078	-18
81	ttcagagtatcatagt	4-10-2	TTCAgagtatcataGT	81_1	1080	-18
82	cttcagagtatcatag	3-9-4	CTTcagagtatcATAG	82_1	1081	-19
83	ttcttcagagtatcata	4-11-2	TTCTtcagagtatcaTA	83_1	1082	-19
84	tttcttcagagtatcat	3-10-4	TTTcttcagagtaTCAT	84_1	1083	-20
85	gagaaaggctaagttt	4-9-3	GAGAaaggctaagTTT	85_1	1099	-19
86	gacactcttgtacatt	2-10-4	GAcactcttgtaCATT	86_1	1213	-19
87	tgagacactcttgtaca	2-13-2	TGagacactcttgtaCA	87_1	1215	-18
88	tgagacactcttgtac	2-11-3	TGagacactcttgTAC	88_1	1216	-18
89	ctttattaaactccat	2-10-4	CTttattaaactCCAT	89_1	1266	-18
90	accaaactttattaaa	4-10-2	ACCAaactttattaAA	90_1	1272	-14
91	aaacctctactaagtg	4-10-2	AAACctctactaagTG	91_1	1288	-16
92	agattaagacagttga	2-11-3	AGattaagacagtTGA	92_1	1310	-16

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SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 1	dG
93	aagtaggagcaagaggc	2-12-3	AAgtaggagcaagaGGC	93_1	1475	-22
94	aaagtaggagcaagagg	4-10-3	AAAGtaggagcaagAGG	94_1	1476	-20
95	gttaagcagccaggag	2-12-2	GTtaagcagccaggAG	95_1	1806	-20
96	agggtaggatgggtag	2-12-2	AGggtaggatgggtAG	96_1	1842	-20
97	aagggtaggatgggta	3-11-2	AAGggtaggatgggTA	97_1	1843	-20
98	caagggtaggatgggt	2-12-2	CAagggtaggatggGT	98_2	1844	-20
98	caagggtaggatgggt	3-11-2	CAAgggtaggatggGT	98_1	1844	-21
99	ccaagggtaggatggg	2-12-2	CCaagggtaggatgGG	99_1	1845	-22
100	tccaagggtaggatgg	2-12-2	TCcaagggtaggatGG	100_1	1846	-20
101	cttccaagggtaggat	4-10-2	CTTCcaagggtaggAT	101_1	1848	-21
102	atcttccaagggtagga	3-12-2	ATCttccaagggtagGA	102_1	1849	-22
103	agaagtgatggctcatt	2-11-4	AGaagtgatggctCATT	103_1	1936	-21
104	aagaagtgatggctcat	3-10-4	AAGaagtgatggcTCAT	104_1	1937	-21
105	gaagaagtgatggctca	3-11-3	GAAgaagtgatggcTCA	105_1	1938	-21
106	atgaaatgtaaactggg	4-9-4	ATGAaatgtaaacTGGG	106_1	1955	-21
107	caatgaaatgtaaactgg	4-10-4	CAATgaaatgtaaaCTGG	107_1	1956	-20
108	gcaatgaaatgtaaactg	4-10-4	GCAAtgaaatgtaaACTG	108_1	1957	-20
109	agcaatgaaatgtaaact	4-10-4	AGCAatgaaatgtaAACT	109_1	1958	-20
110	gagcaatgaaatgtaaac	4-10-4	GAGCaatgaaatgtAAAC	110_1	1959	-19
111	tgaattcccatatccga	2-12-3	TGaattcccatatcCGA	111_1	1992	-22
112	agaattatgaccatat	2-11-3	AGaattatgaccaTAT	112_1	2010	-15
113	aggtaagaattatgacc	3-10-4	AGGtaagaattatGACC	113_1	2014	-21
114	tcaggtaagaattatgac	4-10-4	TCAGgtaagaattaTGAC	114_1	2015	-22
115	cttcaggtaagaattatg	4-10-4	CTTCaggtaagaatTATG	115_1	2017	-21
116	tcttcaggtaagaatta	4-9-4	TCTTcaggtaagaATTA	116_1	2019	-20
117	cttcttcaggtaagaat	4-9-4	CTTCttcaggtaaGAAT	117_1	2021	-21
118	tcttcttcaggtaagaa	4-10-3	TCTTcttcaggtaaGAA	118_1	2022	-20
119	tcttcttcaggtaaga	3-10-3	TCTtcttcaggtaAGA	119_1	2023	-20
120	tggtctaagagaagaag	3-10-4	TGGtctaagagaaGAAG	120_1	2046	-20
121	gttggtctaagagaag	4-9-3	GTTGgtctaagagAAG	121_1	2049	-19
123	cagttggtctaagagaa	2-11-4	CAgttggtctaagAGAA	123_1	2050	-20
124	gcagttggtctaagagaa	3-13-2	GCAgttggtctaagagAA	124_1	2050	-22
122	agttggtctaagagaa	3-9-4	AGTtggtctaagAGAA	122_1	2050	-20
126	gcagttggtctaagaga	2-13-2	GCagttggtctaagaGA	126_1	2051	-21
125	cagttggtctaagaga	4-10-2	CAGTtggtctaagaGA	125_1	2051	-21
127	gcagttggtctaagag	2-11-3	GCagttggtctaaGAG	127_1	2052	-21
128	ctcatatcagggcagt	2-10-4	CTcatatcagggCAGT	128_1	2063	-24
129	cacacatgttctttaac	4-11-2	CACAcatgttctttaAC	129_1	2087	-18
130	taaatacacacatgttct	3-11-4	TAAatacacacatgTTCT	130_1	2092	-19
131	gtaaatacacacatgttc	4-11-3	GTAAatacacacatgTTC	131_1	2093	-19
132	tgtaaatacacacatgtt	4-10-4	TGTAaatacacacaTGTT	132_1	2094	-22
133	gatcatgtaaatacacac	4-10-4	GATCatgtaaatacACAC	133_1	2099	-20

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134	agatcatgtaaatacaca	4-10-4	AGATcatgtaaataCACA	134_1	2100	-21
135	caaagatcatgtaaatacac	4-12-4	CAAAgatcatgtaaatACAC	135_1	2101	-19
136	acaaagatcatgtaaataca	4-12-4	ACAAagatcatgtaaaTACA	136_1	2102	-20
137	gaatacaaagatcatgta	4-10-4	GAATacaaagatcaTGTA	137_1	2108	-20
138	agaatacaaagatcatgt	4-10-4	AGAAtacaaagatcATGT	138_1	2109	-20
139	cagaatacaaagatcatg	4-10-4	CAGAatacaaagatCATG	139_1	2110	-21
140	gcagaatacaaagatca	4-9-4	GCAGaatacaaagATCA	140_1	2112	-22
141	aggcagaatacaaagat	4-11-2	AGGCagaatacaaagAT	141_1	2114	-19
142	aaggcagaatacaaaga	4-10-3	AAGGcagaatacaaAGA	142_1	2115	-19
143	attagtgagggacgaa	3-10-3	ATTagtgagggacGAA	143_1	2132	-18
144	cattagtgagggacga	2-11-3	CAttagtgagggaCGA	144_1	2133	-20
145	gagggtgatggattag	2-11-3	GAgggtgatggatTAG	145_1	2218	-19
146	ttaggagtaataaagg	2-10-4	TTaggagtaataAAGG	146_1	2241	-14
147	ttaatgaatttggttg	3-11-2	TTAatgaatttggtTG	147_1	2263	-13
148	ctttaatgaatttggt	2-12-2	CTttaatgaatttgGT	148_1	2265	-14
149	catggattacaactaa	4-10-2	CATGgattacaactAA	149_1	2322	-16
150	tcatggattacaacta	2-11-3	TCatggattacaaCTA	150_1	2323	-16
151	gtcatggattacaact	3-11-2	GTCatggattacaaCT	151_1	2324	-18
152	cattaaatctagtcat	2-10-4	CAttaaatctagTCAT	152_1	2335	-16
153	gacattaaatctagtca	4-10-3	GACAttaaatctagTCA	153_1	2336	-19
154	agggacattaaatcta	4-10-2	AGGGacattaaatcTA	154_1	2340	-18
155	caaagcattataacca	4-9-3	CAAAgcattataaCCA	155_1	2372	-18
156	acttactaggcagaag	2-10-4	ACttactaggcaGAAG	156_1	2415	-19
157	cagagttaactgtaca	4-10-2	CAGAgttaactgtaCA	157_1	2545	-20
158	ccagagttaactgtac	4-10-2	CCAGagttaactgtAC	158_1	2546	-20
159	gccagagttaactgta	2-12-2	GCcagagttaactgTA	159_1	2547	-20
160	tgggccagagttaact	2-12-2	TGggccagagttaaCT	160_1	2550	-21
161	cagcatctatcagact	2-12-2	CAgcatctatcagaCT	161_1	2576	-19
162	tgaaataacatgagtcat	3-11-4	TGAaataacatgagTCAT	162_1	2711	-19
163	gtgaaataacatgagtc	3-10-4	GTGaaataacatgAGTC	163_1	2713	-19
164	tctgtttatgtcactg	4-10-2	TCTGtttatgtcacTG	164_1	2781	-20
165	gtctgtttatgtcact	4-10-2	GTCTgtttatgtcaCT	165_1	2782	-22
166	tggtctgtttatgtca	2-10-4	TGgtctgtttatGTCA	166_1	2784	-21
167	ttggtctgtttatgtc	4-10-2	TTGGtctgtttatgTC	167_1	2785	-20
168	tcacccattgtttaaa	2-12-2	TCacccattgtttaAA	168_1	2842	-15
169	ttcagcaaatattcgt	2-10-4	TTcagcaaatatTCGT	169_1	2995	-17
170	gtgtgttcagcaaatat	3-10-4	GTGtgttcagcaaATAT	170_1	2999	-21
171	tctattgttaggtatc	3-10-3	TCTattgttaggtATC	171_1	3053	-18
172	attgcccatcttactg	2-12-2	ATtgcccatcttacTG	172_1	3118	-19
173	tattgcccatcttact	3-11-2	TATtgcccatcttaCT	173_1	3119	-21
174	aaatattgcccatctt	2-11-3	AAatattgcccatCTT	174_1	3122	-17
175	ataaccttatcataca	3-11-2	ATAaccttatcataCA	175_1	3174	-16

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176	tataaccttatcatac	2-11-3	TAtaaccttatcaTAC	176_1	3175	-14
177	ttataaccttatcata	3-11-2	TTAtaaccttatcaTA	177_1	3176	-14
178	tttataaccttatcat	3-10-3	TTTataaccttatCAT	178_1	3177	-16
179	actgctattgctatct	2-11-3	ACtgctattgctaTCT	179_1	3375	-19
180	aggactgctattgcta	2-11-3	AGgactgctattgCTA	180_1	3378	-21
181	gaggactgctattgct	3-11-2	GAGgactgctattgCT	181_1	3379	-22
182	acgtagaataataaca	2-12-2	ACgtagaataataaCA	182_1	3561	-11
183	ccaagtgatataatgg	2-10-4	CCaagtgatataATGG	183_1	3613	-19
184	ttagcagaccaagtga	2-10-4	TTagcagaccaaGTGA	184_1	3621	-21
185	gtttagcagaccaagt	2-12-2	GTttagcagaccaaGT	185_1	3623	-19
186	tgacagtgattatatt	2-12-2	TGacagtgattataTT	186_1	3856	-13
187	tgtccaagatattgac	4-10-2	TGTCcaagatattgAC	187_1	3868	-18
188	gaatatcctagattgt	3-10-3	GAAtatcctagatTGT	188_1	4066	-18
189	caaactgagaatatcc	2-11-3	CAaactgagaataTCC	189_1	4074	-16
190	gcaaactgagaatatc	3-11-2	GCAaactgagaataTC	190_1	4075	-16
191	tcctattacaatcgta	3-11-2	TCCtattacaatcgTA	191_1	4214	-19
192	ttcctattacaatcgt	4-10-2	TTCCtattacaatcGT	192_1	4215	-19
193	actaatgggaggattt	2-12-2	ACtaatgggaggatTT	193_1	4256	-15
194	tagttcagagaataag	2-12-2	TAgttcagagaataAG	194_1	4429	-13
195	taacatatagttcaga	2-11-3	TAacatatagttcAGA	195_1	4436	-15
196	ataacatatagttcag	3-11-2	ATAacatatagttcAG	196_1	4437	-14
197	cataacatatagttca	2-12-2	CAtaacatatagttCA	197_1	4438	-13
198	tcataacatatagttc	2-12-2	TCataacatatagtTC	198_1	4439	-12
199	tagctcctaacaatca	4-10-2	TAGCtcctaacaatCA	199_1	4507	-22
200	ctccaatctttgtata	4-10-2	CTCCaatctttgtaTA	200_1	4602	-20
201	tctccaatctttgtat	4-10-2	TCTCcaatctttgtAT	201_1	4603	-19
202	tctatttcagccaatc	2-12-2	TCtatttcagccaaTC	202_1	4708	-17
203	cggaagtcagagtgaa	3-10-3	CGGaagtcagagtGAA	203_1	4782	-19
204	ttaagcatgaggaata	4-10-2	TTAAgcatgaggaaTA	204_1	4798	-16
205	tgattgagcacctctt	3-10-3	TGAttgagcacctCTT	205_1	4831	-22
206	gactaattatttcgtt	3-11-2	GACtaattatttcgTT	206_1	4857	-15
207	tgactaattatttcgt	3-10-3	TGActaattatttCGT	207_1	4858	-17
208	gtgactaattatttcg	3-10-3	GTGactaattattTCG	208_1	4859	-17
209	ctgcttgaaatgtgac	4-10-2	CTGCttgaaatgtgAC	209_1	4870	-20
210	cctgcttgaaatgtga	2-11-3	CCtgcttgaaatgTGA	210_1	4871	-21
211	atcctgcttgaaatgt	2-10-4	ATcctgcttgaaATGT	211_1	4873	-20
212	attataaatctattct	3-10-3	ATTataaatctatTCT	212_1	5027	-13
213	gctaaatactttcatc	2-11-3	GCtaaatactttcATC	213_1	5151	-16
214	cattgtaacataccta	2-10-4	CAttgtaacataCCTA	214_1	5251	-19
215	gcattgtaacatacct	2-12-2	GCattgtaacatacCT	215_1	5252	-18
216	taatattgcaccaaat	2-12-2	TAatattgcaccaaAT	216_1	5295	-13
217	gataatattgcaccaa	2-11-3	GAtaatattgcacCAA	217_1	5297	-16

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218	agataatattgcacca	2-12-2	AGataatattgcacCA	218_1	5298	-16
219	gccaagaagataatat	2-10-4	GCcaagaagataATAT	219_1	5305	-17
220	cacagccacataaact	4-10-2	CACAgccacataaaCT	220_1	5406	-21
221	ttgtaattgtggaaac	2-12-2	TTgtaattgtggaaAC	221_1	5463	-12
222	tgacttgtaattgtgg	2-11-3	TGacttgtaattgTGG	222_1	5467	-18
223	tctaactgaaatagtc	2-12-2	TCtaactgaaatagTC	223_1	5503	-13
224	gtggttctaactgaaa	3-11-2	GTGgttctaactgaAA	224_1	5508	-16
225	caatatgggacttggt	2-12-2	CAatatgggacttgGT	225_1	5522	-18
226	atgacaatatgggact	3-11-2	ATGacaatatgggaCT	226_1	5526	-17
227	tatgacaatatgggac	4-10-2	TATGacaatatgggAC	227_1	5527	-17
228	atatgacaatatggga	4-10-2	ATATgacaatatggGA	228_1	5528	-17
229	cttcacttaataatta	2-11-3	CTtcacttaataaTTA	229_1	5552	-13
230	ctgcttcacttaataa	4-10-2	CTGCttcacttaatAA	230_1	5555	-18
231	aagactgcttcactta	2-11-3	AAgactgcttcacTTA	231_1	5559	-17
232	gaatgccctaattatg	4-10-2	GAATgccctaattaTG	232_1	5589	-19
233	tggaatgccctaatta	3-11-2	TGGaatgccctaatTA	233_1	5591	-19
234	gcaaatgccagtaggt	3-11-2	GCAaatgccagtagGT	234_1	5642	-23
235	ctaatggaaggatttg	3-11-2	CTAatggaaggattTG	235_1	5673	-15
236	aatatagaacctaatg	2-12-2	AAtatagaacctaaTG	236_1	5683	-10
237	gaaagaatagaatgtt	3-10-3	GAAagaatagaatGTT	237_1	5769	-12
238	atgggtaatagattat	3-11-2	ATGggtaatagattAT	238_1	5893	-15
239	gaaagagcacagggtg	2-12-2	GAaagagcacagggTG	239_1	6103	-18
240	ctacatagagggaatg	4-10-2	CTACatagagggaaTG	240_1	6202	-18
241	gcttcctacatagagg	2-10-4	GCttcctacataGAGG	241_1	6207	-24
242	tgcttcctacatagag	4-10-2	TGCTtcctacatagAG	242_1	6208	-22
243	tgggcttgaaatatgt	2-11-3	TGggcttgaaataTGT	243_1	6417	-19
244	cattatatttaagaac	3-11-2	CATtatatttaagaAC	244_1	6457	-11
245	tcggttatgttatcat	2-10-4	TCggttatgttaTCAT	245_1	6470	-19
246	cactttatctggtcgg	2-10-4	CActttatctggTCGG	246_1	6482	-22
247	aaattggcacagcgtt	3-10-3	AAAttggcacagcGTT	247_1	6505	-18
248	accgtgacagtaaatg	4-9-3	ACCGtgacagtaaATG	248_1	6577	-20
249	tgggaaccgtgacagta	2-13-2	TGggaaccgtgacagTA	249_1	6581	-22
250	ccacatataggtcctt	2-11-3	CCacatataggtcCTT	250_1	6597	-21
251	catattgctaccatac	2-11-3	CAtattgctaccaTAC	251_1	6617	-18
252	tcatattgctaccata	3-10-3	TCAtattgctaccATA	252_1	6618	-19
253	caattgtcatattgct	4-8-4	CAATtgtcatatTGCT	253_1	6624	-21
254	cattcaattgtcatattg	3-12-3	CATtcaattgtcataTTG	254_1	6626	-18
255	tttctactgggaatttg	4-9-4	TTTCtactgggaaTTTG	255_1	6644	-20
256	caattagtgcagccag	3-10-3	CAAttagtgcagcCAG	256_1	6672	-21
257	gaataatgttcttatcc	4-10-3	GAATaatgttcttaTCC	257_1	6704	-20
258	cacaaattgaataatgttct	4-13-3	CACAaattgaataatgtTCT	258_1	6709	-20
259	catgcacaaattgaataat	4-11-4	CATGcacaaattgaaTAAT	259_1	6714	-20

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261	ccaccatagctgatca	2-12-2	CCaccatagctgatCA	261_1	6868	-22
262	accaccatagctgatca	2-12-3	ACcaccatagctgaTCA	262_1	6868	-23
263	caccaccatagctgatc	2-13-2	CAccaccatagctgaTC	263_1	6869	-21
264	tagtcggcaccaccat	2-12-2	TAgtcggcaccaccAT	264_1	6877	-22
265	cttgtagtcggcaccac	1-14-2	CttgtagtcggcaccAC	265_1	6880	-21
266	cttgtagtcggcacca	1-13-2	CttgtagtcggcacCA	266_1	6881	-21
267	cgcttgtagtcggcac	2-12-2	CGcttgtagtcggcAC	267_1	6883	-21
268	tcaataaagatcaggc	3-11-2	TCAataaagatcagGC	268_1	6942	-17
269	tggacttacaagaatg	2-12-2	TGgacttacaagaaTG	269_1	6986	-14
270	atggacttacaagaat	3-11-2	ATGgacttacaagaAT	270_1	6987	-15
271	gctcaagaaattggat	4-10-2	GCTCaagaaattggAT	271_1	7073	-19
272	tactgtagaacatggc	4-10-2	TACTgtagaacatgGC	272_1	7133	-21
273	gcaattcatttgatct	4-9-3	GCAAttcatttgaTCT	273_1	7239	-20
274	tgaagggaggagggacac	2-14-2	TGaagggaggagggacAC	274_1	7259	-20
275	agtggtgaagggaggag	2-13-2	AGtggtgaagggaggAG	275_1	7265	-21
276	tagtggtgaagggaggag	2-14-2	TAgtggtgaagggaggAG	276_1	7265	-21
277	atagtggtgaagggaggag	1-16-2	AtagtggtgaagggaggAG	277_1	7265	-20
278	tagtggtgaagggagga	2-13-2	TAgtggtgaagggagGA	278_1	7266	-21
279	atagtggtgaagggagga	2-14-2	ATagtggtgaagggagGA	279_1	7266	-21
280	tagtggtgaagggagg	3-11-2	TAGtggtgaagggaGG	280_1	7267	-21
281	atagtggtgaagggagg	3-12-2	ATAgtggtgaagggaGG	281_1	7267	-22
282	gatagtggtgaagggagg	2-14-2	GAtagtggtgaagggaGG	282_1	7267	-21
283	atagtggtgaagggag	4-10-2	ATAGtggtgaagggAG	283_1	7268	-20
284	gatagtggtgaagggag	2-12-3	GAtagtggtgaaggGAG	284_1	7268	-21
285	gagatagtggtgaagg	2-10-4	GAgatagtggtgAAGG	285_1	7271	-20
286	catgggagatagtggt	4-10-2	CATGggagatagtgGT	286_1	7276	-22
287	acaaataatggttactct	4-10-4	ACAAataatggttaCTCT	287_1	7302	-20
288	acacacaaataatggtta	4-10-4	ACACacaaataatgGTTA	288_1	7306	-20
289	gagggacacacaaataat	3-11-4	GAGggacacacaaaTAAT	289_1	7311	-21
290	atatagagaggctcaa	4-8-4	ATATagagaggcTCAA	290_1	7390	-21
291	ttgatatagagaggct	2-10-4	TTgatatagagaGGCT	291_1	7393	-20
292	gcatttgatatagaga	4-9-3	GCATttgatatagAGA	292_1	7397	-20
293	tttgcatttgatatag	2-11-3	TTtgcatttgataTAG	293_1	7400	-15
294	ctggaagaataggttc	3-11-2	CTGgaagaataggtTC	294_1	7512	-17
295	actggaagaataggtt	4-10-2	ACTGgaagaataggTT	295_1	7513	-18
296	tactggaagaataggt	4-10-2	TACTggaagaatagGT	296_1	7514	-18
297	tggcttatcctgtact	4-10-2	TGGCttatcctgtaCT	297_1	7526	-25
298	atggcttatcctgtac	2-10-4	ATggcttatcctGTAC	298_1	7527	-22
299	tatggcttatcctgta	4-10-2	TATGgcttatcctgTA	299_1	7528	-22
300	gtatggcttatcctgt	3-10-3	GTAtggcttatccTGT	300_1	7529	-23
301	atgaatatatgcccagt	2-11-4	ATgaatatatgccCAGT	301_1	7547	-22

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303	caagatgaatatatgcc	3-10-4	CAAgatgaatataTGCC	303_1	7551	-21
304	gacaacatcagtataga	4-9-4	GACAacatcagtaTAGA	304_1	7572	-22
305	caagacaacatcagta	4-8-4	CAAGacaacatcAGTA	305_1	7576	-20
306	cactcctagttccttt	3-10-3	CACtcctagttccTTT	306_1	7601	-22
307	aacactcctagttcct	3-10-3	AACactcctagttCCT	307_1	7603	-22
308	taacactcctagttcc	2-11-3	TAacactcctagtTCC	308_1	7604	-20
309	ctaacactcctagttc	2-12-2	CTaacactcctagtTC	309_1	7605	-18
310	tgataacataactgtg	2-12-2	TGataacataactgTG	310_1	7637	-13
311	ctgataacataactgt	2-10-4	CTgataacataaCTGT	311_1	7638	-18
312	tttgaactcaagtgac	4-10-2	TTTGaactcaagtgAC	312_1	7654	-16
313	tcctttacttagctag	4-9-3	TCCTttacttagcTAG	313_1	7684	-23
314	gagtttggattagctg	2-11-3	GAgtttggattagCTG	314_1	7764	-20
315	tgggatatgacaggga	2-11-3	TGggatatgacagGGA	315_1	7838	-21
316	tgtgggatatgacagg	4-10-2	TGTGggatatgacaGG	316_1	7840	-22
317	atatggaagggatatc	4-10-2	ATATggaagggataTC	317_1	7875	-17
318	acaggatatggaaggg	3-10-3	ACAggatatggaaGGG	318_1	7880	-21
319	atttcaacaggatatgg	4-9-4	ATTTcaacaggatATGG	319_1	7885	-20
320	gagtaatttcaacagg	2-11-3	GAgtaatttcaacAGG	320_1	7891	-17
321	agggagtaatttcaaca	4-9-4	AGGGagtaatttcAACA	321_1	7893	-22
322	attagggagtaatttca	4-9-4	ATTAgggagtaatTTCA	322_1	7896	-21
323	cttactattagggagt	2-10-4	CTtactattaggGAGT	323_1	7903	-20
324	cagcttactattaggg	2-11-3	CAgcttactattaGGG	324_1	7906	-20
326	atttcagcttactattag	3-11-4	ATTtcagcttactaTTAG	326_1	7908	-20
325	tcagcttactattagg	3-10-3	TCAgcttactattAGG	325_1	7907	-20
327	ttcagcttactattag	2-10-4	TTcagcttactaTTAG	327_1	7908	-17
328	cagatttcagcttact	4-10-2	CAGAtttcagcttaCT	328_1	7913	-21
329	gactacaactagaggg	3-11-2	GACtacaactagagGG	329_1	7930	-19
330	agactacaactagagg	4-10-2	AGACtacaactagaGG	330_1	7931	-19
331	aagactacaactagag	2-12-2	AAgactacaactagAG	331_1	7932	-13
332	atgatttaattctagtcaaa	4-12-4	ATGAtttaattctagtCAAA	332_1	7982	-20
333	tttaattctagtcaaa	3-10-3	TTTaattctagtcAAA	333_1	7982	-12
334	gatttaattctagtca	4-8-4	GATTtaattctaGTCA	334_1	7984	-20
771	tgatttaattctagtca	3-10-4	TGAtttaattctaGTCA	771_1	7984	-20
335	atgatttaattctagtca	4-11-3	ATGAtttaattctagTCA	335_1	7984	-20
336	gatgatttaattctagtca	4-13-2	GATGatttaattctagtCA	336_1	7984	-20
337	gatttaattctagtca	2-10-4	GAtttaattctaGTCA	337_1	7984	-18
338	gatgatttaattctagtc	4-11-3	GATGatttaattctaGTC	338_1	7985	-20
339	tgatttaattctagtc	2-12-2	TGatttaattctagTC	339_1	7985	-13
340	gagatgatttaattcta	4-9-4	GAGAtgatttaatTCTA	340_1	7988	-20
341	gagatgatttaattct	3-10-3	GAGatgatttaatTCT	341_1	7989	-16
342	cagattgatggtagtt	4-10-2	CAGAttgatggtagTT	342_1	8030	-19

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SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 1	dG
343	ctcagattgatggtag	2-10-4	CTcagattgatgGTAG	343_1	8032	-20
344	gttagccctcagattg	3-10-3	GTTagccctcagaTTG	344_1	8039	-23
345	tgtattgttagccctc	2-10-4	TGtattgttagcCCTC	345_1	8045	-24
346	acttgtattgttagcc	2-10-4	ACttgtattgttAGCC	346_1	8048	-22
347	agccagtatcagggac	3-11-2	AGCcagtatcagggAC	347_1	8191	-23
348	ttgacaatagtggcat	2-10-4	TTgacaatagtgGCAT	348_1	8213	-20
349	acaagtggtatcttct	3-10-3	ACAagtggtatctTCT	349_1	8228	-19
350	aatctactttacaagt	4-10-2	AATCtactttacaaGT	350_1	8238	-16
351	cacagtagatgcctgata	2-12-4	CAcagtagatgcctGATA	351_1	8351	-24
352	gaacacagtagatgcc	2-11-3	GAacacagtagatGCC	352_1	8356	-21
353	cttggaacacagtagat	4-11-2	CTTGgaacacagtagAT	353_1	8359	-20
354	atatcttggaacacag	3-10-3	ATAtcttggaacaCAG	354_1	8364	-18
355	tctttaatatcttggaac	3-11-4	TCTttaatatcttgGAAC	355_1	8368	-19
356	tgatttctttaatatcttg	2-13-4	TGatttctttaatatCTTG	356_1	8372	-19
357	tgatgatttctttaatatc	2-13-4	TGatgatttctttaaTATC	357_1	8375	-18
358	aggctaagtcatgatg	3-11-2	AGGctaagtcatgaTG	358_1	8389	-19
359	ttgatgaggctaagtc	4-10-2	TTGAtgaggctaagTC	359_1	8395	-19
360	ccaggattatactctt	3-11-2	CCAggattatactcTT	360_1	8439	-20
361	gccaggattatactct	2-10-4	GCcaggattataCTCT	361_1	8440	-23
362	ctgccaggattatact	3-11-2	CTGccaggattataCT	362_1	8442	-21
363	cagaaacttatactttatg	4-13-2	CAGAaacttatactttaTG	363_1	8473	-19
364	aagcagaaacttatact	4-9-4	AAGCagaaacttaTACT	364_1	8478	-20
365	gaagcagaaacttatact	3-11-4	GAAgcagaaacttaTACT	365_1	8478	-20
366	tggaagcagaaacttatact	3-15-2	TGGaagcagaaacttataCT	366_1	8478	-21
367	tggaagcagaaacttatac	3-13-3	TGGaagcagaaacttaTAC	367_1	8479	-20
368	aagcagaaacttatac	2-11-3	AAgcagaaacttaTAC	368_1	8479	-13
369	tggaagcagaaacttata	3-11-4	TGGaagcagaaactTATA	369_1	8480	-21
370	aagggatattatggag	4-10-2	AAGGgatattatggAG	370_1	8587	-18
371	tgccggaagatttcct	2-12-2	TGccggaagatttcCT	371_1	8641	-21
372	atggattgggagtaga	4-10-2	ATGGattgggagtaGA	372_1	8772	-21
373	agatggattgggagta	2-12-2	AGatggattgggagTA	373_1	8774	-18
374	aagatggattgggagt	3-11-2	AAGatggattgggaGT	374_1	8775	-18
375	acaagatggattggga	2-10-4	ACaagatggattGGGA	375_1	8777	-20
375	acaagatggattggga	2-12-2	ACaagatggattggGA	375_2	8777	-17
376	agaaggttcagacttt	3-9-4	AGAaggttcagaCTTT	376_1	8835	-20
377	gcagaaggttcagact	2-11-3	GCagaaggttcagACT	377_1	8837	-21
377	gcagaaggttcagact	3-11-2	GCAgaaggttcagaCT	377_2	8837	-22
378	tgcagaaggttcagac	4-10-2	TGCAgaaggttcagAC	378_1	8838	-22
379	agtgcagaaggttcag	2-11-3	AGtgcagaaggttCAG	379_1	8840	-20
379	agtgcagaaggttcag	4-10-2	AGTGcagaaggttcAG	379_2	8840	-21
380	aagtgcagaaggttca	4-10-2	AAGTgcagaaggttCA	380_1	8841	-20
381	taagtgcagaaggttc	2-10-4	TAagtgcagaagGTTC	381_1	8842	-19

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SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 1	dG
382	tctaagtgcagaaggt	2-10-4	TCtaagtgcagaAGGT	382_1	8844	-21
383	ctcaggagttctacttc	3-12-2	CTCaggagttctactTC	383_1	8948	-20
384	ctcaggagttctactt	3-10-3	CTCaggagttctaCTT	384_1	8949	-21
385	atggaggtgactcaggag	1-15-2	AtggaggtgactcaggAG	385_1	8957	-20
386	atggaggtgactcagga	2-13-2	ATggaggtgactcagGA	386_1	8958	-21
387	atggaggtgactcagg	2-11-3	ATggaggtgactcAGG	387_1	8959	-21
388	tatggaggtgactcagg	2-12-3	TAtggaggtgactcAGG	388_1	8959	-21
389	atatggaggtgactcagg	2-14-2	ATatggaggtgactcaGG	389_1	8959	-21
390	tatggaggtgactcag	4-10-2	TATGgaggtgactcAG	390_1	8960	-21
391	atatggaggtgactcag	2-11-4	ATatggaggtgacTCAG	391_1	8960	-22
392	catatggaggtgactcag	2-14-2	CAtatggaggtgactcAG	392_1	8960	-20
393	atatggaggtgactca	3-10-3	ATAtggaggtgacTCA	393_1	8961	-20
394	catatggaggtgactca	2-12-3	CAtatggaggtgacTCA	394_1	8961	-21
395	catatggaggtgactc	2-10-4	CAtatggaggtgACTC	395_1	8962	-20
396	gcatatggaggtgactc	2-13-2	GCatatggaggtgacTC	396_1	8962	-21
397	tgcatatggaggtgactc	2-14-2	TGcatatggaggtgacTC	397_1	8962	-21
398	ttgcatatggaggtgactc	1-16-2	TtgcatatggaggtgacTC	398_1	8962	-20
399	tttgcatatggaggtgactc	1-17-2	TttgcatatggaggtgacTC	399_1	8962	-21
400	gcatatggaggtgact	2-12-2	GCatatggaggtgaCT	400_1	8963	-20
401	tgcatatggaggtgact	2-13-2	TGcatatggaggtgaCT	401_1	8963	-20
402	ttgcatatggaggtgact	3-13-2	TTGcatatggaggtgaCT	402_1	8963	-22
403	tttgcatatggaggtgact	1-16-2	TttgcatatggaggtgaCT	403_1	8963	-20
404	tgcatatggaggtgac	3-11-2	TGCatatggaggtgAC	404_1	8964	-20
405	ttgcatatggaggtgac	3-11-3	TTGcatatggaggtGAC	405_1	8964	-21
406	tttgcatatggaggtgac	4-12-2	TTTGcatatggaggtgAC	406_1	8964	-21
407	tttgcatatggaggtga	4-11-2	TTTGcatatggaggtGA	407_1	8965	-21
408	tttgcatatggaggtg	2-10-4	TTtgcatatggaGGTG	408_1	8966	-21
409	aagtgaagttcaacagc	2-11-4	AAgtgaagttcaaCAGC	409_1	8997	-20
410	tgggaagtgaagttca	2-10-4	TGggaagtgaagTTCA	410_1	9002	-20
411	atgggaagtgaagttc	2-11-3	ATgggaagtgaagTTC	411_1	9003	-17
412	gatgggaagtgaagtt	4-9-3	GATGggaagtgaaGTT	412_1	9004	-21
413	ctgtgatgggaagtgaa	3-11-3	CTGtgatgggaagtGAA	413_1	9007	-20
414	attgagtgaatccaaa	3-10-3	ATTgagtgaatccAAA	414_1	9119	-14
415	aattgagtgaatccaa	2-10-4	AAttgagtgaatCCAA	415_1	9120	-16
416	gataattgagtgaatcc	4-10-3	GATAattgagtgaaTCC	416_1	9122	-20
417	gtgataattgagtgaa	3-10-3	GTGataattgagtGAA	417_1	9125	-16
418	aagaaaggtgcaataa	3-10-3	AAGaaaggtgcaaTAA	418_1	9155	-14
419	caagaaaggtgcaata	2-10-4	CAagaaaggtgcAATA	419_1	9156	-15
420	acaagaaaggtgcaat	4-10-2	ACAAgaaaggtgcaAT	420_1	9157	-16
421	atttaaactcacaaac	2-12-2	ATttaaactcacaaAC	421_1	9171	-10
422	ctgttaggttcagcga	2-10-4	CTgttaggttcaGCGA	422_1	9235	-24
423	tctgaatgaacatttcg	4-9-4	TCTGaatgaacatTTCG	423_1	9260	-20

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424	ctcattgaaggttctg	2-10-4	CTcattgaaggtTCTG	424_1	9281	-20
425	ctaatctcattgaagg	3-11-2	CTAatctcattgaaGG	425_1	9286	-17
426	cctaatctcattgaag	2-12-2	CCtaatctcattgaAG	426_1	9287	-16
427	actttgatctttcagc	3-10-3	ACTttgatctttcAGC	427_1	9305	-20
428	actatgcaacactttg	2-12-2	ACtatgcaacacttTG	428_1	9315	-15
429	caaatagctttatcgg	3-10-3	CAAatagctttatCGG	429_1	9335	-17
430	ccaaatagctttatcg	2-10-4	CCaaatagctttATCG	430_1	9336	-19
431	tccaaatagctttatc	4-10-2	TCCAaatagctttaTC	431_1	9337	-18
432	gatccaaatagcttta	4-10-2	GATCcaaatagcttTA	432_1	9339	-18
433	atgatccaaatagctt	2-10-4	ATgatccaaataGCTT	433_1	9341	-19
434	tatgatccaaatagct	4-10-2	TATGatccaaatagCT	434_1	9342	-18
435	taaacagggctgggaat	4-9-4	TAAAcagggctggGAAT	435_1	9408	-22
436	acttaaacagggctgg	2-10-4	ACttaaacagggCTGG	436_1	9412	-21
437	acacttaaacagggct	2-10-4	ACacttaaacagGGCT	437_1	9414	-22
438	gaacacttaaacaggg	4-8-4	GAACacttaaacAGGG	438_1	9416	-20
439	agagaacacttaaacag	4-9-4	AGAGaacacttaaACAG	439_1	9418	-20
440	ctacagagaacactta	4-8-4	CTACagagaacaCTTA	440_1	9423	-20
441	atgctacagagaacact	3-10-4	ATGctacagagaaCACT	441_1	9425	-22
442	ataaatgctacagagaaca	4-11-4	ATAAatgctacagagAACA	442_1	9427	-20
443	agataaatgctacagaga	2-12-4	AGataaatgctacaGAGA	443_1	9430	-20
444	tagagataaatgctaca	4-9-4	TAGAgataaatgcTACA	444_1	9434	-21
445	tagatagagataaatgct	4-11-3	TAGAtagagataaatGCT	445_1	9437	-20
446	caatatactagatagaga	4-10-4	CAATatactagataGAGA	446_1	9445	-21
447	tacacaatatactagatag	4-11-4	TACAcaatatactagATAG	447_1	9448	-21
448	ctacacaatatactag	3-10-3	CTAcacaatatacTAG	448_1	9452	-16
449	gctacacaatatacta	4-8-4	GCTAcacaatatACTA	449_1	9453	-21
450	atatgctacacaatatac	4-10-4	ATATgctacacaatATAC	450_1	9455	-20
451	tgatatgctacacaat	4-8-4	TGATatgctacaCAAT	451_1	9459	-20
452	atgatatgatatgctac	4-9-4	ATGAtatgatatgCTAC	452_1	9464	-21
453	gaggagagagacaataaa	4-10-4	GAGGagagagacaaTAAA	453_1	9495	-20
454	ctaggaggagagagaca	3-11-3	CTAggaggagagagACA	454_1	9500	-22
455	tattctaggaggagaga	4-10-3	TATTctaggaggagAGA	455_1	9504	-21
456	ttatattctaggaggag	4-10-3	TTATattctaggagGAG	456_1	9507	-21
457	gtttatattctaggag	3-9-4	GTTtatattctaGGAG	457_1	9510	-20
458	tggagtttatattctagg	2-12-4	TGgagtttatattcTAGG	458_1	9512	-22
459	cgtaccaccactctgc	2-11-3	CGtaccaccactcTGC	459_1	9590	-25
460	tgaggaaatcattcattc	4-10-4	TGAGgaaatcattcATTC	460_1	9641	-22
461	tttgaggaaatcattcat	4-10-4	TTTGaggaaatcatTCAT	461_1	9643	-20
462	aggctaatcctatttg	4-10-2	AGGCtaatcctattTG	462_1	9657	-22
463	tttaggctaatcctat	4-8-4	TTTAggctaatcCTAT	463_1	9660	-22
464	tgctccagtgtaccct	3-11-2	TGCtccagtgtaccCT	464_1	9755	-27
465	tagtagtactcgatag	2-10-4	TAgtagtactcgATAG	465_1	9813	-18

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SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 1	dG
466	ctaattgtagtagtactc	3-12-3	CTAattgtagtagtaCTC	466_1	9818	-20
467	tgctaattgtagtagt	2-10-4	TGctaattgtagTAGT	467_1	9822	-19
468	agtgctaattgtagta	4-10-2	AGTGctaattgtagTA	468_1	9824	-19
469	gcaagtgctaattgta	4-10-2	GCAAgtgctaattgTA	469_1	9827	-20
470	gaggaaatgaactaattta	4-13-2	GAGGaaatgaactaattTA	470_1	9881	-18
471	caggaggaaatgaacta	4-11-2	CAGGaggaaatgaacTA	471_1	9886	-19
472	ccctagagtcatttcc	2-11-3	CCctagagtcattTCC	472_1	9902	-24
473	atcttacatgatgaagc	3-11-3	ATCttacatgatgaAGC	473_1	9925	-20
475	agacacactcagatttcag	2-15-2	AGacacactcagatttcAG	475_1	9967	-20
474	gacacactcagatttcag	3-13-2	GACacactcagatttcAG	474_1	9967	-20
476	aagacacactcagatttcag	3-15-2	AAGacacactcagatttcAG	476_1	9967	-21
477	agacacactcagatttca	2-13-3	AGacacactcagattTCA	477_1	9968	-20
478	aagacacactcagatttca	3-13-3	AAGacacactcagattTCA	478_1	9968	-21
479	aaagacacactcagatttca	2-14-4	AAagacacactcagatTTCA	479_1	9968	-20
480	gaaagacacactcagatttc	3-14-3	GAAagacacactcagatTTC	480_1	9969	-20
481	aagacacactcagatttc	4-11-3	AAGAcacactcagatTTC	481_1	9969	-21
482	aaagacacactcagatttc	4-11-4	AAAGacacactcagaTTTC	482_1	9969	-20
483	tgaaagacacactcagattt	4-14-2	TGAAagacacactcagatTT	483_1	9970	-20
484	tgaaagacacactcagatt	2-13-4	TGaaagacacactcaGATT	484_1	9971	-21
485	tgaaagacacactcagat	3-12-3	TGAaagacacactcaGAT	485_1	9972	-20
486	attgaaagacacactca	4-10-3	ATTGaaagacacacTCA	486_1	9975	-19
487	tcattgaaagacacact	2-11-4	TCattgaaagacaCACT	487_1	9977	-18
488	ttccatcattgaaaga	3-9-4	TTCcatcattgaAAGA	488_1	9983	-18
489	ataataccacttatcat	4-9-4	ATAAtaccacttaTCAT	489_1	10010	-20
490	ttacttaatttctttgga	2-12-4	TTacttaatttcttTGGA	490_1	10055	-20
491	ttagaactagctttatca	3-12-3	TTAgaactagctttaTCA	491_1	10101	-20
492	gaggtacaaatatagg	3-10-3	GAGgtacaaatatAGG	492_1	10171	-18
493	cttatgatacaactta	3-10-3	CTTatgatacaacTTA	493_1	10384	-15
494	tcttatgatacaactt	2-11-3	TCttatgatacaaCTT	494_1	10385	-15
495	ttcttatgatacaact	3-11-2	TTCttatgatacaaCT	495_1	10386	-15
496	cagtttcttatgatac	2-11-3	CAgtttcttatgaTAC	496_1	10390	-16
497	gcagtttcttatgata	3-11-2	GCAgtttcttatgaTA	497_1	10391	-19
498	tacaaatgtctattaggtt	4-12-3	TACAaatgtctattagGTT	498_1	10457	-21
499	tgtacaaatgtctattag	4-11-3	TGTAcaaatgtctatTAG	499_1	10460	-20
500	agcatcacaattagta	3-11-2	AGCatcacaattagTA	500_1	10535	-18
501	ctaatgatagtgaagc	3-11-2	CTAatgatagtgaaGC	501_1	10548	-17
502	agctaatgatagtgaa	3-11-2	AGCtaatgatagtgAA	502_1	10550	-16
503	atgccttgacatatta	4-10-2	ATGCcttgacatatTA	503_1	10565	-20
504	ctcaagattattgacac	4-9-4	CTCAagattattgACAC	504_1	10623	-20
505	acctcaagattattga	2-10-4	ACctcaagattaTTGA	505_2	10626	-18
505	acctcaagattattga	3-9-4	ACCtcaagattaTTGA	505_1	10626	-20
506	aacctcaagattattg	4-10-2	AACCtcaagattatTG	506_1	10627	-17

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SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 1	dG
507	cacaaacctcaagattatt	4-13-2	CACAaacctcaagattaTT	507_1	10628	-20
508	gtacttaattagacct	3-9-4	GTActtaattagACCT	508_1	10667	-21
509	agtacttaattagacc	4-9-3	AGTActtaattagACC	509_1	10668	-20
510	gtatgaggtggtaaac	4-10-2	GTATgaggtggtaaAC	510_1	10688	-18
511	aggaaacagcagaagtg	2-11-4	AGgaaacagcagaAGTG	511_1	10723	-21
512	gcacaacccagaggaa	2-12-2	GCacaacccagaggAA	512_1	10735	-20
513	caagcacaacccagag	3-11-2	CAAgcacaacccagAG	513_1	10738	-20
514	ttcaagcacaacccag	3-10-3	TTCaagcacaaccCAG	514_1	10740	-21
515	aattcaagcacaaccc	2-10-4	AAttcaagcacaACCC	515_1	10742	-20
516	taataattcaagcacaacc	4-13-2	TAATaattcaagcacaaCC	516_1	10743	-20
517	actaataattcaagcac	4-9-4	ACTAataattcaaGCAC	517_1	10747	-20
518	ataatactaataattcaagc	4-12-4	ATAAtactaataattcAAGC	518_1	10749	-19
519	tagatttgtgaggtaa	2-10-4	TAgatttgtgagGTAA	519_1	11055	-18
520	agccttaattctccat	4-10-2	AGCCttaattctccAT	520_1	11091	-24
521	aatgatctagagcctta	4-9-4	AATGatctagagcCTTA	521_1	11100	-22
522	ctaatgatctagagcc	3-10-3	CTAatgatctagaGCC	522_1	11103	-22
523	actaatgatctagagc	3-9-4	ACTaatgatctaGAGC	523_1	11104	-21
524	cattaacatgttcttatt	3-11-4	CATtaacatgttctTATT	524_1	11165	-19
525	acaagtacattaacatgttc	4-12-4	ACAAgtacattaacatGTTC	525_1	11170	-22
526	ttacaagtacattaacatg	4-11-4	TTACaagtacattaaCATG	526_1	11173	-20
527	gctttattcatgtttat	4-9-4	GCTTtattcatgtTTAT	527_1	11195	-22
528	gctttattcatgttta	3-11-2	GCTttattcatgttTA	528_1	11196	-18
529	agagctttattcatgttt	3-13-2	AGAgctttattcatgtTT	529_1	11197	-20
530	ataagagctttattcatg	4-10-4	ATAAgagctttattCATG	530_1	11200	-21
531	cataagagctttattca	4-9-4	CATAagagctttaTTCA	531_1	11202	-21
532	agcataagagctttat	4-8-4	AGCAtaagagctTTAT	532_1	11205	-22
533	tagattgtttagtgca	3-10-3	TAGattgtttagtGCA	533_1	11228	-20
534	gtagattgtttagtgc	2-10-4	GTagattgtttaGTGC	534_1	11229	-21
535	gacaattctagtagatt	4-9-4	GACAattctagtaGATT	535_1	11238	-21
536	ctgacaattctagtag	3-9-4	CTGacaattctaGTAG	536_1	11241	-20
537	gctgacaattctagta	4-10-2	GCTGacaattctagTA	537_1	11242	-21
538	aggattaagatacgta	2-12-2	AGgattaagatacgTA	538_1	11262	-15
539	caggattaagatacgt	2-11-3	CAggattaagataCGT	539_1	11263	-17
540	tcaggattaagatacg	3-11-2	TCAggattaagataCG	540_1	11264	-16
541	ttcaggattaagatac	2-10-4	TTcaggattaagATAC	541_1	11265	-15
542	aggaagaaagtttgattc	4-10-4	AGGAagaaagtttgATTC	542_1	11308	-21
543	tcaaggaagaaagtttga	4-10-4	TCAAggaagaaagtTTGA	543_1	11311	-20
544	ctcaaggaagaaagtttg	4-10-4	CTCAaggaagaaagTTTG	544_1	11312	-20
545	tgctcaaggaagaaagt	3-10-4	TGCtcaaggaagaAAGT	545_1	11315	-21
546	aattatgctcaaggaaga	4-11-3	AATTatgctcaaggaAGA	546_1	11319	-20
547	taggataccacattatga	4-12-2	TAGGataccacattatGA	547_1	11389	-22
548	cataatttattccattcctc	2-15-3	CAtaatttattccattcCTC	548_1	11449	-22

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SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start ID NO: 1	dG
549	tgcataatttattccat	4-10-3	TGCAtaatttattcCAT	549_1	11454	-22
550	actgcataatttattcc	4-10-3	ACTGcataatttatTCC	550_1	11456	-21
551	ctaaactgcataatttatt	4-11-4	CTAAactgcataattTATT	551_1	11458	-20
552	ataactaaactgcata	2-10-4	ATaactaaactgCATA	552_1	11465	-16
553	ttattaataactaaactgc	3-12-4	TTAttaataactaaaCTGC	553_1	11468	-19
554	tagtacattattaataact	4-13-2	TAGTacattattaataaCT	554_1	11475	-18
555	cataactaaggacgtt	4-10-2	CATAactaaggacgTT	555_1	11493	-17
556	tcataactaaggacgt	2-11-3	TCataactaaggaCGT	556_1	11494	-16
557	cgtcataactaaggac	4-10-2	CGTCataactaaggAC	557_1	11496	-17
558	tcgtcataactaagga	2-12-2	TCgtcataactaagGA	558_1	11497	-16
559	atcgtcataactaagg	2-10-4	ATcgtcataactAAGG	559_1	11498	-17
560	gttagtatcttacatt	2-11-3	GTtagtatcttacATT	560_1	11525	-15
561	ctctattgttagtatc	3-10-3	CTCtattgttagtATC	561_1	11532	-17
562	agtatagagttactgt	3-10-3	AGTatagagttacTGT	562_1	11567	-19
563	ttcctggtgatacttt	4-10-2	TTCCtggtgatactTT	563_1	11644	-21
564	gttcctggtgatactt	4-10-2	GTTCctggtgatacTT	564_1	11645	-21
565	tgttcctggtgatact	2-12-2	TGttcctggtgataCT	565_1	11646	-20
566	ataaacatgaatctctcc	2-12-4	ATaaacatgaatctCTCC	566_1	11801	-20
567	ctttataaacatgaatctc	3-12-4	CTTtataaacatgaaTCTC	567_1	11804	-19
568	ctgtctttataaacatg	3-10-4	CTGtctttataaaCATG	568_1	11810	-19
569	ttgttataaatctgtctt	2-12-4	TTgttataaatctgTCTT	569_1	11820	-18
570	ttaaatttattcttggata	3-12-4	TTAaatttattcttgGATA	570_1	11849	-19
571	cttaaatttattcttgga	2-12-4	CTtaaatttattctTGGA	571_1	11851	-19
572	cttcttaaatttattcttg	4-13-2	CTTCttaaatttattctTG	572_1	11853	-18
573	tatgtttctcagtaaag	4-9-4	TATGtttctcagtAAAG	573_1	11877	-19
574	gaattatctttaaacca	3-10-4	GAAttatctttaaACCA	574_1	11947	-18
575	cccttaaatttctaca	3-11-2	CCCttaaatttctaCA	575_1	11980	-20
576	acactgctcttgtacc	4-10-2	ACACtgctcttgtaCC	576_1	11995	-23
577	tgacaacactgctctt	3-10-3	TGAcaacactgctCTT	577_1	12000	-21
578	tacatttattgggctc	4-10-2	TACAtttattgggcTC	578_1	12081	-19
579	gtacatttattgggct	2-10-4	GTacatttattgGGCT	579_1	12082	-23
580	ttggtacatttattgg	3-10-3	TTGgtacatttatTGG	580_1	12085	-18
581	catgttggtacatttat	4-10-3	CATGttggtacattTAT	581_1	12088	-21
582	aatcatgttggtacat	4-10-2	AATCatgttggtacAT	582_1	12092	-16
583	aaatcatgttggtaca	2-12-2	AAatcatgttggtaCA	583_1	12093	-14
584	gacaagtttggattaa	3-11-2	GACaagtttggattAA	584_1	12132	-14
585	aatgttcagatgcctc	2-10-4	AAtgttcagatgCCTC	585_1	12197	-21
586	gcttaatgttcagatg	2-12-2	GCttaatgttcagaTG	586_1	12201	-17
587	cgtacatagcttgatg	4-10-2	CGTAcatagcttgaTG	587_1	12267	-20
588	gtgaggaattaggata	3-11-2	GTGaggaattaggaTA	588_1	12753	-17
589	gtaacaatatggtttg	3-11-2	GTAacaatatggttTG	589_1	12780	-15
590	gaaatattgtagacta	2-11-3	GAaatattgtagaCTA	590_1	13151	-14

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591	ttgaaatattgtagac	3-11-2	TTGaaatattgtagAC	591_1	13153	-12
592	aagtctagtaatttgc	2-10-4	AAgtctagtaatTTGC	592_1	13217	-17
593	gctcagtagattataa	4-10-2	GCTCagtagattatAA	593_1	13259	-17
594	catacactgttgctaa	3-10-3	CATacactgttgcTAA	594_1	13296	-19
595	atggtctcaaatcatt	3-10-3	ATGgtctcaaatcATT	595_1	13314	-17
596	caatggtctcaaatca	4-10-2	CAATggtctcaaatCA	596_1	13316	-18
597	ttcctattgattgact	4-10-2	TTCCtattgattgaCT	597_1	13568	-20
598	tttctgttcacaacac	4-10-2	TTTCtgttcacaacAC	598_1	13600	-17
599	aggaacccactaatct	2-11-3	AGgaacccactaaTCT	599_1	13702	-20
600	taaatggcaggaaccc	3-11-2	TAAatggcaggaacCC	600_1	13710	-19
601	gtaaatggcaggaacc	4-10-2	GTAAatggcaggaaCC	601_1	13711	-20
602	ttgtaaatggcaggaa	2-11-3	TTgtaaatggcagGAA	602_1	13713	-16
603	ttatgagttaggcatg	2-10-4	TTatgagttaggCATG	603_1	13835	-19
604	ccaggtgaaactttaa	3-11-2	CCAggtgaaactttAA	604_1	13935	-17
605	cccttagtcagctcct	3-10-3	CCCttagtcagctCCT	605_1	13997	-30
606	acccttagtcagctcc	2-10-4	ACccttagtcagCTCC	606_1	13998	-27
607	cacccttagtcagctc	2-11-3	CAcccttagtcagCTC	607_1	13999	-24
608	tctcttactaggctcc	3-10-3	TCTcttactaggcTCC	608_1	14091	-24
609	cctatctgtcatcatg	2-11-3	CCtatctgtcatcATG	609_1	14178	-20
610	tcctatctgtcatcat	3-11-2	TCCtatctgtcatcAT	610_1	14179	-20
611	gagaagtgtgagaagc	3-11-2	GAGaagtgtgagaaGC	611_1	14808	-19
612	catccttgaagtttag	4-10-2	CATCcttgaagtttAG	612_1	14908	-19
613	taataagatggctccc	3-10-3	TAAtaagatggctCCC	613_1	15046	-21
614	caaggcataataagat	3-11-2	CAAggcataataagAT	614_1	15053	-14
615	ccaaggcataataaga	2-10-4	CCaaggcataatAAGA	615_1	15054	-18
616	tgatccaattctcacc	2-12-2	TGatccaattctcaCC	616_1	15151	-19
617	atgatccaattctcac	3-10-3	ATGatccaattctCAC	617_1	15152	-19
618	cgcttcatcttcaccc	3-11-2	CGCttcatcttcacCC	618_1	15260	-26
619	tatgacactgcatctt	2-10-4	TAtgacactgcaTCTT	619_1	15317	-19
620	gtatgacactgcatct	3-10-3	GTAtgacactgcaTCT	620_1	15318	-21
621	tgtatgacactgcatc	2-10-4	TGtatgacactgCATC	621_1	15319	-20
622	ttctcttctgtaagtc	4-10-2	TTCTcttctgtaagTC	622_1	15363	-19
623	ttctacagaggaacta	2-10-4	TTctacagaggaACTA	623_1	15467	-17
624	actacagttctacaga	3-10-3	ACTacagttctacAGA	624_1	15474	-19
625	ttcccacaggtaaatg	4-10-2	TTCCcacaggtaaaTG	625_1	15561	-21
626	attatttgaatatactcatt	4-12-4	ATTAtttgaatatactCATT	626_1	15594	-20
627	tgggaggaaattatttg	4-10-3	TGGGaggaaattatTTG	627_1	15606	-20
628	tgactcatcttaaatg	4-10-2	TGACtcatcttaaaTG	628_1	15621	-17
629	ctgactcatcttaaat	3-11-2	CTGactcatcttaaAT	629_1	15622	-16
630	tttactctgactcatc	3-10-3	TTTactctgactcATC	630_1	15628	-17
631	tattggaggaattatt	3-11-2	TATtggaggaattaTT	631_1	15642	-14
632	gtattggaggaattat	3-11-2	GTAttggaggaattAT	632_1	15643	-16

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633	tggtatacttctctaagtat	2-15-3	TGgtatacttctctaagTAT	633_1	15655	-22
634	gatctcttggtatact	4-10-2	GATCtcttggtataCT	634_1	15666	-20
635	cagacaactctatacc	2-12-2	CAgacaactctataCC	635_1	15689	-18
636	aacatcagacaactcta	4-9-4	AACAtcagacaacTCTA	636_1	15693	-21
637	taacatcagacaactc	4-10-2	TAACatcagacaacTC	637_1	15695	-16
638	tttaacatcagacaactc	4-10-4	TTTAacatcagacaACTC	638_1	15695	-20
639	atttaacatcagacaa	2-12-2	ATttaacatcagacAA	639_1	15698	-11
640	cctatttaacatcagac	2-11-4	CCtatttaacatcAGAC	640_1	15700	-20
641	tccctatttaacatca	3-10-3	TCCctatttaacaTCA	641_1	15703	-21
642	tcaacgactattggaat	4-9-4	TCAAcgactattgGAAT	642_1	15737	-20
643	cttatattctggctat	4-9-3	CTTAtattctggcTAT	643_1	15850	-20
644	atccttatattctggc	4-10-2	ATCCttatattctgGC	644_1	15853	-23
645	gatccttatattctgg	2-10-4	GAtccttatattCTGG	645_1	15854	-21
646	tgatccttatattctg	3-10-3	TGAtccttatattCTG	646_1	15855	-19
647	attgaaacttgatcct	4-8-4	ATTGaaacttgaTCCT	647_1	15864	-21
648	actgtcattgaaactt	2-10-4	ACtgtcattgaaACTT	648_1	15870	-16
649	tcttactgtcattgaa	3-11-2	TCTtactgtcattgAA	649_1	15874	-16
650	aggatcttactgtcatt	2-11-4	AGgatcttactgtCATT	650_1	15877	-21
651	gcaaatcaactccatc	3-10-3	GCAaatcaactccATC	651_1	15896	-20
652	gtgcaaatcaactcca	3-10-3	GTGcaaatcaactCCA	652_1	15898	-22
653	caattatttctttgtgc	4-10-3	CAATtatttctttgTGC	653_1	15910	-21
654	tggcaacaattatttctt	3-11-4	TGGcaacaattattTCTT	654_1	15915	-21
655	gctggcaacaattatt	3-9-4	GCTggcaacaatTATT	655_1	15919	-21
656	atccatttctactgcc	4-10-2	ATCCatttctactgCC	656_1	15973	-24
657	taatatctattgatttcta	4-11-4	TAATatctattgattTCTA	657_1	15988	-20
658	tcaatagtgtagggca	2-12-2	TCaatagtgtagggCA	658_1	16093	-18
659	ttcaatagtgtagggc	3-11-2	TTCaatagtgtaggGC	659_1	16094	-19
660	aggttaattaattcaatag	4-11-4	AGGTtaattaattcaATAG	660_1	16102	-21
661	catttgtaatccctag	3-10-3	CATttgtaatcccTAG	661_2	16163	-20
661	catttgtaatccctag	3-9-4	CATttgtaatccCTAG	661_1	16163	-22
662	acatttgtaatcccta	3-10-3	ACAtttgtaatccCTA	662_1	16164	-20
663	aacatttgtaatccct	2-10-4	AAcatttgtaatCCCT	663_2	16165	-21
663	aacatttgtaatccct	3-9-4	AACatttgtaatCCCT	663_1	16165	-22
664	taaatttcaagttctg	2-11-3	TAaatttcaagttCTG	664_1	16184	-14
665	gtttaaatttcaagttct	3-11-4	GTTtaaatttcaagTTCT	665_1	16185	-19
666	ccaagtttaaatttcaag	4-10-4	CCAAgtttaaatttCAAG	666_1	16189	-21
667	acccaagtttaaatttc	4-9-4	ACCCaagtttaaaTTTC	667_1	16192	-22
668	catacagtgacccaagttt	2-14-3	CAtacagtgacccaagTTT	668_1	16199	-23
669	acatcccatacagtga	2-11-3	ACatcccatacagTGA	669_1	16208	-21
670	agcacagctctacatc	2-10-4	AGcacagctctaCATC	670_1	16219	-22
671	atatagcacagctcta	3-9-4	ATAtagcacagcTCTA	671_1	16223	-21
672	tccatatagcacagct	3-11-2	TCCatatagcacagCT	672_1	16226	-22

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674	tttatttccatatagca	4-9-4	TTTAtttccatatAGCA	674_1	16231	-22
675	tttatttccatatagc	3-10-3	TTTatttccatatAGC	675_1	16232	-18
676	aaggagaggagattatg	4-9-4	AAGGagaggagatTATG	676_1	16409	-21
677	agttcttgtgttagct	3-11-2	AGTtcttgtgttagCT	677_1	16456	-21
678	gagttcttgtgttagc	2-12-2	GAgttcttgtgttaGC	678_1	16457	-20
679	attaattatccatccac	3-10-4	ATTaattatccatCCAC	679_1	16590	-21
680	atcaattaattatccatc	3-11-4	ATCaattaattatcCATC	680_1	16593	-19
681	agaatcaattaattatcc	3-12-3	AGAatcaattaattaTCC	681_1	16596	-18
682	tgagataccgtgcatg	2-12-2	TGagataccgtgcaTG	682_1	16656	-18
683	aatgagataccgtgca	2-10-4	AAtgagataccgTGCA	683_1	16658	-21
684	ctgtggttaggctaat	3-11-2	CTGtggttaggctaAT	684_1	16834	-19
685	aagagtaagggtctgtggtt	1-17-2	Aagagtaagggtctgtgg TT	685_1	16842	-21
686	gatgggttaagagtaa	4-9-3	GATGggttaagagTAA	686_1	16854	-19
687	agcagatgggttaaga	3-11-2	AGCagatgggttaaGA	687_1	16858	-20
688	tgtaaacatttgtagc	2-10-4	TGtaaacatttgTAGC	688_1	16886	-19
689	cctgcttataaatgta	3-11-2	CCTgcttataaatgTA	689_1	16898	-19
690	tgccctgcttataaat	4-10-2	TGCCctgcttataaAT	690_1	16901	-23
691	tcttcttagttcaata	2-12-2	TCttcttagttcaaTA	691_1	16935	-15
692	tggtttctaactacat	2-10-4	TGgtttctaactACAT	692_1	16980	-18
693	agtttggtttctaacta	2-12-3	AGtttggtttctaaCTA	693_1	16983	-19
694	gaatgaaacttgcctg	3-10-3	GAAtgaaacttgcCTG	694_1	17047	-18
695	attatccttacatgat	3-10-3	ATTatccttacatGAT	695_1	17173	-17
696	gtacccaattatcctt	2-11-3	GTacccaattatcCTT	696_1	17180	-21
697	tgtacccaattatcct	3-10-3	TGTacccaattatCCT	697_1	17181	-24
698	ttgtacccaattatcc	2-11-3	TTgtacccaattaTCC	698_1	17182	-20
699	tttgtacccaattatc	3-11-2	TTTgtacccaattaTC	699_1	17183	-17
700	agcagcaggttatatt	4-10-2	AGCAgcaggttataTT	700_1	17197	-22
701	tgggaagtggtctggg	3-10-3	TGGgaagtggtctGGG	701_1	17292	-25
702	ctggagagtgataata	3-11-2	CTGgagagtgataaTA	702_1	17322	-17
703	aatgctggattacgtc	4-10-2	AATGctggattacgTC	703_1	17354	-19
704	caatgctggattacgt	2-11-3	CAatgctggattaCGT	704_1	17355	-19
705	ttgttcagaagtatcc	2-10-4	TTgttcagaagtATCC	705_1	17625	-19
706	gatgatttgcttggag	2-10-4	GAtgatttgcttGGAG	706_1	17646	-21
707	gaaatcattcacaacc	3-10-3	GAAatcattcacaACC	707_1	17860	-17
708	ttgtaacatctactac	3-10-3	TTGtaacatctacTAC	708_1	17891	-16
709	cattaagcagcaagtt	3-11-2	CATtaagcagcaagTT	709_1	17923	-17
710	ttactagatgtgagca	3-11-2	TTActagatgtgagCA	710_1	17942	-18
711	tttactagatgtgagc	2-11-3	TTtactagatgtgAGC	711_1	17943	-18
712	gaccaagcaccttaca	3-11-2	GACcaagcaccttaCA	712_1	17971	-22
713	agaccaagcaccttac	3-10-3	AGAccaagcacctTAC	713_1	17972	-22
714	atgggttaaataaagg	2-10-4	ATgggttaaataAAGG	714_1	18052	-15

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715	tcaaccagagtattaa	2-12-2	TCaaccagagtattAA	715_1	18067	-13
716	gtcaaccagagtatta	3-11-2	GTCaaccagagtatTA	716_1	18068	-18
717	attgtaaagctgatat	2-11-3	ATtgtaaagctgaTAT	717_1	18135	-14
718	cacataattgtaaagc	2-10-4	CAcataattgtaAAGC	718_1	18141	-16
719	gaggtctgctatttac	2-11-3	GAggtctgctattTAC	719_1	18274	-19
720	tgtagattcaatgcct	2-11-3	TGtagattcaatgCCT	720_1	18404	-20
721	cctcattatactatga	2-11-3	CCtcattatactaTGA	721_1	18456	-19
722	ccttatgctatgacac	2-12-2	CCttatgctatgacAC	722_1	18509	-18
723	tccttatgctatgaca	4-10-2	TCCTtatgctatgaCA	723_1	18510	-22
724	aagatgtttaagtata	3-10-3	AAGatgtttaagtATA	724_1	18598	-13
725	ctgattattaagatgt	2-10-4	CTgattattaagATGT	725_1	18607	-17
726	tggaaaggtatgaatt	2-12-2	TGgaaaggtatgaaTT	726_1	18808	-13
727	acttgaatggcttgga	2-12-2	ACttgaatggcttgGA	727_1	18880	-18
728	aacttgaatggcttgg	3-10-3	AACttgaatggctTGG	728_1	18881	-19
729	caatgtgttactattt	4-10-2	CAATgtgttactatTT	729_1	19004	-16
730	acaatgtgttactatt	3-10-3	ACAatgtgttactATT	730_1	19005	-15
731	catctgctatataaga	4-10-2	CATCtgctatataaGA	731_1	19063	-18
732	cctagagcaaatactt	4-10-2	CCTAgagcaaatacTT	732_1	19223	-20
733	cagagttaataataag	3-10-3	CAGagttaataatAAG	733_1	19327	-13
734	gttcaagcacaacgaa	4-10-2	GTTCaagcacaacgAA	734_1	19493	-18
735	agggttcaagcacaac	2-11-3	AGggttcaagcacAAC	735_1	19496	-18
736	tgttggagacactgtt	2-12-2	TGttggagacactgTT	736_1	19677	-17
737	aaggaggagttaggac	3-11-2	AAGgaggagttaggAC	737_1	19821	-18
738	ctatgccatttacgat	4-10-2	CTATgccatttacgAT	738_1	19884	-21
739	tcaaatgcagaattag	2-12-2	TCaaatgcagaattAG	739_1	19913	-12
740	agtgacaatcaaatgc	2-10-4	AGtgacaatcaaATGC	740_1	19921	-18
741	aagtgacaatcaaatg	2-11-3	AAgtgacaatcaaATG	741_1	19922	-12
742	gtgtaccaagtaacaa	3-11-2	GTGtaccaagtaacAA	742_1	19978	-16
743	tgggatgttaaactga	3-10-3	TGGgatgttaaacTGA	743_1	20037	-20

Motif sequences represent the contiguous sequence of nucleobases present in the oligonucleotide.

Designs refer to the gapmer design, F-G-F', where each number represents the number of consecutive modified nucleosides, e.g. 2' modified nucleosides (first number=5' flank), followed by the number of DNA nucleosides (second number= gap region), followed by the number of modified nucleosides, e.g. 2' modified nucleosides (third number=3' flank), optionally preceded by or followed by further repeated regions of DNA and LNA, which are not necessarily part of the contiguous sequence that is complementary to the target nucleic acid.

Oligonucleotide compounds represent specific designs of a motif sequence. Capital letters represent beta-D-oxy LNA nucleosides, lowercase letters represent DNA nucleosides, all LNA C are 5-methyl cytosine, all internucleoside linkages are phosphorothioate internucleoside linkages.

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Table 6: Oligonucleotides targeting mouse PD-L1 transcript (SEQ ID NO: 4) designs of these, as well as specific oligonucleotide compounds (indicated by CMP ID NO) designed based on the motif sequence.

SEQ ID NO	Motif sequence	Design	Oligonucleotide Compound	CMP ID NO	Start on SEQ ID NO: 4	dG
744	agtttacattttctgc	3-10-3	AGTttacattttcTGC	744_1	4189	-20
745	tatgtgaagaggagag	3-10-3	TATgtgaagaggaGAG	745_1	7797	-19
746	caccttaaaacccca	3-10-3	CACctttaaaaccCCA	746_1	9221	-23
747	tcctttataatcacac	3-10-3	TCCtttataatcaCAC	747_1	10386	-19
748	acggtattttcacagg	3-10-3	ACGgtattttcacAGG	748_1	12389	-21
749	gacactacaatgagga	3-10-3	GACactacaatgaGGA	749_1	15088	-20
750	tggtttttaggactgt	3-10-3	TGGtttttaggacTGT	750_1	16410	-21
751	cgacaaattctatcct	3-10-3	CGAcaaattctatCCT	751_1	18688	-20
752	tgatatacaatgctac	3-10-3	TGAtatacaatgcTAC	752_1	18735	-16
753	tcgttgggtaaattta	3-10-3	TCGttgggtaaatTTA	753_1	19495	-17
754	tgctttataaatggtg	3-10-3	TGCtttataaatgGTG	754_1	19880	-19

Motif sequences represent the contiguous sequence of nucleobases present in the oligonucleotide.

Designs refer to the gapmer design, F-G-F', where each number represents the number of consecutive modified nucleosides, e.g. 2' modified nucleosides (first number=5' flank), followed by the number of DNA nucleosides (second number= gap region), followed by the number of modified nucleosides, e.g. 2' modified nucleosides (third number=3' flank), optionally preceded by or followed by further repeated regions of DNA and LNA, which are not necessarily part of the contiguous sequence that is complementary to the target nucleic acid.

Oligonucleotide compounds represent specific designs of a motif sequence. Capital letters represent beta-D-oxy LNA nucleosides, lowercase letters represent DNA nucleosides, all LNA C are 5-methyl cytosine, all internucleoside linkages are phosphorothioate internucleoside linkages.

Table 7: Oligonucleotide motif sequences and antisense compounds with 5' ca biocleavable linker.

SEQ ID NO	motif sequence	oligonucleotide compound with ca linker	CMP ID NO
755	caagtttacattttctgc	c_oa_oAGTttacattttcTGC	755_1
756	catatgtgaagaggagag	c_oa_oTATgtgaagaggaGAG	756_1
757	caccttaaaacccca	c_oa_oCACctttaaaaccCCA	757_1
758	catcctttataatcacac	c_oa_oTCCtttataatcaCAC	758_1
759	caacggtattttcacagg	c_oa_oACGgtattttcacAGG	759_1
760	cagacactacaatgagga	c_oa_oGACactacaatgaGGA	760_1
761	catggtttttaggactgt	c_oa_oTGGtttttaggacTGT	761_1
762	cacgacaaattctatcct	c_oa_oCGAcaaattctatCCT	762_1
763	catgatatacaatgctac	c_oa_oTGAtatacaatgcTAC	763_1
764	catcgttgggtaaattta	c_oa_oTCGttgggtaaatTTA	764_1
765	catgctttataaatggtg	c_oa_oTGCtttataaatgGTG	765_1

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SEQ ID NO	motif sequence	oligonucleotide compound with ca linker	CMP ID NO
766	caacaaataatggttactct	c_oa_oACAAataatggttaCTCT	766_1
767	cacagattgatggtagtt	c_oa_oCAGAttgatggtagTT	767_1
768	cacctatttaacatcagac	c_oa_oCCtatttaacatcAGAC	768_1
769	cactaattgtagtagtactc	c_oa_oCTAattgtagtagtaCTC	769_1
770	caataaacatgaatctctcc	c_oa_oATaaacatgaatctCTCC	770_1

Capital letters represent beta-D-oxy LNA nucleosides, lowercase letters represent DNA nucleosides, all LNA C are 5-methyl cytosine, subscript o represent a phosphodiester internucleoside linkage and unless otherwise indicated other internucleoside linkages are phosphorothioate internucleoside linkages.

Table 8: GalNAc conjugated antisense oligonucleotide compounds.

antisense oligonucleotide conjugate	**CMP ID NO**
GN2-C6$_o$c$_o$a$_o$AGTttacattttcTGC	755_2
GN2-C6$_o$c$_o$a$_o$TATgtgaagaggaGAG	756_2
GN2-C6$_o$c$_o$a$_o$CACctttaaaaccCCA	757_2
GN2-C6$_o$c$_o$a$_o$TCCtttataatcaCAC	758_2
GN2-C6$_o$c$_o$a$_o$ACGgtattttcacAGG	759_2
GN2-C6$_o$c$_o$a$_o$GACactacaatgaGGA	760_2
GN2-C6$_o$c$_o$a$_o$TGGtttttaggacTGT	761_2
GN2-C6$_o$c$_o$a$_o$CGAcaaattctatCCT	762_2
GN2-C6$_o$c$_o$a$_o$TGAtatacaatgcTAC	763_2
GN2-C6$_o$c$_o$a$_o$TCGttgggtaaatTTA	764_2
GN2-C6$_o$c$_o$a$_o$TGCtttataaatgGTG	765_2
GN2-C6$_o$c$_o$a$_o$ACAAataatggttaCTCT	766_2
GN2-C6$_o$c$_o$a$_o$CAGAttgatggtagTT	767_2
GN2-C6$_o$c$_o$a$_o$CCtatttaacatcAGAC	768_2
GN2-C6$_o$c$_o$a$_o$CTAattgtagtagtaCTC	769_2
GN2-C6$_o$c$_o$a$_o$ATaaacatgaatctCTCC	770_2

GN2 represents the trivalent GalNAc cluster shown in Figure 3, C6 represents an amino alkyl group with 6 carbons, capital letters represent beta-D-oxy LNA nucleosides, lowercase letters represent DNA nucleosides, all LNA C are 5-methyl cytosine, subscript o represent a phosphodiester nucleoside linkage and unless otherwise indicated internucleoside linkages are phosphorothioate internucleoside linkages. Chemical drawings representing some of the molecules are shown in figures 4 to 8.

AAV/HBV mouse models

Pasteur model:

HLA-A2.1-/HLA-DR1-transgenic H-2 class I-/class II-knockout (here referred to as HLA-A2/DR1) mice were created and bred at the Institut Pasteur. These mice represent an *in vivo* experimental model for human immune function studies without any interference with mouse MHC response (Pajot et al 2004 Eur J Immunol. 34(11):3060-9.

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Adeno-associated virus (AAV) vector, AAV serotype 2/8 carrying a replication competent HBV DNA genome was used in these studies. The AAV-HBV vector (batch GVPN #6163) was diluted in sterile Phosphate buffered Saline (PBS) to reach a titer of 5×10^{11} vg/mL. Mice were injected intravenously (i.v.) with 100µL of this diluted solution (dose/mouse: 5×10^{10} vg) in a tail vein. Complete viral particles containing HBV DNA were detected in the blood of HBV-carrier mice. HBcAg was detected for up to one year in the liver together with HBV circulating proteins HBeAg and HBsAg in the blood. In all AAV2/8-HBV-transduced mice, HBsAg, HBeAg, and HBV DNA persisted in serum for at least one year (Dion et al 2013 J Virol 87:5554-5563).

Shanghai model:

In this model, mice infected with a recombinant adeno-associated virus (AAV) carrying the HBV genome (AAV/HBV) maintains stable viremia and antigenimia for more than 30 weeks (Dan Yang, et al. 2014 Cellular & Molecular Immunology 11, 71–78).

Male C57BL/6 mice (4-6 weeks old), specific pathogen free, were purchased from SLAC (Shanghai Laboratory Animal Center of Chinese Academy of Sciences) and housed in an animal care facility in individually ventilated cages. Guidelines were followed for the care and use of animals as indicated by WuXi IACUC (Institutional Animal Care and Use Committee, WUXI IACUC protocol number R20131126-Mouse). Mice were allowed to acclimate to the new environment for 3 days and are grouped according to the experimental design.

Recombinant AAV-HBV was diluted in PBS, 200 µL per injection. This recombinant virus carries 1.3 copies of the HBV genome (genotype D, serotype ayw).

On day 0, all mice were injected through tail vein with 200 µL AAV-HBV. On days 6, 13 and 20 after AAV injection, all mice in were submandibularly bled (0.1 ml blood/mouse) for serum collection. On day 22 post injection, mice with stable viremia were ready for oligonucleotide treatment. The oligonucleotides can be unconjugated or GalNAc conjugated.

DNA vaccine

Plasmid DNA were endotoxin-free and manufactured by Plasmid-Factory (Germany). pCMV-S2.S ayw encodes the preS2 and S domains of the HBsAg (genotype D), and its expression is controlled by the cytomegalovirus immediate early gene promoter (Michel et al 1995 Proc Natl Acad Sci U S A 92:5307-5311). pCMV-HBc encodes the HBV capsid carrying the hepatitis core (HBc) Ag (Dion et al 2013 J Virol 87:5554-5563).

Treatment with DNA vaccine was conducted as described here. Five days prior to vacciantion cardiotoxine (CaTx, Latoxan refL81-02, 50 µl/ muscle) was injected into the muscle of the mice. CaTx depolarizees the muscular fibers to induce cell degeneration, 5 days post injection, new muscular fibers will appear and will receive the DNA vaccine for a better efficacy for transfection. The pCMV-S2.S ayw and pCMVCore at 1 mg/ml each were mixed in equal amount

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and each mouse received a total of 100 µg by bilateral intramuscular injection into cardiotoxin-treated tibialis anterior muscles as previously described in Michel et al 1995 Proc Natl Acad Sci U S A 92:5307-5311, under anesthesia (100 µL of 12.5 mg/mL ketamine, 1.25 mg/mL xylazine).

Anti-PD-L1 antibody

This is a mouse anti mouse PD-L1 IgG1 antibody clone 6E11 internally produced at Genetech. It is a surrogate antibody that cross blocks Atezolizumab and has similar in vitro blocking activity Atezolizumabproduced internally at Roche. The antibody was adminstredadministered by intraperitoneal (i.p.) injection at a dose of 12.5 µg/g.

Oligonucleotide synthesis

Oligonucleotide synthesis is generally known in the art. Below is a protocol which may be applied. The oligonucleotides of the present invention may have been produced by slightly varying methods in terms of apparatus, support and concentrations used.

Oligonucleotides are synthesized on uridine universal supports using the phosphoramidite approach on an Oligomaker 48 at 1 µmol scale. At the end of the synthesis, the oligonucleotides are cleaved from the solid support using aqueous ammonia for 5-16hours at 60°C. The oligonucleotides are purified by reverse phase HPLC (RP-HPLC) or by solid phase extractions and characterized by UPLC, and the molecular mass is further confirmed by ESI-MS.

Elongation of the oligonucleotide:

The coupling of β-cyanoethyl- phosphoramidites (DNA-A(Bz), DNA- G(ibu), DNA- C(Bz), DNA-T, LNA-5-methyl-C(Bz), LNA-A(Bz), LNA- G(dmf), or LNA-T) is performed by using a solution of 0.1 M of the 5'-O-DMT-protected amidite in acetonitrile and DCI (4,5–dicyanoimidazole) in acetonitrile (0.25 M) as activator. For the final cycle a phosphoramidite with desired modifications can be used, e.g. a C6 linker for attaching a conjugate group or a conjugate group as such. Thiolation for introduction of phosphorthioate linkages is carried out by using xanthane hydride (0.01 M in acetonitrile/pyridine 9:1). Phosphordiester linkages can be introduced using 0.02 M iodine in THF/Pyridine/water 7:2:1. The rest of the reagents are the ones typically used for oligonucleotide synthesis.

For post solid phase synthesis conjugation a commercially available C6 amino linker phosphoramidite can be used in the last cycle of the solid phase synthesis and after deprotection and cleavage from the solid support the aminolinked deprotected oligonucleotide is isolated. The conjugates are introduced via activation of the functional group using standard synthesis methods.

Alternatively, the conjugate moiety can be added to the oligonucleotide while still on the solid support by using a GalNAc- or GalNAc-cluster phosphoramidite as described in PCT/EP2015/073331 or in EP appl. NO. 15194811.4.

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Purification by RP-HPLC:

The crude compounds are purified by preparative RP-HPLC on a Phenomenex Jupiter C18 10µ 150x10 mm column. 0.1 M ammonium acetate pH 8 and acetonitrile is used as buffers at a flow rate of 5 mL/min. The collected fractions are lyophilized to give the purified compound typically as a white solid.

Abbreviations:

DCI: 4,5-Dicyanoimidazole

DCM: Dichloromethane

DMF: Dimethylformamide

DMT: 4,4'-Dimethoxytrityl

THF: Tetrahydrofurane

Bz: Benzoyl

Ibu: Isobutyryl

RP-HPLC: Reverse phase high performance liquid chromatography

T_m Assay

Oligonucleotide and RNA target (phosphate linked, PO) duplexes are diluted to 3 mM in 500 ml RNase-free water and mixed with 500 ml 2x T_m-buffer (200mM NaCl, 0.2mM EDTA, 20mM Naphosphate, pH 7.0). The solution is heated to 95ºC for 3 min and then allowed to anneal in room temperature for 30 min. The duplex melting temperatures (T_m) is measured on a Lambda 40 UV/VIS Spectrophotometer equipped with a Peltier temperature programmer PTP6 using PE Templab software (Perkin Elmer). The temperature is ramped up from 20ºC to 95ºC and then down to 25ºC, recording absorption at 260 nm. First derivative and the local maximums of both the melting and annealing are used to assess the duplex T_m.

Tissue specific In vitro linker cleavage assay

FAM-labeled oligonucleotides with the biocleavable linker to be tested (e.g. a DNA phosphodiester linker (PO linker)) are subjected to *in vitro* cleavage using homogenates of the relevant tissues (e.g. liver or kidney) and Serum.

The tissue and serum samples are collected from a suitable animal (e.g. mice, monkey, pig or rat) and homogenized in a homogenisation buffer (0,5% Igepal CA-630, 25 mM Tris pH 8.0, 100 mM NaCl, pH 8.0 (adjusted with 1 N NaOH).The tissue homogenates and Serum are spiked with oligonucleotide to concentrations of 200 µg/g tissue. The samples are incubated for 24 hours at 37°C and thereafter the samples are extracted with phenol - chloroform. The solutions are subjected to AIE HPLC analyses on a Dionex Ultimate 3000 using an Dionex DNApac p-100 column and a gradient ranging from 10mM – 1 M sodium perchlorate at pH 7.5. The content of cleaved and non-cleaved oligonucleotide are determined against a standard using both a fluorescence detector at 615 nm and a uv detector at 260 nm.

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S1 nuclease cleavage assay

FAM-labelled oligonucleotides with S1 nuclease susceptible linkers (e.g. a DNA phosphodiester linker (PO linker)) are subjected to *in vitro* cleavage in S1 nuclease extract or Serum.

100 µM of the oligonucleotides are subjected to *in vitro* cleavage by S1 nuclease in nuclease buffer (60 U pr. 100 µL) for 20 and 120 minutes. The enzymatic activity is stopped by adding EDTA to the buffer solution. The solutions are subjected to AIE HPLC analyses on a Dionex Ultimate 3000 using an Dionex DNApac p-100 column and a gradient ranging from 10mM – 1 M sodium perchlorate at pH 7.5. The content of cleaved and non-cleaved oligonucleotide is determined against a standard using both a fluorescence detector at 615 nm and a uv detector at 260 nm.

Preparation of liver mononuclear cells

Liver cells from AAV/HBV mice were prepared as described below and according to a method described by Tupin et al 2006 Methods Enzymol 417:185-201 with minor modifications. After mouse euthanasia, the liver was perfused with 10 ml of sterile PBS via hepatic portal vein using syringe with G25 needle. When organ is pale, the organ was harvested in Hank's Balanced Salt Solution (HBSS) (GIBCO® HBSS, 24020) + 5 % decomplemented fetal calf serum (FCS). The harvested liver was gently pressed through 100 µm cell-strainer (BD Falcon, 352360) and cells were suspended in 30 ml of HBSS + 5 % FCS. Cell suspension was centrifuged at 50 g for 5 min. Supernatants were then centrifuged at 289 g for 10 min at 4 °C. After centrifugation, supernatants were discarded and pellets were re-suspended in 15 mL at room temperature in a 35 % isotonic Percoll solution (GE Healthcare Percoll #17-0891-01 diluted into RPMI 1640 (GIBCO, 31870)) and transferred to a 15 ml tube. Cells were further centrifuged at 1360g for 25 min at room temperature. The supernatant was discarded by aspiration and the pellet containing mononuclear cells was washed twice with HBSS + 5 % FCS.

Cells were cultured in complete medium (α-minimal essential medium (Gibco, 22571) supplemented with 10 % FCS (Hyclone, # SH30066, lot APG21570), 100 U/mL penicillin + 100 µg/mL streptomycin + 0.3 mg/mL L-glutamine (Gibco, 10378), 1X non-essential amino acids (Gibco, 11140), 10 mM Hepes (Gibco, 15630), 1 mM sodium pyruvate (Gibco, 11360) and 50 µM β-mercaptoethanol (LKB, 1830)).

Surface labeling of cells

Cells were seeded in U-bottom 96-well plates and washed with PBS FACS (PBS containing 1 % bovine serum albumin and 0.01% sodium azide). Cells were incubated with 5 µL of PBS FACS containing a rat anti-mouse CD16/CD32 antibody and a viability marker LD fixable yellow, Thermofisher, L34959 for 10 min in the dark at 4°C. Then, cells were stained for 20 min in the dark at 4°C with 25 µL of PBS FACS containing monoclonal antibodies (Mab) against NK P46 BV421 (Rat Mab anti mouse NK P46, Biolegend, 137612) and F4/80 (rat Mab anti-mouse F4/80

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FITC, BD Biolegend, 123108) and two supplemental surface markers: PD1 (rat Mab anti-mouse PD1 PE, BD Biosciences, 551892) and PDL1 (rat Mab anti-mouse PDL1 BV711, Biolegend, 124319) were also added.

Intracelluar cytokine staining (ICS) assay

ICS assays were performed on both splenocytes and liver mononuclear cells. Cells were seeded in Ubottom 96-well plates. Plates with cells were incubated overnight at 37 °C either in complete medium alone as negative control or with the peptides described in Table 9 at a concentration of 2 µg/ml. Brefeldin A at 2µg/mL (Sigma, B6542) was added after one hour of incubation.

After the overnight culture, cells were washed with PBS FACS and incubated with 5 µL of PBS FACS containing rat anti-mouse CD16/CD32 antibody and a viability marker LD fixable yellow, Thermofisher, L34959 for 10 min in the dark at 4°C. Then, cells were stained for 20 min in the dark at 4°C with 25 µL of PBS FACS containing Mab. The mix was composed of monoclonal antibodies against CD3 (hamster Mab anti-mouse CD3-PerCP, BD Biosciences, 553067), CD8 (rat Mab anti-mouse CD8-APC-H7, BD Biosciences, 560182), CD4 (rat Mab anti-mouse CD4-PE-Cy7, BD Biosciences, 552775), and NK cells (Rat Mab anti mouse NK P46 BV421, Biolegend, 137612). Cells were fixed after several washes and permeabilized for 20 min in the dark at room temperature with Cytofix/Cytoperm, washed with Perm/Wash solution (BD Biosciences, 554714) at 4 °C.

Intracellular cytokine staining with antibodies against IFNγ (rat Mab anti-mouse IFNγ-APC, clone XMG1.2, BD Biosciences, 554413) and tumor necrosis factor alpha (TNFα) (rat Mab anti-mouse TNFα-FITC, clone MP6-XT22; 1/250 (BD Biosciences 554418) was performed for 30 min in the dark at 4 °C. Before analysis by flow cytometry using the MACSQuant Analyzer, cells were washed with Perm/Wash and re-suspended in PBS FACS containing 1% Formaldehyde.

Live CD3+CD8+CD4- and cells CD3+CD8-CD4+ were gated and presented on dot-plot. Two regions were defined to gate for positive cells for each cytokine. Numbers of events found in these gates were divided by total number of events in parental population to yield percentages of responding T cells. For each mouse, the percentage obtained in medium alone was considered as background and subtracted from the percentage obtained with peptide stimulations.

Threshold of positivity was defined according to experiment background i.e. the mean percentage of stained cells obtained for each group in medium alone condition plus two standard deviations. Only percentage of cytokine represented at least 5 events were considered as positive.

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Table 9: HLA-A2/DR1 restricted epitopes contained in the HBV Core protein and the Envelope domains of the HBsAg (S2+S).

Protein	Start Position	End Position	Sequence	HLA restriction	References
Core	18	27	FLPSDFFPSV (SEQ ID NO: 773)	A2	Bertoletti et al Gastroenterology 1997;112:193-199
	111	125	GRETVLEYLVSFGVW (SEQ ID NO: 774)	DR1	(Bertoletti et al Gastroenterology 1997;112:193-199
Envelope (S2+S)	114	128	TTFHQTLQDPRVRGL (SEQ ID NO: 775)	DR1	Pajot et al Microbes Infect 2006;8:2783-2790.
	179	194	QAGFFLLTRILTIPQS (SEQ ID NO: 776)	A2 + DR1	Pajot et al Microbes Infect 2006;8:2783-2790.
	183	191	FLLTRILTI (SEQ ID NO: 777)	A2	Sette et al J Immunol 1994;153:5586-5592.
	200	214	TSLNFLGGTTVCLGQ (SEQ ID NO: 778)	A2 + DR1	Pajot et al Microbes Infect 2006;8:2783-2790.
	204	212	FLGGTTVCL (SEQ ID NO: 779)	A2	Rehermann et al J Exp Med 1995;181: 1047-1058.
	335	343	WLSLLVPFV (SEQ ID NO: 780)	A2	Nayersina et alJ Immunol 1993;150: 4659-4671.
	337	357	SLLVPFVQWFVGLSPTVWLSV (SEQ ID NO: 781)	A2 + DR1	Loirat et al J Immunol 2000;165: 4748-4755
	348	357	GLSPTVWLSV (SEQ ID NO: 782)	A2	Loirat et al J Immunol 2000;165: 4748-4755
	370	379	SILSPFLPLL (SEQ ID NO: 783)	A2	Mizukoshi et al J Immunol 2004;173: 5863-5871.

Example 1 Testing in vitro efficacy

A gene walk was performed across the human PD-L1 transcript primarily using 16 to 20mer gapmers. Efficacy testing was performed in an *in vitro* experiment in the human leukemia monocytic cell line THP1 and in the human non-Hodgkin's K lymphoma cell line (KARPAS-299).

Cell lines

THP1 and Karpas-299 cell line were originally purchased from European Collection of Authenticated Cell Cultures (ECACC) and maintained as recommended by the supplier in a humidified incubator at 37°C with 5% CO_2.

Oligonucleotide efficacy

THP-1 cells (3.104 in RPMI-GLutamax, 10% FBS, 1% Pen-Strep (Thermo Fisher Scientific) were added to the oligonucleotides (4-5 ul) into 96-well round bottom plates and cultured for 6 days in a final volume of 100 µl/well. Oligonucleotides were screened at one single concentration (20 µM) and in dose-range concentrations from 25 µM to 0.004 µM (1:3 dilution in water). Total mRNA was extracted using the MagNA Pure 96 Cellular RNA Large Volume Kit on the MagNA Pure 96 System (Roche Diagnostics) according to the manufacturer's instructions.

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For gene expression analysis, RT-qPCR was performed using the TaqMan RNA-to-ct 1-Step kit (Thermo Fisher Scientific) on the QuantStudio machine (Applied Biosystems) with pre-designed Taqman primers targeting human PDL1 and ACTB used as endogenous control (Thermo Fisher Scientific). The relative PD-L1 mRNA expression level was calculated using 2(-Delta Delta C(T)) method and the percentage of inhibition as the % compared to the control sample (non-treated cells).

Karpas-299 cells were cultured in RPMI 1640, 2 mM Glutamine and 20% FBS (Sigma). The cells were plated at 10000 cell/well in 96 wells plates incubated for 24 hours before addition of oligonucleotides dissolved in PBS. Final concentration of oligonucleotides was in a single dose of 5 µM, in a final culture volume was 100 µl/well or added in a dose response ranging from 50 µM, 15.8 µM, 5.0 µM, 1.58 µM, 0.5 µM, 0.158 µM, 0.05 µM, to 0.0158 µM in 100 µL culture volume. The cells were harvested 3 days after addition of oligonucleotide compounds and RNA was extracted using the PureLink Pro 96 RNA Purification kit (Ambion), according to the manufacturer's instructions. cDNA was synthesized using M-MLT Reverse Transcriptase, random decamers RETROscript, RNase inhibitor (Ambion) and 100 mM dNTP set (Invitrogen, PCR Grade) according to the manufacturer's instruction. For gene expressions analysis, qPCR was performed using TaqMan Fast Advanced Master Mix (2X) (Ambion) in a duplex set up with TaqMan primer assays for the PD-L1 (Applied Biosystems; Hs01125299_m1) and TBP (Applied Biosystems; 4325803). The relative PD-L1 mRNA expression level is shown in table 10 as % of control sample (PBS-treated cells).

Table 10: *in vitro* efficacy of anti-PD-L1 compounds in THP1 and KARPAS-299 cell lines (Average from n=3 experiments). PD-L1 mRNA levels are normalized to TBP in KARPAS-299 cells or ACTB in THP1 cells and shown as % of control (PBS treated cells).

CMP ID NO	KARPAS-299 cells 5 µM CMP		THP1 cells 20 µM CMP		Compound (CMP)	Start on SEQ ID NO 1
	% mRNA of control	sd	% mRNA of control	sd		
4_1	50	1	32	11	TAattggctctacTGC	236
5_1	25	5	9	6	TCGCataagaatgaCT	371
6_1	29	2	15	5	TGaacacacagtcgCA	382
7_1	27	7	3	1	CTGaacacacagtCGC	383
8_1	23	4	11	3	TCTgaacacacagtCG	384
9_1	32	3	19	6	TTCtgaacacacagTC	385
10_1	57	5	39	16	ACaagtcatgttaCTA	463
11_1	75	5	37	12	ACacaagtcatgttAC	465
12_1	22	2	10	3	CTtacttagatgcTGC	495
13_1	33	4	23	11	ACttacttagatgCTG	496
14_1	33	7	21	6	GACttacttagatgCT	497
15_1	41	6	18	10	AGacttacttagaTGC	498
16_1	96	14	40	7	GCAggaagagactTAC	506

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CMP ID NO	KARPAS-299 cells 5 µM CMP		THP1 cells 20 µM CMP		Compound (CMP)	Start on SEQ ID NO 1
	% mRNA of control	sd	% mRNA of control	sd		
17_1	22	2	9	3	AATAaattccgttCAGG	541
18_1	34	6	21	9	GCAAataaattcCGTT	545
18_2	51	4	27	11	GCAaataaattccGTT	545
19_1	38	5	23	7	AGCAaataaattcCGT	546
20_1	73	8	56	15	CAGAgcaaataaatTCC	548
21_1	83	8	65	10	TGGAcagagcaaataAAT	551
22_1	86	6	80	8	ATGGacagagcaAATA	554
23_1	44	4	30	2	CAgaatggacagaGCA	558
24_1	63	10	40	11	TTCtcagaatggacAG	562
25_1	31	1	39	5	CTGAactttgacATAG	663
26_1	60	4	56	19	AAgacaaacccagacTGA	675
27_1	36	4	34	10	TATAagacaaacccAGAC	678
28_1	40	4	28	13	TTATaagacaaaccCAGA	679
29_1	30	2	18	6	TGTTataagacaaaCCC	682
30_1	77	3	67	10	TAGAacaatggtaCTTT	708
31_1	81	17	20	14	GTAGaacaatggtaCT	710
32_1	29	5	14	8	AGGtagaacaatgGTA	712
33_1	32	1	43	20	AAGAggtagaacaATGG	714
34_1	70	4	35	13	GCatccacagtaaaTT	749
35_1	83	2	66	21	GAaggttatttaaTTC	773
36_1	18	2	15	5	CTAAtcgaatgcaGCA	805
37_1	64	7	35	10	TACccaatctaatCGA	813
38_1	69	1	49	13	TAGttacccaatcTAA	817
39_1	49	5	26	9	CATttagttacccAAT	821
40_1	23	7	8	2	TCAtttagttaccCAA	822
41_1	24	6	12	3	TTcatttagttaCCCA	823
42_1	51	7	40	5	GAATtaatttcattTAGT	829
43_1	71	9	45	3	CAGTgaggaattaATTT	837
44_1	60	5	45	17	CCAAcagtgaggAATT	842
45_1	63	1	37	15	CCCaacagtgaggAAT	843
46_1	31	3	29	12	TAtacccaacagtgAGG	846
47_1	44	3	27	0	TTatacccaacagTGAG	847
48_1	38	3	26	6	TTTatacccaacagTGA	848
49_1	20	4	7	1	CCTttatacccaaCAG	851
50_1	22	3	6	2	TAACctttatacCCAA	854
51_1	28	1	29	16	AATaacctttataCCCA	855
52_1	80	11	48	10	GTAaataacctttaTA	859
53_1	54	4	37	14	ACTGtaaataacctTTAT	860
54_1	81	4	53	15	ATAtatatgcaatgAG	903
55_1	86	12	70	15	AGatatatatgcaaTG	905
56_1	56	8	27	7	GAGatatatatgcAAT	906

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	% mRNA of control	sd	% mRNA of control	sd		
57_1	28	7	13	5	CCagagatatataTGC	909
58_1	88	13	69	23	CAATattccagagATAT	915
59_1	29	3	14	6	GCAAtattccagagATA	916
60_1	25	3	14	3	AGCaatattccagaGAT	917
61_1	29	4	17	2	CAGcaatattccAGAG	919
62_1	27	3	14	3	AATCagcaatattCCAG	921
63_1	23	6	12	6	ACAAtcagcaataTTCC	923
64_1	53	9	43	15	ACtaagtagttacactTCT	957
65_1	32	5	14	6	CTAAgtagttacactTC	958
66_1	35	4	31	6	GACtaagtagttacaCTT	959
67_1	64	10	55	14	TGActaagtagtTACA	962
68_1	62	11	57	16	CTTTgactaagtagTTA	964
69_1	42	9	59	13	CTCtttgactaagTAG	967
70_1	81	6	56	12	GCTCtttgactaagTA	968
71_1	27	3	39	9	CCttaaatactgtTGAC	1060
72_1	75	5	36	7	CTtaaatactgttgAC	1060
73_1	35	6	43	13	TCCttaaatactgTTG	1062
74_1	57	4	79	25	TCTCcttaaatactgTT	1063
75_1	53	6	28	6	TAtcatagttctCCTT	1073
76_1	26	4	9	2	AGTatcatagttcTCC	1075
77_1	74	5	39	12	GAgtatcatagttCTC	1076
78_1	49	5	35	6	AGagtatcatagTTCT	1077
78_2	74	6	36	8	AGAgtatcatagtTCT	1077
79_1	19	2	19	13	CAGagtatcatagTTC	1078
80_1	23	2	26	2	TTCAgagtatcataGT	1080
81_1	35	3	36	11	CTTcagagtatcATAG	1081
82_1	24	6	20	7	TTCTtcagagtatcaTA	1082
83_1	20	2	16	2	TTTcttcagagtaTCAT	1083
84_1	33	4	37	10	GAGAaaggctaagTTT	1099
85_1	42	2	35	18	GAcactcttgtaCATT	1213
86_1	50	4	54	8	TGagacactcttgtaCA	1215
87_1	50	8	28	8	TGagacactcttgTAC	1216
88_1	61	4	33	6	CTttattaaactCCAT	1266
89_1	71	8	43	12	ACCAaactttattaAA	1272
90_1	62	5	42	9	AAACctctactaagTG	1288
91_1	22	3	12	5	AGattaagacagtTGA	1310
92_1	46	3	ND	ND	AAgtaggagcaagaGGC	1475
93_1	42	4	60	24	AAAGtaggagcaagAGG	1476
94_1	86	15	46	10	GTtaagcagccaggAG	1806
95_1	66	6	82	27	AGggtaggatgggtAG	1842
96_1	83	19	62	36	AAGggtaggatgggTA	1843

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	% mRNA of control	sd	% mRNA of control	sd		
97_1	60	9	69	5	CAAgggtaggatggGT	1844
97_2	76	13	34	7	CAagggtaggatggGT	1844
98_1	65	8	76	28	CCaagggtaggatgGG	1845
99_1	61	2	75	17	TCcaagggtaggatGG	1846
100_1	83	4	82	13	CTTCcaagggtaggAT	1848
101_1	45	3	52	14	ATCttccaagggtagGA	1849
102_1	29	2	17	7	AGaagtgatggctCATT	1936
103_1	26	3	22	1	AAGaagtgatggcTCAT	1937
104_1	34	6	22	2	GAAgaagtgatggcTCA	1938
105_1	41	5	21	5	ATGAaatgtaaacTGGG	1955
106_1	40	8	29	6	CAATgaaatgtaaaCTGG	1956
107_1	24	3	16	4	GCAAtgaaatgtaaACTG	1957
108_1	30	4	20	6	AGCAatgaaatgtaAACT	1958
109_1	44	4	34	14	GAGCaatgaaatgtAAAC	1959
110_1	18	1	13	3	TGaattcccatatcCGA	1992
111_1	69	8	35	8	AGaattatgaccaTAT	2010
112_1	77	7	38	10	AGGtaagaattatGACC	2014
113_1	97	10	56	13	TCAGgtaagaattaTGAC	2015
114_1	69	8	54	21	CTTCaggtaagaatTATG	2017
115_1	91	7	115	42	TCTTcaggtaagaATTA	2019
116_1	88	6	104	36	CTTCttcaggtaaGAAT	2021
117_1	85	6	118	17	TCTTcttcaggtaaGAA	2022
118_1	105	14	102	9	TCTtcttcaggtaAGA	2023
119_1	37	2	76	18	TGGtctaagagaaGAAG	2046
120_1	46	6	81	11	GTTGgtctaagagAAG	2049
121_1	74	11	64	4	AGTtggtctaagAGAA	2050
122_1	74	9	55	21	CAgttggtctaagAGAA	2050
123_1	65	9	95	21	GCAgttggtctaagagAA	2050
124_1	63	7	ND	ND	CAGTtggtctaagaGA	2051
125_1	65	6	ND	ND	GCagttggtctaagaGA	2051
126_1	67	14	104	34	GCagttggtctaaGAG	2052
127_1	22	6	10	3	CTcatatcagggCAGT	2063
128_1	50	4	46	9	CACAcatgttctttaAC	2087
129_1	22	4	12	12	TAAatacacacatgTTCT	2092
130_1	24	2	43	28	GTAAatacacacatgTTC	2093
131_1	33	3	20	12	TGTAaatacacacaTGTT	2094
132_1	73	17	57	21	GATCatgtaaatacACAC	2099
133_1	47	5	28	14	AGATcatgtaaatacACA	2100
134_1	35	6	26	11	CAAAgatcatgtaaatACAC	2101
135_1	30	2	14	3	ACAAagatcatgtaaaTACA	2102
136_1	52	6	24	18	GAATacaaagatcaTGTA	2108

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	% mRNA of control	sd	% mRNA of control	sd		
137_1	33	5	20	6	AGAAtacaaagatcATGT	2109
138_1	37	1	22	15	CAGAatacaaagatCATG	2110
139_1	85	6	53	8	GCAGaatacaaagATCA	2112
140_1	79	4	40	6	AGGCagaatacaaagAT	2114
141_1	56	2	53	20	AAGGcagaatacaaAGA	2115
142_1	28	5	20	5	ATTagtgagggacGAA	2132
143_1	26	2	22	10	CAttagtgagggaCGA	2133
144_1	29	6	16	4	GAgggtgatggatTAG	2218
145_1	45	6	22	5	TTaggagtaataAAGG	2241
146_1	65	7	44	9	TTAatgaatttggtTG	2263
147_1	84	8	43	10	CTttaatgaatttgGT	2265
148_1	32	0	15	3	CATGgattacaactAA	2322
149_1	33	2	20	4	TCatggattacaaCTA	2323
150_1	29	1	11	3	GTCatggattacaaCT	2324
151_1	64	2	40	9	CAttaaatctagTCAT	2335
152_1	97	8	63	22	GACAttaaatctagTCA	2336
153_1	92	7	ND	ND	AGGGacattaaatcTA	2340
154_1	35	4	25	15	CAAAgcattataaCCA	2372
155_1	34	3	24	6	ACttactaggcaGAAG	2415
156_1	102	6	113	18	CAGAgttaactgtaCA	2545
157_1	102	10	103	15	CCAGagttaactgtAC	2546
158_1	88	7	95	18	GCcagagttaactgTA	2547
159_1	78	10	ND	ND	TGggccagagttaaCT	2550
160_1	59	5	26	5	CAgcatctatcagaCT	2576
161_1	78	8	42	10	TGAaataacatgagTCAT	2711
162_1	31	6	ND	ND	GTGaaataacatgAGTC	2713
163_1	18	2	11	3	TCTGtttatgtcacTG	2781
164_1	56	5	29	9	GTCTgtttatgtcaCT	2782
165_1	37	8	12	5	TGgtctgtttatGTCA	2784
166_1	39	1	19	3	TTGGtctgtttatgTC	2785
167_1	41	3	35	14	TCacccattgtttaAA	2842
168_1	18	3	14	4	TTcagcaaatatTCGT	2995
169_1	36	8	13	2	GTGtgttcagcaaATAT	2999
170_1	18	2	11	4	TCTattgttaggtATC	3053
171_1	67	4	26	12	ATtgcccatcttacTG	3118
172_1	71	2	33	9	TATtgcccatcttaCT	3119
173_1	47	4	20	5	AAatattgcccatCTT	3122
174_1	74	4	34	7	ATAaccttatcataCA	3174
175_1	98	19	44	12	TAtaaccttatcaTAC	3175
176_1	100	10	64	11	TTAtaaccttatcaTA	3176
177_1	72	38	28	5	TTTataaccttatCAT	3177

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	% mRNA of control	sd	% mRNA of control	sd		
178_1	47	6	34	6	ACtgctattgctaTCT	3375
179_1	41	3	23	6	AGgactgctattgCTA	3378
180_1	32	6	27	7	GAGgactgctattgCT	3379
181_1	83	1	46	20	ACgtagaataataaCA	3561
182_1	94	4	52	9	CCaagtgatataATGG	3613
183_1	49	2	16	3	TTagcagaccaaGTGA	3621
184_1	96	3	26	5	GTttagcagaccaaGT	3623
185_1	78	3	46	10	TGacagtgattataTT	3856
186_1	88	5	45	21	TGTCcaagatattgAC	3868
187_1	46	6	23	6	GAAtatcctagatTGT	4066
188_1	79	3	45	14	CAaactgagaataTCC	4074
189_1	63	5	27	8	GCAaactgagaataTC	4075
190_1	77	9	37	11	TCCtattacaatcgTA	4214
191_1	74	10	36	9	TTCCtattacaatcGT	4215
192_1	91	8	51	28	ACtaatgggaggatTT	4256
193_1	95	14	67	24	TAgttcagagaataAG	4429
194_1	86	5	47	16	TAacatatagttcAGA	4436
195_1	87	4	81	20	ATAacatatagttcAG	4437
196_1	101	6	67	20	CAtaacatatagttCA	4438
197_1	91	6	60	13	TCataacatatagtTC	4439
198_1	61	3	31	10	TAGCtcctaacaatCA	4507
199_1	79	12	49	11	CTCCaatctttgtaTA	4602
200_1	74	2	58	13	TCTCcaatctttgtAT	4603
201_1	53	3	33	10	TCtatttcagccaaTC	4708
202_1	25	4	30	9	CGGaagtcagagtGAA	4782
203_1	32	5	21	7	TTAAgcatgaggaaTA	4798
204_1	34	10	26	11	TGAttgagcacctCTT	4831
205_1	81	12	62	12	GACtaattatttcgTT	4857
206_1	57	7	37	7	TGActaattatttCGT	4858
207_1	26	5	21	6	GTGactaattattTCG	4859
208_1	48	3	33	13	CTGCttgaaatgtgAC	4870
209_1	32	1	34	13	CCtgcttgaaatgTGA	4871
210_1	60	5	50	19	ATcctgcttgaaATGT	4873
211_1	111	8	110	26	ATTataaatctatTCT	5027
212_1	107	1	67	12	GCtaaatactttcATC	5151
213_1	26	3	19	6	CAttgtaacataCCTA	5251
214_1	33	2	20	4	GCattgtaacatacCT	5252
215_1	89	8	53	16	TAatattgcaccaaAT	5295
216_1	25	2	29	9	GAtaatattgcacCAA	5297
217_1	27	1	27	6	AGataatattgcacCA	5298
218_1	79	6	45	11	GCcaagaagataATAT	5305

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	% mRNA of control	sd	% mRNA of control	sd		
219_1	159	16	68	14	CACAgccacataaaCT	5406
220_1	90	2	72	12	TTgtaattgtggaaAC	5463
221_1	10	2	11	5	TGacttgtaattgTGG	5467
222_1	82	1	67	18	TCtaactgaaatagTC	5503
223_1	30	1	32	9	GTGgttctaactgaAA	5508
224_1	53	7	53	15	CAatatgggacttgGT	5522
225_1	44	1	33	10	ATGacaatatgggaCT	5526
226_1	49	1	41	14	TATGacaatatgggAC	5527
227_1	77	1	54	15	ATATgacaatatggGA	5528
228_1	100	3	98	29	CTtcacttaataaTTA	5552
229_1	90	12	80	19	CTGCttcacttaatAA	5555
230_1	91	0	79	23	AAgactgcttcacTTA	5559
231_1	49	8	77	34	GAATgccctaattaTG	5589
232_1	17	7	88	33	TGGaatgccctaatTA	5591
233_1	40	5	35	10	GCAaatgccagtagGT	5642
234_1	81	6	72	25	CTAatggaaggattTG	5673
235_1	97	17	87	25	AAtatagaacctaaTG	5683
236_1	98	4	83	21	GAAagaatagaatGTT	5769
237_1	93	2	102	26	ATGggtaatagattAT	5893
238_1	110	24	44	14	GAaagagcacagggTG	6103
239_1	66	5	36	10	CTACatagagggaaTG	6202
240_1	70	4	34	8	GCttcctacataGAGG	6207
241_1	64	NA	33	6	TGCTtcctacatagAG	6208
242_1	30	NA	19	7	TGggcttgaaataTGT	6417
243_1	88	6	69	15	CATtatatttaagaAC	6457
244_1	8	2	5	2	TCggttatgttaTCAT	6470
245_1	18	9	12	4	CActttatctggTCGG	6482
246_1	37	2	19	5	AAAttggcacagcGTT	6505
247_1	46	12	29	8	ACCGtgacagtaaATG	6577
248_1	31	2	25	2	TGggaaccgtgacagTA	6581
249_1	17	2	23	9	CCacatataggtcCTT	6597
250_1	15	6	23	7	CAtattgctaccaTAC	6617
251_1	4	2	9	2	TCAtattgctaccATA	6618
252_1	65	12	85	14	CAATtgtcatatTGCT	6624
253_1	20	2	51	7	CATtcaattgtcataTTG	6626
254_1	48	8	91	41	TTTCtactgggaaTTTG	6644
255_1	11	5	23	8	CAAttagtgcagcCAG	6672
256_1	43	7	62	13	GAATaatgttcttaTCC	6704
257_1	28	2	36	19	CACAaattgaataatgtTCT	6709
258_1	64	4	78	22	CATGcacaaattgaaTAAT	6714
259_1	53	8	104	73	ATCctgcaatttcaCAT	6832

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	% mRNA of control	sd	% mRNA of control	sd		
260_1	54	5	59	14	CCaccatagctgatCA	6868
261_1	42	8	52	22	ACcaccatagctgaTCA	6868
262_1	68	5	118	66	CAccaccatagctgaTC	6869
263_1	40	2	73	20	TAgtcggcaccaccAT	6877
264_1	64	6	72	35	CttgtagtcggcaccAC	6880
265_1	56	4	82	35	CttgtagtcggcacCA	6881
266_1	41	5	46	21	CGcttgtagtcggcAC	6883
267_1	51	4	33	14	TCAataaagatcagGC	6942
268_1	61	2	49	10	TGgacttacaagaaTG	6986
269_1	45	7	40	9	ATGgacttacaagaAT	6987
270_1	51	12	36	12	GCTCaagaaattggAT	7073
271_1	17	0	14	5	TACTgtagaacatgGC	7133
272_1	15	3	11	3	GCAAttcatttgaTCT	7239
273_1	64	11	ND	ND	TGaagggaggagggacAC	7259
274_1	52	6	50	28	AGtggtgaagggaggAG	7265
275_1	79	7	ND	ND	TAgtggtgaagggaggAG	7265
276_1	81	6	ND	ND	AtagtggtgaagggaggAG	7265
277_1	70	9	ND	ND	TAgtggtgaagggagGA	7266
278_1	84	9	ND	ND	ATagtggtgaagggagGA	7266
279_1	40	6	64	53	TAGtggtgaagggaGG	7267
280_1	42	10	ND	ND	ATAgtggtgaagggaGG	7267
281_1	63	7	ND	ND	GAtagtggtgaagggaGG	7267
282_1	27	7	38	11	ATAGtggtgaagggAG	7268
283_1	60	22	ND	ND	GAtagtggtgaaggGAG	7268
284_1	23	3	97	54	GAgatagtggtgAAGG	7271
285_1	51	6	72	19	CATGggagatagtgGT	7276
286_1	7	1	21	9	ACAAataatggttaCTCT	7302
287_1	66	8	48	20	ACACacaaataatgGTTA	7306
288_1	67	6	58	20	GAGggacacacaaaTAAT	7311
289_1	46	2	50	21	ATATagagaggcTCAA	7390
290_1	22	6	ND	ND	TTgatatagagaGGCT	7393
291_1	11	2	17	3	GCATttgatatagAGA	7397
292_1	70	18	44	8	TTtgcatttgataTAG	7400
293_1	30	1	30	9	CTGgaagaataggtTC	7512
294_1	53	5	42	10	ACTGgaagaataggTT	7513
295_1	56	2	41	15	TACTggaagaatagGT	7514
296_1	80	8	53	13	TGGCttatcctgtaCT	7526
297_1	73	6	52	14	ATggcttatcctGTAC	7527
298_1	75	7	89	25	TATGgcttatcctgTA	7528
299_1	52	5	50	11	GTAtggcttatccTGT	7529
300_1	27	3	31	6	ATgaatatatgccCAGT	7547

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	% mRNA of control	sd	% mRNA of control	sd		
301_1	41	8	33	9	GAtgaatatatgCCCA	7549
302_1	8	2	ND	ND	CAAgatgaatataTGCC	7551
303_1	32	5	37	14	GACAacatcagtaTAGA	7572
304_1	28	5	30	23	CAAGacaacatcAGTA	7576
305_1	47	5	41	9	CACtcctagttccTTT	7601
306_1	39	6	33	7	AACactcctagttCCT	7603
307_1	68	3	42	14	TAacactcctagtTCC	7604
308_1	115	5	69	22	CTaacactcctagtTC	7605
309_1	97	16	57	14	TGataacataactgTG	7637
310_1	36	1	23	10	CTgataacataaCTGT	7638
311_1	38	5	24	5	TTTGaactcaagtgAC	7654
312_1	42	3	39	5	TCCTttacttagcTAG	7684
313_1	15	2	14	3	GAgtttggattagCTG	7764
314_1	49	28	ND	ND	TGggatatgacagGGA	7838
315_1	34	6	ND	ND	TGTGggatatgacaGG	7840
316_1	47	3	37	8	ATATggaagggataTC	7875
317_1	11	3	ND	ND	ACAggatatggaaGGG	7880
318_1	48	4	ND	ND	ATTTcaacaggatATGG	7885
319_1	18	2	16	4	GAgtaatttcaacAGG	7891
320_1	74	6	44	5	AGGGagtaatttcAACA	7893
321_1	38	5	56	28	ATTAgggagtaatTTCA	7896
322_1	66	9	32	11	CTtactattaggGAGT	7903
323_1	13	1	15	5	CAgcttactattaGGG	7906
324_1	26	4	20	9	TCAgcttactattAGG	7907
325_1	43	4	17	2	ATTtcagcttactaTTAG	7908
326_1	54	5	57	16	TTcagcttactaTTAG	7908
327_1	28	3	8	2	CAGAtttcagcttaCT	7913
328_1	43	4	37	16	GACtacaactagagGG	7930
329_1	45	12	36	10	AGACtacaactagaGG	7931
330_1	99	8	94	32	AAgactacaactagAG	7932
331_1	59	4	52	19	ATGAtttaattctagtCAAA	7982
332_1	100	2	84	23	TTTaattctagtcAAA	7982
333_1	91	9	60	19	GATTtaattctaGTCA	7984
771_1	74	6	50	5	TGAtttaattctaGTCA	7984
334_1	73	5	54	12	ATGAtttaattctagTCA	7984
335_1	15	1	26	3	GATGatttaattctagtCA	7984
336_1	71	22	49	16	GAtttaattctaGTCA	7984
337_1	43	5	30	11	GATGatttaattctaGTC	7985
338_1	98	5	90	27	TGatttaattctagTC	7985
339_1	87	21	86	2	GAGAtgatttaatTCTA	7988
340_1	92	5	85	27	GAGatgatttaatTCT	7989

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	% mRNA of control	sd	% mRNA of control	sd		
341_1	7	1	7	1	CAGAttgatggtagTT	8030
342_1	7	2	24	11	CTcagattgatgGTAG	8032
343_1	3	1	14	9	GTTagccctcagaTTG	8039
344_1	14	5	20	7	TGtattgttagcCCTC	8045
345_1	10	2	11	5	ACttgtattgttAGCC	8048
346_1	52	4	52	17	AGCcagtatcagggAC	8191
347_1	33	3	18	8	TTgacaatagtgGCAT	8213
348_1	7	2	13	5	ACAagtggtatctTCT	8228
349_1	63	8	44	15	AATCtactttacaaGT	8238
350_1	36	2	ND	ND	CAcagtagatgcctGATA	8351
351_1	24	2	30	9	GAacacagtagatGCC	8356
352_1	23	4	103	14	CTTGgaacacagtagAT	8359
353_1	20	2	45	2	ATAtcttggaacaCAG	8364
354_1	25	3	24	6	TCTttaatatcttgGAAC	8368
355_1	39	2	41	10	TGatttctttaatatCTTG	8372
356_1	54	5	88	43	TGatgatttctttaaTATC	8375
357_1	31	4	45	27	AGGctaagtcatgaTG	8389
358_1	18	3	43	20	TTGAtgaggctaagTC	8395
359_1	6	2	11	2	CCAggattatactcTT	8439
360_1	43	5	40	14	GCcaggattataCTCT	8440
361_1	56	8	73	13	CTGccaggattataCT	8442
362_1	23	1	33	7	CAGAaacttatactttaTG	8473
363_1	49	8	45	14	AAGCagaaacttaTACT	8478
364_1	39	6	37	4	GAAgcagaaacttaTACT	8478
365_1	26	4	45	13	TGGaagcagaaacttataCT	8478
366_1	21	4	44	5	TGGaagcagaaacttaTAC	8479
367_1	97	4	70	22	AAgcagaaacttaTAC	8479
368_1	34	3	32	11	TGGaagcagaaactTATA	8480
369_1	71	7	46	19	AAGGgatattatggAG	8587
370_1	51	9	79	38	TGccggaagatttcCT	8641
371_1	45	6	52	25	ATGGattgggagtaGA	8772
372_1	27	7	30	8	AGatggattgggagTA	8774
373_1	13	3	28	6	AAGatggattgggaGT	8775
374_1	42	10	44	11	ACaagatggattGGGA	8777
374_2	41	3	45	14	ACaagatggattggGA	8777
375_1	83	9	88	32	AGAaggttcagaCTTT	8835
376_1	40	5	33	3	GCAgaaggttcagaCT	8837
376_2	28	5	20	4	GCagaaggttcagACT	8837
377_1	70	2	43	8	TGCAgaaggttcagAC	8838
378_1	23	3	55	17	AGtgcagaaggttCAG	8840
378_2	51	6	41	8	AGTGcagaaggttcAG	8840

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	% mRNA of control	sd	% mRNA of control	sd		
379_1	34	6	35	7	AAGTgcagaaggttCA	8841
380_1	44	11	24	6	TAagtgcagaagGTTC	8842
381_1	37	5	45	9	TCtaagtgcagaAGGT	8844
382_1	75	5	147	26	CTCaggagttctactTC	8948
383_1	90	10	141	55	CTCaggagttctaCTT	8949
384_1	73	8	234	116	AtggaggtgactcaggAG	8957
385_1	33	4	42	7	ATggaggtgactcagGA	8958
386_1	24	3	29	14	ATggaggtgactcAGG	8959
387_1	37	2	65	15	TAtggaggtgactcAGG	8959
388_1	50	10	81	19	ATatggaggtgactcaGG	8959
389_1	42	5	61	10	TATGgaggtgactcAG	8960
390_1	36	2	76	50	ATatggaggtgacTCAG	8960
391_1	52	6	64	6	CAtatggaggtgactcAG	8960
392_1	63	5	57	6	ATAtggaggtgacTCA	8961
393_1	53	7	64	12	CAtatggaggtgacTCA	8961
394_1	51	5	56	24	CAtatggaggtgACTC	8962
395_1	23	3	41	34	GCatatggaggtgacTC	8962
396_1	34	3	54	10	TGcatatggaggtgacTC	8962
397_1	54	5	71	24	TtgcatatggaggtgacTC	8962
398_1	61	11	59	13	TttgcatatggaggtgacTC	8962
399_1	25	2	30	6	GCatatggaggtgaCT	8963
400_1	34	4	25	9	TGcatatggaggtgaCT	8963
401_1	25	4	31	20	TTGcatatggaggtgaCT	8963
402_1	51	6	37	11	TttgcatatggaggtgaCT	8963
403_1	26	1	33	5	TGCatatggaggtgAC	8964
404_1	25	2	69	19	TTGcatatggaggtGAC	8964
405_1	26	4	24	4	TTTGcatatggaggtgAC	8964
406_1	19	3	20	7	TTTGcatatggaggtGA	8965
407_1	16	5	46	16	TTtgcatatggaGGTG	8966
408_1	9	2	9	6	AAgtgaagttcaaCAGC	8997
409_1	26	8	109	52	TGggaagtgaagTTCA	9002
410_1	31	5	24	5	ATgggaagtgaagTTC	9003
411_1	49	9	19	10	GATGggaagtgaaGTT	9004
412_1	28	10	17	9	CTGtgatgggaagtGAA	9007
413_1	54	4	34	8	ATTgagtgaatccAAA	9119
414_1	11	1	14	2	AAttgagtgaatCCAA	9120
415_1	58	6	14	2	GATAattgagtgaaTCC	9122
416_1	5	1	16	3	GTGataattgagtGAA	9125
417_1	73	5	61	14	AAGaaaggtgcaaTAA	9155
418_1	86	6	64	13	CAagaaaggtgcAATA	9156
419_1	75	19	64	14	ACAAgaaaggtgcaAT	9157

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	% mRNA of control	sd	% mRNA of control	sd		
420_1	75	8	50	13	ATttaaactcacaaAC	9171
421_1	21	8	23	6	CTgttaggttcaGCGA	9235
422_1	54	10	30	5	TCTGaatgaacatTTCG	9260
423_1	11	4	15	5	CTcattgaaggtTCTG	9281
424_1	87	3	52	8	CTAatctcattgaaGG	9286
425_1	95	1	85	13	CCtaatctcattgaAG	9287
426_1	31	7	22	7	ACTttgatctttcAGC	9305
427_1	64	7	49	16	ACtatgcaacacttTG	9315
428_1	18	6	21	3	CAAatagctttatCGG	9335
429_1	19	6	17	4	CCaaatagctttATCG	9336
430_1	35	4	27	8	TCCAaatagctttaTC	9337
431_1	75	8	43	7	GATCcaaatagcttTA	9339
432_1	67	11	32	8	ATgatccaaataGCTT	9341
433_1	53	5	43	6	TATGatccaaatagCT	9342
434_1	97	9	66	29	TAAAcagggctggGAAT	9408
435_1	58	12	44	17	ACttaaacagggCTGG	9412
436_1	58	10	30	12	ACacttaaacagGGCT	9414
437_1	87	38	41	3	GAACacttaaacAGGG	9416
438_1	70	4	59	33	AGAGaacacttaaACAG	9418
439_1	83	17	28	9	CTACagagaacaCTTA	9423
440_1	49	12	27	4	ATGctacagagaaCACT	9425
441_1	53	10	24	13	ATAAatgctacagagAACA	9427
442_1	23	6	20	10	AGataaatgctacaGAGA	9430
443_1	48	6	27	7	TAGAgataaatgcTACA	9434
444_1	51	3	32	8	TAGAtagagataaatGCT	9437
445_1	38	5	ND	ND	CAATatactagataGAGA	9445
446_1	52	3	31	1	TACAcaatatactagATAG	9448
447_1	65	6	48	11	CTAcacaatatacTAG	9452
448_1	67	9	29	2	GCTAcacaatatACTA	9453
449_1	103	17	65	15	ATATgctacacaatATAC	9455
450_1	71	13	129	22	TGATatgctacaCAAT	9459
451_1	19	4	9	1	ATGAtatgatatgCTAC	9464
452_1	75	10	45	21	GAGGagagagacaaTAAA	9495
453_1	68	6	43	10	CTAggaggagagagACA	9500
454_1	72	7	79	25	TATTctaggaggagAGA	9504
455_1	31	3	29	9	TTATattctaggagGAG	9507
456_1	38	5	62	17	GTTtatattctaGGAG	9510
457_1	15	6	15	8	TGgagtttatattcTAGG	9512
458_1	34	3	21	3	CGtaccaccactcTGC	9590
459_1	41	5	55	22	TGAGgaaatcattcATTC	9641
460_1	81	8	47	22	TTTGaggaaatcatTCAT	9643

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	% mRNA of control	sd	% mRNA of control	sd		
461_1	76	8	39	5	AGGCtaatcctattTG	9657
462_1	93	12	216	12	TTTAggctaatcCTAT	9660
463_1	15	6	30	9	TGCtccagtgtaccCT	9755
464_1	27	3	25	6	TAgtagtactcgATAG	9813
465_1	9	2	7	3	CTAattgtagtagtaCTC	9818
466_1	52	3	32	6	TGctaattgtagTAGT	9822
467_1	68	11	36	16	AGTGctaattgtagTA	9824
468_1	35	6	32	3	GCAAgtgctaattgTA	9827
469_1	91	9	ND	ND	GAGGaaatgaactaattTA	9881
470_1	92	5	ND	ND	CAGGaggaaatgaacTA	9886
471_1	67	5	42	6	CCctagagtcattTCC	9902
472_1	35	5	20	8	ATCttacatgatgaAGC	9925
473_1	13	1	20	5	GACacactcagatttcAG	9967
474_1	24	4	20	2	AGacacactcagatttcAG	9967
475_1	25	4	24	7	AAGacacactcagatttcAG	9967
476_1	26	6	19	4	AGacacactcagattTCA	9968
477_1	28	4	32	13	AAGacacactcagattTCA	9968
478_1	31	8	37	6	AAagacacactcagatTTCA	9968
479_1	63	7	51	26	GAAagacacactcagatTTC	9969
480_1	37	10	ND	ND	AAGAcacactcagatTTC	9969
481_1	41	4	ND	ND	AAAGacacactcagaTTTC	9969
482_1	19	5	48	14	TGAAagacacactcagatTT	9970
483_1	60	8	68	10	TGaaagacacactcaGATT	9971
484_1	42	8	63	22	TGAaagacacactcaGAT	9972
485_1	48	9	41	20	ATTGaaagacacacTCA	9975
486_1	27	6	27	12	TCattgaaagacaCACT	9977
487_1	88	13	121	33	TTCcatcattgaAAGA	9983
488_1	80	12	ND	ND	ATAAtaccacttaTCAT	10010
489_1	13	4	27	15	TTacttaatttcttTGGA	10055
490_1	32	5	60	24	TTAgaactagctttaTCA	10101
491_1	58	10	55	17	GAGgtacaaatatAGG	10171
492_1	4	1	12	3	CTTatgatacaacTTA	10384
493_1	37	6	35	5	TCttatgatacaaCTT	10385
494_1	30	0	27	6	TTCttatgatacaaCT	10386
495_1	27	8	18	3	CAgtttcttatgaTAC	10390
496_1	25	10	25	6	GCAgtttcttatgaTA	10391
497_1	77	6	72	29	TACAaatgtctattagGTT	10457
498_1	66	5	69	17	TGTAcaaatgtctatTAG	10460
499_1	27	10	20	4	AGCatcacaattagTA	10535
500_1	31	10	25	5	CTAatgatagtgaaGC	10548
501_1	21	7	30	8	AGCtaatgatagtgAA	10550

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	% mRNA of control	sd	% mRNA of control	sd		
502_1	35	5	39	8	ATGCcttgacatatTA	10565
503_1	64	11	79	26	CTCAagattattgACAC	10623
504_2	25	4	83	32	ACctcaagattaTTGA	10626
504_1	94	7	22	6	ACCtcaagattaTTGA	10626
505_1	31	6	34	10	AACCtcaagattatTG	10627
506_1	55	6	62	17	CACAaacctcaagattaTT	10628
507_1	66	12	40	4	GTActtaattagACCT	10667
508_1	78	5	80	10	AGTActtaattagACC	10668
509_1	36	5	42	15	GTATgaggtggtaaAC	10688
510_1	40	4	48	22	AGgaaacagcagaAGTG	10723
511_1	27	7	13	6	GCacaacccagaggAA	10735
512_1	54	5	ND	ND	CAAgcacaacccagAG	10738
513_1	35	7	ND	ND	TTCaagcacaaccCAG	10740
514_1	49	6	52	15	AAttcaagcacaACCC	10742
515_1	72	4	106	49	TAATaattcaagcacaaCC	10743
516_1	43	4	57	21	ACTAataattcaaGCAC	10747
517_1	37	3	60	12	ATAAtactaataattcAAGC	10749
518_1	9	3	6	1	TAgatttgtgagGTAA	11055
519_1	59	10	31	5	AGCCttaattctccAT	11091
520_1	41	4	34	9	AATGatctagagcCTTA	11100
521_1	34	6	34	7	CTAatgatctagaGCC	11103
522_1	52	6	52	17	ACTaatgatctaGAGC	11104
523_1	60	4	54	10	CATtaacatgttctTATT	11165
524_1	57	4	55	8	ACAAgtacattaacatGTTC	11170
525_1	53	6	44	5	TTACaagtacattaaCATG	11173
526_1	54	11	49	17	GCTTtattcatgtTTAT	11195
527_1	34	7	17	5	GCTttattcatgttTA	11196
528_1	11	2	21	4	AGAgctttattcatgtTT	11197
529_1	22	4	33	7	ATAAgagctttattCATG	11200
530_1	30	5	32	15	CATAagagctttaTTCA	11202
531_1	77	8	24	4	AGCAtaagagctTTAT	11205
532_1	8	3	15	6	TAGattgtttagtGCA	11228
533_1	4	2	10	2	GTagattgtttaGTGC	11229
534_1	41	6	33	11	GACAattctagtaGATT	11238
535_1	50	1	37	7	CTGacaattctaGTAG	11241
536_1	49	7	36	6	GCTGacaattctagTA	11242
537_1	59	2	42	11	AGgattaagatacgTA	11262
538_1	28	11	28	4	CAggattaagataCGT	11263
539_1	96	5	20	6	TCAggattaagataCG	11264
540_1	70	11	59	11	TTcaggattaagATAC	11265
541_1	53	5	28	4	AGGAagaaagtttgATTC	11308

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	% mRNA of control	sd	% mRNA of control	sd		
542_1	92	13	59	12	TCAAggaagaaagtTTGA	11311
543_1	44	3	67	7	CTCAaggaagaaagTTTG	11312
544_1	43	4	32	4	TGCtcaaggaagaAAGT	11315
545_1	41	7	44	20	AATTatgctcaaggaAGA	11319
546_1	11	4	26	8	TAGGataccacattatGA	11389
547_1	25	4	26	12	CAtaatttattccattcCTC	11449
548_1	64	6	ND	ND	TGCAtaatttattcCAT	11454
549_1	48	17	49	7	ACTGcataatttatTCC	11456
550_1	91	10	92	15	CTAAactgcataattTATT	11458
551_1	85	8	38	9	ATaactaaactgCATA	11465
552_1	86	4	ND	ND	TTAttaataactaaaCTGC	11468
553_1	91	13	92	21	TAGTacattattaataaCT	11475
554_1	50	4	37	7	CATAactaaggacgTT	11493
555_1	41	5	30	7	TCataactaaggaCGT	11494
556_1	80	7	55	13	CGTCataactaaggAC	11496
557_1	86	3	59	11	TCgtcataactaagGA	11497
558_1	51	9	33	12	ATcgtcataactAAGG	11498
559_1	91	6	65	26	GTtagtatcttacATT	11525
560_1	30	3	41	8	CTCtattgttagtATC	11532
561_1	59	8	18	6	AGTatagagttacTGT	11567
562_1	65	11	41	11	TTCCtggtgatactTT	11644
563_1	57	13	45	13	GTTCctggtgatacTT	11645
564_1	57	15	30	7	TGttcctggtgataCT	11646
565_1	17	4	35	4	ATaaacatgaatctCTCC	11801
566_1	16	3	30	4	CTTtataaacatgaaTCTC	11804
567_1	60	5	45	11	CTGtctttataaaCATG	11810
568_1	20	2	19	5	TTgttataaatctgTCTT	11820
569_1	68	9	44	4	TTAaatttattcttgGATA	11849
570_1	76	8	48	12	CTtaaatttattctTGGA	11851
571_1	62	5	66	5	CTTCttaaatttattctTG	11853
572_1	28	4	44	10	TATGtttctcagtAAAG	11877
573_1	29	6	36	11	GAATtatctttaaACCA	11947
574_1	74	6	34	7	CCCttaaatttctaCA	11980
575_1	37	8	30	9	ACACtgctcttgtaCC	11995
576_1	45	14	27	6	TGAcaacactgctCTT	12000
577_1	2	1	12	5	TACAtttattgggcTC	12081
578_1	65	14	39	9	GTacatttattgGGCT	12082
579_1	34	4	53	12	TTGgtacatttatTGG	12085
580_1	41	7	35	6	CATGttggtacattTAT	12088
581_1	11	4	12	5	AATCatgttggtacAT	12092
582_1	96	16	48	9	AAatcatgttggtaCA	12093

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	% mRNA of control	sd	% mRNA of control	sd		
583_1	71	15	42	13	GACaagtttggattAA	12132
584_1	46	34	39	6	AAtgttcagatgCCTC	12197
585_1	37	26	28	12	GCttaatgttcagaTG	12201
586_1	75	8	43	12	CGTAcatagcttgaTG	12267
587_1	41	10	28	5	GTGaggaattaggaTA	12753
588_1	41	5	27	9	GTAacaatatggttTG	12780
589_1	67	10	37	7	GAaatattgtagaCTA	13151
590_1	97	10	80	12	TTGaaatattgtagAC	13153
591_1	64	10	47	9	AAgtctagtaatTTGC	13217
592_1	84	7	60	9	GCTCagtagattatAA	13259
593_1	42	8	32	9	CATacactgttgcTAA	13296
594_1	101	6	79	17	ATGgtctcaaatcATT	13314
595_1	53	14	46	7	CAATggtctcaaatCA	13316
596_1	47	6	36	6	TTCCtattgattgaCT	13568
597_1	97	12	41	6	TTTCtgttcacaacAC	13600
598_1	85	1	49	11	AGgaacccactaaTCT	13702
599_1	56	3	34	7	TAAatggcaggaacCC	13710
600_1	15	4	24	8	GTAAatggcaggaaCC	13711
601_1	40	6	26	8	TTgtaaatggcagGAA	13713
602_1	59	12	26	6	TTatgagttaggCATG	13835
603_1	62	2	42	10	CCAggtgaaacttAA	13935
604_1	77	9	55	18	CCCttagtcagctCCT	13997
605_1	82	13	42	11	ACccttagtcagCTCC	13998
606_1	74	1	39	10	CAcccttagtcagCTC	13999
607_1	76	9	30	8	TCTcttactaggcTCC	14091
608_1	82	5	50	13	CCtatctgtcatcATG	14178
609_1	82	1	48	12	TCCtatctgtcatcAT	14179
610_1	41	6	50	13	GAGaagtgtgagaaGC	14808
611_1	70	5	84	19	CATCcttgaagtttAG	14908
612_1	64	14	61	16	TAAtaagatggctCCC	15046
613_1	85	2	51	14	CAAggcataataagAT	15053
614_1	47	1	35	10	CCaaggcataatAAGA	15054
615_1	74	8	53	11	TGatccaattctcaCC	15151
616_1	63	4	41	11	ATGatccaattctCAC	15152
617_1	46	7	42	9	CGCttcatcttcacCC	15260
618_1	104	4	15	4	TAtgacactgcaTCTT	15317
619_1	8	3	8	5	GTAtgacactgcaTCT	15318
620_1	21	3	27	10	TGtatgacactgCATC	15319
621_1	37	7	38	11	TTCTcttctgtaagTC	15363
622_1	49	7	36	11	TTctacagaggaACTA	15467
623_1	47	1	32	10	ACTacagttctacAGA	15474

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CMP ID NO	KARPAS-299 cells 5 µM CMP		THP1 cells 20 µM CMP		Compound (CMP)	Start on SEQ ID NO 1
	% mRNA of control	sd	% mRNA of control	sd		
624_1	78	8	69	6	TTCCcacaggtaaaTG	15561
625_1	70	7	ND	ND	ATTAtttgaatatactCATT	15594
626_1	73	7	49	25	TGGGaggaaattatTTG	15606
627_1	80	5	64	11	TGACtcatcttaaaTG	15621
628_1	71	6	66	19	CTGactcatcttaaAT	15622
629_1	31	6	41	6	TTTactctgactcATC	15628
630_1	88	2	68	18	TATtggaggaattaTT	15642
631_1	53	2	27	6	GTAttggaggaattAT	15643
632_1	23	3	39	7	TGgtatacttctctaagTAT	15655
633_1	42	9	33	3	GATCtcttggtataCT	15666
634_1	38	1	30	16	CAgacaactctataCC	15689
635_1	10	2	19	3	AACAtcagacaacTCTA	15693
636_1	13	1	11	3	TAACatcagacaacTC	15695
637_1	14	2	27	2	TTTAacatcagacaACTC	15695
638_1	101	14	81	16	ATttaacatcagacAA	15698
639_1	14	1	17	1	CCtatttaacatcAGAC	15700
640_1	65	2	ND	ND	TCCctatttaacaTCA	15703
641_1	41	6	42	12	TCAAcgactattgGAAT	15737
642_1	37	2	29	5	CTTAtattctggcTAT	15850
643_1	31	7	35	4	ATCCttatattctgGC	15853
644_1	13	3	8	1	GAtccttatattCTGG	15854
645_1	25	5	20	4	TGAtccttatattCTG	15855
646_1	33	6	54	10	ATTGaaacttgaTCCT	15864
647_1	43	3	27	6	ACtgtcattgaaACTT	15870
648_1	54	7	32	12	TCTtactgtcattgAA	15874
649_1	12	1	25	2	AGgatcttactgtCATT	15877
650_1	13	4	11	3	GCAaatcaactccATC	15896
651_1	10	5	16	3	GTGcaaatcaactCCA	15898
652_1	7	0	36	18	CAATtatttctttgTGC	15910
653_1	21	3	31	7	TGGcaacaattattTCTT	15915
654_1	75	9	73	24	GCTggcaacaatTATT	15919
655_1	21	6	39	6	ATCCatttctactgCC	15973
656_1	25	3	38	8	TAATatctattgattTCTA	15988
657_1	14	2	11	5	TCaatagtgtagggCA	16093
658_1	11	4	10	3	TTCaatagtgtaggGC	16094
659_1	18	1	32	12	AGGTtaattaattcaATAG	16102
660_1	33	7	25	10	CATttgtaatccCTAG	16163
660_2	64	14	31	8	CATttgtaatcccTAG	16163
661_1	48	6	34	6	ACAtttgtaatccCTA	16164
662_2	29	6	23	5	AAcatttgtaatCCCT	16165
662_1	30	6	18	6	AACatttgtaatCCCT	16165

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CMP ID NO	KARPAS-299 cells 5 µM CMP		THP1 cells 20 µM CMP		Compound (CMP)	Start on SEQ ID NO 1
	% mRNA of control	sd	% mRNA of control	sd		
663_1	49	1	26	6	TAaatttcaagttCTG	16184
664_1	17	3	30	10	GTTtaaatttcaagTTCT	16185
665_1	22	7	40	9	CCAAgtttaaatttCAAG	16189
666_1	89	11	ND	ND	ACCCaagtttaaaTTTC	16192
667_1	60	16	87	8	CAtacagtgacccaagTTT	16199
668_1	65	9	50	12	ACatcccatacagTGA	16208
669_1	83	8	103	4	AGcacagctctaCATC	16219
670_1	80	9	150	36	ATAtagcacagcTCTA	16223
671_1	57	14	ND	ND	TCCatatagcacagCT	16226
672_1	53	10	106	8	ATTtccatatagCACA	16229
673_1	78	3	96	14	TTTAtttccatatAGCA	16231
674_1	77	9	31	7	TTTatttccatatAGC	16232
675_1	32	6	ND	ND	AAGGagaggagatTATG	16409
676_1	32	5	24	6	AGTtcttgtgttagCT	16456
677_1	19	4	17	4	GAgttcttgtgttaGC	16457
678_1	14	3	25	3	ATTaattatccatCCAC	16590
679_1	11	2	20	6	ATCaattaattatcCATC	16593
680_1	31	5	40	11	AGAatcaattaattaTCC	16596
681_1	8	3	30	10	TGagataccgtgcaTG	16656
682_1	11	3	ND	ND	AAtgagataccgTGCA	16658
683_1	15	3	33	10	CTGtggttaggctaAT	16834
684_1	45	7	38	7	AagagtaagggtctgtggTT	16842
685_1	24	5	ND	ND	GATGggttaagagTAA	16854
686_1	11	2	ND	ND	AGCagatgggttaaGA	16858
687_1	ND	ND	51	7	TGtaaacatttgTAGC	16886
688_1	83	1	54	11	CCTgcttataaatgTA	16898
689_1	103	4	73	14	TGCCctgcttataaAT	16901
690_1	104	2	64	22	TCttcttagttcaaTA	16935
691_1	ND	ND	60	9	TGgtttctaactACAT	16980
692_1	ND	ND	94	22	AGtttggtttctaaCTA	16983
693_1	8	2	17	5	GAAtgaaacttgcCTG	17047
694_1	98	6	51	9	ATTatccttacatGAT	17173
695_1	48	4	18	4	GTacccaattatcCTT	17180
696_1	94	2	48	9	TGTacccaattatCCT	17181
697_1	31	5	42	13	TTgtacccaattaTCC	17182
698_1	41	4	39	6	TTTgtacccaattaTC	17183
699_1	63	0	28	12	AGCAgcaggttataTT	17197
700_1	99	6	43	12	TGGgaagtggtctGGG	17292
701_1	103	2	28	5	CTGgagagtgataaTA	17322
702_1	52	6	27	9	AATGctggattacgTC	17354
703_1	67	3	37	7	CAatgctggattaCGT	17355

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CMP ID NO	KARPAS-299 cells 5 µM CMP		THP1 cells 20 µM CMP		Compound (CMP)	Start on SEQ ID NO 1
	% mRNA of control	sd	% mRNA of control	sd		
704_1	36	10	80	12	TTgttcagaagtATCC	17625
705_1	19	9	47	9	GAtgatttgcttGGAG	17646
706_1	44	NA	60	9	GAAatcattcacaACC	17860
707_1	46	9	32	9	TTGtaacatctacTAC	17891
708_1	56	0	79	17	CATtaagcagcaagTT	17923
709_1	30	9	46	7	TTActagatgtgagCA	17942
710_1	29	4	36	6	TTtactagatgtgAGC	17943
711_1	41	13	41	6	GACcaagcaccttaCA	17971
712_1	36	19	49	11	AGAccaagcacctTAC	17972
713_1	30	6	34	7	ATgggttaaataAAGG	18052
714_1	70	2	24	8	TCaaccagagtattAA	18067
715_1	11	4	26	8	GTCaaccagagtatTA	18068
716_1	126	56	26	6	ATtgtaaagctgaTAT	18135
717_1	73	1	42	10	CAcataattgtaAAGC	18141
718_1	23	9	55	18	GAggtctgctattTAC	18274
719_1	50	1	42	11	TGtagattcaatgCCT	18404
720_1	79	3	39	10	CCtcattatactaTGA	18456
721_1	27	6	30	8	CCttatgctatgacAC	18509
722_1	26	7	50	13	TCCTtatgctatgaCA	18510
723_1	59	1	48	12	AAGatgtttaagtATA	18598
724_1	54	2	50	13	CTgattattaagATGT	18607
725_1	92	10	84	19	TGgaaaggtatgaaTT	18808
726_1	24	8	61	16	ACttgaatggcttgGA	18880
727_1	8	4	51	14	AACttgaatggctTGG	18881
728_1	35	4	35	10	CAATgtgttactatTT	19004
729_1	36	9	53	11	ACAatgtgttactATT	19005
730_1	70	2	41	11	CATCtgctatataaGA	19063
731_1	38	NA	42	9	CCTAgagcaaatacTT	19223
732_1	102	15	15	4	CAGagttaataatAAG	19327
733_1	37	10	8	5	GTTCaagcacaacgAA	19493
734_1	13	1	38	11	AGggttcaagcacAAC	19496
735_1	49	NA	36	11	TGttggagacactgTT	19677
736_1	48	NA	32	10	AAGgaggagttaggAC	19821
737_1	36	NA	64	11	CTATgccatttacgAT	19884
738_1	105	19	66	19	TCaaatgcagaattAG	19913
739_1	44	NA	41	6	AGtgacaatcaaATGC	19921
740_1	107	NA	68	18	AAgtgacaatcaaATG	19922
741_1	102	4	27	6	GTGtaccaagtaacAA	19978
742_1	110	10	30	16	TGGgatgttaaacTGA	20037

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Example 2 - Testing in vitro efficacy in a dose response curve

A selection of oligonucleotides from Table 10 were tested in KARPAS-299 cells using half-log serial dilutions in in PBS (50 µM, 15.8 µM, 5.0 µM, 1.58 µM, 0.5 µM, 0.158 µM, 0.05 µM, to 0.0158 µM oligonucleotide) in the in vitro efficacy assay described in Example 1. IC 50 and max inhibition (% residual PD-L1 expression) was assessed for the oligonucleotides.

EC50 calculations were performed in GraphPad Prism6. The IC50 and maximum PD-L1 knock down level is shown in table 11 as % of control (PBS) treated cells.

Table 11: Max inhibition as % of saline and EC50 in KARPAS-299 cell line.

CMP ID NO	Max Inhibition (% residual PD-L1 expression; % of saline-treated)		EC50 (µM)		Compound CMP	Start on SEQ ID NO: 1
	Avg	SD	Avg	SD		
6_1	11	3.3	0.69	0.11	TCGCataagaatgaCT	371
8_1	29	1.7	0.06	0.01	CTGaacacacagtCGC	383
9_1	19	1.7	0.23	0.02	TCTgaacacacagtCG	384
13_1	14	4.7	0.45	0.12	CTtacttagatgcTGC	495
41_1	10	1.8	0.19	0.02	TCAtttagttaccCAA	822
42_1	17	1.3	0.19	0.02	TTcatttagttaCCCA	823
58_1	23	1.5	0.17	0.01	CCagagatatataTGC	909
77_1	24	2.4	0.16	0.02	AGTatcatagttcTCC	1075
92_1	12	2.4	0.25	0.03	AGattaagacagtTGA	1310
111_1	3	2.0	0.27	0.03	TGaattcccatatcCGA	1992
128_1	11	1.8	0.25	0.03	CTcatatcagggCAGT	2063
151_1	16	2.7	0.28	0.05	GTCatggattacaaCT	2324
164_1	19	1.6	0.15	0.01	TCTGtttatgtcacTG	2781
166_1	36	1.7	0.11	0.02	TGgtctgtttatGTCA	2784
169_1	10	1.6	0.22	0.02	TTcagcaaatatTCGT	2995
171_1	12	2.0	0.21	0.02	TCTattgttaggtATC	3053
222_1	1	2.0	0.21	0.02	TGacttgtaattgTGG	5467
233_1	1	4.3	0.89	0.17	TGGaatgccctaatTA	5591
245_1	4	2.0	0.17	0.02	TCggttatgttaTCAT	6470
246_1	7	2.1	0.25	0.03	CActttatctggTCGG	6482
250_1	0	2.5	0.23	0.03	CCacatataggtcCTT	6597
251_1	0	2.8	0.75	0.10	CAtattgctaccaTAC	6617
252_1	3	2.2	0.19	0.02	TCAtattgctaccATA	6618
256_1	5	2.2	0.32	0.03	CAAttagtgcagcCAG	6672
272_1	1	3.2	0.69	0.10	TACTgtagaacatgGC	7133
273_1	3	2.8	0.28	0.04	GCAAttcatttgaTCT	7239
287_1	1	1.4	0.13	0.01	ACAAataatggttaCTCT	7302
292_1	2	2.1	0.21	0.02	GCATttgatatagAGA	7397
303_1	0	1.2	0.21	0.01	CAAgatgaatataTGCC	7551
314_1	3	2.1	0.39	0.04	GAgtttggattagCTG	7764

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CMP ID NO	Max Inhibition (% residual PD-L1 expression; % of saline-treated)		EC50 (μM)		Compound CMP	Start on SEQ ID NO: 1
	Avg	SD	Avg	SD		
318_1	3	1.4	0.14	0.01	ACAggatatggaaGGG	7880
320_1	2	2.4	0.22	0.03	GAgtaatttcaacAGG	7891
324_1	0	2.4	0.44	0.05	CAgcttactattaGGG	7906
336_1	0	2.5	0.21	0.03	GATGatttaattctagtCA	7984
342_1	1	2.2	0.12	0.01	CAGAttgatggtagTT	8030
343_1	4	1.8	0.11	0.01	CTcagattgatgGTAG	8032
344_1	0	0.9	0.12	0.01	GTTagccctcagaTTG	8039
345_1	0	2.3	0.36	0.04	TGtattgttagcCCTC	8045
346_1	1	2.1	0.22	0.02	ACttgtattgttAGCC	8048
349_1	4	2.9	0.21	0.03	ACAagtggtatctTCT	8228
359_1	6	2.9	0.39	0.05	TTGAtgaggctaagTC	8395
360_1	0	1.7	0.18	0.02	CCAggattatactcTT	8439
374_1	5	1.7	0.33	0.03	AAGatggattgggaGT	8775
408_1	3	1.8	0.21	0.02	TTtgcatatggaGGTG	8966
409_1	0	1.8	0.21	0.02	AAgtgaagttcaaCAGC	8997
415_1	0	1.4	0.23	0.02	AAttgagtgaatCCAA	9120
417_1	7	0.9	0.15	0.01	GTGataattgagtGAA	9125
424_1	6	3.2	0.19	0.03	CTcattgaaggtTCTG	9281
429_1	5	2.5	0.48	0.05	CAAatagctttatCGG	9335
430_1	1	2.7	0.68	0.09	CCaaatagctttATCG	9336
458_1	0	4.1	0.35	0.07	TGgagtttatattcTAGG	9512
464_1	0	4.1	0.56	0.10	TGCtccagtgtaccCT	9755
466_1	1	2.1	0.21	0.02	CTAattgtagtagtaCTC	9818
474_1	0	2.4	0.27	0.03	GACacactcagatttcAG	9967
490_1	0	1.9	0.29	0.03	TTacttaatttcttTGGA	10055
493_1	3	1.8	0.20	0.02	CTTatgatacaacTTA	10384
512_1	0	3.3	0.63	0.10	GCacaacccagaggAA	10735
519_1	5	1.5	0.15	0.01	TAgatttgtgagGTAA	11055
529_1	0	2.7	0.24	0.03	AGAgctttattcatgtTT	11197
533_1	6	1.5	0.14	0.01	TAGattgtttagtGCA	11228
534_1	5	0.9	0.06	0.00	GTagattgtttaGTGC	11229
547_1	1	1.6	0.26	0.02	TAGGataccacattatGA	11389
566_1	0	3.0	0.40	0.06	ATaaacatgaatctCTCC	11801
567_1	2	2.5	0.34	0.04	CTTtataaacatgaaTCTC	11804
578_1	2	1.3	0.09	0.01	TACAtttattgggcTC	12081
582_1	1	1.6	0.20	0.02	AATCatgttggtacAT	12092
601_1	1	2.1	0.47	0.05	GTAAatggcaggaaCC	13711
619_1	4	3.4	0.44	0.08	TAtgacactgcaTCTT	15317
620_1	1	1.2	0.12	0.01	GTAtgacactgcaTCT	15318
636_1	0	1.3	0.19	0.01	AACAtcagacaacTCTA	15693

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CMP ID NO	Max Inhibition (% residual PD-L1 expression; % of saline-treated)		EC50 (µM)		Compound CMP	Start on SEQ ID NO: 1
	Avg	SD	Avg	SD		
638_1	0	2.2	0.36	0.04	TAACatcagacaacTC	15695
637_1	0	2.1	0.21	0.02	TTTAacatcagacaACTC	15695
640_1	2	3.3	0.42	0.06	CCtatttaacatcAGAC	15700
645_1	1	2.9	0.34	0.04	GAtccttatattCTGG	15854
650_1	0	2.4	0.24	0.03	AGgatcttactgtCATT	15877
651_1	4	3.4	0.33	0.05	GCAaatcaactccATC	15896
652_1	0	1.3	0.16	0.01	GTGcaaatcaactCCA	15898
653_1	4	2.0	0.09	0.01	CAATtatttctttgTGC	15910
658_1	3	1.6	0.32	0.02	TCaatagtgtagggCA	16093
659_1	5	1.4	0.20	0.01	TTCaatagtgtaggGC	16094
660_1	4	2.1	0.22	0.02	AGGTtaattaattcaATAG	16102
665_1	3	1.8	0.18	0.02	GTTtaaatttcaagTTCT	16185
678_1	3	2.1	0.43	0.04	GAgttcttgtgttaGC	16457
679_1	0	3.5	0.31	0.05	ATTaattatccatCCAC	16590
680_1	4	1.6	0.12	0.01	ATCaattaattatcCATC	16593
682_1	3	2.4	0.27	0.03	TGagataccgtgcaTG	16656
683_1	0	3.2	0.16	0.03	AAtgagataccgTGCA	16658
684_1	2	2.3	0.25	0.03	CTGtggttaggctaAT	16834
687_1	5	1.3	0.13	0.01	AGCagatgggttaaGA	16858
694_1	0	1.7	0.16	0.02	GAAtgaaacttgcCTG	17047
706_1	15	3.6	0.27	0.06	GAtgatttgcttGGAG	17646
716_1	10	2.1	0.15	0.02	GTCaaccagagtatTA	18068
728_1	5	1.2	0.09	0.01	AACttgaatggctTGG	18881
733_1	0	12.7	8.01	3.62	CAGagttaataatAAG	19327
734_1	0	14.6	3.49	2.39	GTTCaagcacaacgAA	19493
735_1	0	2.5	0.30	0.04	AGggttcaagcacAAC	19496

A selection of oligonucleotides from Table 6 were tested in THP-1 cells using 1:3 serial in water from 25 µM to 0.004 µM in the in vitro efficacy assay described in Example 1. IC 50 and max inhibition (Percent residual PD-L1 expresson) was assessed for the oligonucleotides.

EC50 calculations were performed in GraphPad Prism6. The IC50 and maximum PD-L1 knock down level is shown in table 12 as % of control (PBS) treated cells.

Table 12: Max inhibition as % of saline and EC50 in THP1 cell line.

CMP ID NO	Max Inhibition (% residual PD-L1 expression; % of saline)		EC50 (µM)		Compound CMP	Start on SEQ ID NO: 1
	Avg	SD	Avg	SD		
6_1	12	11.5	0.73	0.38	TCGCataagaatgaCT	371
8_1	6	5.6	0.11	0.04	CTGaacacacagtCGC	383
9_1	1	14.3	0.36	0.27	TCTgaacacacagtCG	384

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CMP ID NO	Max Inhibition (% residual PD-L1 expression; % of saline)		EC50 (μM)		Compound CMP	Start on SEQ ID NO: 1
	Avg	SD	Avg	SD		
13_1	2	12.4	0.49	0.31	CTtacttagatgcTGC	495
41_1	14	14.6	0.38	0.27	TCAtttagttaccCAA	822
42_1	21	10.4	0.22	0.10	TTcatttagttaCCCA	823
58_1	6	19.8	0.97	0.81	CCagagatatataTGC	909
77_1	5	4.8	0.14	0.04	AGTatcatagttcTCC	1075
92_1	0	12.9	0.57	0.39	AGattaagacagtTGA	1310
128_1	15	10.1	0.23	0.13	CTcatatcagggCAGT	2063
151_1	9	14.4	0.18	0.15	GTCatggattacaaCT	2324
164_1	16	22.0	0.57	0.60	TCTGtttatgtcacTG	2781
166_1	13	11.9	0.17	0.11	TGgtctgtttatGTCA	2784
169_1	0	9.3	0.22	0.11	TTcagcaaatatTCGT	2995
171_1	11	12.9	0.28	0.20	TCTattgttaggtATC	3053
222_1	16	19.7	0.68	0.64	TGacttgtaattgTGG	5467
245_1	14	6.1	0.26	0.08	TCggttatgttaTCAT	6470
246_1	28	7.3	0.10	0.20	CActttatctggTCGG	6482
252_1	19	8.0	0.29	0.12	TCAtattgctaccATA	6618
272_1	3	9.7	0.25	0.14	TACTgtagaacatgGC	7133
314_1	13	9.6	0.31	0.15	GAgtttggattagCTG	7764
344_1	11	8.0	0.14	0.06	GTTagccctcagaTTG	8039
349_1	12	12.5	0.18	0.14	ACAagtggtatctTCT	8228
415_1	11	9.6	0.26	0.12	AAttgagtgaatCCAA	9120
493_1	15	16.5	0.48	0.34	CTTatgatacaacTTA	10384
512_1	43	14.1	0.31	0.68	GCacaacccagaggAA	10735
519_1	9	12.2	0.45	0.26	TAgatttgtgagGTAA	11055
533_1	11	13.6	0.29	0.21	TAGattgtttagtGCA	11228
534_1	9	6.5	0.09	0.03	GTagattgtttaGTGC	11229
582_1	0	12.3	0.33	0.23	AATCatgttggtacAT	12092
619_1	8	10.4	0.32	0.18	TAtgacactgcaTCTT	15317
620_1	12	24.6	1.10	1.08	GTAtgacactgcaTCT	15318
638_1	2	5.4	0.00	0.00	TAACatcagacaacTC	15695
645_1	20	29.6	1.10	1.50	GAtccttatattCTGG	15854
651_1	0	11.2	0.14	0.09	GCAaatcaactccATC	15896
658_1	11	13.8	0.48	0.32	TCaatagtgtagggCA	16093
659_1	0	8.2	0.11	0.06	TTCaatagtgtaggGC	16094
733_1	0	69.6	11.03	26.95	CAGagttaataatAAG	19327
734_1	36	16.8	2.84	2.12	GTTCaagcacaacgAA	19493

The results in table 7 and 8 are also shown in figure 2 in relation to their position where they target the PD-L1 pre mRNA of SEQ ID NO: 1.

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From this it can be seen that almost all of the compounds have EC50 values below 1 µM and a target knock down below 25% of the PD-L1 expression level in the control cells (treated with saline).

Example 3 – *In vitro* potency and efficacy and *in vivo* PD-L1 reduction in poly(I:C) induced mice using naked and GalNAc conjugated PD-L1 antisense oligonucleotides

Efficacy and potency testing was performed in an *in vitro* experiment in in dose-response studies in MCP-11 cells using the oligonucleotides in table 6. The same oligonucleotides as well as GalNAc conjugated versions (Table 8 CMP ID NO 755_2 – 765_2) were tested *in vivo* in poly(I:C) induced C57BL/6J female mice for their ability to reduce PD-L1 mRNA and protein expression

In vitro assay

MCP-11 cells (originally purchased from ATCC) suspended in DMEM (Sigma cat. no. D0819) supplemented with 10% horse serum, 2 mM L-glutamine, 0.025 mg/ml gentamicin and 1 mM sodium pyruvate were added at a density of 8000 cells/well to the oligonucleotides (10 µl) in 96-well round bottom plates and cultured for 3 days in a final volume of 200 µl/well in a humidified incubator at 37°C with 5% CO_2. Oligonucleotides were screened in dose-range concentrations (50 µM, 15.8 µM, 5.0 µM, 1.58 µM, 0.5 µM, 0.158 µM, 0.05 µM and 0.0158 µM).

Total mRNA was extracted using the PureLink Pro 96 RNA Purification kit (Ambion), according to the manufacturer's instructions. cDNA was synthesized using M-MLT Reverse Transcriptase, random decamers RETROscript, RNase inhibitor (Ambion) and 100 mM dNTP set (Invitrogen, PCR Grade) according to the manufacturer's instruction. For gene expressions analysis, qPCR was performed using TaqMan Fast Advanced Master Mix (2X) (Ambion) in a duplex set up with TaqMan primer assays for the PD-L1 (Thermo Fisher Scientific; FAM-MGB Mm00452054-m1) and Gusb (Thermo Fisher Scientific; VIC-MGB-PL Mm01197698-m1). The relative PD-L1 mRNA expression level is shown in table 9 as % of residual PD-L1 expression in % of PBS control samples (PBS-treated cells). EC50 calculations were performed in GraphPad Prism6. The EC50 and maximum PD-L1 knockdown level is shown in table 13 as % of control (PBS) cells.

In vivo assay

C57BL/6J female mice (20-23 g; 5 mice per group) were injected s.c. with 5 mg/kg unconjugated oligonucleotides to mouse PD-L1 or 2.8 mg/kg GalNAc-conjugated oligonucleotides to mouse PD-L1. Three days later, the mice were injected i.v. with 10 mg/kg poly(I:C) (LWM, Invivogen). The mice were sacrificed 5 h after poly(I:C) injection and liver samples were placed in RNAlater (Thermo Fisher Scientific) for RNA extraction or frozen at dry ice for protein extraction.

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Total mRNA was extracted from homogenized liver samples using the PureLink Pro 96 RNA Purification kit (Ambion), according to the manufacturer's instructions. cDNA was synthesized using M-MLT Reverse Transcriptase, random decamers RETROscript, RNase inhibitor (Ambion) and 100 mM dNTP set (Invitrogen, PCR Grade) according to the manufacturer's instruction. For gene expressions analysis, qPCR was performed using TaqMan® Fast Advanced Master Mix TaqMan Fast Advanced Master Mix (2X) (Ambion) in a duplex set up with TaqMan primer assays for the PD-L1 mRNA (Thermo Fisher Scientific; FAM-MGB Mm00452054-m1) and TBP (Thermo Fisher Scientific; VIC-MGB-PL Mm00446971_m1). The relative PD-L1 mRNA expression level is shown in table 13 as % of control samples from mice injected with saline and poly(I:C).

Liver homogenates were prepared by homogenizing liver samples in 2 ml per 100 mg tissue T-PER® Tissue Protein Extraction Reagent (Thermo Fisher Scientific) mixed with 1x Halt Protease Inhibitor Cocktail, EDTA-Free (Thermo Fisher Scientific). Protein concentrations in liver homogenates were measured using Coomassie Plus (Bradford) Assay Reagent (Thermo Scientific) according to the manufacturer's instructions. Liver homogenates (40 µg protein) were separated on 4-12% Bis-Tris Plus polyacrylamide gels (Thermo Fisher Scientific) in 1xMOPS running buffer and transferred to nitrocellulose membranes using iBLOT Dry blotting system (Thermo Fisher Scientific) according to the manufacturer's instructions. Each blot was cut in to two parts horizontally at the 64 kDa band. Following blocking in TBS containing 5% skim milk and 0.05% Tween20, the membranes were incubated overnight at 4°C with rabbit monoclonal anti-vinculin (Abcam cat. no. ab129002) diluted 1:10000 (upper membranes) or goat polyclonal anti-mPD-L1 (R&D Systems cat. no. AF1019) diluted 1:1000 (lower membranes) in TBS containing 5% skim milk and 0.05% Tween20. The membranes were washed in TBS containing 0.05% Tween20 and exposed for 1 h at room temperature to HRP-conjugated swine anti-rabbit IgG (DAKO) diluted 1:3000 (upper membranes) or HRP-conjugated rabbit anti-goat IgG (DAKO) diluted 1:2000 in TBS containing 5% skim milk and 0.05% Tween20. Following washing of the membranes, the reactivity was detected using ECL select (Amersham GE Healthcare). For each group of mice treated with oligonucleotides, the intensity of the PD-L1 bands in relation to vinculin bands were evaluated by comparison with the PD-L1/vinculin band intensities of mice injected with saline and poly(I:C) (control). Results are shown in table 13, and westernblots with pairs of naked and conjugated oligonucleotides are shown in figure 9 A-E.

Table 13: In vitro and in vivo efficacy of oligonucleotides to mouse PD-L1

CMP ID NO	Compound CMP	Max Inhibition (%of PBS)	EC50 (µM)	PD-L1 mRNA (% of control)	PD-L1 protein (relative to control)
744_1	AGTttacattttcTGC	9.1	0.56	86	++
746_1	CACctttaaaaccCCA	5.0	0.46	181	nd
747_1	TCCtttataatcaCAC	4.4	0.52	104	++

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CMP ID NO	Compound CMP	Max Inhibition (%of PBS)	EC50 (µM)	PD-L1 mRNA (% of control)	PD-L1 protein (relative to control)
748_1	ACGgtattttcacAGG	1.8	0.26	102	+++
749_1	GACactacaatgaGGA	7.6	1.21	104	nd
750_1	TGGtttttaggacTGT	12.4	0.74	84	nd
751_1	CGAcaaattctatCCT	9.9	0.69	112	nd
752_1	TGAtatacaatgcTAC	10.5	1.11	142	+++
753_1	TCGitgggtaaatTTA	5.7	0.53	116	+++
754_1	TGCttataaatgGTG	5.2	0.35	98	nd
755_2	5'-GN2-C6-caAGTttacattttcTGC	nd	nd	58	+
757_2	5'-GN2-C6-caCACctttaaaaccCCA	nd	nd	62	nd
758_2	5'-GN2-C6-caTCCtttataatcaCAC	nd	nd	53	+
759_2	5'-GN2-C6-caACGgtattttcacAGG	nd	nd	66	+
760_2	5'-GN2-C6-caGACactacaatgaGGA	nd	nd	101	nd
761_2	5'-GN2-C6-caTGGtttttaggacTGT	nd	nd	99	nd
762_2	5'-GN2-C6-caCGAcaaattctatCCT	nd	nd	84	nd
763_2	5'-GN2-C6-caTGAtatacaatgcTAC	nd	nd	93	+++
764_2	5'-GN2-C6-caTCGttgggtaaatTTA	nd	nd	53	+
765_2	5'-GN2-C6-caTGCtttataaatgGTG	nd	nd	106	nd

+++: similar to PD-L1/vinculin band intensity of control; ++: weaker than PD-L1/vinculin band intensity of control; +: much weaker than PD-L1/vinculin band intensity of control; nd= not determined.

From the data in table 13 it can be seen that GalNAc conjugation of the oligonucleotides clearly improves the *in vivo* PD-L1 reduction. The reduction of mRNA generally correlates with a reduction in PD-L1 protein. Except for CMP ID NO: 754_1, a low *in vitro* EC50 value generally reflects a good *in vivo* PD-L1 mRNA reduction once the oligonucleotide is conjugated to GalNAc.

Example 4 – *In vivo* PK/PD in sorted hepatocytes and non-parenchymal cells from poly(I:C) induced mice

The distribution of naked and GalNAc conjugated oligonucleotides as well as PD-L1 mRNA reduction was investigated in hepatocytes and non-parenchymal cells isolated from poly(I:C) induced mice.

C57BL/6J female mice (n=3 per group) were injected s.c. with 5 mg/kg unconjugated oligonucleotide (748_1) or 7 mg/kg GalNAc-conjugated oligonucleotides (759_2) targeting mouse PD-L1 mRNA. Two days later, the mice were injected i.p. with 15 mg/kg poly(I:C) (LWM, Invivogen). The mice were anesthesized 18-20 h after poly(I:C) injection and the liver was perfused at a flow rate of 7 ml per min through the vena cava using Hank's balanced salt solution containing 15 mM Hepes and 0.38 mM EGTA for 5 min followed by collagenase solution (Hank's balanced salt solution containing 0.17 mg/ml Collagenase type 2 (Worthington 4176), 0.03% BSA, 3.2 mM $CaCl_2$ and 1.6 g/l $NaHCO_3$) for 12 min. Following perfusion , the

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liver was removed and the liver capsule was opened, the liver suspension was filtered through 70 µm cell strainer using William E medium and an aliquot of the cell suspension (= mixed liver cells) was removed for later analysis. The rest of the cell suspension was centrifuged for 3 min at 50xg. The supernatant was collected for later purification of non-parenchymal cells. The pellet was resuspended in 25 ml William E medium (Sigma cat. no. W1878 complemented with 1x Pen/Strep, 2 mM L-glutamine and 10% FBS (ATCC #30-2030)), mixed with 25 ml William E medium containing 90% percoll and the hepatocytes were precipitated by centrifugation at 50xg for 10 min. Following 2x washing in William E medium, the precipitated hepatocytes were resuspended in Williams E medium. The supernant containing non-parenchymal cells was centrifuged at 500xg 7 min and the cells were resuspended in 4 ml RPMI medium and centrifugated through two layers of percoll (25% and 50% percoll) at 1800xg for 30 min. Following collection of the non-parenchymal cells between the two percoll layers, the cells were washed and resuspended in RPMI medium.

Total mRNA was extracted from purified hepatocytes, non-parenchymal cells and total liver suspension (non-fractionated liver cells) using the PureLink Pro 96 RNA Purification kit (Ambion), according to the manufacturer's instructions. cDNA was synthesized using M-MLT Reverse Transcriptase, random decamers RETROscript, RNase inhibitor (Ambion) and 100 mM dNTP set (Invitrogen, PCR Grade) according to the manufacturer's instruction. For gene expressions analysis, qPCR was performed using TaqMan Fast Advanced Master Mix (2X) (Ambion) in a duplex set up with TaqMan primer assays for the PD-L1 (Thermo Fisher Scientific; FAM-MGB Mm00452054-m1) and TBP (Thermo Fisher Scientific; VIC-MGB-PL Mm00446971_m1). The relative PD-L1 mRNA expression level is shown in table 10 as % of control samples from mice injected with saline and poly(I:C).

Oligonucleotide content analysis was performed using ELISA employing a biotinylated capture probe with the sequence 5´-TACCGT-s-Bio-3' and a digoxigenin conjugated detection probe with the sequence 5´- DIG-C12-S1-CCTGTG – 3´. The probes consisted of only LNA with a phosphodiester backbone. Liver samples (approximately 50 mg) were homogenized in 1.4 mL MagNa pure lysis buffer (Roche Cat. No 03604721001) in a 2 mL Eppendorf tube containing one 5mm stainless steel bead. Samples were homogenized on Retsch MM400 homogenizer (Merck Eurolab) until a uniform lysate was obtained. The samples were incubated for 30 min at room temperature. Standards were generated by spiking the unconjugated antisense oligonucleotide compound (CMP ID NO 748_1) in defined concentrations into an untreated liver sample and processing them as the samples. Spike-in concentrations are chosen to match the expected sample oligo content (within ~10-fold).

The homogenized samples were diluted a minimum of 10 times in 5 x SSCT buffer (750 mM NaCl, and 75 mM sodium citrate, containing 0.05 % (v/v) Tween-20, pH 7.0) and a dilution series of 6 times 2 fold dilutions using capture-detection solution (35 nM capture probe and 35

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nM detection probe in 5xSSCT buffer) were made and incubated for 30 min at room temperature. The samples were transferred to a 96 well streptavidin coated plate (Nunc Cat. No. 436014) with 100 µL in each well. The plates were incubated for 1 hour at room temperature with gentle agitation. Wash three times with 2 x SSCT buffer and add 100 µL anti-DIG-AP Fab fragment (Roche Applied Science, Cat. No. 11 093 274 910) diluted 1:4000 in PBST (Phosphate buffered saline, containing 0.05 % (v/v) Tween-20, pH 7.2, freshly made) was added to each well and incubated for 1 hour at room temperature under gentle agitation. Wash three times with 2 x SSCT buffer and add 100 µL of alkaline phosphatase (AP) substrate solution (Blue Phos Substrate, KPL product code 50-88-00, freshly prepared). The intensity of the color was measured spectrophotometrically at 615 nm after 30 minutes incubation with gentle agitation. Raw data were exported from the readers (Gen5 2.0 software) to excel format and further analyzed in excel. Standard curves were generated using GraphPad Prism 6 software and a logistic 4PL regression model.

Table 14: PD-L1 expression and oligo content in total liver suspension, hepatocytes and non-parenchymal cells from poly(I:C) mice treated with unconjugated and GalNAc-conjugated oligonucleotides, n=3.

Cell type	CMP ID no	PD-L1 expression (% of saline- poly(I:C))		oligo content (ng/10^5 cells)	
		Avg	SD	Avg	SD
Total liver	748_1	31	12.4	2.3	0.3
	759_2	28	5.3	8.3	1.1
Hepatocytes	748_1	33	8.0	5.1	3.7
	759_2	7	1.0	43.8	18.9
Non-parenchymal cells	748_1	31	10.1	2.2	0.7
	759_2	66	1.6	1.7	0.9

The results show that naked (CMP ID NO: 748_1) and conjugated (CMP ID NO: 759_2) oligonucleotide reduce PD-L1 mRNA equally well in total liver cells. In isolated hepatocytes, the effect of the conjugated oligonucleotide is almost 5 fold stronger than the effect of the naked oligonucleotide, while naked oligonucleotides showed two fold stronger effect than GalNAc-conjugated oligonucleotides in non-parenchymal cells. In hepatocytes and non- parenchymal cells the reduction of PD-L1 mRNA expression correlates to some extent with the oligonucleotide content in these cell types.

Example 5 - *In vivo* PD-L1 knock down in AAV/HBV mice using naked and GalNAc conjugated PD-L1 antisense oligonucleotides

In the present study AAV/HBV mice were treated with naked or conjugated to GalNAc PD-L1 antisense oligonucleotides, and the PD-L1 mRNA expression and HBV gene expression was evaluated in the liver.

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Female HLA-A2/DR1 mice 5-8 weeks old (5 animals pr. group) were pretreated at week -1 vehicle (saline), naked PD-L1 antisense oligonucleotides (CMP ID NO 752_1 at 5 mg/kg s.c.) and GalNAc PD-L1 antisense oligonucleotides (CMP ID NO 763_2 at 7 mg/kg s.c.), these doses correspond to equimolar concentrations of the oligonucleotides. The mice were transduced by 5x 10^{10} vg AAV-HBV at week 0 (for further details see description AAV/HBV mouse model in the Materials and Methods section). From W1 post AAV-HBV transduction to W4, mice received 4 additional s.c. injections of PD-L1 oligonucleotides or vehicle (saline solution), given one week apart.

Blood samples were taken one week before transduction and one week after each injection.

Mice were sacrificed two weeks after the last injections and their liver were removed following PBS perfusion. The liver was cut in smaller pieces and directly frozen.

To measure HBV gene expression, DNA was extracted from serum with Qiagen Biorobot using the QIAamp One for all nucleic acid kit, Cat.# 965672, serum was diluted 1:20 dilution in PBS a total of 100 µl was lysed in 200ul Buffer AL. DNA was eluted from the kit in 100 µl.

For the Real-Time qPCR the TaqMan Gene Expression Master Mix (cat.#4369016, Applied Biosystems) was used together with a primer mix prepared by adding 1:1:0.5 of the following primers F3_core, R3_core, P3_core (Integrated DNA Technologies, all reconstituted at 100uM each)

Forward (F3_core): CTG TGC CTT GGG TGG CTT T (SEQ ID NO: 784)
Reverse (R3_core): AAG GAA AGA AGT CAG AAG GCA AAA (SEQ ID NO: 785)
Probe (P3_core):56-FAM-AGC TCC AAA/ZEN/TTC TTT ATA AGG GTC GAT GTC CAT G-3IABkFQ (SEQ ID NO: 786)

A standard curve using HBV plasmid (Genotype D, GTD) was prepared using 10-fold dilutions starting with 1x10^9 copies/µl down to 1 copy/µl and used in 5µl per reaction.

For each reaction 10µl Gene Expression Master Mix, 4.5µl water, 0.5µl Primer mix and 5µl sample or standard was added and the qPCR was run.

For the analysis the copy number / ml / well was calculated using the standard curve. The results are shown in table 15.

PD-L1 mRNA expression was measured using qPCR.

mRNA was extracted from frozen liver pieces that were added to 2ml tubes containing ceramic beads (Lysing Matrix D tubes, 116913500, mpbio) and 1ml of Trizol.

The liver piece was homogenized using the Precellys Tissue Disruptor.200µl Chloroform was added to the homogenate, vortexed and centrifuged at 4°C for 20min at 10000rpm.The RNA containing clear phase (around 500ul) was transferred into a fresh tube and the same volume of

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70% EtOH was added. After mixing well the solution was transferred onto a RNeasy spin column and RNA was further extracted following the RNeasy Kit's manual RNeasy Mini Kit, cat.# 74104, Qiagen (including the RNA digestion RNase-free DNase Set, cat.# 79254). Elution in 50µl H_2O. The final RNA concentration was measured and adjusted to 100ng/ul for all samples.

The qPCR was conducted on 7.5µl RNA using the Taqman RNA-to-ct 1-step Kit, cat.# 4392938, Thermo Fisher according to the manufactures instructions. The fprimer mixed used contained PD-L1_1-3 (Primer number Mm00452054_m1, Mm03048247_m1 and Mm03048248_m1) and endogounous controls (ATCB Mm00607939_s1, CANX Mm00500330_m1, YWHAZ Mm03950126_s1 and GUSB Mm01197698_m1)

Data were analysed using the 2^-ddct method. The mean of all four endogenous controls was used to calculate dct values. The PD-L1 expression relative to mean of the endogenous controls and in % of saline

Table 15: PD-L1 mRNA expression and HBV DNA in AAV/HBV mice treated with unconjugated and GalNAc-conjugated oligonucleotides, n=5.

	CMP ID no	PD-L1 mRNA expression (% of saline)		HBV DNA expression (% of saline)	
		Avg	SD	Avg	SD
Naked	752_1	55	35	72	16
GalNAc conjugated	763_2	34	3	79	9

From these results it can be seen that both naked and GalNAc conjugated oligonucleotides are capable of reducing PD-L1 mRNA expression in the liver of an AAV/HBV mouse, with the GalNAc conjugated oligonucleotide being somewhat better. Both oligonucleotides also resulted in some reduction in HBV DNA in the serum.

Example 6 – *In vivo* effect on T cell response in AAV/HBV mice

In the present study AAV/HBV mice from Pasteur were treated with an antibody or antisense oligonucleotides targeting PD-L1. The antisense oligonucleotides were either naked or conjugated to GalNAc. During the treatment the animals were immunized with a DNA vaccine against HBs and HBc antigens (see Materials and Methods section) to ensure efficient T cell priming by the antigen presenting cells. It was evaluated how the treatment affected the cell population in liver and spleen, as well as the PD-L1 expression on these populations and whether a HBV specific T cell response could be identified.

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Treatment protocol:

Female HLA-A2/DR1 mice were treated according to the protocols below. The study was conducted in two separate sub-studies, with slight differences in the administration regimens as indicated in Table 16 and 17 below.

DNA vaccine and anti-PD-L1 antibody was administered as described in the materials and method section. The antisense oligonucleotides used were CMP ID NO 748_1 (naked) at 5 mg/kg and CMP ID NO: 759_2 (GalNAc conjugated) at 7mg/kg, both where administered as subcutaneous injections (s.c.).

Table 16: AAV/HBV mouse treatment protocol with DNA vaccine and DNA vaccine + anti-PD-L1 antibody, 6 mice in each group

Day	Vehicle (Group 10)	DNA vaccine (Group 11)	DNA vaccine + anti-PDL-1 Ab (Group 13)
0	AAV/HBV		
29*	Animal randomization		
34	Saline+Isotype	-	Ab
41	Saline+Isotype	-	Ab
48	Saline+Isotype	-	Ab
50	-	CaTx	CaTx
55*	PBS+Isotype	DNA	DNA+Ab
62	Saline+Isotype	-	Ab
69	PBS+Isotype	DNA	DNA+Ab
76*	Saline+Isotype	-	Ab
83	Saline+Isotype	-	Ab
97*	Sacrifice		

Isotype= mouse IgG control Ab, CaTx = cardiotoxine, DNA = DNA vaccine, Ab=anti-PD-L1 Ab and *= serum collection

Table 17: AAV/HBV mouse treatment protocol with DNA vaccine and DNA vaccine + naked or conjugated PD-L1 oligonucleotide (ASO), 7 mice in each group

Day	Vehicle (Group 1)	DNA vaccine (Group 2)	DNA vaccine + PDL-1 ASO (Group 7)	DNA vaccine + GN-PDL-1 ASO (Group 8)
0	AAV/HBV			
29*	Animal randomization			
39	Saline	Saline		
41		Saline	ASO	GN-ASO
46	Saline	Saline		
49		Saline	ASO	GN-ASO
53	Saline	Saline		
55	CaTx	CaTx	CaTx	CaTx
56		Saline	ASO	GN-ASO
59	PBS+ Saline	DNA+PBS	DNA	DNA
62*		Saline	ASO	GN-ASO
67	Saline	Saline		
70		Saline	ASO	GN-ASO
74	PBS+ Saline	DNA+PBS	DNA	DNA

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Day	Vehicle (Group 1)	DNA vaccine (Group 2)	DNA vaccine + PDL-1 ASO (Group 7)	DNA vaccine + GN-PDL-1 ASO (Group 8)
77		Saline	ASO	GN-ASO
81	Saline	Saline		
84*		Saline	ASO	GN-ASO
88	Saline	Saline		
91		Saline	ASO	GN-ASO
102	Sacrifice			

DNA = DNA vaccine, CaTx = cardiotoxine, Ab=anti-PD-L1 Ab, ASO= naked PDL-1 oligonucleotide, GN-ASO= GalNAc-PDL-1 oligonucleotide and *= serum collection

At the time of sacrifice blood, spleen and liver mononuclear cells of each mouse from each group were collected and depleted of red blood cells (Lysing Buffer, BD biosciences, 555899). The liver mononuclear cells required a specific preparation as described in the materials and method section.

Cell populations:

In the liver the cell population was analyzed by surface labeling on liver mononuclear cells (see materials and methods) using cytometry.

No significant changes were noticed in the frequencies of NK cells in the spleen and liver of treated mice compared to control groups (i.e. vehicle and DNA-immunized groups). Table 18 show that in the liver, groups treated with naked PD-L1 oligonucleotide (CMP ID NO 748_1) and GalNAc conjugated PD-L1 oligonucleotide (CMP ID NO: 759_2) had a significant increase in T cell numbers compared to either control groups (i.e. vehicle and DNA-immunized groups) also presented in figure 10 A. This increase was due to an increase in both CD4+ and CD8+ T cell populations (Table 18 and figure 10B and 10C, respectively).

Table 18: T-cells in the liver following treatment in millions of cells

	T-cells (millinons)		CD4+ T-cells (millions)		CD8+ T-cells (millions)	
	Avg	Std	Avg	Std	Avg	Std
Vehicle (Group 1)	0.77	0.44	0.51	0.35	0.11	0.05
DNA vaccine (Group 2)	0.90	0.24	0.58	0.16	0.16	0.08
DNA vaccine + anti-PD-L1 Ab (Group 13)	1.98	0.90	1.40	0.81	0.41	0.23
Vehicle (Group 10)	1.73	0.87	1.13	0.55	0.40	0.25
DNA vaccine (Group 11)	1.27	0.97	0.79	0.58	0.32	0.32
DNA vaccine + PD-L1 ASO (Group 7)	3.78	1.31	2.46	0.72	0.79	0.39
DNA vaccine + GN-PD-L1 ASO (Group 8)	3.33	0.66	2.18	0.40	0.67	0.17

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PD-L1 expression:

The expression of PD-L1 protein was evaluated on macrophages, B and T cells from spleen and liver at time of sacrifice. The presence of PD-L1 antibody in the surface labeling antibody mix (see materials and methods) allowed quantification of PD-L1 expressing cells by cytometry.

In spleen, no significant difference between the treatments was observed in the % of macrophages, B cells and CD4+ T cells expressing PD-L1. The % of the CD8+ T cells expressing PD-L1 was lower in mice treated with naked PD-L1 oligonucleotide (CMP ID NO 748_1) and GalNAc conjugated PD-L1 oligonucleotide (CMP ID NO: 759_2) when compared to the other treatments (data not shown).

In liver, PD-L1 was expressed mainly on CD8+ T cells with a mean frequency of 32% and 41% in the control groups (the two vehicle and DNA vaccination groups combined, respectively, figure 11A). Treatment with naked PD-L1 oligonucleotide or GalNAc PD-L1 oligonucleotide resulted in a decrease of the frequency of CD8+ T cells expressing PD-L1 (see table 19 figure 11A). Significant differences in the % of cells expressing PD-L1 were also noticed for B cells and CD4+ T-cells after ASO treatment, although these cell types express significantly less PD-L1 than the CD8+ T cells (see table 19 and figure 11B and C). Treatment with anti-PD-L1 Ab, also resulted in an apparent decrease in the PD-L1 expression in all cell types. It is, however, possible that this decrease is due to partly blockage of the PD-L1 epitope by the anti-PD-L1 antibody used for treatment, so that the PD-L1 detection antibody in the surface labeling antibody mix is prevented from binding to PD-L1. Therefore what appears to be a PD-L1 down regulation by the anti-PD-L1 antibody used for treatment may be the result of epitope competition between the treatment antibody and the detection antibody.

Table 19: % of liver cell population with PD-L1 expression

	% of CD8+ T-cells		% of CD4+ T-cells		% of B-cells	
	Avg	Std	Avg	Std	Avg	Std
Vehicle (Group 10)	35.5	4.7	0.75	0.52	5.9	1.5
DNA vaccine (Group 11)	36.8	7.7	0.61	0.08	5.5	1.1
DNA vaccine + anti-PD-L1 Ab (Group 13)	18.6	12.3	0.33	0.10	2.9	1.7
Vehicle (Group 1)	28.5	11.5	0.64	0.21	5.9	1.7
DNA vaccine (Group 2)	44.9	14.4	1.43	0.69	8.7	3.1
DNA vaccine + PD-L1 ASO (Group 7)	9.6	2.4	0.37	0.21	2.9	0.8
DNA vaccine + GN-PD-L1 ASO Group 8)	14.6	3.3	0.31	0.11	2.8	0.8

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HBV specific T cell response:

NK cells and CD4+ and CD8+ T cells producing pro-inflammatory cytokines were detected using the intracellular cytokine staining assays (see Materials and Methods section) detecting IFNγ and TNFα production.

In the spleen no NK cells and few CD4+ T cells secreting IFNγ- and TNFα were detectable (frequency < 0.1%) at sacrifice. IFNγ-producing CD8+ T cells targeting the two HBV antigens were detected in mice treated with naked PD-L1 oligonucleotide or GalNAc PD-L1 oligonucleotide as well as in mice from this study which received only DNA vaccine (data not shown).

In the livers of DNA-immunized HBV-carrier mice, no IFNγ-producing NK cells were detected at sacrifice, whereas IFNγ-secreting CD4+ T cells specific for Core or for S2+S were detected in the liver of a few DNA-immunized mice at a low frequency (< 0.4%, data not shown). HBV S2+S -specific CD8+ T cells producing IFNγ were detected in the majority of DNA-immunized mice. The frequency of IFNγ-secreting CD8+ T cells increased in mice treated with combination of DNA vaccine and naked PD-L1 oligonucleotide or GalNAc PD-L1 oligonucleotide, whereas treatment with anti-PD-L1 antibody did not add any apparent additional effect to the DNA vaccination (figure 12). IFNγ-producing CD8+ T cells targeting the envelope and core antigens were detected in most DNA-immunized groups (except anti-PD-L1 antibody) (figure 12B). Most of the S2-S specific T cells produced both IFNγ and TNFα (figure 12C). The results are also shown in Table 20.

Table 20: % of HBV antigen (S2-S or core) specific CD8+ T cells from total IFNγ or IFNγ + TNFα cell population

	PreS2-S specific T cells (% of IFNγ cells)		Core specific T cells (% of IFNγ cells)		S2-S specific T cells (% of IFNγ + TNFα)	
	Avg	Std	Avg	Std	Avg	Std
Vehicle (Group 10)	0.15	0.37	0.18	0.43	0.00	0.00
DNA vaccine (Group 11)	1.48	1.10	0.47	0.53	0.42	1.02
DNA vaccine + anti-PDL-1 Ab	1.18	0.95	0	0	0.38	0.49
Vehicle (Group 1)	0.17	0.45	0.11	0.28	0.00	0.00
DNA vaccine (Group 2)	1.70	1.02	0.27	0.51	0.98	0.90
DNA vaccine + PDL-1 ASO	2.56	1.60	0.78	0.80	1.44	1.55
DNA vaccine + GN-PDL-1 ASO	3.83	2.18	0.68	1.16	2.62	1.62

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Example 7 – *In vivo* effect on HBV antigen and HBV DNA in the serum of AAV/HBV mice

In the present study AAV/HBV mice from Shanghai (see Materials and Methods section) were treated with the GalNAc conjugated PD-L1 antisense oligonucleotide CMP ID NO 759_2.

It was evaluated how the treatment affected the HBe and HBs antigens and HBV DNA levels in the serum compared to vehicle treated animals.

Treatment protocol:

Male C57BL/6 mice infected with recombinant adeno-associated virus (AAV) carrying the HBV genome (AAV/HBV) as described under the Shanghai model in the materials and method section were used in this study. The mice (6 mice pr. group) were injected once a week for 8 weeks with the antisense oligonucleotide CMP ID NO: 759_2 at 5 mg/kg or vehicle (saline) both where administered as subcutaneous injections (s.c.). Blood samples were collected each week during treatment as well as 6 weeks post treatment. HBV DNA, HBsAg and HBeAg levels were measured in the serum samples as described below. The results for the first 10 weeks are shown in table 21 and in figure 13. The study was still ongoing at the time of filing the application therefore data for the remaining 4 weeks have not been obtained.

HBsAg and HBeAg detection:

Serum HBsAg and HBeAg levels were determined in the serum of infected AAV-HBV mouse using the HBsAg chemoluminescence immunoassay (CLIA) and the HBeAg CLIA kit (Autobio diagnostics Co. Ltd., Zhengzhou,China, Cat. no.CL0310-2 and CL0312-2 respectively), according to the manufacturer's protocol. Briefly, 50µl of serum was transferred to the respective antibody coated microtiter plate and 50µl of enzyme conjugate reagent was added. The plate was incubated for 60 min on a shaker at room temperature before all wells were washed six times with washing buffer using an automatic washer. 25µl of substrate A and then 25µl of substrate B was added to each well. The plate was incubated for 10 min at RT before luminescence was measured using an Envision luminescence reader. HBsAg is given in the unit IU/ml; where 1 ng HBsAg =1.14 IU. HBeAg is given in the unit NCU/ml serum.

HBV DNA extraction and qPCR:

Initially mice serum was diluted by a factor of 10 (1:10) with Phosphate buffered saline (PBS). DNA was extracted using the MagNA Pure 96 (Roche) robot. 50µl of the diluted serum was mixed in a processing cartridge with 200ul MagNA Pure 96 external lysis buffer (Roche, Cat. no. 06374913001) and incubated for 10 minutes. DNA was then extracted using the "MagNA Pure 96 DNA and Viral Nucleic Acid Small Volume Kit" (Roche, Cat. no. 06543588001) and the "Viral NA Plasma SV external lysis 2.0" protocol. DNA elution volume was 50µl.

Quantification of extracted HBV DNA was performed using a Taqman qPCR machine (ViiA7, life technologies). Each DNA sample was tested in duplicate in the PCR. 5µl of DNA sample was

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added to 15µl of PCR mastermix containing 10µl TaqMan Gene Expression Master Mix (Applied Biosystems, Cat. no. 4369016), 0.5 µl PrimeTime XL qPCR Primer/Probe (IDT) and 4.5µl distilled water in a 384 well plate and the PCR was performed using the following settings: UDG Incubation (2min, 50°C), Enzyme Activation (10min, 95°C) and PCR (40 cycles with 15sec, 95° for Denaturing and 1min, 60°C for annealing and extension). DNA copy numbers were calculated from C_t values based on a HBV plasmid DNA standard curve by the ViiA7 software.

Sequences for TaqMan primers and probes (IDT):
Forward core primer (F3_core): CTG TGC CTT GGG TGG CTT T (SEQ ID NO: 784)
Reverse primer (R3_core): AAG GAA AGA AGT CAG AAG GCA AAA (SEQ ID NO: 785)
Taqman probe (P3_core): 56-FAM/AGC TCC AAA /ZEN/TTC TTT ATA AGG GTC GAT GTC CAT G/3IABkFQ (SEQ ID NO: 786).

Table 21: HBV-DNA, HBsAg and HBeAg levels in serum from AAV/HBV mice following treatment with GalNAc conjugated PD-L1 antisense oligonucleotide.

	Saline						**CMP ID NO: 759_2 at 5 mg/kg**					
	HBV-DNA		**HBsAg**		**HBeAg**		**HBV-DNA**		**HBsAg**		**HBeAg**	
Day	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std	Avg	Std
0	7.46	0.35	3.96	0.48	3.23	0.14	7.44	0.29	3.87	0.40	3.17	0.13
7	7.53	0.23	4.17	0.45	3.35	0.10	7.53	0.20	3.91	0.42	3.19	0.18
14	7.57	0.24	4.12	0.49	3.19	0.11	7.45	0.22	3.90	0.50	2.99	0.27
21	7.47	0.27	3.93	0.51	3.12	0.05	7.33	0.47	3.71	0.76	2.78	0.26
28	7.68	0.26	3.88	0.67	3.18	0.13	7.45	0.46	3.65	0.93	2.67	0.38
35	7.69	0.21	4.03	0.54	2.95	0.08	7.13	0.75	2.98	1.05	2.04	0.38
42	7.58	0.23	3.89	0.65	3.34	0.10	6.69	0.89	2.60	1.05	1.98	0.45
49	7.77	0.17	3.54	1.06	3.08	0.26	6.56	1.26	2.19	0.70	1.47	0.37
56	7.71	0.24	3.99	0.86	3.28	0.05	6.21	1.48	2.28	0.84	1.38	0.30
63	7.59	0.28	3.67	1.07	3.25	0.13	6.08	1.39	2.08	0.71	1.35	0.30

From this study it can be seen that GalNAc conjugated PD-L1 antisense oligonucleotide CMP NO 759_2 has a significant effect on the reduction of HBV-DNA, HBsAg and HBeAg levels in serum after 6 weeks of treatment, and effect that is sustained for at least 2 weeks after the treatment has ended.

Example 8 – *In vitro* PD-L1 knock down in human primary hepatocytes using GalNAc conjugated PD-L1 oligonucleotides

The ability of GalNAc conjugated PD-L1 antisense oligonucleotide compounds to reduce the PD-L1 transcript in primary human hepatocytes was investigated using genomics.

Cell culture

Cryopreserved human hepatocytes were suspended in WME supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (0.1 mg/ml) and L-glutamine (0.292 mg/ml) at a

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density of approx. 5 x 10^6 cells/ml and seeded into collagen-coated 24-well plates (Becton Dickinson AG, Allschwil, Switzerland) at a density of 2 x 10^5 cells/well. Cells were pre-cultured for 4h allowing for attachment to cell culture plates before start of treatment with oligonucleotides at a final concentration of 100 µM. The oligonucleotides used are shown in table 21 and table 8, vehicle was PBS. Seeding medium was replaced by 315 µl of serum free WME (supplemented with penicillin (100 U/ml), streptomycin (0.1 mg/ml), L-glutamine (0.292 mg/ml)) and 35 µl of 1 mM oligonucleotide stock solutions in PBS were added to the cell culture and left on the cells for 24 hours or 66 hours.

Library preparation

Transcript expression profiling was performed using Illumina Stranded mRNA chemistry on the Illumina sequencing platform with a sequencing strategy of 2 x 51 bp paired end reads and a minimum read depth of 30M per specimen (Q squared EA). Cells were lysed in the wells by adding 350 µl of Qiagen RLT buffer and were accessioned in a randomization scheme.

mRNA was purified using the Qiagen RNeasy Mini Kit. mRNA was quantitated and integrity was assessed using an Agilent Bioanalyzer. Upon initial quality assessment of the isolated RNA, it was observed that all samples met the input quality metric of 100ng with RIN scores >7.0.

Sequencing libraries were generated for all samples using the Illumina TruSeq Stranded mRNA Library Preparation, starting with 100 ng of total RNA. Final cDNA libraries were analyzed for size distribution and using an Agilent Bioanalyzer (DNA 1000 kit), quantitated by qPCR (KAPA Library Quant Kit) and normalized to 2 nM in preparation for sequencing. The Standard Cluster Generation Kit v5 was used to bind the cDNA libraries to the flow cell surface and the cBot isothermally to amplify the attached cDNA constructs up to clonal clusters of ~1000 copies each. The DNA sequence was determined by sequencing-by-synthesis technology using the TruSeq SBS Kit.

Data processing

Illumina paired-end sequencing reads of length 2x51bp were mapped on the human reference genome hg19 using the GSNAP short read alignment program. SAM-format alignments were converted into sorted alignment BAM-format files using the SAMTOOLS program. Gene read counts were estimated for PD-L1 based on the exon annotation from NCBI RefSeq, specified by the corresponding GTF file for hg19. A normalization step accounting for the different library size of each sample was applied using the DESeq2 R package.

The reduction in PD-L1 transcript after incubation with GalNAc conjugated PD-L1 antisense oligonucleotide compounds are shown in table 22.

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Table 22: PD-L1 transcript reduction in human primary hepatocytes following treatment with GalNAc conjugated oligonucleotides, n=4

Compound	PD-L1 expression level 24 h (library size adjusted counts)	PD-L1 expression level 66 h (library size adjusted counts)
Vehicle	259	156
	159	168
	192	136
	202	211
767_2	7	7
	11	14
	22	9
	28	15
766_2	16	13
	15	10
	17	11
	29	13
769_2	15	21
	18	18
	25	18
	26	25
768_2	41	25
	27	48
	31	25
	34	22
770_2	21	16
	44	62
	67	51
	38	63

All five GalNAc conjugated antisense compounds showed significant PD-L1 transcript reduction after 24 and 66 hour incubation when compared to samples treated with vehicle.

Example 9 - EC50 of conjugated and naked PD-L1 antisense oligonucleotides in HBV infected ASGPR-HepaRG cells

The potency of two naked and the equivalent GalNAc conjugated PD-L1 antisense oligonucleotides were compared in HBV infected ASGPR-HepaRG cells.

Cell line

HepaRG cells (Biopredic International, Saint-Gregoire, France) were cultured in Williams E medium (supplemented with 10% HepaRG growth supplement (Biopredic). From this cell line a HepaRG cell line stably overexpressing human ASGPR1 and ASGPR2 was generated using a lentiviral method. Proliferating HepaRG cells were transduced at MOI 300 with a lentivirus produced on demand by Sirion biotech (CLV-CMV-ASGPR1-T2a_ASGPR2-IRES-Puro) coding for Human ASGPR1 and 2 under the control of a CMV promoter and a puromycin resistance gene. Transduced cells were selected for 11 days with 1µg/ml puromycin and then maintained in the same concentration of antibiotic to ensure stable expression of the transgenes. ASGPR1/2 overexpression was confirmed both at mRNA level by RT-qPCR

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(ASGPR1: 8560 fold vs non-transduced, ASGPR2: 2389 fold vs non transduced), and at protein level by flow cytometry analysis.

The cells were differentiated using 1.8% DMSO for at least 2 weeks before infection. HBV genotype D was derived from HepG2.2.15 cell culture supernatant and was concentrated using PEG precipitation. To evaluate activity of test compounds against HBV, differentiated ASGPR-HepaRG cells in 96 well plates were infected with HBV at an MOI of 20 to 30 for 20 h, before the cells were washed 4 times with PBS to remove the HBV inoculum.

Oligonucleotide potency

The following oligonucleotides

Naked PD-L1 ASO	Equivalent GalNAc conjugated PD-L1 ASO
CPM ID NO: 640_1	CPM ID NO: 768_2
CPM ID NO: 466_1	CPM ID NO: 769_2

were added to the HBV infected ASGPR-HepaRG cells on day 7 and day 10 post infection using serial dilutions from 25 µM to 0.4 nM (1:4 dilutions in PBS). Cells were harvested on day 13 post infection.

Total mRNA was extracted using the MagNA Pure 96 Cellular RNA Large Volume Kit on the MagNA Pure 96 System (Roche Diagnostics) according to the manufacturer's instructions. For gene expression analysis, RT-qPCR was performed as described in Example 5.

Data were analysed using the 2^-ddct method. ActinB was used as the endogenous control to calculate dct values. The PD-L1 expression is relative to the endogenous controls and to the saline vehicle.

EC50 calculations were performed in GraphPad Prism6 and is shown in table 23.

Table 23: EC50 in ASGPR-HepaRG HBV infected cells, n=4.

CMP ID NO	EC50 (µM)
640_1	2.25
768_2	0.10
466_1	5.82
769_2	0.13

These data clearly shows that GalNAc conjugation of the PD-L1 antisense oligonucleotides improves the EC50 values significantly.

Example 10 – Stimulation T cell function in PBMCs derived from chronic HBV patients

It was investigated whether naked PD-L1 antisense compounds could increase the T cells function of chronically infected HBV (CHB) patients after ex-vivo HBV antigen stimulation of the peripheral blood mononuclear cells (PBMCs).

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Frozen PBMCs from three chronic HBV infected patients were thawed and seeded at a density of 200'000 cells/well in 100µl medium (RPMI1640 + GlutaMax+ 8% Human Serum + 25mM Hepes + 1% PenStrep). The next day, cells were stimulated with 1µM PepMix HBV Large Envelope Protein or 1µM PepMix HBV Core Protein (see table 9) with or without 5µM of CMP ID NO: 466_1 or CMP ID NO: 640_1 in 100µl medium containing 100pg/ml IL-12 and 5ng/ml IL-7 (Concanavalin stimulation was only applied at day 8). Four days later PD-L1 antisense oligonucleotide treatment was renewed with medium containing 50IU IL-2. At day 8 after the first stimulation the cells were re-stimulated with PepMix or 5µg/ml Concanavalin A plus PD-L1 antisense oligonucleotide for 24h. For the last 5h of the stimulation, 0.1µl Brefeldin A, 0.1µl Monensin and 3µl anti-human CD-107 (APC) were added.

After 24h the cells were washed with Stain Buffer (PBS + 1% BSA + 0.09% Sodium Azide + EDTA) and surface staining was applied for 30min at 4°C [anti-human CD3 (BV 605), anti-human CD4 (FITC), anti-human CD8 (BV711), anti-human PDL1 (BV421), anti-human PD1 (PerCP-Cy5.5) and Live and Dead stain (BV510) (BD Biosciences)]. Cells were fixed in BD Fixation Buffer for 15min at 4°C. The next morning, cells were permeabilized with BD Perm/Wash Buffer for 15min at 4°C and intracellular staining was done for 30min at 4°C [anti-human INFγ (PE)]. After washing in Perm/Wash Buffer cells were dissolved in 250µl stain buffer.

FACS measurement was performed on a BD Fortessa (BD Biosciences). For the analysis, the whole cell population was first gated on live cells (Live and Death stain, BV510), and then on CD3+ (BV605) cells. The CD3+ cells were then graphed as CD107a+ (APC) vs IFNγ+ (PE).

The results are shown in table 24.

Table 24: Effect of PD-L1 ASO treatment on CD3+ T cell from PBMCs isolated from three chronically HBV infected patients.

	No antigen stimulation			Envelope antigen			Core antigen		
	Saline	CMP 466_1	CMP 640_1	Saline	CMP 466_1	CMP 640_1	Saline	CMP 466_1	CMP 640_1
INFγ-/ CD107+	1.16	4.95	4.81	4.7	9.12	8.62	3.84	9.66	7.31
	2.7	3.59	2.74	2.57	3.69	3.2	3.25	3.34	2.92
	3	3.87	3.98	4.59	12.5	10.9	9.23	6.11	6.88
INFγ+/ CD107+	0.12	1.03	1.15	3.19	17.3	18.9	2.38	15.1	5.75
	0.49	3.12	1.75	2.73	7	5.34	1.63	2.35	1.9
	0.24	1.13	1.5	1.6	8.16	3.06	1.68	1.9	1.91
INFγ+/ CD107-	0.33	1.43	1.08	5.11	7.74	9.47	3.14	7.76	2.83
	0.61	2.9	2.26	7.84	5.79	5.78	2.33	2.82	2.95
	0.17	1.57	1.72	1.22	2.58	0.99	0.1	0.61	1.04

From these data it can be seen that the antigen stimulation by itself is capable of inducing T cell activation (increase % of CD3+ cells expressing INFγ and/or CD107a) in the PBMCs of CHB patients (n=3). The addition of PD-L1 antisense oligonucleotide CMP 466_1 or 640_1 resulted

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in an additional increase of CD3+ T cell response. This increase was mainly observed in the HBV envelop stimulated group.

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CLAIMS

1. An antisense oligonucleotide conjugate comprising:
   a. an oligonucleotide comprising a contiguous nucleotide sequence of 10 to 30 nucleotides in length with at least 90% complementarity to a PD-L1 target nucleic acid; and
   b. at least one asialoglycoprotein receptor targeting conjugate moiety covalently attached to the oligonucleotide in a), wherein the contiguous nucleotide sequence is complementary to a sub-sequence of the target nucleic acid, wherein the subsequence is position 5467-12107 on SEQ ID NO: 1.

2. The antisense oligonucleotide conjugate of claim 1, wherein the oligonucleotide comprises the sequence SEQ ID NO: 466.

3. The antisense oligonucleotide conjugate of claim 1 or claim 2, wherein the contiguous nucleotide sequence comprises one or more modified nucleosides.

4. The antisense oligonucleotide of claim 3, wherein the one or more modified nucleosides are 2' sugar modified nucleosides.

5. The antisense oligonucleotide conjugate of claim 4, wherein the one or more 2' sugar modified nucleoside is 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 conjugate of any one of claims 3 to 5, wherein all the modified nucleosides are LNA nucleosides.

7. The antisense oligonucleotide conjugate of any one of claims 1 to 6, wherein the contiguous nucleotide sequence comprises at least one modified internucleoside linkage.

8. The antisense oligonucleotide conjugate of claim 7, wherein the at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.

9. The antisense oligonucleotide conjugate of any one of claims 1 to 8, wherein the oligonucleotide is a gapmer.

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10. The antisense oligonucleotide conjugate of any one of claims 1 to 9, wherein the asialoglycoprotein receptor targeting conjugate moiety comprises at least one carbohydrate moiety selected from group consisting of galactose, galactosamine, N-formyl-galactosamine, N- acetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine and N- isobutanoylgalactosamine.

11. The antisense oligonucleotide conjugate of any one of claims 1 to 10, wherein the asialoglycoprotein receptor targeting conjugate moiety is mono-valent, di-valent, tri-valent or tetra-valent.

12. The antisense oligonucleotide conjugate of any one of claims 1 to 11, wherein the asialoglycoprotein receptor targeting conjugate moiety is a tri-valent N-acetylgalactosamine (GalNAc) moiety.

13. An antisense oligonucleotide of formula CTAattgtagtagtaCTC, wherein capital letters represent beta-D-oxy LNA nucleosides, lowercase letters represent DNA nucleosides, all LNA C are 5-methyl cytosine and all internucleoside linkages are phosphorothioate internucleoside linkages.

14. An antisense oligonucleotide conjugate comprising the oligonucleotide of claim 13 and a conjugate moiety covalently attached to said oligonucleotide.

15. The antisense oligonucleotide conjugate of claim 14, wherein a linker is present between the oligonucleotide and the conjugate moiety.

16. The antisense oligonucleotide conjugate of claim 14 or claim 15, wherein the conjugate moiety is an asialoglycoprotein receptor targeting moiety.

17. The antisense oligonucleotide conjugate of claim 16, wherein the asialoglycoprotein receptor targeting moiety is a tri-valent N-acetylgalactosamine (GalNAc) moiety.

18. The antisense oligonucleotide conjugate of any one of claims 15 to 17, wherein the linker is a physiologically labile linker.

19. The antisense oligonucleotide conjugate of claim 18, wherein the physiologically labile linker is a nuclease susceptible linker.

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20. The antisense oligonucleotide conjugate of claim 18 or claim 19 wherein the physiologically labile linker comprises a cytidine-adenosine dinucleotide.

21. The antisense oligonucleotide conjugate of claim 14 wherein a linker is present between the oligonucleotide and the conjugate moiety; further wherein the conjugate moiety is an asialoglycoprotein receptor targeting moiety that is a tri-valent N-acetylgalactosamine (GalNAc) moiety; wherein the linker is a physiologically labile linker; further wherein the physiologically labile linker comprises a cytidine-adenosine dinucleotide.

22. The antisense oligonucleotide conjugate of any one of claims 14 to 21, wherein the antisense oligonucleotide conjugate is of formula GN2-C6$_o$c$_o$a$_o$CTAattgtagtagtaCTC, wherein C6 represents an amino alkyl group with 6 carbons, capital letters represent beta-D-oxy LNA nucleosides, lowercase letters represent DNA nucleosides, all LNA C are 5-methyl cytosine, subscript o represent a phosphodiester nucleoside linkage and unless otherwise indicated, all internucleoside linkages are phosphorothioate internucleoside linkages, and wherein GN2 represents the trivalent GalNAc cluster shown in Figure 3, further wherein the wavy line in Figure 3 illustrates the site of conjugation of the cluster to the C6 amino alkyl group.

23. The antisense oligonucleotide conjugate of any one of claims 14 to 22, wherein the oligonucleotide conjugate is CMP ID NO: 769_2.

24. The antisense oligonucleotide conjugate shown in Figure 7.

25. A pharmaceutical composition comprising the antisense oligonucleotide or antisense oligonucleotide conjugate of any one of claims 1 to 24 and a pharmaceutically acceptable diluent, solvent, carrier, salt and/or adjuvant.

26. The pharmaceutical composition according to claim 25 wherein the pharmaceutically acceptable diluent is sterile phosphate buffered saline.

27. The pharmaceutical composition according to claim 25 or claim 26 wherein the pharmaceutically acceptable salt is sodium.

28. The pharmaceutical composition according to claim 25 or claim 26 wherein the

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pharmaceutically acceptable salt is potassium.

29. An *in vivo* or *in vitro* method for modulating PD-L1 expression in a target cell which is expressing PD-L1, said method comprising administering an antisense oligonucleotide, antisense oligonucleotide conjugate or pharmaceutical composition of any one claims 1 to 28 in an effective amount to said cell.

30. The antisense oligonucleotide, antisense oligonucleotide conjugate or the pharmaceutical composition of any one of claims 1 to 29 for use in restoration of immune response against a virus.

31. The antisense oligonucleotide, antisense oligonucleotide conjugate or pharmaceutical composition for use according to claim 30, wherein the virus is HBV.

32. The antisense oligonucleotide, antisense oligonucleotide conjugate or the pharmaceutical composition of any one of claims 1 to 29 for use in restoration of immune response against a parasite.

33. The antisense oligonucleotide, antisense oligonucleotide conjugate or the pharmaceutical composition for use according to any one of claims 30 to 32, wherein the restoration of the immune response is an increase in the liver of CD8+ T cells specific to one or more HBV antigens when compared to a control.

34. The antisense oligonucleotide, antisense oligonucleotide conjugate or the pharmaceutical composition of any one of claims 1 to 28 for use as a medicament.

35. The antisense oligonucleotide, antisense oligonucleotide conjugate or the pharmaceutical composition of any one of claims 1 to 28 for use in the treatment or prevention of HBV infection.

36. Use of the antisense oligonucleotide, antisense oligonucleotide conjugate or the pharmaceutical composition of any one of claims 1 to 28, for the preparation of a medicament for treatment or prevention of HBV infection.

37. A method for treating or preventing a disease comprising administering a therapeutically or prophylactically effective amount of an antisense oligonucleotide,

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antisense oligonucleotide conjugate or pharmaceutical composition of any one of claims 1 to 28 to a subject suffering from or susceptible to the disease.

38. A method for treating or preventing HBV infection comprising administering a therapeutically or prophylactically effective amount of an antisense oligonucleotide, antisense oligonucleotide conjugate or pharmaceutical composition of any one of claims 1 to 28 to a subject suffering from or susceptible to HBV infection.

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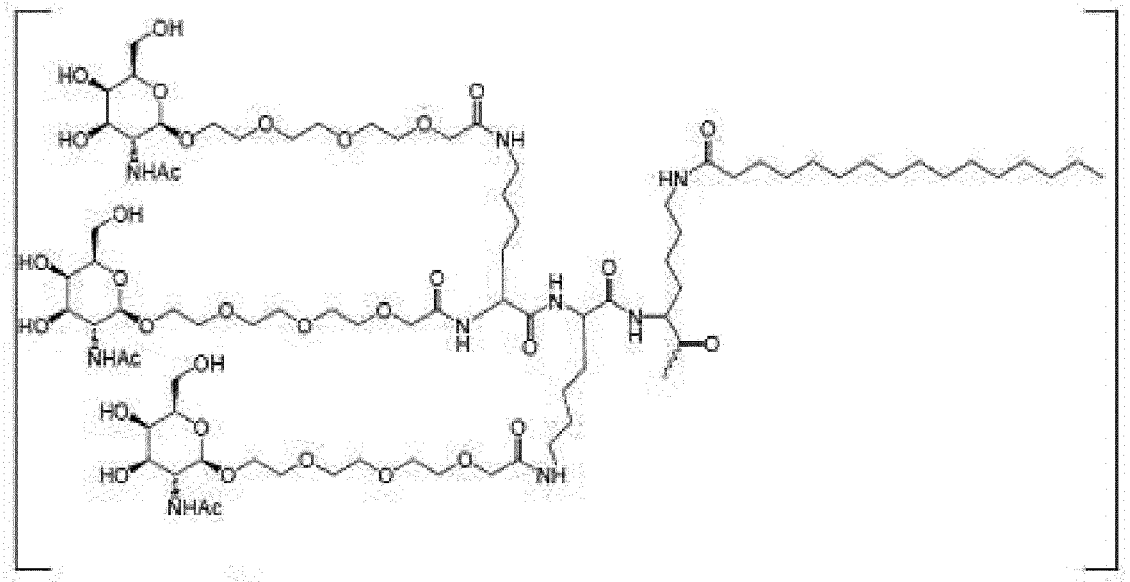
FIGURES

Figure 1

A



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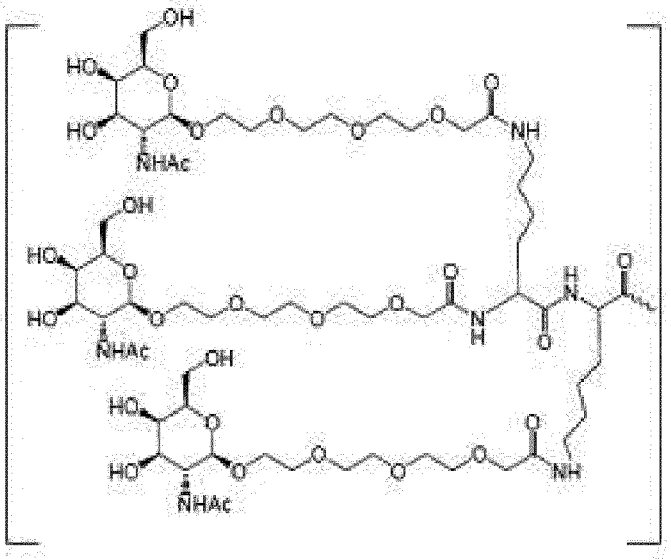

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Figure 1 Cont'd

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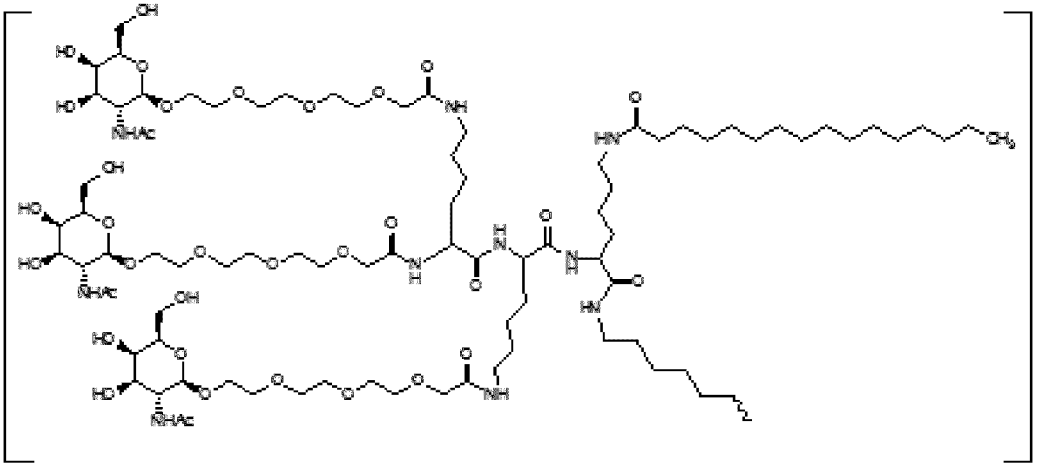

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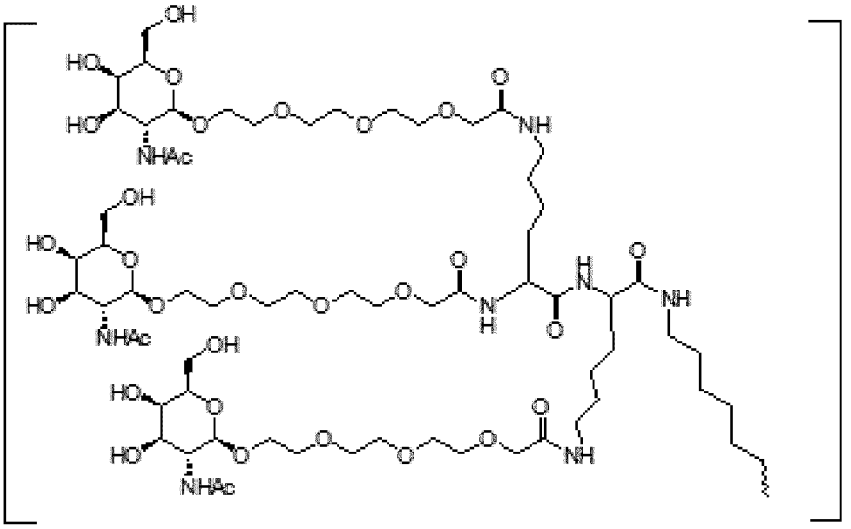

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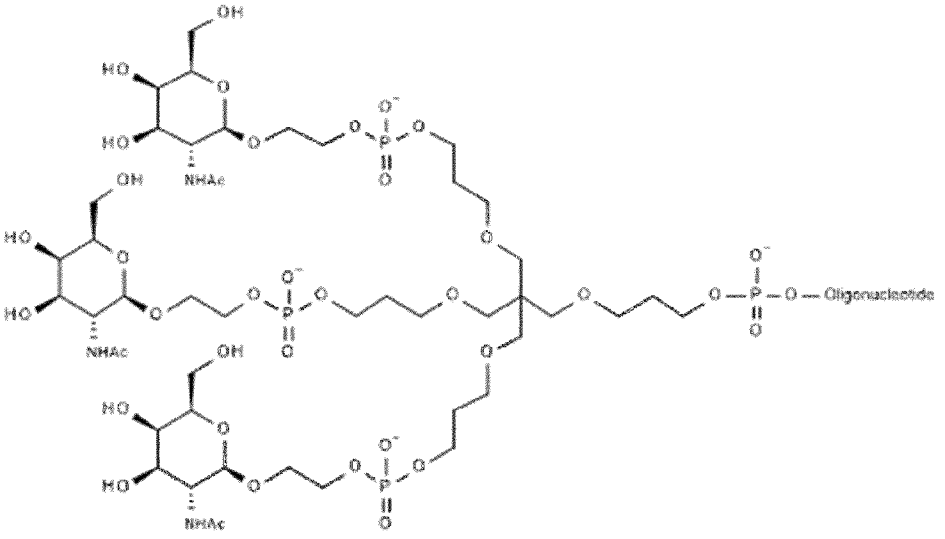

Figure 1 Cont'd

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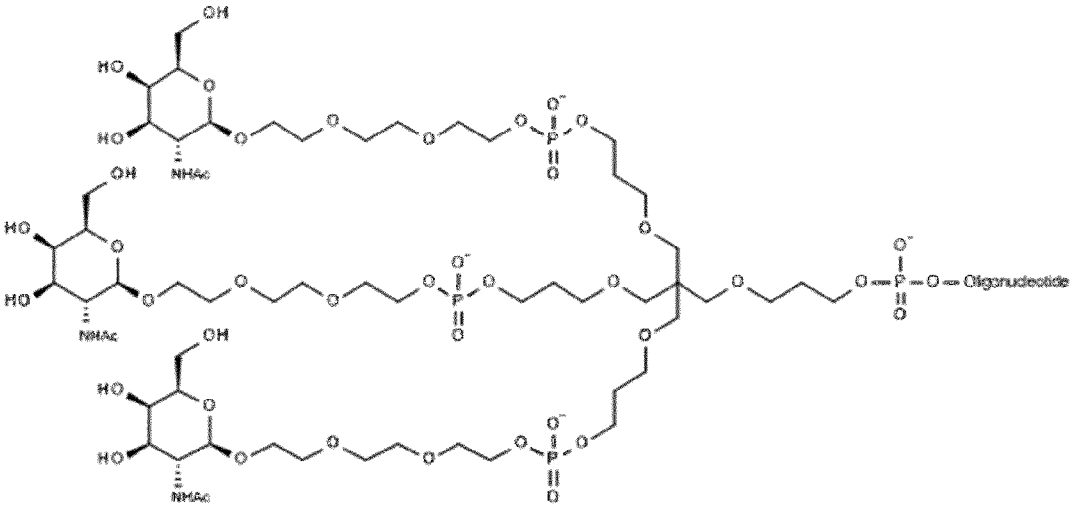
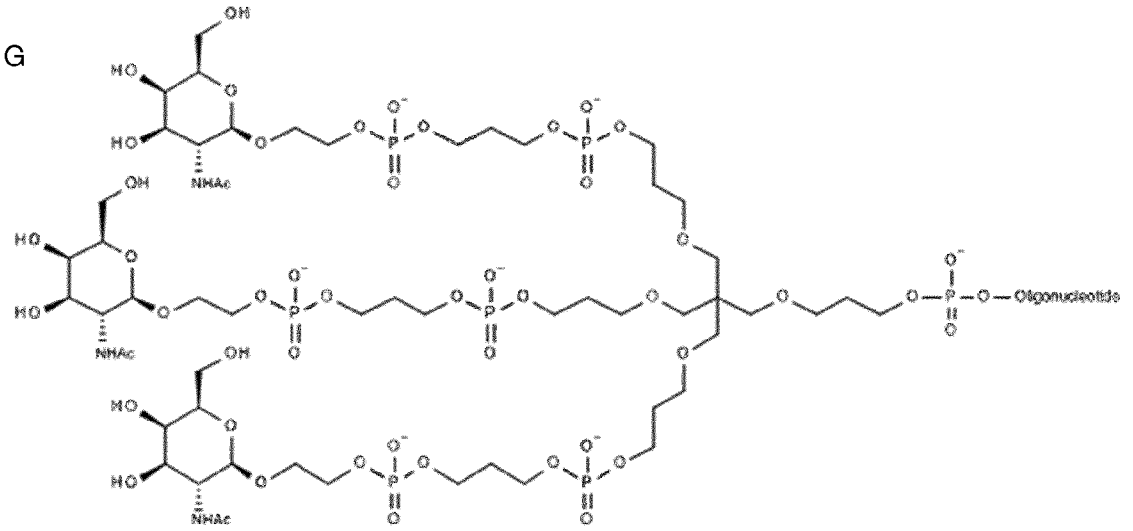


G



H

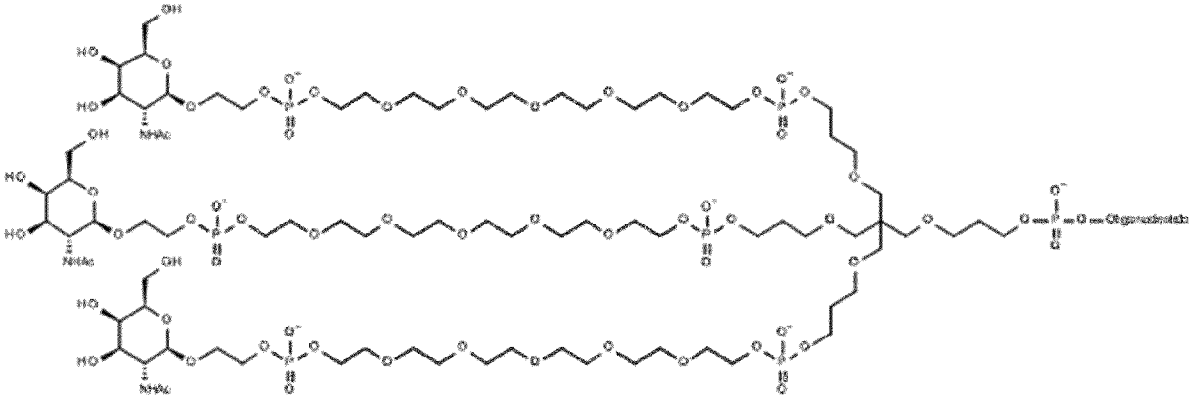


Figure 1 Cont'd

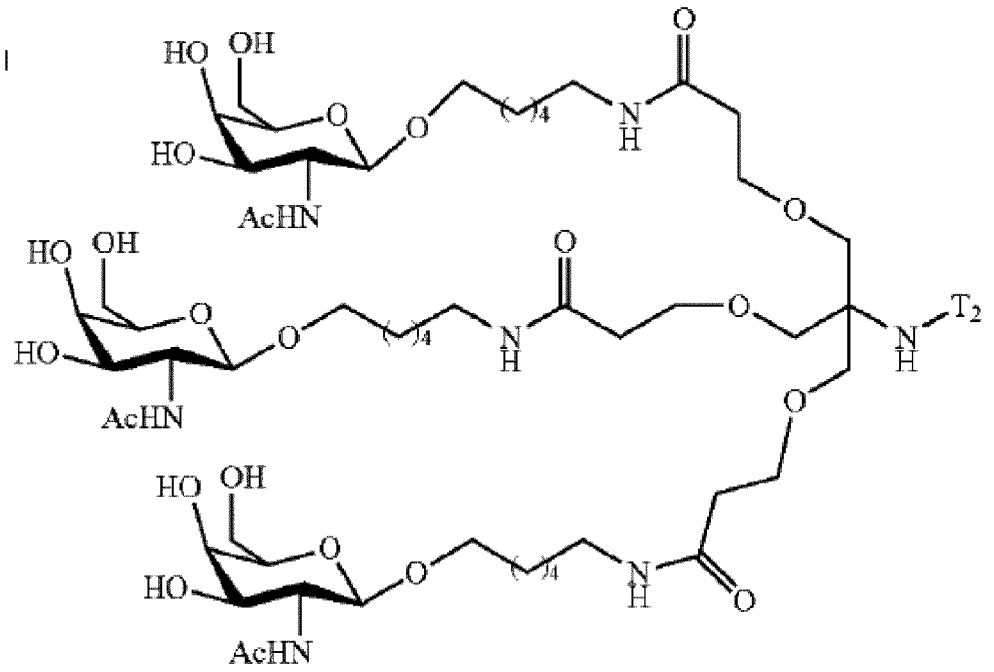


FIGURE 2

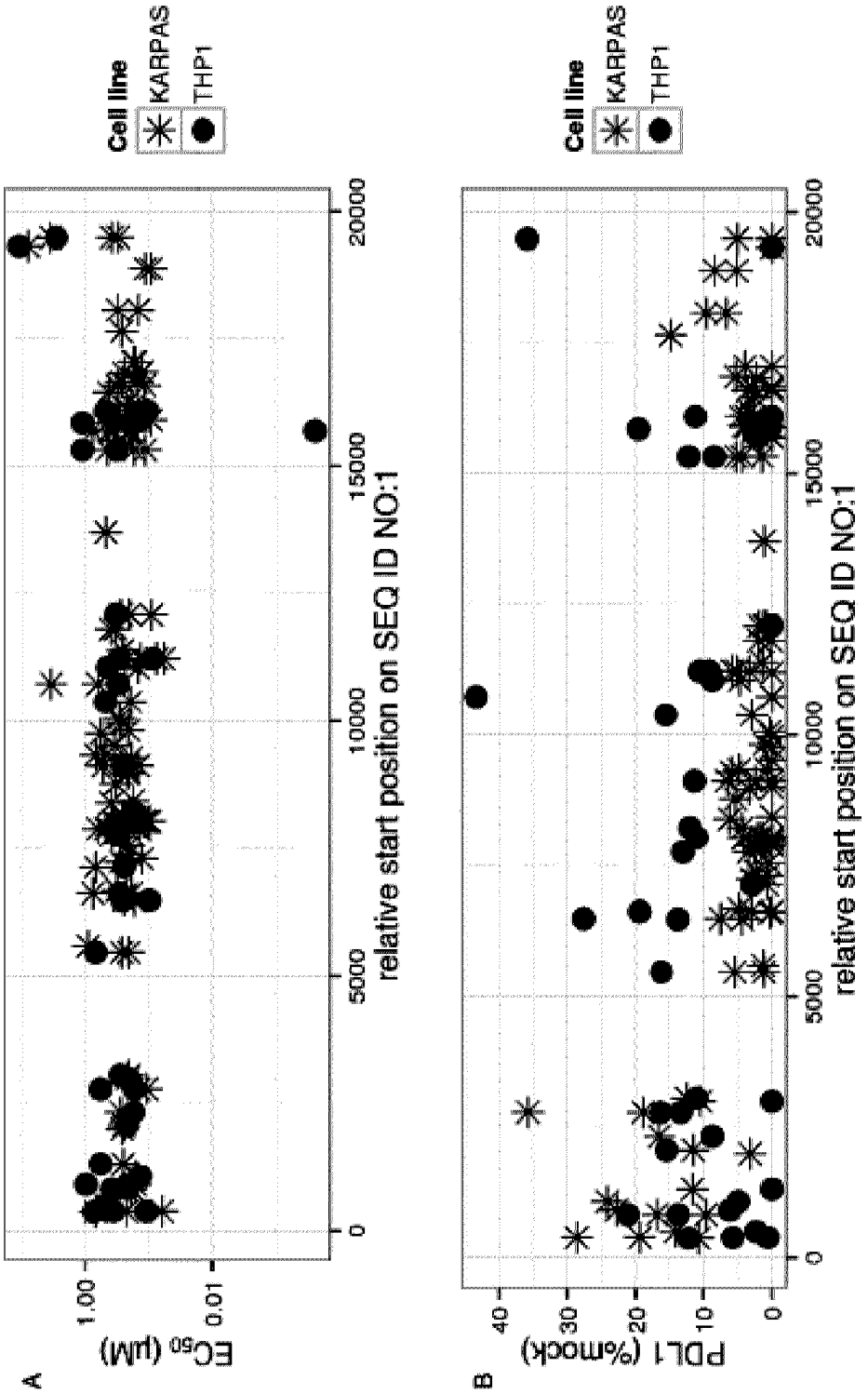

A
Cell line
KARPAS
THP1
EC50 (μM)
1.00
0.01
0
5000
10000
15000
20000
relative start position on SEQ ID NO:1
B
Cell line
KARPAS
THP1
PDL1 (%mock)
0
10
20
30
40
0
5000
10000
15000
20000
relative start position on SEQ ID NO:1

Figure 3

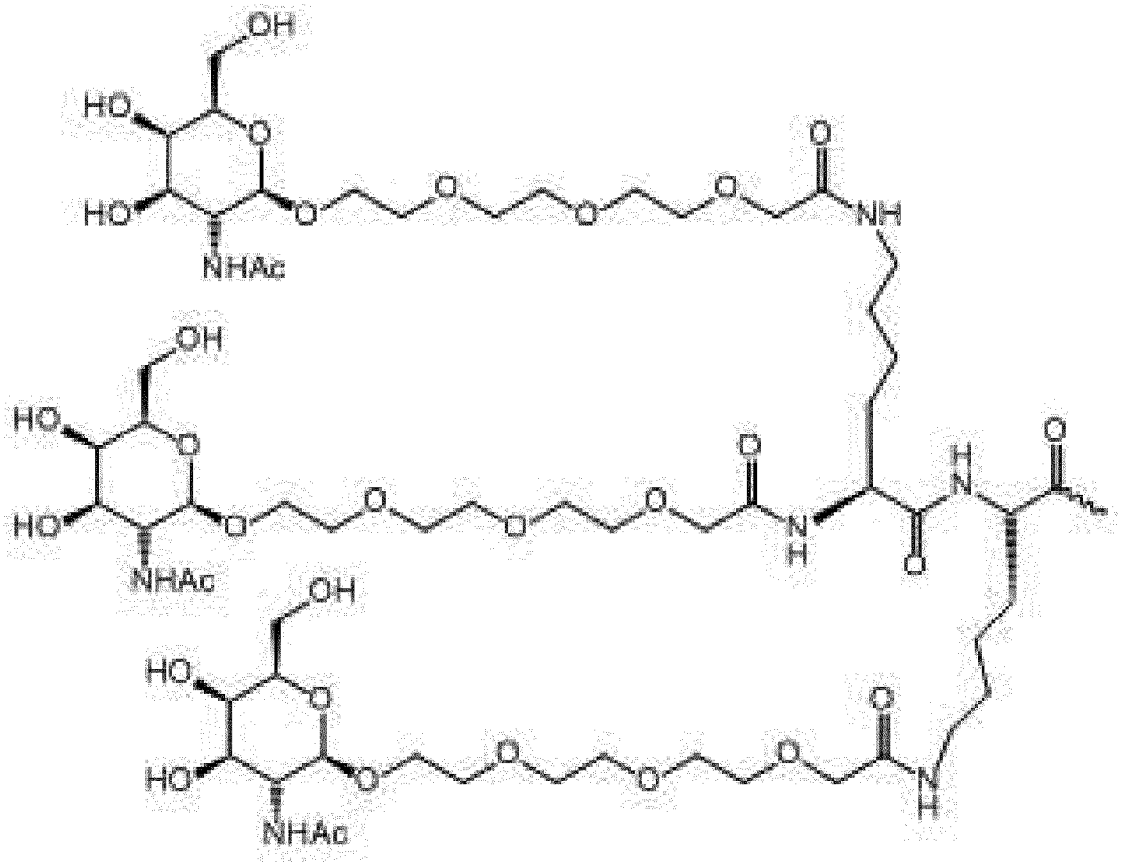


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Figure 4

CMP ID NO 766_2

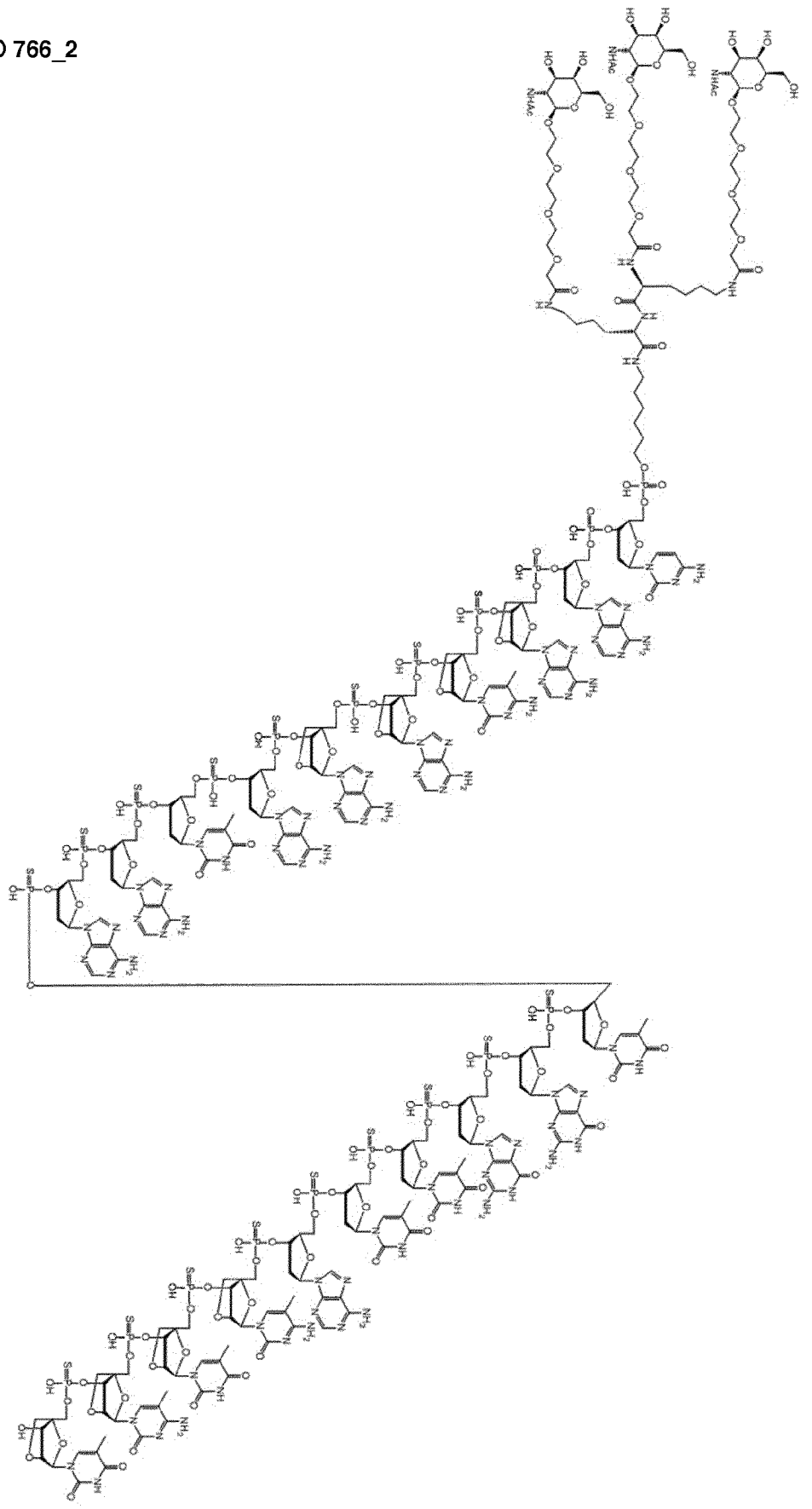


Figure 5

CMP ID NO 767_2

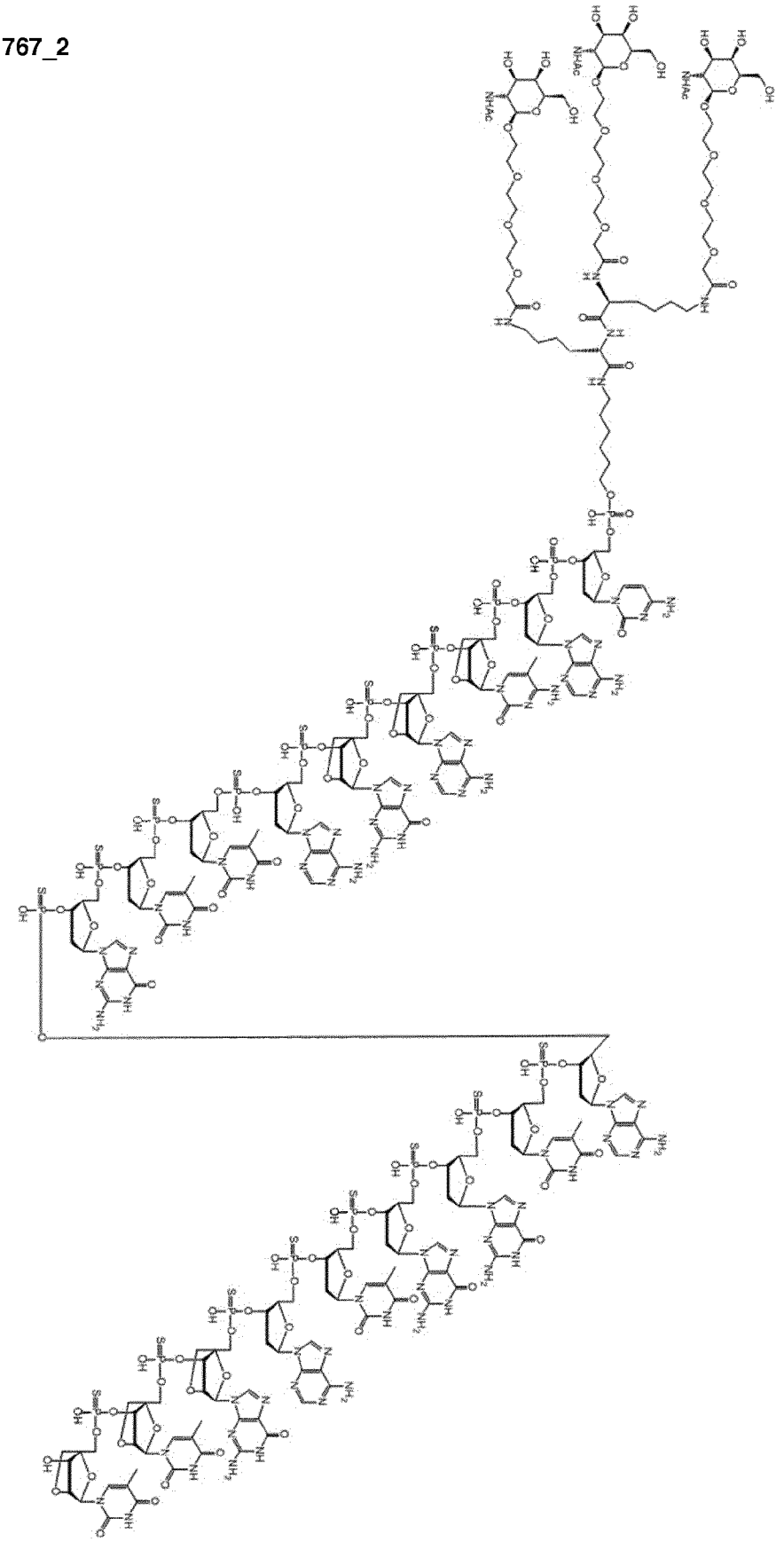


Figure 6

CMP ID NO 768_2

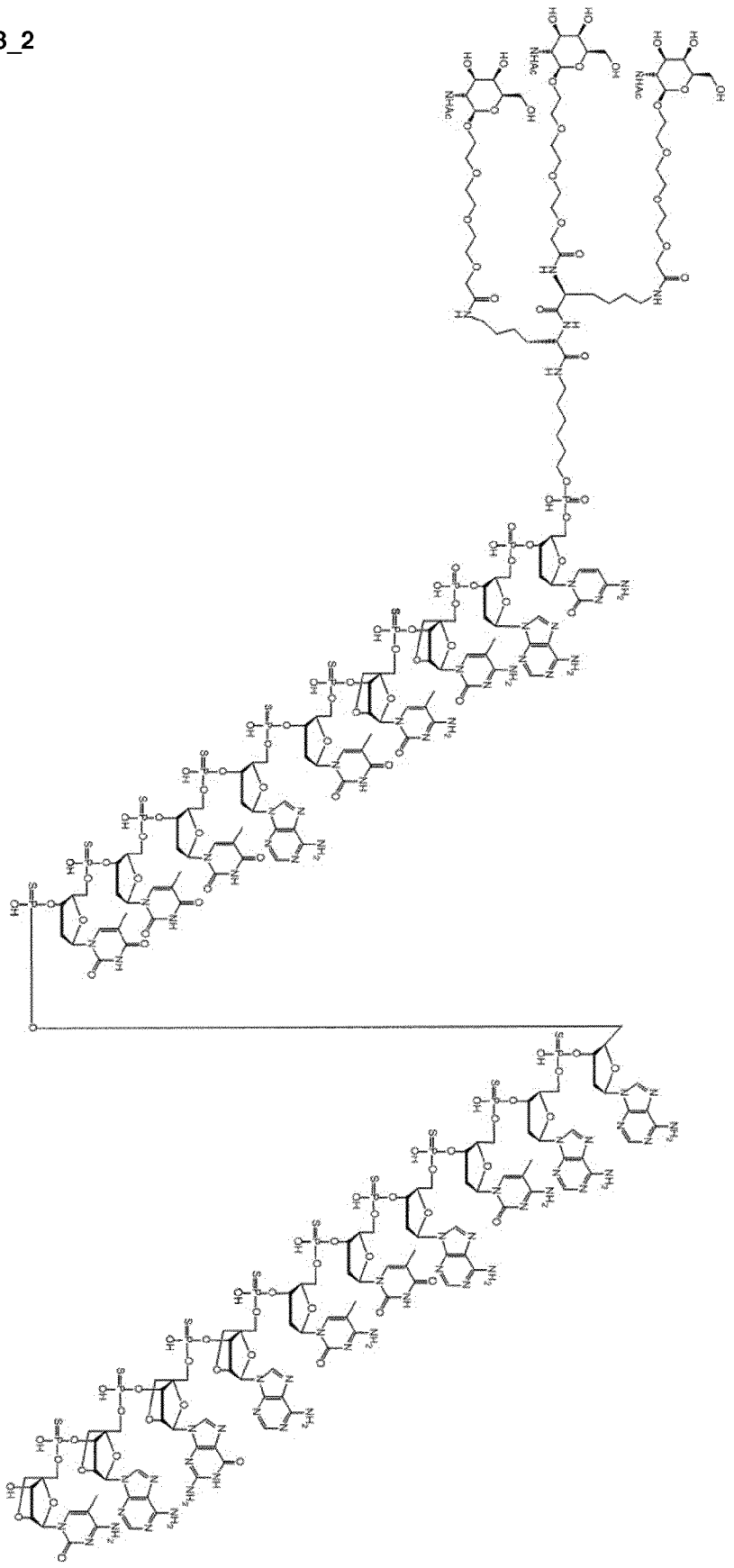


Figure 7

CMP ID NO 769_2

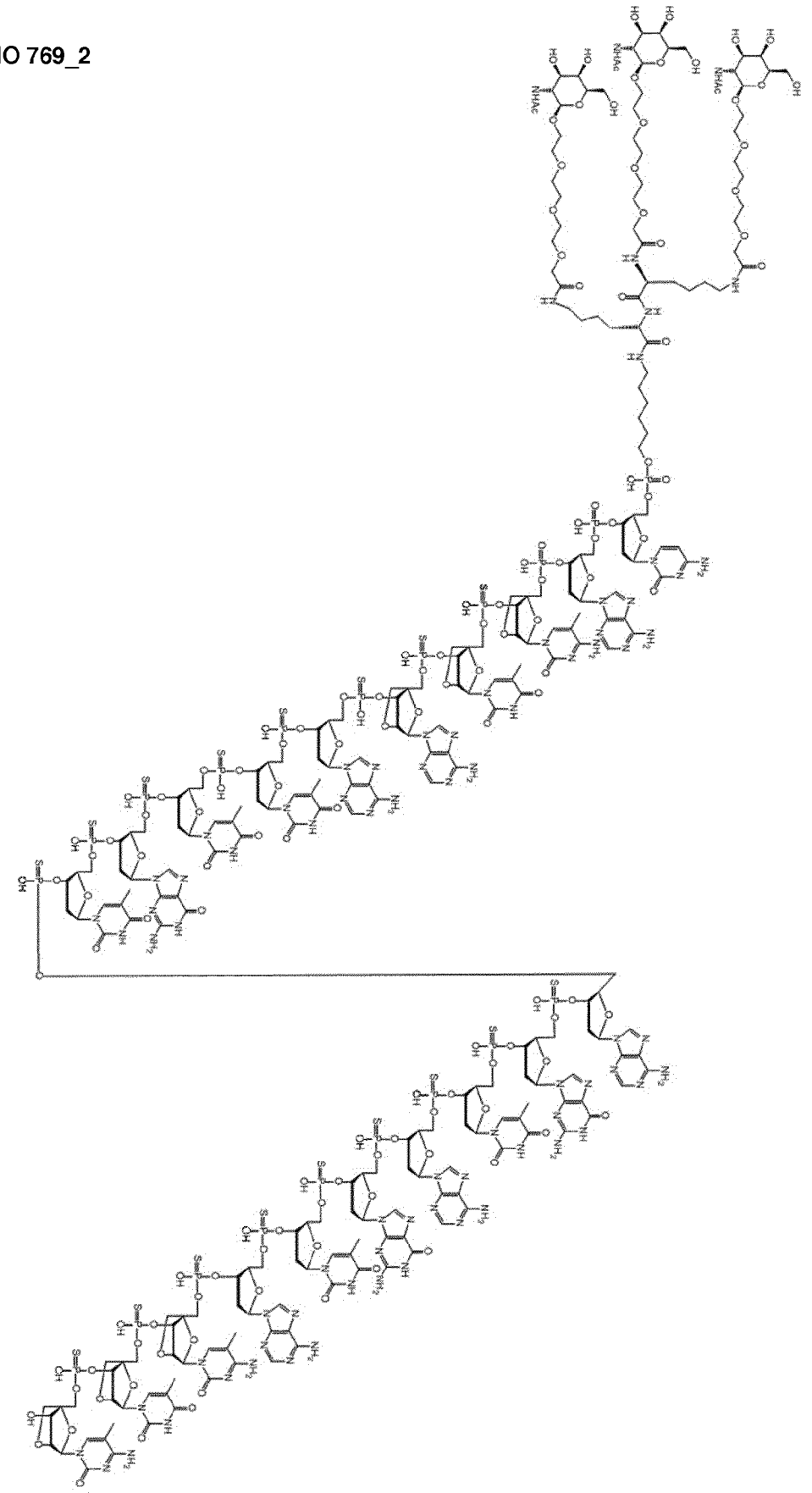


Figure 8

CMP ID NO 770_2

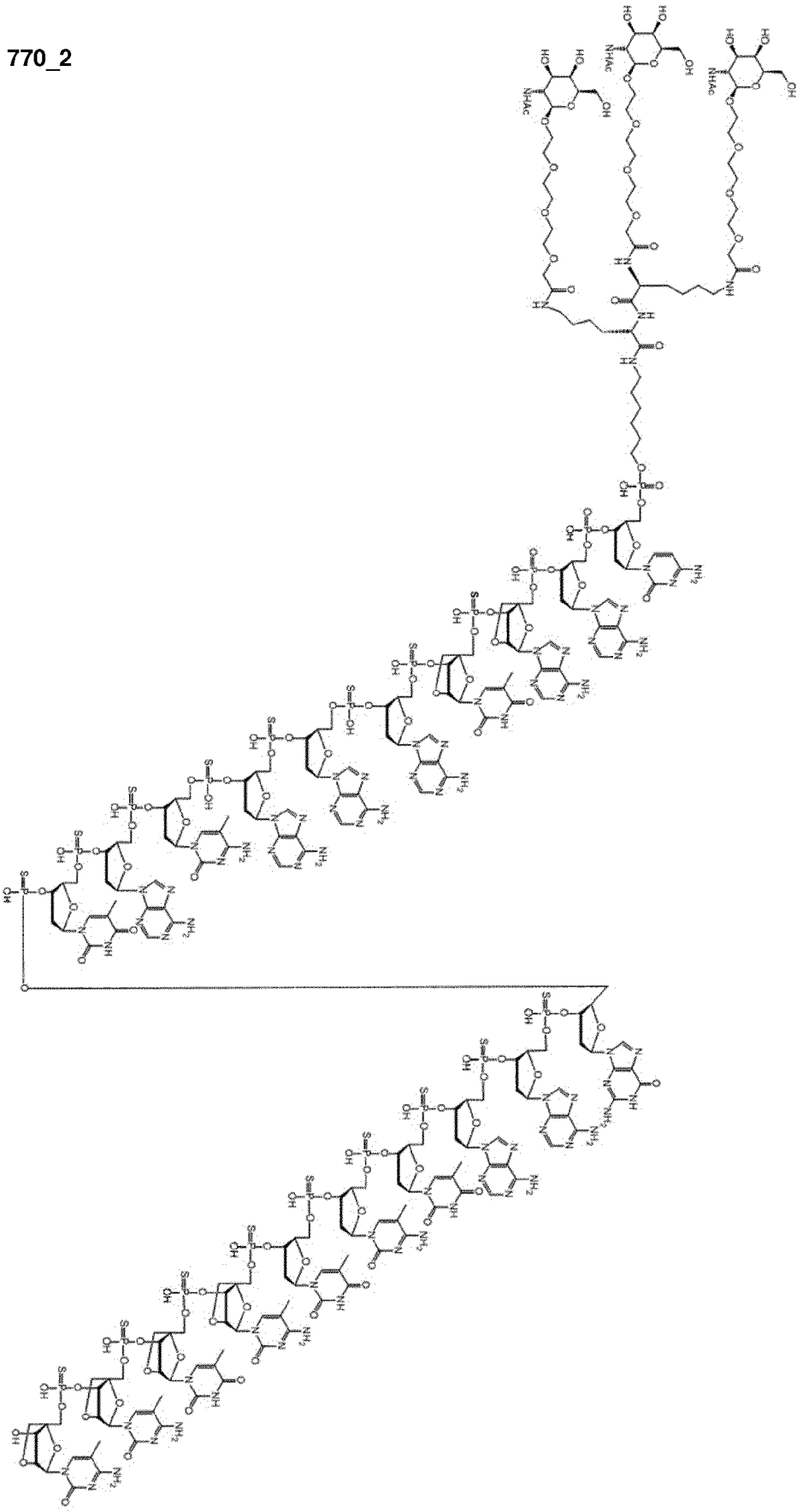
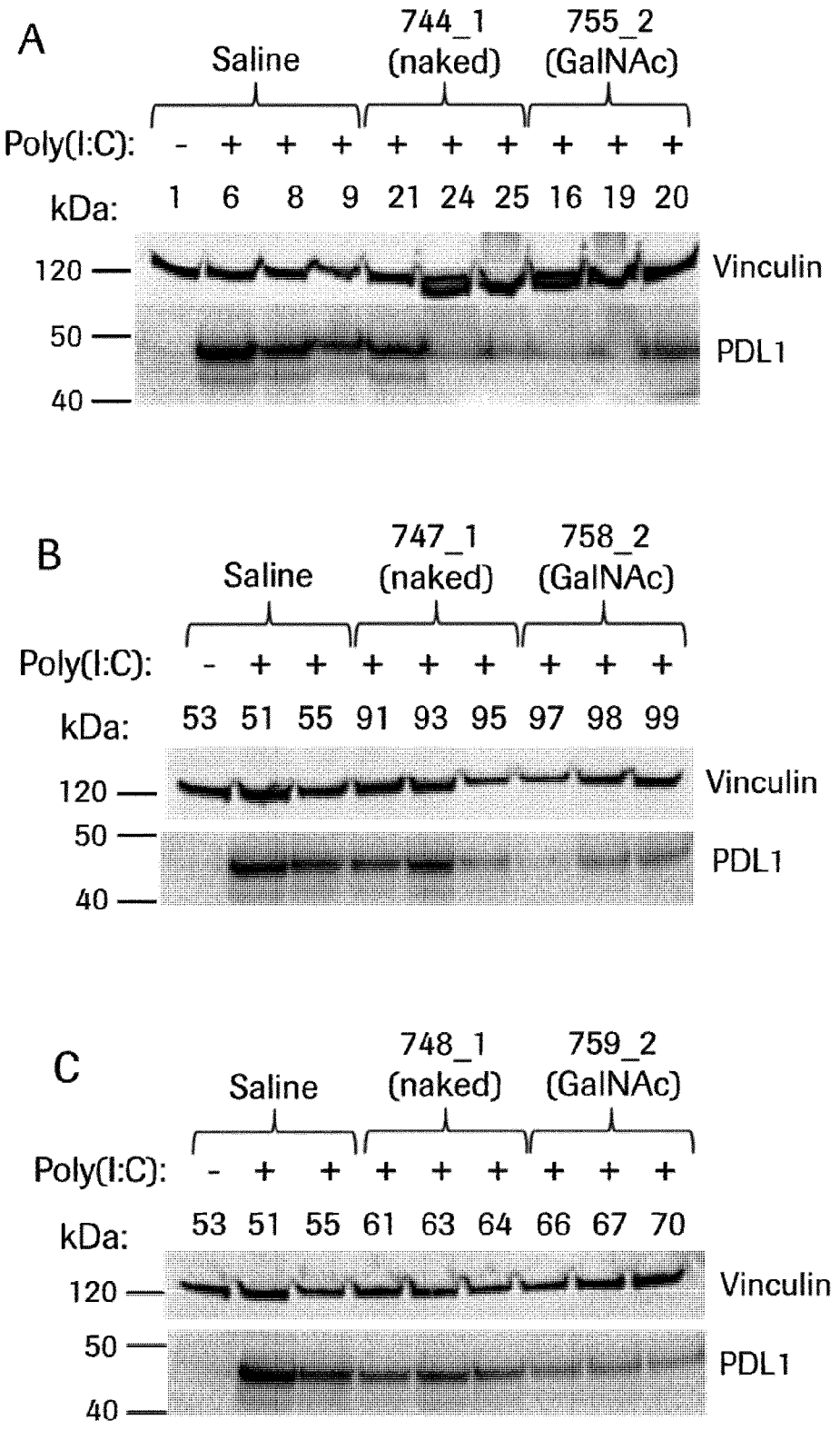
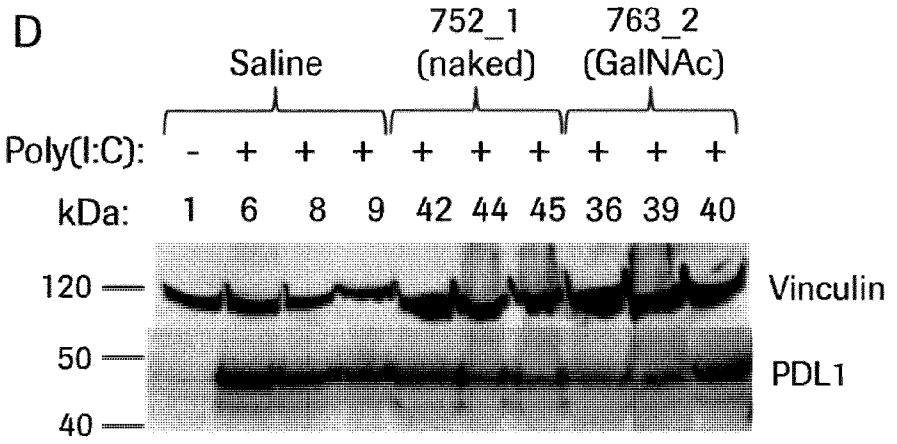


Figure 9



Date Reçue/Date Received 2021-06-02

Figure 10 cont'd



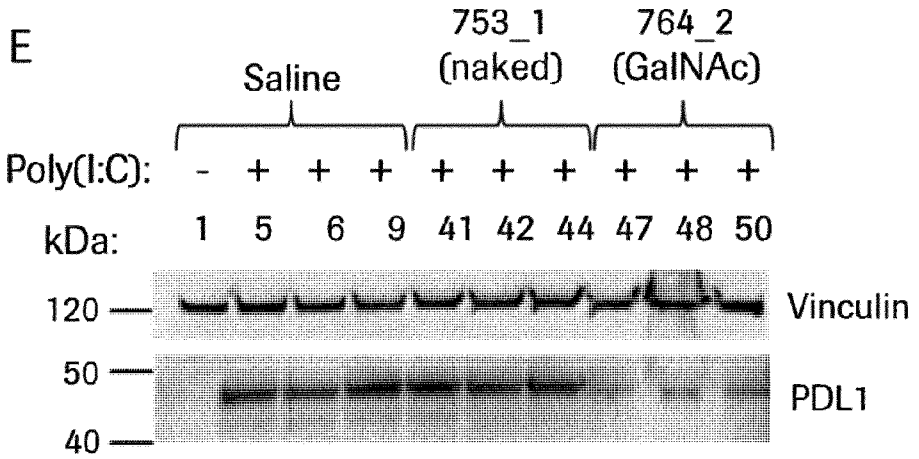
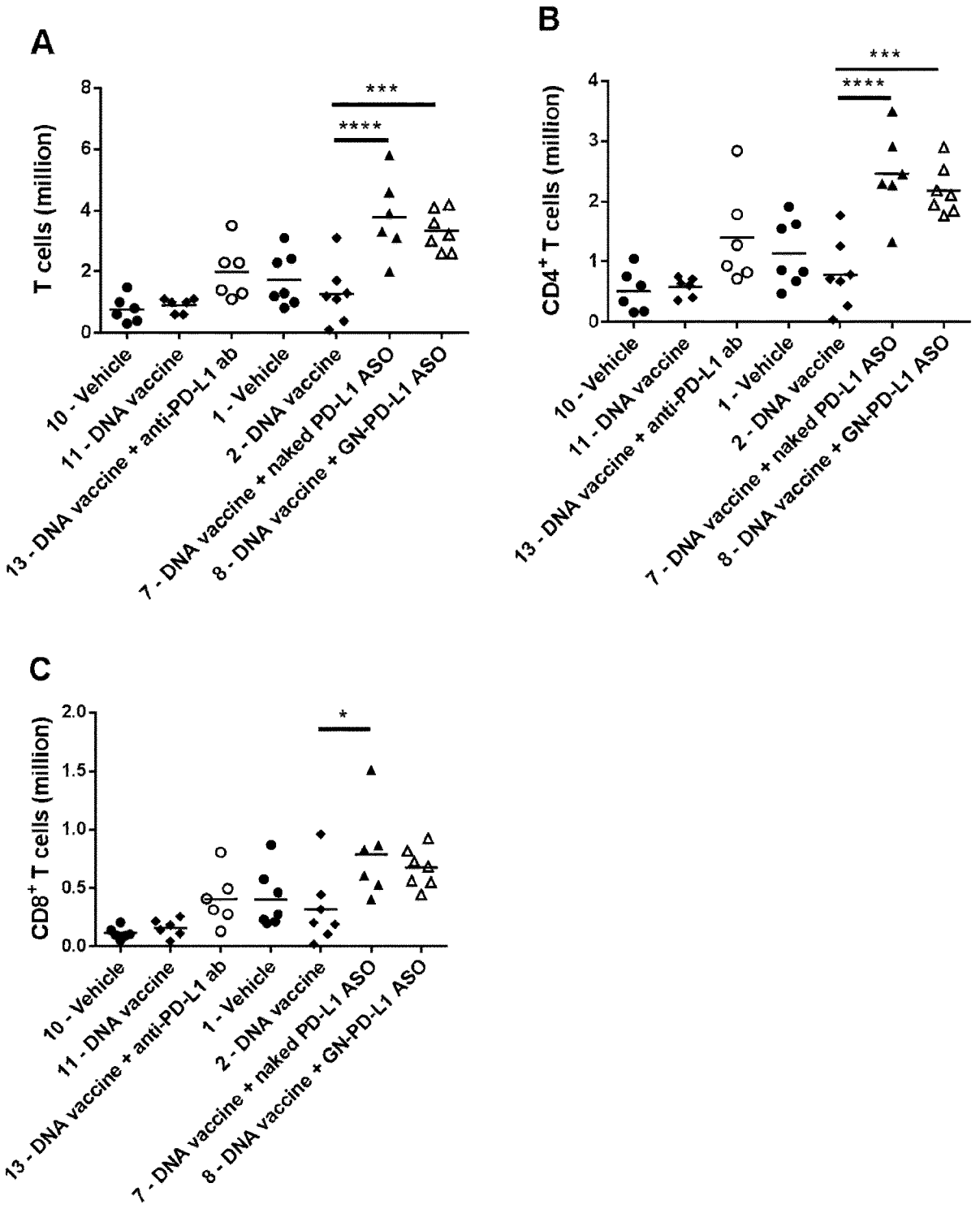


Figure 10



Date Reçue/Date Received 2021-06-02

FIGURE 11

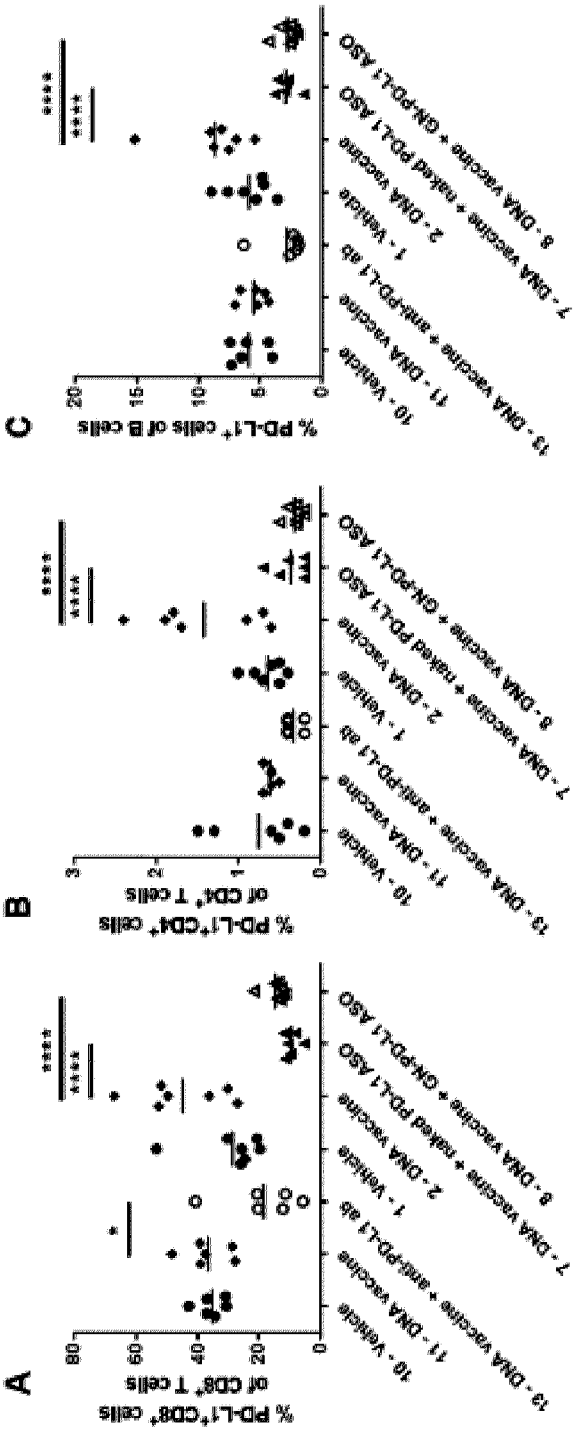

A
% PD-L1+CD8+ cells of CD8+ T cells
B
% PD-L1+CD4+ cells of CD4+ T cells
C
% PD-L1+ cells of B cells
10 - Vehicle
11 - DNA vaccine + anti-PD-L1 ab
1 - Vehicle
2 - DNA vaccine
7 - DNA vaccine + naked PD-L1 ASO
8 - DNA vaccine + GN-PD-L1 ASO

FIGURE 12

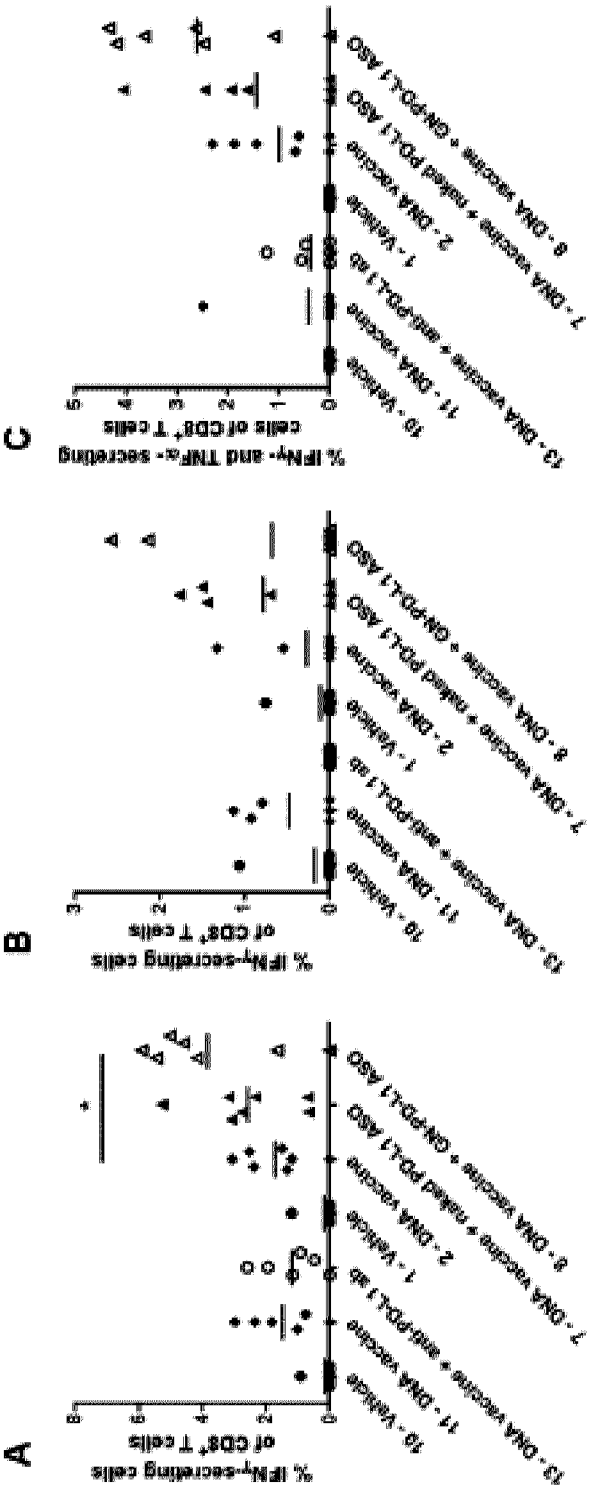
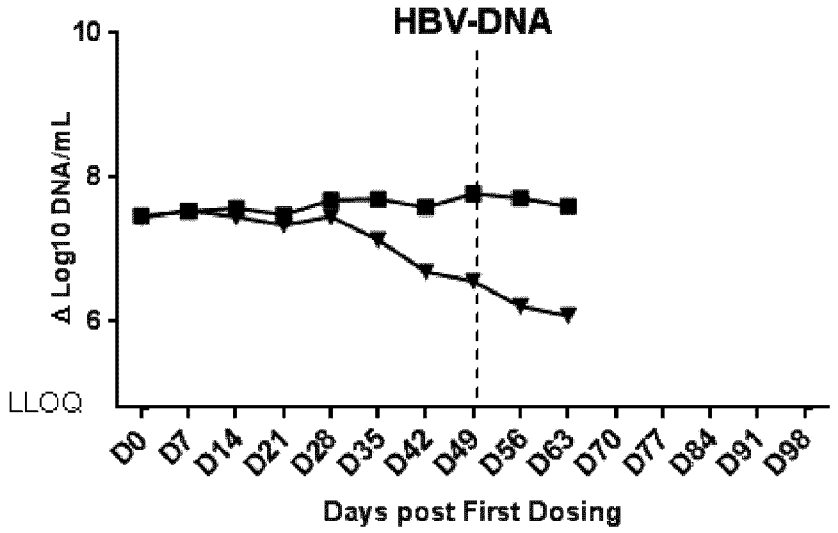
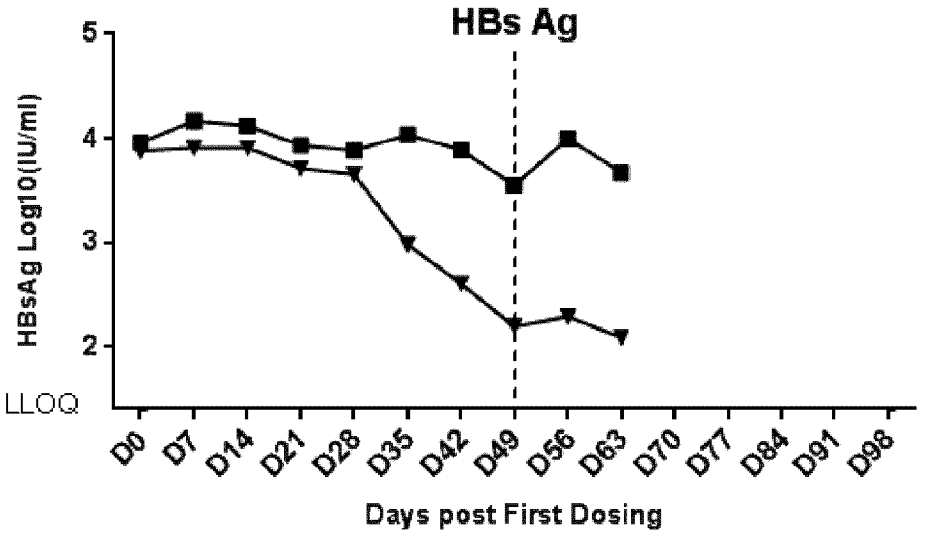
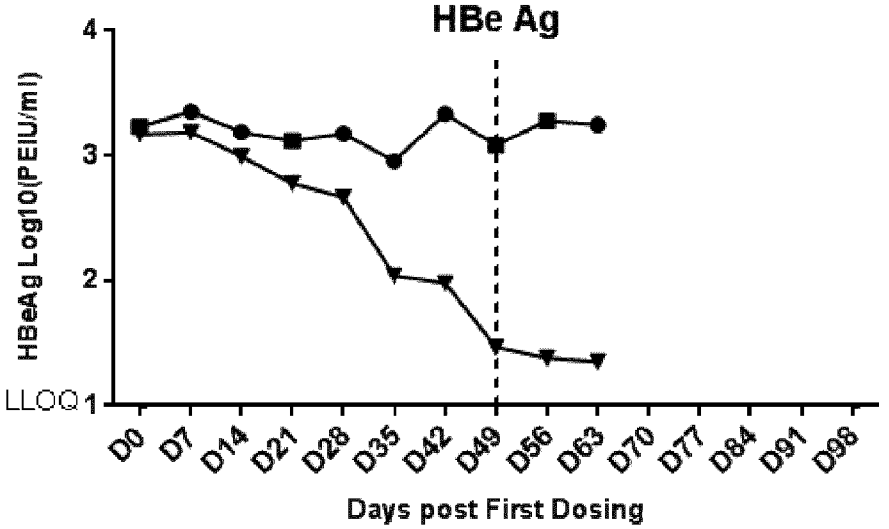

A
% IFNγ-secreting cells of CD8+ T cells
B
% IFNγ-secreting cells of CD8+ T cells
C
% IFNγ- and TNFα- secreting cells of CD8+ T cells
1 - Vehicle
2 - DNA Vaccine
6 - DNA Vaccine + naked PD-L1 ASO
7 - DNA Vaccine + GN-PD-L1 ASO
10 - Vehicle
11 - DNA Vaccine + anti-PD-L1 Ab
13 - DNA Vaccine + anti-PD-L1 ASO

Figure 13


HBV-DNA
10
8
6
LLOQ
Δ Log10 DNA/mL
D0 D7 D14 D21 D28 D35 D42 D49 D56 D63 D70 D77 D84 D91 D98
Days post First Dosing


HBs Ag
5
4
3
2
LLOQ
HBsAg Log10(IU/ml)
D0 D7 D14 D21 D28 D35 D42 D49 D56 D63 D70 D77 D84 D91 D98
Days post First Dosing


HBe Ag
4
3
2
1
LLOQ
HBeAg Log10(PEIU/ml)
D0 D7 D14 D21 D28 D35 D42 D49 D56 D63 D70 D77 D84 D91 D98
Days post First Dosing