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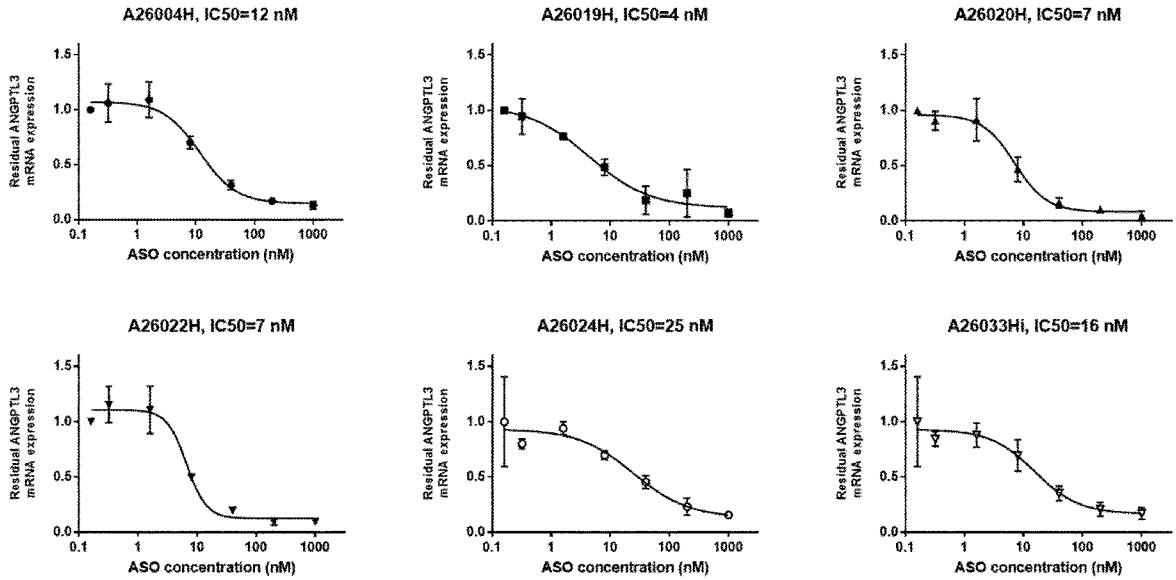
(54) Title: ANGPTL 3 OLIGONUCLEOTIDES INFLUENCING THE REGULATION OF THE FATTY ACID METABOLISM

Fig. 4: IC_{50} determination of selected human ANGPTL3-specific antisense oligonucleotides



(57) **Abstract:** The present invention refers to an ANGPTL3 inhibitor consisting of an oligonucleotide comprising 12 to 22 nucleotides, wherein at least one of the nucleotides is modified, and the oligonucleotide hybridizes with a nucleic acid sequence of ANGPTL3 of SEQ ID NO.1 (human), ANGPTL3 of SEQ ID NO. 2 (human) or both, and/or with a nucleic acid sequence of ANGPTL3 of SEQ ID NO.47 (mouse), wherein the oligonucleotide inhibits the expression of ANGPTL3, and a pharmaceutical composition comprising such inhibitor and a pharmaceutically acceptable carrier, excipient, dilutant or a combination thereof.

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ANGPTL 3 oligonucleotides influencing the regulation of the fatty acid metabolism

The present invention refers to an inhibitor of ANGPTL3 such as an antisense oligonucleotide and a pharmaceutical composition comprising such an inhibitor as well as its use for treating a cardiovascular disease, obesity, diabetes type II, homozygote familial hypercholesterolemia (HoFH), heterozygote familial hypercholesterolemia (HeFH) or dislipidemia.

Technical background

Disturbed plasma lipids are well-known risk factors in cardiometabolic disease. Successful treatment for elevated LDL-cholesterol has been given since the mid-80's and the following decades focus has broadened towards other lipid classes such as HDL-cholesterol and triglycerides. Epidemiological studies have revealed that increased plasma triacylglycerol (TG) and concomitant remnant cholesterol is an independent risk factor for coronary heart disease (Cullen, 2000). Furthermore, hypertriglyceridemia (HTG) is a hallmark of the metabolic syndrome (MS) and is often accompanied by obesity and insulin resistance (Reaven, 1995). The increased risk of type 2 diabetes and cardiovascular disease (CVD) associated with metabolic syndrome and HTG suggests that maintenance of plasma TG homeostasis is highly desirable.

Patients with severe hypertriglyceridemia can also develop pancreatitis (Athyros, 2002), particularly when TG levels exceed 1000–1500 mg/dl (Tsuang, 2009). As many as 40 different genes are now known to regulate plasma TG (Johansen, 2011), but only a few monogenetic disorders are known to markedly increase TG (Nordestgaard and Varbo, 2014). These comprises familial chylomicronemia syndrome (FCS) which is described in more detail below.

Lipolysis is a key step in clearance of TG-rich lipoproteins that takes place on the luminal surface of capillaries of heart, skeletal muscle, and adipose tissues. LPL synthesized in muscle and adipocytes is translocated to capillary endothelial cells. Rare

genetic defects in lipoprotein lipase (LPL) (Benlian, 1996), the main enzyme responsible for the hydrolysis of TG on lipoproteins, can lead to FCS characterized by plasma TG levels well over >10 mmol. Homozygous defects in apolipoprotein C-II (apoC-II), a key protein activator of LPL, can also lead to a similar hypertriglyceridemic phenotype (Breckenridge, 1978). More recently, defects in GPIHBP1, a protein that links LPL to the surface of endothelial cells (Beigneux, 2007), and mutations in apolipoprotein A-V (ApoA-V) (Ishihara, 2005) have also been described to cause hypertriglyceridemia in humans. Genetic defects in the lipase specific chaperone LMF1 has also been found to promote FCS. Taken together about 2-3:1 000 000 patients have FCS.

Not only LPL activating factors affect the LPL system; loss of function mutations in LPL negative regulators such as apoC3 and ANGPTL3, ANGPTL4 or ANGPTL8 has been shown to promote a favorable plasma lipid profile and a reduced risk for metabolic diseases. ANGPTL3 is a regulator of different lipases and LPL in particular. The protein is unfolding chaperones that break up the dimeric catalytically active form of LPL into inactive monomers which is an irreversible event. The ANGPTLs are the only known factors to regulate LPL in this manner, compared to e.g. apoC3 which displaces LPL from lipid substrates. In addition ANGPTL3 affects endothelial lipase thus affecting not only the TG moiety of plasma lipids but also LDL-c and HDL-c. ANGPTL3 is mainly expressed by the liver and regulates postprandial plasma lipids in concert with co-factor ANGPTL8. Animal models deficient for these ANGPTLs show increased LPL activity and decreased plasma lipids and mice with transgenic overexpression for the human variants show the opposite. The findings from animal studies are supported by human deficiency and loss of function mutations which for ANGPTL3 correlates with plasma TG levels and LDL-c. ANGPTL3 gene shows a link to cardiometabolic diseases.

Thus, information to date provides new insights into the coordinate activities of LPL, GPIHBP1, ANGPTLs and apoA-V in plasma TG homeostasis. Among these factors, ANGPTL3s also regulates plasma cholesterol levels, i.e., LDL-c, HDL-c and remnant-c intriguingly without being all dependent on the LDL-receptor which in most cases is non-functional in homozygote familial hypercholesterolemia (HoFH) and heterozygote familial hypercholesterolemia (HeFH). This provides an opportunity for an “all-purpose” plasma lipid drug while targeting ANGPTL3.

Oligonucleotides often naturally accumulate in the liver, which is advantageous in the targeting of ANGPTL3, mainly expressed in the liver. ANGPTL3 regulates the activity of the lipoprotein lipase that plays an important role in the intake of free fatty acids into the liver. Dysregulation of lipoprotein lipase can lead to a lipid excess in the cells, which results for example in obesity, diabetes type II or cardiovascular diseases. ANGPTL3 *Loss-of-Function* mutations correlate with reduced plasma triglycerides and a reduced LDL cholesterol level. Further effects of ANGPTL3 *Loss-of-Function* mutations are increased activity of lipoprotein lipase and endothelial lipase, increased insulin sensitivity and reduced amounts of fatty acids in the serum.

Oligonucleotides of the present invention inhibiting the expression of ANGPTL3 reduce for example the plasma lipid level independent of LDL receptor functionality, which is relevant for example for use of the oligonucleotides in treating homozygote familial hypercholesterolemia (HoFH) or heterozygote familial hypercholesterolemia (HeFH), where the LDL receptor is defect.

Studies with siRNA to inhibit ANGPTL3 expression showed that *in vivo* inhibition is only possible if siRNA is packed in suitable packaging material. Even if siRNA is packed the efficiency on the inhibition of mRNA expression can often not be improved.

An oligonucleotide of the present invention is very successful in the inhibition of the expression of ANGPTL3. The mode of action of an oligonucleotide differs from the mode of action of an antibody or small molecule, and oligonucleotides are highly advantageous regarding for example

(i) the penetration into tissues,
(ii) the blocking of multiple functions and activities, respectively, of a target,
(iii) the combination of oligonucleotides with each other or an antibody or a small molecule, and
(iv) the inhibition of intracellular effects which are not accessible or not specifically accessible for an antibody or inhibitable via a small molecule.

Summary of the invention

The present invention refers to an ANGPTL3 inhibitor consisting of an oligonucleotide comprising or consisting of for example 12 to 22 nucleotides, 15 to 20 nucleotides, or 15,

16, 17, 18, 19 or 20 nucleotides, wherein at least one of the nucleotides is modified. The ANGPTL3 oligonucleotide hybridizes for example with a nucleic acid sequence of ANGPTL3 of SEQ ID NO.1 (human; NM_014495.3), ANGPTL3 of SEQ ID NO. 2 (human; GRCh38:1:62597487:62606159) or both, and/or with a nucleic acid sequence of ANGPTL3 of SEQ ID NO.47 (mouse; NM_013913.4), wherein the oligonucleotide inhibits the expression of ANGPTL3. The modified nucleotide is for example selected from the group consisting of a bridged nucleic acid such as LNA, cET, ENA, 2´Fluoro modified nucleotide, 2´O-Methyl modified nucleotide, 2' O-Methoxyethyl modified nucleotide and a combination thereof.

The ANGPTL3 oligonucleotide of the present invention hybridizes for example with an active area selected from position 45-72 (e.g., A26004H, SEQ ID NO.6) and/or from position 1130-1170 (e.g., A26019H, SEQ ID NO.20; A26020H, SEQ ID NO.20; A26021H, SEQ ID NO. 21; A26022H, SEQ ID NO.22; A26023H, SEQ ID NO. 23; A26024H, SEQ ID NO.24) of SEQ ID NO. 1 and/or from position 3060-3086 (e.g., A26033Hi, SEQ ID NO.33) and/or from position 5768-5794 (e.g., A26036Hi, SEQ ID NO.36; A26037Hi, SEQ ID NO.36) of SEQ ID NO.2. It inhibits the expression of ANGPTL3 for example at a nanomolar or micromolar concentration.

The present invention is further directed to a pharmaceutical composition comprising an ANGPTL3 inhibitor of the present invention and a pharmaceutically acceptable carrier, excipient, dilutant or a combination thereof. The inhibitor and the pharmaceutical composition, respectively, are for use in a method of preventing and/or treating a disorder, where an ANGPTL3 imbalance is involved. Such disorder is for example a cardiometabolic disease, obesity, diabetes such as type 2 diabetes mellitus, hypercholesterolemia, hypertriglyceridemia (HTG), dyslipidemia, pancreatitis, metabolic syndrome, familial chylomicronemia syndrome (FCS) and/or cancer. Hypercholesterolemia is for example homozygote familial hypercholesterolemia (HoFH) and heterozygote familial hypercholesterolemia (HeFH), cancer is for example breast cancer, lung cancer, malignant melanoma, lymphoma, skin cancer, bone cancer, prostate cancer, liver cancer, brain cancer, cancer of the larynx, gall bladder, pancreas, testicular, rectum, parathyroid, thyroid, adrenal, neural tissue, head and neck, colon, stomach, bronchi, kidneys, basal cell carcinoma, squamous cell carcinoma, metastatic skin carcinoma, osteo sarcoma, Ewing's sarcoma, reticulum cell sarcoma, liposarcoma, myeloma, giant cell tumor, small-cell lung tumor, islet cell tumor, primary brain tumor,

meningioma, acute and chronic lymphocytic and granulocytic tumors, acute and chronic myeloid leukemia, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, intestinal ganglioneuromas, Wilm's tumor, seminoma, ovarian tumor, leiomyomater tumor, cervical dysplasia, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic sarcoma, malignant hypercalcemia, renal cell tumor, polycythermia vera, adenocarcinoma, anaplastic astrocytoma, glioblastoma multiforma, leukemia, or epidermoid carcinoma.

The ANGPTL3 inhibitor or the pharmaceutical composition of the present invention comprising the ANGPTL3 inhibitor is administered locally or systemically.

All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

Description of the figures

Fig. 1 depicts a single dose efficacy screen of human ANGPTL3-specific antisense oligonucleotides in Hep3B cells.

Fig. 2 shows a single dose efficacy screen of 25 selected human ANGPTL3-specific antisense oligonucleotides in primary human hepatocytes.

Fig. 3 depicts a human TLR9 assay using hTLR9-HEK 293 cells, where HEK-Blue™ hTLR9 cells were used to study the stimulation of human TLR9 by monitoring the activation of nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-kB)-dependent secreted embryonic alkaline phosphatase (SEAP)-production *in vitro*.

Fig. 4 shows nine human ANGPTL3-specific antisense oligonucleotides (A26004H (SEQ ID NO.6), A26019H (SEQ ID NO.20), A26020H (SEQ ID NO.20), A26022H (SEQ ID

NO.22), A26023H (SEQ ID NO23), A26024H (SEQ ID NO.24), A26033Hi (SEQ ID NO.33), A26036Hi (SEQ ID NO.36) and A26037Hi (SEQ ID NO.36)) with high knockdown efficacy in primary human hepatocytes that were selected for determination of half maximal inhibitory concentration (IC_{50}) values. For graphic representation mock-treated cells were set as 0.16 nM.

Fig. 5 depicts a single dose efficacy screen of mouse ANGPTL3-specific antisense oligonucleotides in AML12 cells. 8,000 AML12 cells / well were seeded in 96-well plates and treated with the respective antisense oligonucleotides at a final concentration of 30 nM by use of Lipofectamine 2000. Three days after start of treatment, cells were lysed and mouse Hprt1 and mouse ANGPTL3 mRNA expression was measured using the QuantiGene RNA Singleplex assay. ANGPTL3-mRNA expression values were normalized to expression of the housekeeping gene Hprt1. Residual ANGPTL3-mRNA expression relative to mock-treated cells ("no oligo" set as 1) is shown. Solid line and dotted lines indicate 70 % and 50 % or 0 % knockdown efficacy, respectively.

Fig. 6 shows a single dose efficacy screen of 13 selected mouse ANGPTL3-specific antisense oligonucleotides in primary mouse hepatocytes. 15,000 primary hepatocytes / well were seeded in 96-well plates and treated with the respective antisense oligonucleotides at a final concentration of 5 µM. Every 24 hours, 70 µl of cell supernatant was replaced by fresh maintenance medium w/o antisense oligonucleotide. Three days after start of treatment, cells were lysed and mouse Hprt1 and mouse ANGPTL3 mRNA expression was measured using the QuantiGene RNA Singleplex assay. ANGPTL3-mRNA expression values were normalized to expression of the housekeeping gene Hprt1. Residual ANGPTL3-mRNA expression relative to mock-treated cells ("no oligo" set as 1) is shown. Solid line and dotted lines indicate 80 % and 0 % knockdown efficacy, respectively.

Fig. 7 shows IC_{50} determination of selected mouse ANGPTL3 antisense oligonucleotides. 15,000 primary mouse hepatocytes / well were seeded in 96-well plates and treated with different concentrations of the respective antisense oligonucleotides. Three days after start of treatment, cells were lysed and Hprt1 and ANGPTL3 mRNA expression were measured using the QuantiGene RNA Singleplex assay. ANGPTL3-mRNA expression values were normalized to expression of the housekeeping gene Hprt1. Residual

ANGPTL3-mRNA expression relative to mock-treated cells (set as 1). Triplicate wells, mean+/-SD.

Fig. 8A and **8B** show an analysis of dose-dependent inhibition of human ANGPTL3-specific ASOs in mouse hepatocytes**.** 15,000 primary mouse hepatocytes / well were seeded in 96-well plates and treated with different concentrations (5000 nM, 1000 nM, 200 nM, 40 nM, 8 nM, 1.6 nM) of the respective ASO. After three days, cells were lysed and Hypoxanthine phosphoribosyltransferase 1 (Hprt1) and Angptl3 mRNA expression were measured using the QuantiGene RNA Singleplex assay. **Fig. 8A**: Angptl3-mRNA expression values were normalized to expression of the housekeeping gene Hprt1. Residual Angptl3-mRNA expression relative to mock-treated cells (set as 1 (n=6), SD=0.23). **Fig. 8B**: Residual Hprt1-mRNA expression relative to mock-treated cells (set as 1 (n=6), SD=0.15). Solid line and dotted lines indicate 70% and 0% (equivalent to residual mRNA level of 0.3 and 0, respectively) knockdown efficacy, respectively. Data are represented as mean of triplicate wells +/-SD.

Fig. 9A and **9B** depicts efficacy of selected ANGPTL3 ASOs on target gene expression after transfection of cynomolgus hepatocytes. 25,000 primary cynomolgus hepatocytes / well were seeded in 96-well plates and transfected with different concentrations (20 nM, 2 nM, 0.2 nM) of the respective ASO. Simultaneously, to induce ANGPTL3 expression, cells were treated with 1 μM LXR or equal amount of DMSO as vehicle control. After 24 h incubation at 37°C, cells were lysed and HPRT1 and ANGPTL3 mRNA expression was measured using the QuantiGene RNA Singleplex assay. **Fig. 9A**: ANGPTL3-mRNA expression values were normalized to expression of the housekeeping gene HPRT1. Residual ANGPTL3-mRNA expression relative to mock-treated cells (set as 1 (n=24), SD=0.17). **Fig. 9B**: Residual HPRT1-mRNA expression relative to mock-treated cells (set as 1 (n=24), SD=0.15). Solid line and dotted lines indicate 70% and 0% (equivalent to residual mRNA level of 0.3 and 0, respectively) knockdown efficacy, respectively. Data are represented as mean of triplicate wells +/-SD.

Detailed description of the invention

The present invention provides a successful inhibitor of ANGPTL3 expression, which is an oligonucleotide hybridizing with human or mouse mRNA and/or pre-mRNA sequences of ANGPTL3 and inhibits the expression and activity, respectively, of ANGPTL3. mRNA

comprises only exons of the ANGPTL3 encoding nucleic acid sequence, whereas pre-mRNA comprises exons and introns of the ANGPTL3 encoding nucleic acid sequence. Thus, the oligonucleotides of the present invention represent an interesting and highly efficient tool for use in a method of preventing and/or treating disorders, where the ANGPTL3 expression and activity, respectively, is increased.

In the following, the elements of the present invention will be described in more detail. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Throughout this specification and the claims, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated member, integer or step or group of members, integers or steps but not the exclusion of any other member, integer or step or group of members, integers or steps. The terms "a" and "an" and "the" and similar reference used in the context of describing the invention (especially in the context of the claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by the context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as", “for example”), provided herein is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

An inhibitor which is an oligonucleotide of the present invention is for example an antisense oligonucleotide (ASO) consisting of or comprising 10 to 25 nucleotides, 12 to 22 nucleotides, 15 to 20 nucleotides or 16 to 18 nucleotides. The oligonucleotides for example consist of or comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 nucleotides. The oligonucleotides of the present invention comprise at least one nucleotide which is modified.

The oligonucleotide of the present invention forms for example a gapmer consisting of or comprising a central block of at least 5 nucleotides, i.e., deoxynucleotides and/or ribonucleotides, which is flanked by for example naturally and/or artificially modified nucleotides such as deoxynucleotides and/or ribonucleotides.

The modified nucleotide is for example a bridged nucleotide such as a locked nucleic acid (LNA, e.g., 2´,4´-LNA), cET, ENA, a 2`Fluoro modified nucleotide, a 2`O-Methyl modified nucleotide, 2' O-Methoxyethyl modified nucleotide or a combination thereof. In some embodiments, the oligonucleotide of the present invention comprises one or more nucleotides having the same or different modifications. In addition, the oligonucleotide of the present invention optionally comprises a modified phosphate backbone, wherein the phosphate is for example a phosphorothioate.

The oligonucleotide of the present invention comprises the one or more modified nucleotide at the 3´- and/or 5´- end of the oligonucleotide and/or at any position within the oligonucleotide, wherein modified nucleotides follow in a row of for example 1, 2, 3, 4, 5, or 6 modified nucleotides, or a modified nucleotide is combined with one or more unmodified nucleotides. The following Tables 1 and 2 present examples of ANGPTL3 oligonucleotides comprising modified nucleotides for example LNA which are indicated by (+) and phosphorothioate (PTO) indicated by (*). The ANGPTL3 oligonucleotides consisting of or comprising the sequences of **Table 1** (human) or **Table 2** (mouse) may comprise any other modified nucleotide and/or any other combination of modified and unmodified nucleotides. ANGPTL3 oligonucleotides of **Table 1** hybridize with mRNA and/or pre-m RNA of human ANGPTL3:

Seq ID	Name	Antisense Sequence 5'-3'	Antisense Sequence 5'-3' with PTO (*) and LNA (+)
3	A26001H	GACCTAGACTTCTTAAC	+G*+A*+C*C*T*A*G*A*C*T*T*C*T*T*A*+A*+C

4	A26002H	GCAGACCTAGACTTCTT	+G*+C*+A*G*A*C*C*T*A*G*A*C*T*T*+C*+T*+T
5	A26003H	AGCAGACCTAGACTTCT	+A*+G*C*A*G*A*C*C*T*A*G*A*C*T*+T*C*+T
6	A26004H	TCAATTTCAAGCAACGT	+T*+C*+A*A*T*T*T*C*A*A*G*C*A*+A*+C*G*+T
7	A26005H	TTTACATCGTCTAACAT	+T*+T*+T*A*C*A*T*C*G*T*C*T*A*+A*+C*+A*+T
8	A26006H	GTCTTATGGACAAAGTC	+G*+T*C*T*T*A*T*G*G*A*C*A*A*A*G*+T*+C
9	A26007H	GTTTGCAGCGATAGAT	+G*+T*+T*T*G*C*A*G*C*G*A*T*A*+G*+A*+T
10	A26008H	GACTTGTAGTTTATATG	+G*+A*+C*T*T*G*T*A*G*T*T*T*A*T*+A*+T*+G
11	A26009H	AGTTAGTTAGTTGCTCT	+A*+G*+T*T*A*G*T*T*A*G*T*T*G*C*T*+C*+T
12	A26010H	AATGTCCATGGACTACC	+A*+A*+T*G*T*C*C*A*T*G*G*A*C*T*+A*+C*+C
13	A26011H	TCCATCTATTCGATGTT	+T*+C*+C*A*T*C*T*A*T*T*C*G*A*T*G*+T*+T
14	A26012H	GTGATCCATCTATTCGA	+G*+T*+G*+A*T*C*C*A*T*C*T*A*T*T*+C*+G*+A
15	A26013H	GTTTTGTGATCCATCTA	+G*+T*+T*T*T*G*T*G*A*T*C*C*A*T*+C*+T*+A
15	A26014H	GTTTTGTGATCCATCTA	+G*+T*T*T*T*G*T*G*A*T*C*C*A*+T*+C*+T*+A
16	A26015H	CCACGTTTCATTGAAGT	+C*+C*+A*C*G*T*T*T*C*A*T*T*G*A*+A*+G*+T
17	A26016H	TTGCTTCACTATGGAGT	+T*+T*+G*C*T*T*C*A*C*T*A*T*G*G*+A*G*+T
18	A26017H	TCCAACTCAATTCGTAA	+T*+C*+C*A*A*C*T*C*A*A*T*T*C*G*+T*+A*+A
19	A26018H	TTCCAACTCAATTCGTA	+T*+T*+C*C*A*A*C*T*C*A*A*T*T*C*+G*+T*+A
20	A26019H	GTTGGTTTCGTGATTTC	+G*+T*+T*G*G*T*T*T*C*G*T*G*A*T*+T*+T*+C
20	A26020H	GTTGGTTTCGTGATTTC	+G*+T*+T*G*G*T*T*T*C*G*T*G*A*T*T*+T*+C
21	A26021H	GTATAGTTGGTTTCGTG	+G*+T*+A*T*A*G*T*T*G*G*T*T*T*C*+G*+T*+G
22	A26022H	CGTATAGTTGGTTTCGT	+C*+G*+T*A*T*A*G*T*T*G*G*T*T*T*+C*+G*+T
23	A26023H	TGTAGCGTATAGTTGGT	+T*+G*+T*A*G*C*G*T*A*T*A*G*T*T*+G*+G*+T
24	A26024H	GATGTAGCGTATAGTT	+G*+A*+T*G*T*A*G*C*G*T*A*T*A*+G*+T*+T
25	A26025H	TTTAACTCGATGCCAC	+T*+T*+T*A*A*C*T*C*G*A*T*G*C*+C*+A*+C
26	A26026H	AGCGTTAATTTCGACA	+A*+G*+C*G*T*T*A*A*T*T*T*C*G*+A*+C*+A
27	A26027H	TCAGCGTTAATTTCGA	+T*+C*+A*G*C*G*T*T*A*A*T*T*T*+C*+G*+A
28	A26028H	ATCAGCGTTAATTTCG	+A*+T*+C*A*G*C*G*T*T*A*A*T*T*+T*+C*+G
29	A26029Hi	GTAATGACATAGTGTTC	+G*+T*+A*A*T*G*A*C*A*T*A*G*T*G*+T*+T*+C
30	A26030Hi	ATCTTGGTCTAAAGAGC	+A*+T*+C*T*T*G*G*T*C*T*A*A*A*G*+A*+G*+C
31	A26031Hi	GGCACACTATTTCTAGT	+G*+G*C*A*C*A*C*T*A*T*T*T*T*C*+T*+A*+G*+T
32	A26032Hi	GACCTTTAATTATGAGG	+G*+A*+C*C*T*T*T*A*A*T*T*A*T*G*A*G*+G
33	A26033Hi	AGTAGTTTATCGTTAGT	+A*+G*+T*A*G*T*T*T*A*T*C*G*T*T*+A*+G*+T
34	A26034Hi	GCTAGGAGTGGTTCTTT	+G*+C*+T*A*G*G*A*G*T*G*G*T*T*C*+T*+T*+T
35	A26035Hi	GACTAATGCTAGGAGTG	+G*+A*+C*T*A*A*T*G*C*T*A*G*G*A*G*+T*+G
36	A26036Hi	GTTAGTAGGAAGCTTCA	+G*+T*+T*A*G*T*A*G*G*A*A*G*C*T*+T*+C*+A
36	A26037Hi	GTTAGTAGGAAGCTTCA	+G*+T*+T*A*G*T*A*G*G*A*A*G*C*T*T*+C*+A
37	A26038Hi	AGTCTCGTCATTGGTAG	+A*+G*+T*C*T*C*G*T*C*A*T*T*G*G*+T*+A*+G
38	A26039Hi	AAGTCTCGTCATTGGTA	+A*+A*+G*T*C*T*C*G*T*C*A*T*T*G*+G*+T*+A

39	A26040Hi	GGTGCAACTAATTGAGT	+G*+G*+T*G*C*A*A*C*T*A*A*T*T*G*+A*+G*+T
40	A26041Hi	GTCTCGTCATTGGTAG	+G*+T*+C*T*C*G*T*C*A*T*T*G*G*+T*+A*+G
41	A26042Hi	ACTCGTATGTAGTTACT	+A*+C*+T*C*G*T*A*T*G*T*A*G*T*T*+A*+C*+T
42	A26043Hi	GACTCGTATGTAGTTAC	+G*+A*+C*T*C*G*T*A*T*G*T*A*G*T*+T*+A*+C
43	A26044Hi	AGACTCGTATGTAGTTA	+A*+G*+A*C*T*C*G*T*A*T*G*T*A*G*+T*+T*+A
44	A26045Hi	CAGACTCGTATGTAGTT	+C*+A*+G*A*C*T*C*G*T*A*T*G*T*A*+G*+T*+T
45	A26046Hi	ACTCGTATGTAGTTAC	+A*+C*+T*C*G*T*A*T*G*T*A*G*T*+T*+A*+C
46	Neg1		+C*+G*+T*T*T*A*G*G*C*T*A*T*G*T*A*+C*+T*+T
100	R01009		+T*+T*+A*G*C*G*C*G*C*G*A*A*T*+A*+T*+G
101	R01019		+G*+A*+C*T*C*G*T*T*A*A*A*C*C*G*+A*+T*+A

Table 1: List of antisense oligonucleotides hybridizing with human ANGPLT3 mRNA and/or pre-mRNA for example of SEQ ID NO. 1 and/or SEQ ID NO. 2; Neg1, R01009 and R01019 are oligonucleotides representing a negative control which is not hybridizing with ANGPTL3 of SEQ ID NO. 1 or SEQ ID NO. 2. Oligonucleotides primarily hybridizing with human ANGPLT3 mRNA are indicated by "H", and oligonucleotides primarily hybridizing with human ANGPLT3 pre-mRNA are indicated by "Hi" as the oligonucleotides hybridize with an intron.

Oligonucleotides of **Table 2** hybridize with mRNA of mouse ANGPTL3:

Seq ID	Name	Antisense Sequence 5'-3'	Antisense Sequence 5'-3' with PTO (*) and LNA (+)
48	A26001M	GCCATTCGCTAAAATTT	+G*C*+C*+A*T*T*C*G*C*T*A*A*A*+A*+T*+T*+T
49	A26002M	GAGGCCATTCGCTAAA	+G*+A*+G*G*C*C*A*T*T*C*G*C*T*+A*+A*+A
50	A26003M	TGTCGTTAATTTGTCC	+T*+G*+T*C*G*T*T*A*A*T*T*T*G*+T*+C*+C
51	A26004M	CTTCTGAAATATGTCGT	+C*+T*+T*C*T*G*A*A*A*T*A*T*G*+T*+C*+G*+T
52	A26005M	GTTCGAAGTGATAGGT	+G*+T*+T*C*G*A*A*G*T*G*A*T*A*+G*+G*+T
53	A26006M	TTGGTTCGAAGTGAT	+T*T*+G*G*T*T*C*G*A*A*G*T*+G*+A*+T
54	A26007M	TTCATTGGTTCGAAGTG	+T*+T*+C*A*T*T*G*G*T*T*C*G*A*A*+G*+T*+G
54	A26008M	TTCATTGGTTCGAAGTG	+T*+T*+C*A*T*T*G*G*T*T*C*G*A*+A*+G*+T*+G
55	A26009M	CGTTTTTAACTTGTAGT	+C*G*+T*T*T*T*T*A*A*C*T*T*G*+T*+A*+G*+T
56	A26010M	TCGTTTTTAACTTGTAG	+T*+C*+G*+T*T*T*T*T*A*A*C*T*T*+G*+T*+A*+G
57	A26011M	AAGTTGGTTAGCTGCTC	+A*+A*G*T*T*G*G*T*T*A*G*C*T*G*C*+T*+C
58	A26012M	TCGTTCAGTTGAAGAGG	+T*+C*G*T*T*C*A*G*T*T*G*A*A*G*+A*+G*+G
59	A26013M	TGTTCGCCTCTGTTATA	+T*+G*+T*T*C*G*C*C*T*C*T*G*T*T*+A*+T*+A
59	A26014M	TGTTCGCCTCTGTTATA	+T*+G*+T*+T*C*G*C*C*T*C*T*G*T*T*A*+T*+A

60	A26015M	TTGTATGTTCGCCTCT	+T*+T*+G*T*A*T*G*T*T*C*G*C*C*+T*+C*+T
61	A26016M	CCACTTGTATGTTCGCC	+C*+C*+A*C*T*T*G*T*A*T*G*T*T*C*+G*+C*+C
62	A26017M	CGCCACTTGTATGTTC	+C*+G*+C*C*A*C*T*T*G*T*A*T*G*+T*+T*+C
63	A26018M	ACGCCACTTGTATGT	+A*+C*+G*C*C*A*C*T*T*G*T*A*+T*+G*+T
64	A26019M	TGTACACGCCACTTG	+T*+G*+T*A*C*A*C*G*C*C*A*C*+T*+T*+G
65	A26020M	ATAGTGTACACGCCAC	+A*+T*+A*G*T*G*T*A*C*A*C*G*C*+C*+A*+C
66	A26021M	AATAGTGTACACGCC	+A*+A*+T*A*G*T*G*T*A*C*A*C*+G*+C*+C
67	A26022M	TCCGGTGTTGAATTAAT	+T*+C*+C*G*G*T*G*T*T*G*A*A*T*T*+A*+A*+T
68	A26023M	TCTTTCCGGTGTTGAAT	+T*+C*+T*T*T*C*C*G*G*T*G*T*T*G*+A*+A*+T
69	A26024M	CATCTTTCCGGTGTTGA	+C*+A*+T*+C*T*T*T*C*C*G*G*T*G*T*T*+G*+A
70	A26025M	TCGTTGAAGTCCTGTG	+T*+C*+G*T*T*G*A*A*G*T*C*C*T*+G*+T*+G
70	A26026M	TCGTTGAAGTCCTGTG	+T*+C*+G*T*T*G*A*A*G*T*C*C*+T*G*+T*+G
71	A26027M	ATGTTTCGTTGAAGTCC	+A*+T*+G*T*T*T*C*G*T*T*G*A*A*G*+T*+C*+C
72	A26028M	AAATTCTCCATCGA*+C	+A*+A*+A*+T*T*C*T*C*C*A*T*C*+G*+A*G*+C
73	A26029M	ACTATAGCATAGATC	+A*+C*+T*A*T*A*G*C*A*T*A*G*+A*+T*+C
74	A26030M	AGTTAGACTGTTGGA	+A*+G*+T*T*A*G*A*C*T*G*T*T*+G*+G*+A
75	A26031M	GAGTCGTAAAATGTAGT	+G*+A*+G*T*C*G*T*A*A*A*A*T*G*T*+A*+G*+T
76	A26032M	GTAGCTCGAGTCGTA	+G*+T*+A*G*C*T*C*G*A*G*T*C*+G*+T*+A
77	A26033M	TTGTAGCTCGAGTCGT	+T*+T*+G*T*A*G*C*T*C*G*A*G*T*+C*+G*+T
78	A26034M	GGAGTATTCAACGTAGT	+G*+G*+A*G*T*A*T*T*C*A*A*C*G*T*+A*+G*+T
79	A26035M	GGAGTATTCAACGTA	+G*+G*+A*G*T*A*T*T*C*A*A*C*+G*+T*+A
80	A26036M	AGGTGAAAGGAGTATTC	+A*+G*+G*T*G*A*A*A*G*G*A*G*T*A*+T*+T*+C
81	A26037M	GTTGGTTTCGTGACTGC	+G*+T*+T*G*G*T*T*T*C*G*T*G*A*C*+T*+G*+C
82	A26038M	GTAGTTGGTTTCGTGAC	+G*+T*+A*G*T*T*G*G*T*T*T*C*G*T*+G*+A*+C
83	A26039M	CGTGTAGTTGGTTTCGT	+C*+G*+T*G*T*A*G*T*T*G*G*T*T*T*+C*+G*+T
84	A26040M	GCGTGTAGTTGGTTTC	+G*+C*+G*T*G*T*A*G*T*T*G*G*T*+T*+T*+C
85	A26041M	TAGCGTGTAGTTGGTTT	+T*+A*+G*C*G*T*G*T*A*G*T*T*G*G*+T*+T*+T
86	A26042M	TAGCGTGTAGTTGGTT	+T*+A*+G*C*G*T*G*T*A*G*T*T*G*+G*+T*+T
87	A26043M	TGTAGCGTGTAGTTGGT	+T*+G*+T*A*G*C*G*T*G*T*A*G*T*T*+G*+G*+T
88	A26044M	CATGTAGCGTGTAGTT	+C*+A*+T*G*T*A*G*C*G*T*G*T*A*+G*+T*+T
88	A26045M	CATGTAGCGTGTAGTT	+C*+A*T*G*T*A*G*C*G*T*G*T*+A*+G*+T*+T
89	A26046M	TTGGATGATTTGATAGC	+T*T*+G*G*A*T*G*A*T*T*T*+G*+A*T*+A*+G*+C
90	A26047M	TACCAGATTACTACAGT	+T*+A*+C*C*A*G*A*T*T*A*C*T*A*C*+A*+G*+T
91	A26048M	ATACCAGATTACTAC	+A*+T*+A*C*C*A*G*A*T*T*A*C*+T*+A*+C
91	A26049M	ATACCAGATTACTAC	+A*+T*+A*+C*C*A*G*A*T*T*A*C*T*+A*+C
92	A26050M	TCGGTGATATCTGATCT	+T*+C*+G*G*T*G*A*T*A*T*C*T*G*A*+T*+C*+T
93	A26051M	TGATTCGGTGATATCT	+T*+G*+A*T*T*C*G*G*T*G*A*T*A*+T*+C*+T
94	A26052M	ACGTTGATTCGGTGATA	+A*+C*+G*T*T*G*A*T*T*C*G*G*T*G*+A*+T*+A

95	A26053M	GTTTACGTTGATTCGGT	+G*+T*+T*T*A*C*G*T*T*G*A*T*T*C*+G*+G*+T
96	A26054M	GTTTACGTTGATTCG	+G*+T*+T*T*A*C*G*T*T*G*A*T*+T*+C*+G
97	A26055M	TGTTTACGTTGATTC	+T*+G*+T*T*T*A*C*G*T*T*G*A*+T*+T*+C
98	A26056M	GACATGTTCTTCACCTCCTC	+G*+A*+C*A*T*G*T*T*C*T*T*C*A*C*C*T*C*+C*+T*+C
98	A26057M	GACATGTTCTTCACCTCCTC	+G*+A*C*A*T*G*T*T*C*T*T*C*A*C*C*T*C*C*+T*+C
46	Neg1		+C*+G*+T*T*T*A*G*G*C*T*A*T*G*T*A*+C*+T*+T
100	R01009		+T*+T*+A*G*C*G*C*G*C*G*A*A*T*+A*+T*+G
101	R01019		+G*+A*+C*T*C*G*T*T*A*A*A*C*C*G*+A*+T*+A

Table 2: List of antisense oligonucleotides hybridizing with mouse ANGPLT3 mRNA for example of SEQ ID NO. 47; Neg1, R01009 and R01019 are oligonucleotides representing a negative control which is not hybridizing with ANGPTL3 of SEQ ID NO. 47.

The oligonucleotides of the present invention hybridize for example with mRNA of human ANGPTL3 of SEQ ID NO. 1 and/or introns of the pre-mRNA of human ANGPTL3 of SEQ ID NO. 2. In addition or alternatively, the oligonucleotides of the present invention hybridize for example with mRNA of mouse ANGPTL3 of SEQ ID NO.47. Such oligonucleotides are called ANGPTL3 antisense oligonucleotides. The oligonucleotides hybridize for example within a hybridizing active area which is one or more region(s) on the ANGPTL3 mRNA, e.g., of SEQ ID NO.1 and/or the ANGPTL3 pre-mRNA, e.g., of SEQ ID NO.2, where hybridization with an oligonucleotide highly likely results in a potent knockdown of the ANGPTL3 expression. In the present invention surprisingly several hybridizing active areas were identified for example from position 45-72 (e.g., A26004H, SEQ ID NO.6) and/or from position 1130-1170 (e.g., A26019H, SEQ ID NO.20; A26020H, SEQ ID NO.20; A26021H, SEQ ID NO. 21; A26022H, SEQ ID NO.22; A26023H, SEQ ID NO. 23; A26024H, SEQ ID NO.24) of SEQ ID NO. 1 and/or from position 3060-3086 (e.g., A26033Hi, SEQ ID NO.33) and/or from position 5768-5794 (e.g., A26036Hi, SEQ ID NO.36; A26037Hi, SEQ ID NO.36) of SEQ ID NO.2. Hybridizing areas enriched for ASOs with high activity on mouse SEQ ID NO.47 are for example from position 303-330 (e.g. A26007M, SEQ ID NO. 54) and/or from position 803-843 (e.g. A26016M, SEQ ID NO. 61; A26019M, SEQ ID NO. 64; A26020M, SEQ ID NO. 65; A26021M, SEQ ID NO. 66) and/or from position 1038-1134 (e.g. A26032M, SEQ ID NO. 76; A26033M, SEQ ID NO. 77; A26034M, SEQ ID NO. 78; A26035M, SEQ ID NO. 79; A26039M, SEQ ID NO. 83) and/or from position 1480-1507 (e.g. A26047M, SEQ ID NO. 90).

The oligonucleotide of the present invention inhibits for example at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 93 %, 94%, 95%, 96%, 97%, 98%, 99% or 100% of ANGPTL3 such as the, e.g., human or mouse, ANGPTL3 expression. The oligonucleotide of the present invention inhibits the expression of ANGPTL3 at a nanomolar or micromolar concentration for example in a concentration range of 0.1 nM to 100 μM, 0.5 nM to 15 nM, 0,6 nM to 10 nM, 1 nM to 10 μM, 5 nM to 5 μM, 10 nM to 1 μM, 15 nM to 950 nM, 20 nM to 900 nM, 25 nM to 850 nM, 30 nM to 800 nM, 35 nM to 750 nM, 40 nM to 700 nM, 45 nM to 650 nM, 50 nM to 500 nM, or 40 nM to 150 nM, or in a concentration of 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900 or 950 nM, or 1, 10 or 100 μM.

The ANGPTL3 oligonucleotide of the present invention is for example used in a concentration range of 1 nM to 10 μM, 5 nM to 6.6 μM, 10 nM to 5 μM, 15 nM to 3 μM, 20 nM to 2.2 μM, 25 nM to 1 μM, 30 nM to 800 nM, 50 nM to 500 nM, 60 nM to 300 nM, 70 nM to 250 nM, 80 nM to 200 nM, 90 nM to 120 nM, or in a concentration of 1, 1.6, 3, 5, 8, 9, 10, 15, 20, 25, 27, 30, 40, 50, 75, 82, 100, 200, 250, 300, 500, or 740 nM, or 1, 2.2, 3, 5, 6.6 or 10 μM.

The ANGPTL3 oligonucleotide of the present invention is for example administered once or repeatedly, e.g., every 12 h, every 24 h, every 48 h for some weeks, months or years, or it is administered every week, every two weeks, every three weeks or every months, every three or every six months.

In some embodiments the present invention refers to a pharmaceutical composition comprising an ANGPTL3 oligonucleotide of the present invention and a pharmaceutically acceptable carrier, excipient and/or dilutant. Optionally, the pharmaceutical composition further comprises a chemotherapeutic, another disease specific active agent such as insulin, angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, another oligonucleotide not of the present invention, an antibody, a HERA fusion protein, a ligand trap, a Fab fragment, a nanobody, a BiTe and/or a small molecule which is for example effective in tumor treatment, treatment of diabetes and its side effects, treatment of a cardiovascular disease, obesity, diabetes type II, hypercholesterolemia such as homozygote familial hypercholesterolemia (HoFH), heterozygote familial hypercholesterolemia (HeFH) or dyslipidemia.

The ANGPTL3 oligonucleotide or the pharmaceutical composition of the present invention is for use in a method of preventing and/or treating a disorder for example a disorder where an ANGPTL3 imbalance is involved. Optionally, the use of the oligonucleotide or the pharmaceutical composition of the present invention in a method of preventing and/or treating a disorder is combined with radiotherapy. The radiotherapy may be further combined with a chemotherapy (e.g., platinum, gemcitabine). The disorder is for example characterized by an ANGPTL3 imbalance, i.e., the ANGPTL3 level is increased in comparison to the level in a normal, healthy cell, tissue, organ or subject. The ANGPTL3 level is for example increased by an increased ANGPTL3 expression and activity, respectively. The ANGPTL3 level is measured by any standard method such as immunohistochemistry, western blot, quantitative real time PCR or QuantiGene assay known to a person skilled in the art.

The ANGPTL3 oligonucleotide or a pharmaceutical composition of the present invention is administered locally or systemically for example orally, sublingually, nasally, subcutaneously, intravenously, intraperitoneally, intramuscularly, intratumoral, intrathecal, transdermal, and/or rectal. Alternatively or in combination *ex vivo* treated immune cells are administered. The ANGPTL3 oligonucleotide is administered alone or in combination with another ANGPTL3 antisense oligonucleotide of the present invention and optionally in combination with another compound such as another oligonucleotide not of the present invention, an antibody, a HERA fusion protein, a ligand trap, a Fab fragment, a nanobody, a BiTe, a small molecule and/or a chemotherapeutic (e.g., platinum, gemcitabine) and/or another disease specific agent such as insulin, angiotensin-converting enzyme inhibitor, and/or angiotensin receptor blocker.

The oligonucleotide not of the present invention, an antibody, a HERA fusion protein, a ligand trap, a Fab fragment, a nanobody, a BiTe, and/or the small molecule are effective in preventing and/or treating a tumor, diabetes such as diabetes type II and its side effects, a cardiovascular disease, obesity, hypercholesterolemia such as homozygote familial hypercholesterolemia (HoFH), heterozygote familial hypercholesterolemia (HeFH) or dyslipidemia. An ANGPTL3 oligonucleotide or a pharmaceutical composition of the present invention is used for example in a method of preventing and/or treating a solid tumor or a hematologic tumor. Examples of cancers preventable and/or treatable by use of the oligonucleotide or pharmaceutical composition of the present invention are

breast cancer, lung cancer, malignant melanoma, lymphoma, skin cancer, bone cancer, prostate cancer, liver cancer, brain cancer, cancer of the larynx, gall bladder, pancreas, testicular, rectum, parathyroid, thyroid, adrenal, neural tissue, head and neck, colon, stomach, bronchi, kidneys, basal cell carcinoma, squamous cell carcinoma, metastatic skin carcinoma, osteo sarcoma, Ewing's sarcoma, reticulum cell sarcoma, liposarcoma, myeloma, giant cell tumor, small-cell lung tumor, islet cell tumor, primary brain tumor, meningioma, acute and chronic lymphocytic and granulocytic tumors, acute and chronic myeloid leukemia, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, intestinal ganglioneuromas, Wilm's tumor, seminoma, ovarian tumor, leiomyomater tumor, cervical dysplasia, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic sarcoma, malignant hypercalcemia, renal cell tumor, polycythermia vera, adenocarcinoma, anaplastic astrocytoma, glioblastoma multiforma, leukemia, or epidermoid carcinoma.

Further examples of diseases preventable and/or treatable by use of the ANGPTL3 oligonucleotide or the pharmaceutical composition of the present invention other than cancer are for example diabetes such as diabetes type II and its side effects, a cardiovascular disease, obesity, hypercholesterolemia such as homozygote familial hypercholesterolemia (HoFH), heterozygote familial hypercholesterolemia (HeFH) or dyslipidemia.

In some examples two or more ANGPTL3 oligonucleotides of the present invention are administered together, at the same time point for example in a pharmaceutical composition or separately, or on staggered intervals. In other examples, one or more oligonucleotides of the present invention are administered together with another compound such as another oligonucleotide not of the present invention, an antibody, a HERA fusion protein, a ligand trap, a Fab fragment, a nanobody, a BiTe, a small molecule and/or a chemotherapeutic, at the same time point for example in a pharmaceutical composition or separately, or on staggered intervals.

A subject of the present invention is for example a mammalian, a bird or a fish.

Examples

The following examples illustrate different embodiments of the present invention, but the invention is not limited to these examples. The following experiments are performed on cells endogenously expressing ANGPTL3, i.e., the cells do not represent an artificial system comprising transfected reporter constructs. Such artificial systems generally show a higher degree of inhibition and lower IC_{50} values than endogenous systems which are closer to therapeutically relevant *in vivo* systems. Further, whenever possible no transfecting agent is used in the following experiments, i.e., gymnotic delivery is performed. Transfecting agents are known to increase the activity of an oligonucleotide which influences the IC_{50} value (see for example Zhang et al., Gene Therapy, 2011, 18, 326-333; Stanton et al., Nucleic Acid Therapeutics, Vol. 22, NO. 5, 2012).

Example 1: Transfection screens of human ANGPTL3-specific antisense oligonucleotides in Hep3B cells

8,000 Hep3B cells / well were seeded in 96-well plates and treated with the respective antisense oligonucleotides at a final concentration of 3 nM by use of Lipofectamine 2000 (Thermo Fisher Scientifc). After three days, cells were lysed and human HPRT1 and human ANGPTL3 mRNA expression was measured using the QuantiGene RNA Singleplex assay (Thermo Fisher Scientifc). The QuantiGene Assay used in the examples is built upon the branched DNA technology (bDNA), which relies on cooperative hybridization between a target mRNA and a specific probe set (part of QuantiGene Reagent System). The assay was performed according to manufacturer's protocol and was used for determination of RNA levels. It combines the QuantiGene Sample Processing Kit that is used for cell lysis and the QuantiGene Reagent System that is used for hybridization, amplification and detection of RNA of interest. The QuantiGene Reagent System is based on an RNA-specific probe set, designed to detect a particular RNA of interest.

Human ANGPTL3-specific antisense-oligonucleotides were designed and 28 antisense oligonucleotides targeting ANGPTL3 mRNA as well as 18 intron-targeting antisense oligonucleotides were tested. A control oligonucleotide that does not have sequence complementarity to any human or mouse mRNA (Neg1) was included as a control. Due to low gymnotic delivery of the human hepatocellular carcinoma cell line Hep3B, cells

were treated with the respective oligonucleotides at a single concentration of 3 nM for three days by using a transfection reagent. Three days after start of treatment, the mRNA levels were determined by QuantiGene RNA Singleplex assay. HPRT1 was used as a housekeeping gene for normalization of ANGPTL3 expression. As shown in **Fig. 1**, 20 antisense oligonucleotides, (A26013H (SEQ ID NO.15), A26004H (SEQ ID NO.6), A26033Hi (SEQ ID NO.33), A26007H (SEQ ID NO.9), A26031Hi (SEQ ID NO.31), A26023H (SEQ ID NO.23), A26035Hi (SEQ ID NO.35), A26022H (SEQ ID NO.22), A26005H (SEQ ID NO.7), A26015H (SEQ ID NO.16), A26019H (SEQ ID NO.20), A26025H (SEQ ID NO.25), A26021H (SEQ ID NO.21), A26020H (SEQ ID NO.20), A26037Hi (SEQ ID NO.36), A26024H (SEQ ID NO.24), A26014H (SEQ ID NO.15), A26029Hi (SEQ ID NO.29), A26036Hi (SEQ ID NO.36), A26016H (SEQ ID NO.17)) reduced the normalized ANGPTL3 expression by more than 80 % (equivalent to residual mRNA level of < 0.2). Further, 16 antisense oligonucleotides, (A26009H (SEQ ID NO.11), A26026H (SEQ ID NO.26), A26012H (SEQ ID NO.14), A26027H (SEQ ID NO.27), A26028H (SEQ ID NO.28), A26010H (SEQ ID NO.12), A26038Hi (SEQ ID NO.37), A26039Hi (SEQ ID NO.38), A26040Hi (SEQ ID NO.39), A26034Hi (SEQ ID NO.34), A26044Hi (SEQ ID NO.43), A26011H (SEQ ID NO.13), A26043Hi (SEQ ID NO.42), A26041Hi (SEQ ID NO.40), A26046Hi (SEQ ID NO.45), A26042Hi (SEQ ID NO.41)), showed knockdown efficacy between 80 % and 50 %, while control oligonucleotide (Neg1) did not reduce ANGPTL3 mRNA expression.

Residual ANGPTL3-mRNA expression relative to mock-treated cells ("no oligo" set as 1) is shown. Solid line and dotted lines (indicate 80 % and 50 % or 0 % knockdown efficacy, respectively. Data are represented as mean of triplicate wells +/-SD.

Example 2: Test of selected human ANGPTL3-specific antisense oligonucleotides in human primary hepatocytes

15,000 primary hepatocytes / well were seeded in 96-well plates and treated with the respective antisense oligonucleotides at a final concentration of 10 μM. Every 24 hours, 70 μl of cell supernatant was replaced by fresh maintenance medium without antisense oligonucleotide. Three days after start of treatment, cells were lysed and human HPRT1 and human ANGPTL3 mRNA expression was measured using the QuantiGene RNA Singleplex assay.

To analyze knockdown efficiency of 25 selected antisense oligonucleotides under less artificial conditions, primary human hepatocytes were treated with 10 μM of respective antisense oligonucleotides without using a transfection reagent. After three days the mRNA levels were determined by QuantiGene RNA Singleplex assay. HPRT1 was used as a housekeeping gene for normalization of ANGPTL3 expression. As shown in **Fig. 2**, 21 of the tested 25 antisense oligonucleotides (A26020H (SEQ ID NO.20), A26019H (SEQ ID NO.20), A26023H (SEQ ID NO.23), A26022H (SEQ ID NO.22), A26033Hi (SEQ ID NO.33), A26036Hi (SEQ ID NO.36), A26037Hi (SEQ ID NO.36), A26004H (SEQ ID NO.6), A26024H (SEQ ID NO.24), A26016H (SEQ ID NO.17), A26040Hi (SEQ ID NO.39), A26021H (SEQ ID NO.21), A26014H (SEQ ID NO.15), A26028H (SEQ ID NO.28), A26029Hi (SEQ ID NO.29), A26025H (SEQ ID NO.25), A26027H (SEQ ID NO.27), A26026H (SEQ ID NO.26), A26007H (SEQ ID NO.9), A26013H (SEQ ID NO.15), A26015H (SEQ ID NO.16)), show more than 80 % knockdown of ANGPTL3 mRNA (equivalent to residual mRNA level of < 0.2), while treatment with Neg1 negative control oligonucleotide did not result in decreased ANGPTL3 mRNA levels.

ANGPTL3-mRNA expression values were normalized to expression of the housekeeping gene HPRT1. Residual ANGPTL3-mRNA expression relative to mock-treated cells ("no oligo" set as 1) is shown. Solid line and dotted lines indicate 80 % and 50 % or 0 % knockdown efficacy, respectively. Data are represented as mean of triplicate wells +/-SD.

Example 3: TLR9 activity assay of selected human ANGPTL3-specific antisense oligonucleotides

Binding of immune stimulatory ligands, e.g. bacterial DNA or immune stimulatory oligonucleotides with or without unmethylated CpG dinucleotides results in TLR activation. As immune activation can lead to severe, possibly life threatening condition of excessive cytokine release[4], there is an urgent need for a preclinical test system that predicts cytokine release in humans enabling a safe estimation for phase I studies.

HEK-Blue-hTLR9 (Invivogen cat. no. hkb-htlr9) cells were seeded in flat-bottom 96-well plates and treated with indicated oligonucleotides for 24 h. Then, cell supernatants were harvested and incubated for 4 h with QUANTI-Blue solution (Invivogen cat. no. rep-qbs). SEAP activity was determined by measurement of the optical density. Means and

standard deviations of OD units relative to OD units from cells stimulated with 5000 nM ODN2006 (set as 100) are depicted.

As shown in **Fig. 3**, none of the tested ANGPTL3-specific antisense oligonucleotides induced TLR9 activation. In contrast, positive control CpG oligonucleotide ODN2006 (5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' PTO-modified –Invivogen cat. no. tlrl-2006; SEQ ID NO.99) clearly stimulated NFκB activation (**Fig. 3**). Data are represented as mean of triplicate wells +/-SD.

Example 4: IC_{50} determination of selected human ANGPTL3-specific antisense oligonucleotides

15,000 primary human hepatocytes / well were seeded in 96-well plates and treated with different concentrations (1000 nM, 200 nM, 40 nM, 8 nM, 1.6 nM, 0.32 nM) of nine antisense oligonucleotides (A26004H (SEQ ID NO.6), A26019H (SEQ ID NO.20), A26020H (SEQ ID NO.20), A26022H (SEQ ID NO.22), A26023H (SEQ ID NO.23), A26024H (SEQ ID NO.24), A26033Hi (SEQ ID NO.33), A26036Hi (SEQ ID NO.36) and A26037Hi (SEQ ID NO.36)). Three days after start of treatment, cells were lysed and HPRT1 and ANGPTL3 mRNA expression was measured using the QuantiGene RNA Singleplex assay. ANGPTL3-mRNA expression values were normalized to expression of the housekeeping gene HPRT1. Residual ANGPTL3-mRNA expression relative to mock-treated cells (set as 1). For graphic representation mock-treated cells were set as 0.16 nM. Data are represented as mean of triplicate wells +/-SD.

Fig. 4 and **Table 3** show that the selected human ANGPTL3-specific antisense oligonucleotides inhibit ANGPTL3 mRNA expression dose-dependently with IC_{50} values in the nanomolar range.

ASO	IC_{50} [nM]	R square
A26004H	12	0.95
A26019H	4	0.91
A26020H	7	0.95
A26022H	7	0.95
A26023H	10	0.73
A26024H	25	0.82
A26036Hi	11	0.82
A26037Hi	25	0.80
A26033Hi	16	0.82

Example 5: Transfection screen of mouse ANGPTL3-specific antisense oligonucleotides in AML12 cells

8,000 AML12 cells / well were seeded in 96-well plates and treated with the indicated mouse ANGPTL3 antisense oligonucleotides (**Fig. 5**) at a final concentration of 3 nM by use of Lipofectamine 2000. Three days after start of treatment, cells were lysed and mouse Hprt1 and mouse ANGPTL3 mRNA expression was measured using the QuantiGene RNA Singleplex assay. ANGPTL3-mRNA expression values were normalized to expression of the housekeeping gene Hprt1. Residual ANGPTL3-mRNA expression relative to mock-treated cells ("no oligo" set as 1) is shown. Solid line and dotted lines indicate 70 % and 50 % or 0 % knockdown efficacy, respectively.

Mouse ANGPTL3-specific antisense-oligonucleotides were designed and 55 antisense oligonucleotides targeting ANGPTL3 mRNA were tested. A control oligonucleotide that does not have sequence complementarity to any human or mouse mRNA (Neg1) was included as a control. Furthermore, two positive control oligonucleotides with varying LNA modification patterns hybridizing with ANGPTL3 mRNA (A26056M (SEQ ID NO.98), A26057M (SEQ ID NO.98)) were also analyzed for ANGPTL3 knockdown efficiency. Due to low gymnotic delivery of the mouse hepatocyte epithelial cell line AML12, cells were treated with the respective oligonucleotides at a single concentration of 3 nM for three days by using a transfection reagent. Three days after start of treatment, the mRNA levels were determined by QuantiGene RNA Singleplex assay. Hprt1 was used as a housekeeping gene for normalization of ANGPTL3 expression. Residual ANGPTL3-mRNA expression relative to mock-treated cells ("no oligo" set as 1) is shown. Data are represented as mean of triplicate wells +/-SD.

As shown in **Fig. 5**, 8 antisense oligonucleotides (A26019M (SEQ ID NO.64), A26012M (SEQ ID NO.58), A26035M (SEQ ID NO.79), A26034M (SEQ ID NO.78), A26010M (SEQ ID NO.56), A26021M (SEQ ID NO.66), A26020M (SEQ ID NO.65), A26032M (SEQ ID NO.76)) reduced the normalized ANGPTL3 expression by more than 70 % (equivalent to residual mRNA level of < 0.3). Further, 28 antisense oligonucleotides (A26016M (SEQ ID NO.61), A26047M (SEQ ID NO.90), A26050M (SEQ ID NO.92), A26007M (SEQ ID NO.54), A26056M (SEQ ID NO.98), A26027M (SEQ ID NO.71), A26033M (SEQ ID NO.77), A26002M (SEQ ID NO.49), A26054M (SEQ ID NO.96), A26039M (SEQ ID NO.83), A26017M (SEQ ID NO.62), A26018M (SEQ ID NO.63), A26005M (SEQ ID NO.52), A26026M (SEQ ID NO.70), A26004M (SEQ ID NO.51), A26015M (SEQ ID NO.60), A26013M (SEQ ID NO.59), A26025M (SEQ ID NO.70), A26022M (SEQ ID NO.67), A26024M (SEQ ID NO.69), A26053M (SEQ ID NO.95), A26037M (SEQ ID NO.81), A26038M (SEQ ID NO.82), A26042M (SEQ ID NO.86), A26055M (SEQ ID NO.97), A26045M (SEQ ID NO.88), A26014M (SEQ ID NO.59), A26031M(SEQ ID NO.75) showed knockdown efficacy between 70 % and 50 %, while control oligonucleotide (Neg1) did not reduce ANGPTL3 mRNA expression. Both positive control oligonucleotides reduced ANGPTL3 mRNA expression levels by 66 % and 47 %, respectively (**Fig. 5**).

Example 6: Test of selected ANGPTL3-specific antisense oligonucleotides in mouse primary hepatocytes

Based on transfection screen in AML12 cells, 13 ANGPTL3-specific antisense oligonucleotides were selected for further analysis (**Fig. 6**) in primary mouse hepatocytes. Cells were treated with 5 μM of respective antisense oligonucleotides without using a transfection reagent. Three days after start of treatment, the mRNA levels were determined by QuantiGene RNA Singleplex assay. Hprt1 was used as a housekeeping gene for normalization of ANGPTL3 expression. Residual ANGPTL3-mRNA expression relative to mock-treated cells ("no oligo" set as 1) is shown. Data are represented as mean of triplicate wells +/-SD.

As shown in **Fig. 6**, 10 of the tested antisense oligonucleotides, (A26019M (SEQ ID NO.64), A26016M (SEQ ID NO.61), A26020M (SEQ ID NO.65); A26007M (SEQ ID NO.54); A26021M (SEQ ID NO.66); A26032M (SEQ ID NO.76); A26034M (SEQ ID NO.78); A26033M (SEQ ID NO.77); A26039M (SEQ ID NO.83); A26035M (SEQ ID

NO.79)), show more than 80 % knockdown of ANGPTL3 mRNA (equivalent to residual mRNA level of < 0.2), while treatment with Neg1 negative control oligonucleotide resulted in 36 % decreased ANGPTL3 mRNA levels. Positive control antisense oligonucleotide A26056M (SEQ ID NO.98) also led to reduced ANGPTL3-mRNA levels by more than 80 % (**Fig. 6**).

Example 7: IC_{50} determination of selected mouse ANGPTL3-specific antisense oligonucleotides

11 antisense oligonucleotides (A26019M (SEQ ID NO.64), A26016M (SEQ ID NO.61), A26020M (SEQ ID NO.65), A26007M (SEQ ID NO.54), A26021M (SEQ ID NO.66), A26032M (SEQ ID NO.76), A26034M (SEQ ID NO.78), A26033M (SEQ ID NO.77), A26039M (SEQ ID NO.83), A26035M (SEQ ID NO.79), A26047M (SEQ ID NO.90)) selected due to high knockdown efficacy in primary mouse hepatocytes (**Fig. 6**) were selected for determination of half maximal inhibitory concentration (IC_{50}) values. Primary mouse hepatocytes were treated with the respective antisense oligonucleotides at different concentrations (5000 nM, 1000 nM, 200 nM, 40 nM, 8 nM, 1.6 nM) for three days and mRNA expression was analyzed using the QuantiGene Singleplex RNA assay. Hprt1 was used as a housekeeping gene for normalization of ANGPTL3 expression. Residual ANGPTL3-mRNA expression relative to mock-treated cells ("no oligo" set as 1) is shown. Data are represented as mean of triplicate wells +/-SD.

Fig. 7 and **Table 4** demonstrate that the selected ANGPTL3-specific antisense oligonucleotides with high knockdown efficiency in mouse hepatocytes (**Fig. 6**) inhibit ANGPTL3 mRNA expression dose-dependently with IC_{50} values in the nanomolar range.

Table 4 in the following shows IC_{50} values and R square of selected ANGPTL3-specific antisense oligonucleotides determined in mouse primary hepatocytes:

ASO	IC_{50} [nM]	R square
A26007M	60	0.92
A26016M	49	0.93
A26019M	46	0.97
A26020M	~ 192	0.93
A26021M	167	0.99
A26032M	64	0.93
A26033M	111	0.96
A26034M	113	0.95
A26035M	~ 199	0.90
A26039M	84	0.74
A26047M	328	0.72

Example 8: *In vitro* efficacy of human ANGPTL3-specific oligonucleotides in mouse hepatocytes

15,000 primary mouse hepatocytes were seeded in BioCoat Collagen I 96-well flat bottom plates in 100 µl Hepatocyte Plating Medium. Supernatant was removed 4-6 h after seeding and antisense oligonucleotides (ASOs) were added at indicated concentrations (5000 nM, 1000 nM, 200 nM, 40 nM, 8 nM, 1.6 nM) diluted in 100 µl Maintenance Medium. Cells were cultured for three days at 37°C.

Human ANGPTL3-specific antisense-oligonucleotides A26004H (SEQ ID NO.6), A26022H (SEQ ID NO.22) and A26023H (SEQ ID NO.23) with verified knockdown efficiency in human cell lines (see **Fig. 1**) and primary hepatocytes (see **Fig.2** and **Fig. 4**) were tested in primary mouse hepatocytes (**Fig. 8A-8B**).

The control oligonucleotide Neg1 that does not have sequence complementarity to any human or mouse mRNA was included as negative control. Mouse primary hepatocytes were treated with the respective oligonucleotides at indicated concentrations for three days without the use of a transfection reagent.

As shown in **Fig. 8A**, treatment with human ANGPTL3-specific ASOs A26022H (SEQ ID NO.22) and A26023H (SEQ ID NO.23) led to dose-dependent reduction of Angptl3 mRNA expression in primary mouse hepatocytes up to approx. 30% and 70% (equivalent to residual Angptl3-mRNA expression of 0.7 and 0.3), respectively, while ASO A26004H

(SEQ ID NO.6) did not decrease mouse Angptl3 mRNA levels. Control oligonucleotides Neg1 did also not diminish Angptl3 mRNA expression.

Example 9: *In vitro* efficacy of human ANGPTL3-specific oligonucleotides in cynomolgus hepatocytes

Human ANGPTL3-specific antisense-oligonucleotides A26004H (SEQ ID NO.6), A26022H (SEQ ID NO.22), A26023H (SEQ ID NO.23), A26033Hi (SEQ ID NO.33), A26036Hi (SEQ ID NO.36) and A26037Hi (SEQ ID NO.36) having 0-1 mismatch to cynomolgus ANGPTL3 sequence (**Table 5**) were tested in primary cynomolgus hepatocytes (**Fig. 9**). All ASOs tested were shown to have verified knockdown efficiency in human cell lines and primary hepatocytes (**Fig.1**-**2**, **Fig.4**).

For efficacy testing in cynomolgus hepatocytes, 25,000 cells were seeded in Collagen Coated 96 well plates (Primacyt) using the Plating and Thawing Kit. After incubation for 6 hours at 37°C, supernatant was replaced by 90 µl Human Hepatocyte Maintenance Medium supplemented with Liver X receptor agonist (LXR) (Sigma-Aldrich, Cat. No. T0901317) at a final concentration of 1 µM to induce ANGPTL3 expression. Therefore, LXR stock solution (10 mM) was prepared by dissolving 5 mg LXR (Molecular weight: 481.33) in 1.04 ml DMSO. For final concentration of 1 µM LXR, cells seeded in 96-well plates were incubated with 100 µl Maintenance Medium supplemented with 0.01 µl LXR stock solution.. As a vehicle control, cells were treated with equal volume of DMSO. ASOs (e.g., A26004H (SEQ ID NO.6), A26022H (SEQ ID NO.22), A26023H (SEQ ID NO.23), A26033Hi (SEQ ID NO.33), A26036Hi (SEQ ID NO.36) and A26037Hi (SEQ ID NO.36)) were transfected at indicated concentrations (20 nM, 2 nM, 0.2 nM) in 10 µl Opti-MEM by use of Lipofectamin2000 according to manufacturer's instructions. Two control oligonucleotides (R01009 (SEQ ID NO.100) and R01019 (SEQ ID NO.101), with different lengths (16 and 17 nucleotides, respectively) that do not have sequence complementarity to any human or mouse mRNA were included as negative controls. Cells were cultured for 24 hours at 37°C.

After one day treatment, mRNA expression was analyzed using the QuantiGene Singleplex RNA assay.

As shown in **Fig. 9A**, treatment with LXR did not induce ANGPTL3 expression (DMSO control). ANGPTL3-specific ASO A26022H (SEQ ID NO.22) and A26023H (SEQ ID NO.23), which are completely cross-reactive to cynomolgus ANGPTL3 mRNA sequence led to up to 70% knock-down of ANGPTL3 (equivalent to residual mRNA expression of 0.3). Human ANGPTL3-specific oligonucleotide A26004H (SEQ ID NO.6) having one mismatch to cynomolgus sequence also reduced cynomolgus ANGPTL3 mRNA by more than 70% (equivalent to residual ANGPTL3-mRNA expression of 0.3). In contrast, human ANGPTL3-specific ASOs targeting intronic regions of human ANGPTL3 RNA did not affect cynomolgus ANGPTL3 mRNA expression. Negative control oligonucleotides R01009 (SEQ ID NO.100) and R01019 (SEQ ID NO.101) did not reduce ANGPTL3 expression in primary cynomolgus hepatocytes.

Conclusion from Examples 8 and 9

In order to use mouse models for *in vivo* testing of human ANGPTL3-specific ASOs, it is sought to identify potent ASOs that inhibit mouse Angptl3. One of the tested candidate ASOs A26023H (SEQ ID NO.23), which is completely cross-reactive to mouse Angptl3 RNA sequence, was able to reduce mouse Angptl3 on the mRNA level by about 70%, while human ANGPTL3-specific ASO A26022H (SEQ ID NO.22) reduced mouse Angptl3 mRNA levels by about 30% (**Fig. 8A**).

Testing a set of 6 human ANGPTL3-specific ASOs with 0-1 mismatches to cynomolgus ANGPTL3 RNA sequences, it was shown that 3 human-specific ASOs can potently reduce the expression of cynomolgus ANGPTL3 in primary cynomolgus hepatocytes on the mRNA level by about 70% (**Fig. 9A**).

Taken together, *in vitro* experiments were conducted that led to the identification of highly potent human ANGPTL3-specific ASOs that are eligible for testing of an ASO-based ANGPTL3-targeting therapeutic in cynomolgus monkeys. Such a drug could is for example be used for the systemic treatment of dyslipidemia patients in order to reduce the ANGPTL3-mediated inhibition of lipoprotein lipase L preventing cellular lipid overload, obesity, diabetes type II and cardiovascular disease.

Table 5 shows human ANGPTL3-specific ASOs with proven knock-down efficiency in human cells that do not lead to increased caspase3/7 induction *in vitro*. Depicted are cross-reactivity (CrossReact) as well as number of mismatches to cynomolgus (Mfa,

Macaca fascicularis) or mouse (Mm, *Mus musculus*) ANGPTL3 sequence, as well as activity in the respective primary hepatocytes *in vitro* (**Fig. 8** and **Fig. 9**).

Target	ASO ID	SEQ ID NO.	CrossReact_Mfa	Mismatches to Mfa sequence	Activity in Mfa hepatocytes	Mismatches to Mm sequence	Activity in Mm hepatocytes
ANGPTL3	A26004H	6	no	1	+	3	-
	A26022H	22	yes	0	+	1	+
	A26023H	23	yes	0	+	1	+
	A26033Hi	33	yes	0	-	NA	NA
	A26036Hi	36	yes	0	-	NA	NA
	A26037Hi	36	yes	0	-	NA	NA

Claims

1. ANGPTL3 inhibitor consisting of an oligonucleotide comprising 12 to 22 nucleotides, wherein at least one of the nucleotides is modified, and the oligonucleotide hybridizes with a nucleic acid sequence of ANGPTL3 of SEQ ID NO.1 (human), ANGPTL3 of SEQ ID NO. 2 (human) or both, and/or with a nucleic acid sequence of ANGPTL3 of SEQ ID NO.47 (mouse), wherein the oligonucleotide inhibits the expression of ANGPTL3.

2. Inhibitor according to claim 1, wherein the hybridizing active area is selected from position 45 to 72 and/or from position 1130 to 1170 of SEQ ID NO. 1 and/or from position 3060 to 3086 and/or from position 5768 to 5794 of SEQ ID NO.2.

3. Inhibitor according to claim 1 or 2, wherein the modified nucleotide is selected from the group consisting of a bridged nucleic acid such as LNA, cET, ENA, 2´Fluoro modified nucleotide, 2´O-Methyl modified nucleotide, 2' O-Methoxyethyl modified nucleotide and a combination thereof.

4. Inhibitor according to any one of claims 1 to 3 hybridizing with ANGPTL3 of SEQ ID NO.1 or with ANGPTL3 of SEQ ID NO.2 or both, and/or ANGPTL3 of SEQ ID NO.47 comprising a sequence selected from the group consisting of SEQ ID NO.6, SEQ ID NO.22, SEQ ID NO.33, SEQ ID NO.36, SEQ ID NO.23, SEQ ID NO.3, SEQ ID NO.4, SEQ ID NO.5, SEQ ID NO.7, SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.13, SEQ ID NO.14, SEQ ID NO.15, SEQ ID NO.16, SEQ ID NO.17, SEQ ID NO.18, SEQ ID NO.19, SEQ ID NO.20, SEQ ID NO.21, SEQ ID NO.23, SEQ ID NO.24, SEQ ID NO.25, SEQ ID NO.26, SEQ ID NO.27, SEQ ID NO.28, SEQ ID NO.29, SEQ ID NO.30, SEQ ID NO.31, SEQ ID NO.32, SEQ ID NO.34, SEQ ID NO.35, SEQ ID NO.37, SEQ ID NO.38, SEQ ID NO.39, SEQ ID NO.40, SEQ ID NO.41, SEQ ID NO.42, SEQ ID NO.43, SEQ ID NO.44, SEQ ID NO.45 and a combination thereof.

5. Inhibitor according to any one of claims 1 to 4, wherein the oligonucleotide is selected from the group consisting of
+T*+C*+A*A*T*T*T*C*A*A*G*C*A*+A*+C*G*+T (SEQ ID NO.6),
+C*+G*+T*A*T*A*G*T*T*G*G*T*T*T*+C*+G*+T (SEQ ID NO.22),

+A*+G*+T*A*G*T*T*T*A*T*C*G*T*T*+A*+G*+T (SEQ ID NO.33),
+G*+T*+T*A*G*T*A*G*G*A*A*G*C*T*+T*+C*+A (SEQ ID NO.36),
+G*+T*+T*A*G*T*A*G*G*A*A*G*C*T*T*+C*+A (SEQ ID NO.36),
+T*+G*+T*A*G*C*G*T*A*T*A*G*T*T*+G*+G*+T (SEQ ID NO.23),
+G*+A*+C*C*T*A*G*A*C*T*T*C*T*T*A*+A*+C (SEQ ID NO.3),
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+G*+A*+C*T*C*G*T*A*T*G*T*A*G*T*+T*+A*+C (SEQ ID NO.42),
+A*+G*+A*C*T*C*G*T*A*T*G*T*A*G*+T*+T*+A (SEQ ID NO:43),
+C*+A*+G*A*C*T*C*G*T*A*T*G*T*A*+G*+T*+T (SEQ ID NO.44),
+A*+C*+T*C*G*T*A*T*G*T*A*G*T*+T*+A*+C (SEQ ID NO.45) and a combination thereof, wherein + indicates an LNA nucleotide and * indicates a phosphorothioate (PTO) linkage between the nucleotides.

6. Inhibitor according to any one of claims 1 to 5, wherein the inhibitor inhibits the expression of ANGPTL3 at a nanomolar or micromolar concentration.

7. A pharmaceutical composition comprising an inhibitor according to any one of claims 1 to 6 and a pharmaceutically acceptable carrier, excipient, dilutant or a combination thereof.

8. Inhibitor according to any one of claims 1 to 6 or the pharmaceutical composition according to claim 7 for use in a method of preventing and/or treating a disorder, where an ANGPTL3 imbalance is involved.

9. Inhibitor or the pharmaceutical composition for use according to claim 8, wherein the disorder is a cardiometabolic disease, obesity, diabetes such as type 2 diabetes, hypercholesterolemia, hypertriglyceridemia (HTG), dyslipidemia, pancreatitis, metabolic syndrome, familial chylomicronemia syndrome (FCS) and/or cancer.

10. Inhibitor or the pharmaceutical composition for use according to claim 8 or 9, wherein the hypercholesterolemia is homozygote familial hypercholesterolemia (HoFH) or heterozygote familial hypercholesterolemia (HeFH).

11. Inhibitor or the pharmaceutical composition for use according to any one of claims 8 to 10, wherein the cancer is breast cancer, lung cancer, malignant melanoma, lymphoma, skin cancer, bone cancer, prostate cancer, liver cancer, brain cancer, cancer of the larynx,

gall bladder, pancreas, testicular, rectum, parathyroid, thyroid, adrenal, neural tissue, head and neck, colon, stomach, bronchi, kidneys, basal cell carcinoma, squamous cell carcinoma, metastatic skin carcinoma, osteo sarcoma, Ewing's sarcoma, reticulum cell sarcoma, liposarcoma, myeloma, giant cell tumor, small-cell lung tumor, islet cell tumor, primary brain tumor, meningioma, acute and chronic lymphocytic and granulocytic tumors, acute and chronic myeloid leukemia, hairy-cell tumor, adenoma, hyperplasia, medullary carcinoma, intestinal ganglioneuromas, Wilm's tumor, seminoma, ovarian tumor, leiomyomater tumor, cervical dysplasia, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic sarcoma, malignant hypercalcemia, renal cell tumor, polycythermia vera, adenocarcinoma, anaplastic astrocytoma, glioblastoma multiforma, leukemia, or epidermoid carcinoma.

12. Inhibitor or the pharmaceutical composition for use according to any one of claims 8 to 11, wherein the inhibitor or the composition is suitable to be administered locally or systemically.

13. Inhibitor or the pharmaceutical composition for use according to any one of claims 8 to 12, wherein the composition is suitable to be administered once or repeatedly.

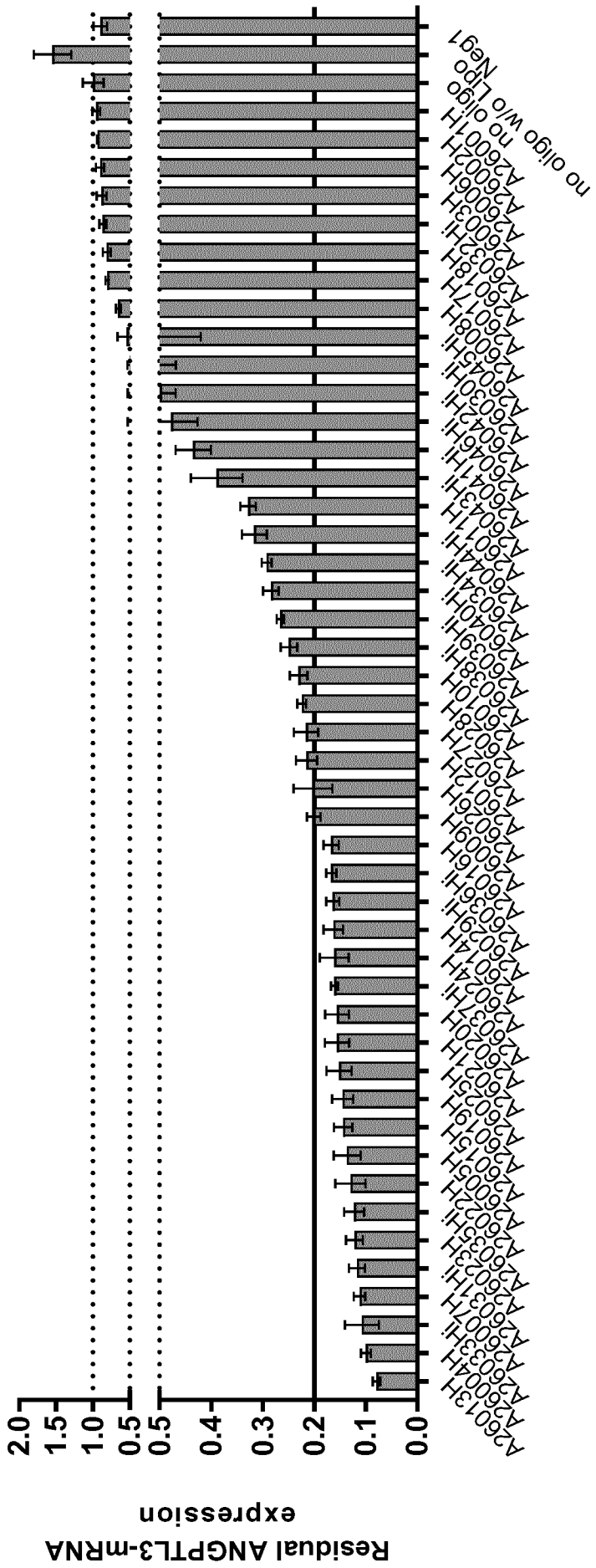


Fig. 1: Single dose efficacy screen of human ANGPTL3-specific antisense oligonucleotides in Hep3B cells

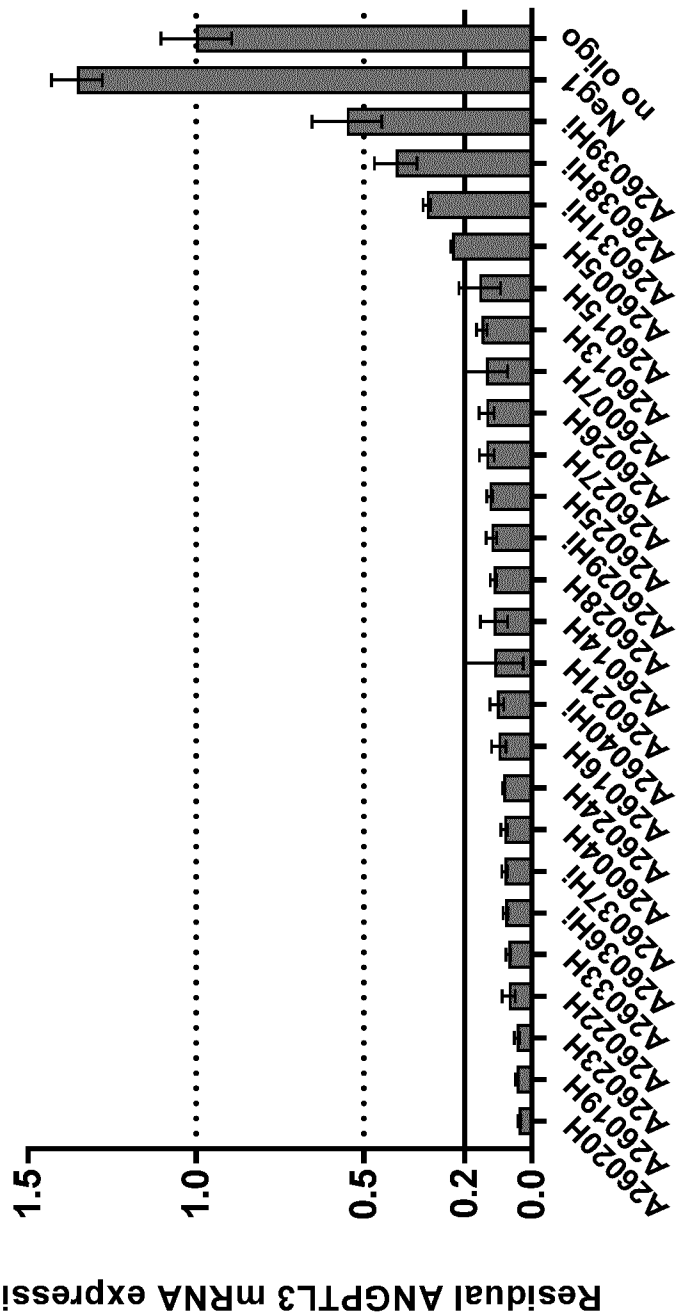
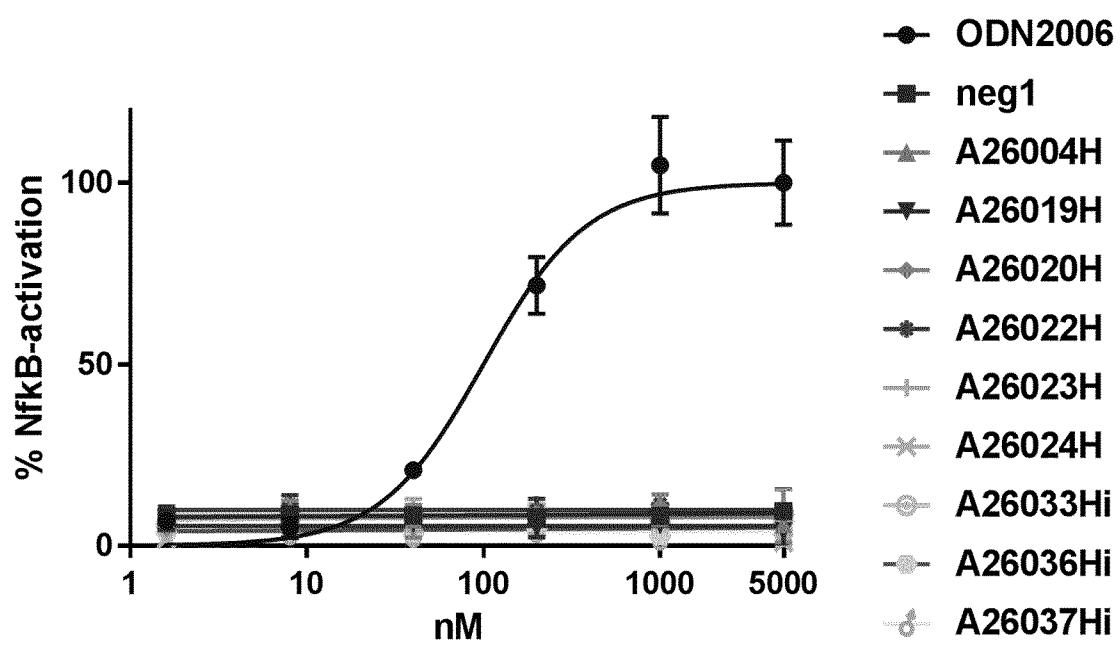


Fig. 2: Single dose efficacy screen of 25 selected human ANGPTL3-specific antisense oligonucleotides in primary human hepatocytes

Fig. 3: TLR9 activity assay of selected human ANGPTL3-specific antisense oligonucleotides



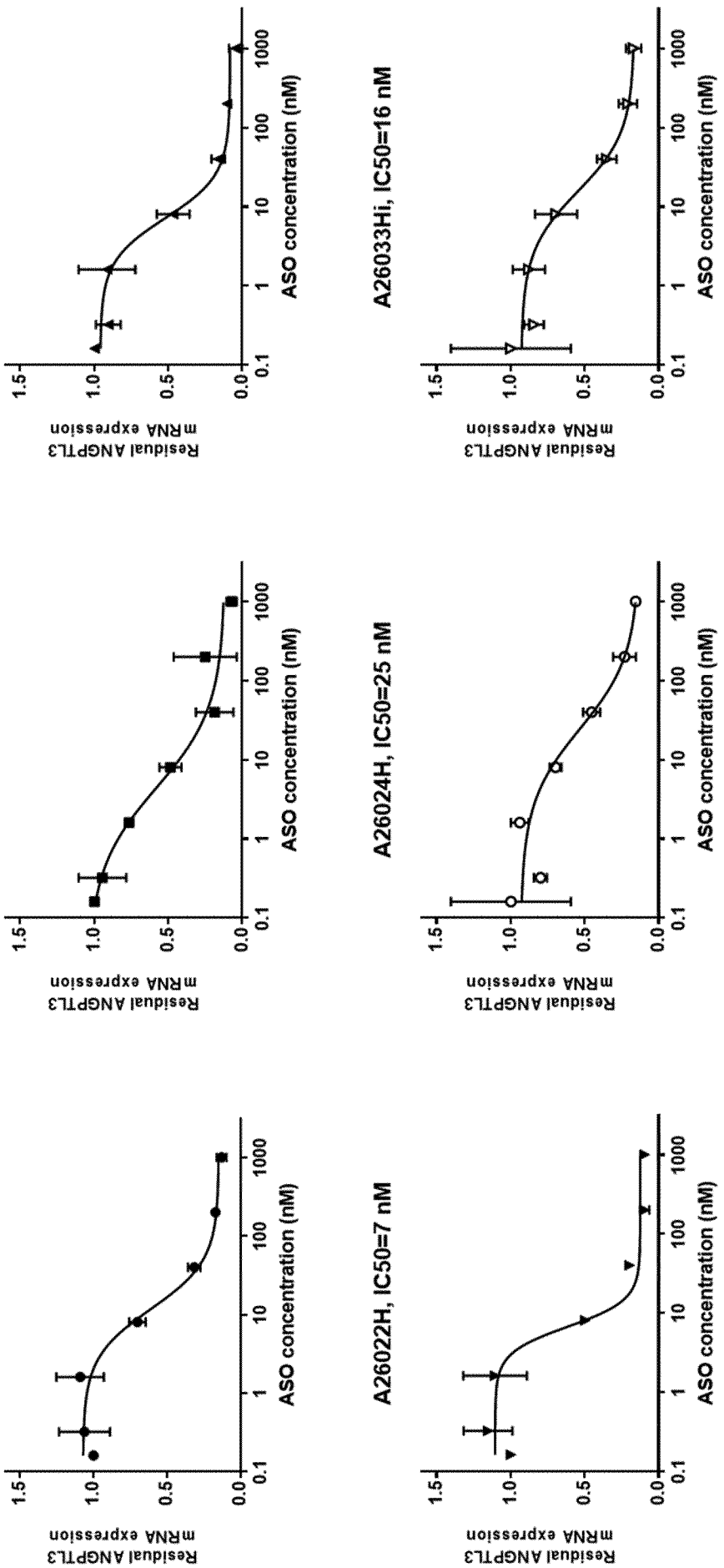


Fig. 4: IC_{50} determination of selected human ANGPTL3-specific antisense oligonucleotides

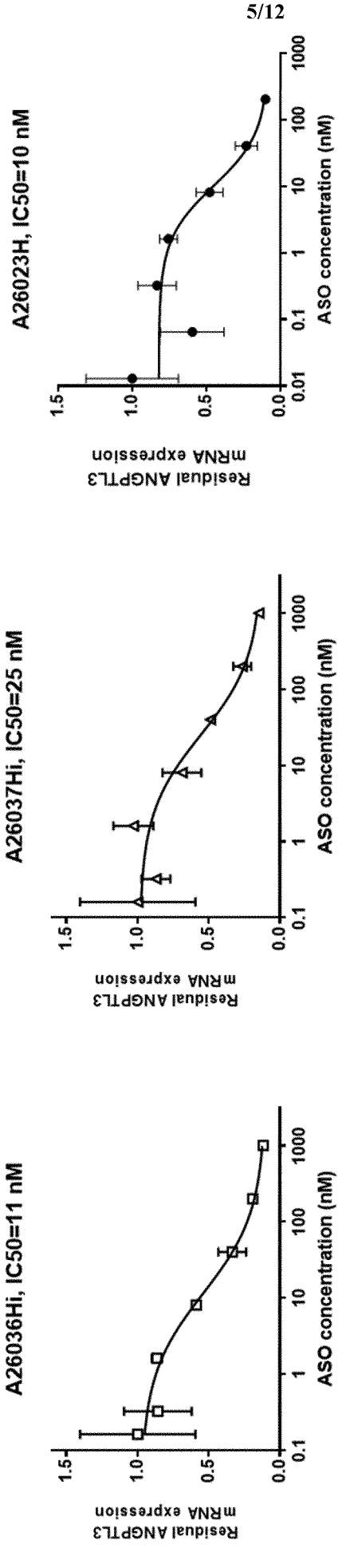


Fig. 4 (continued): IC_{50} determination of selected human ANGPTL3-specific antisense oligonucleotides

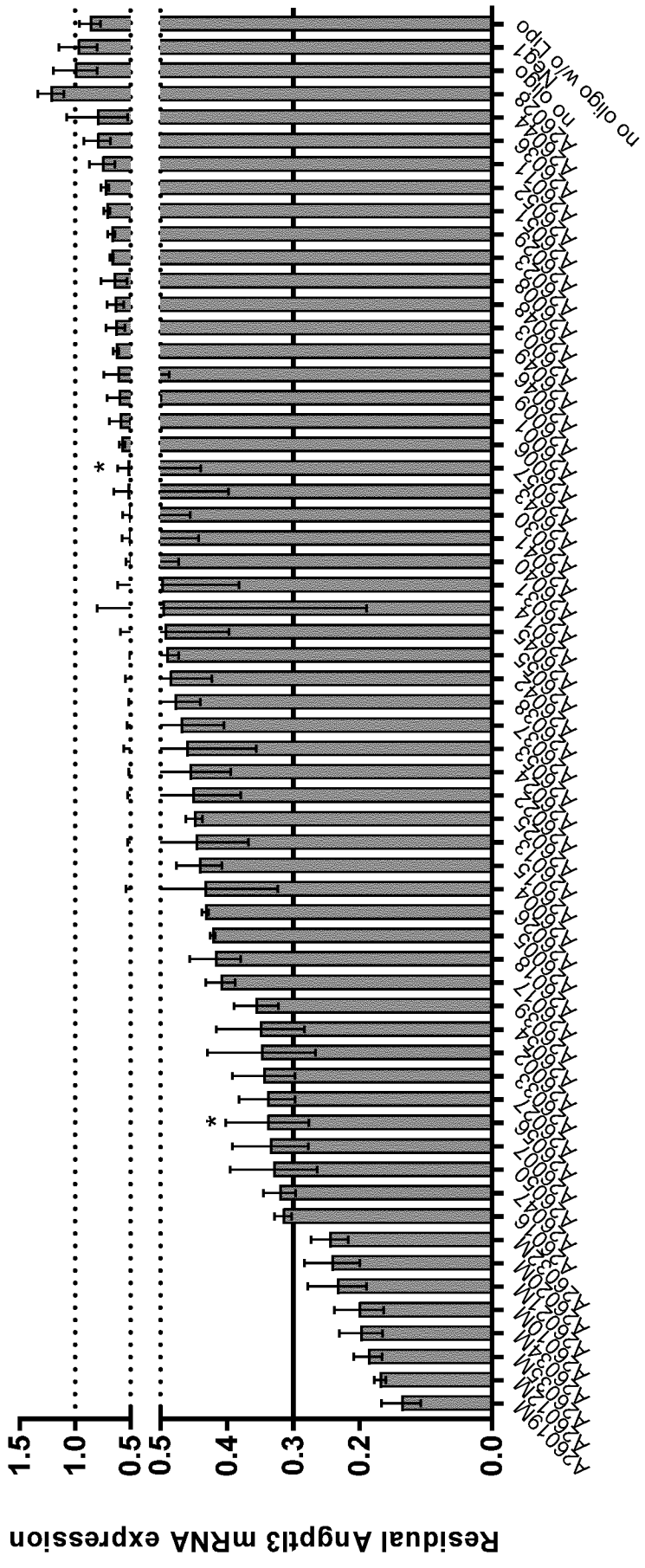
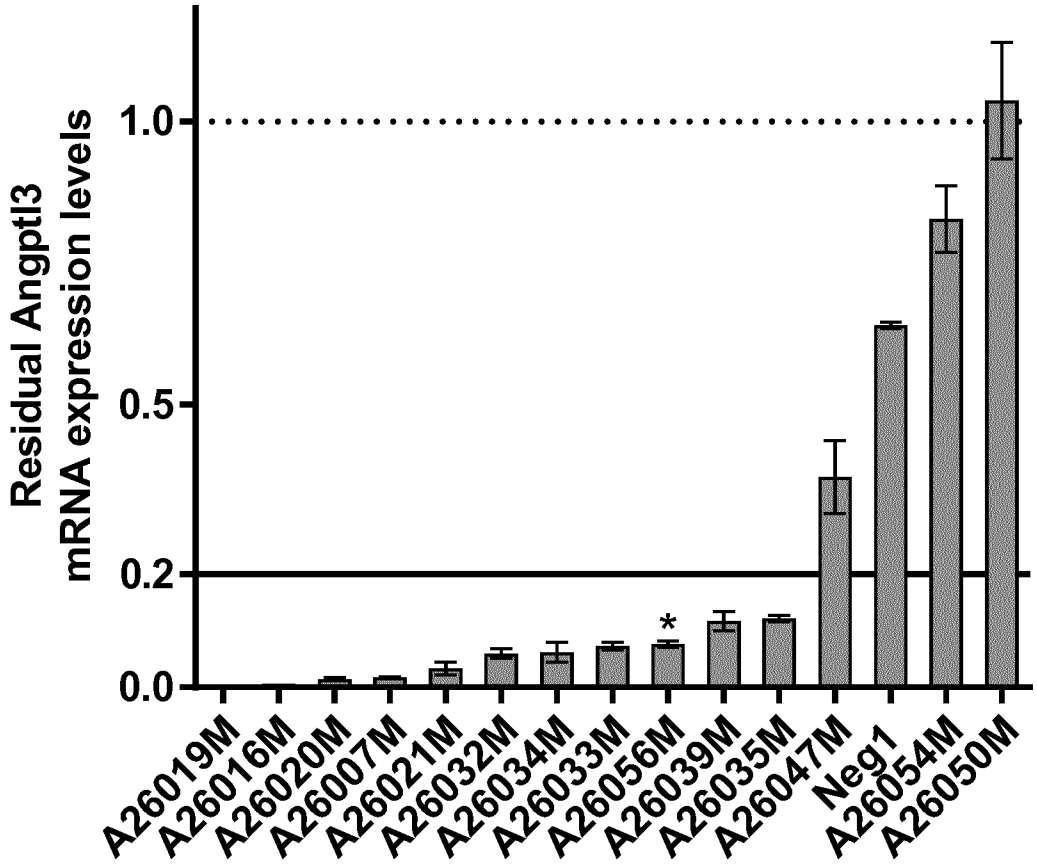


Fig. 5: Single dose efficacy screen of mouse ANGPTL3 -specific antisense oligonucleotides in AML12 cells

Fig. 6: Single dose efficacy screen of 13 selected mouse ANGPTL3-specific antisense oligonucleotides in primary mouse hepatocytes



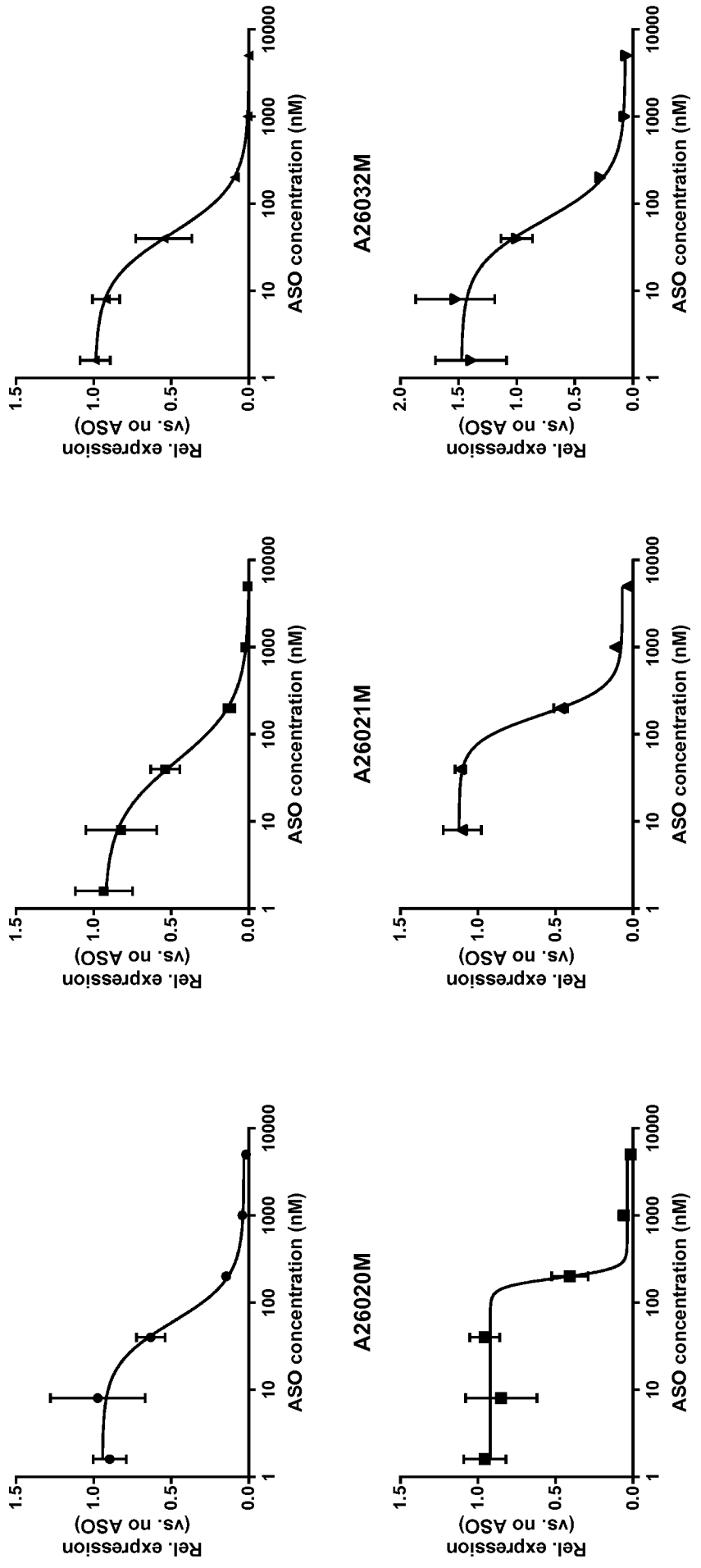


Fig. 7: IC_{50} determination of selected mouse ANGPTL3-specific antisense oligonucleotides

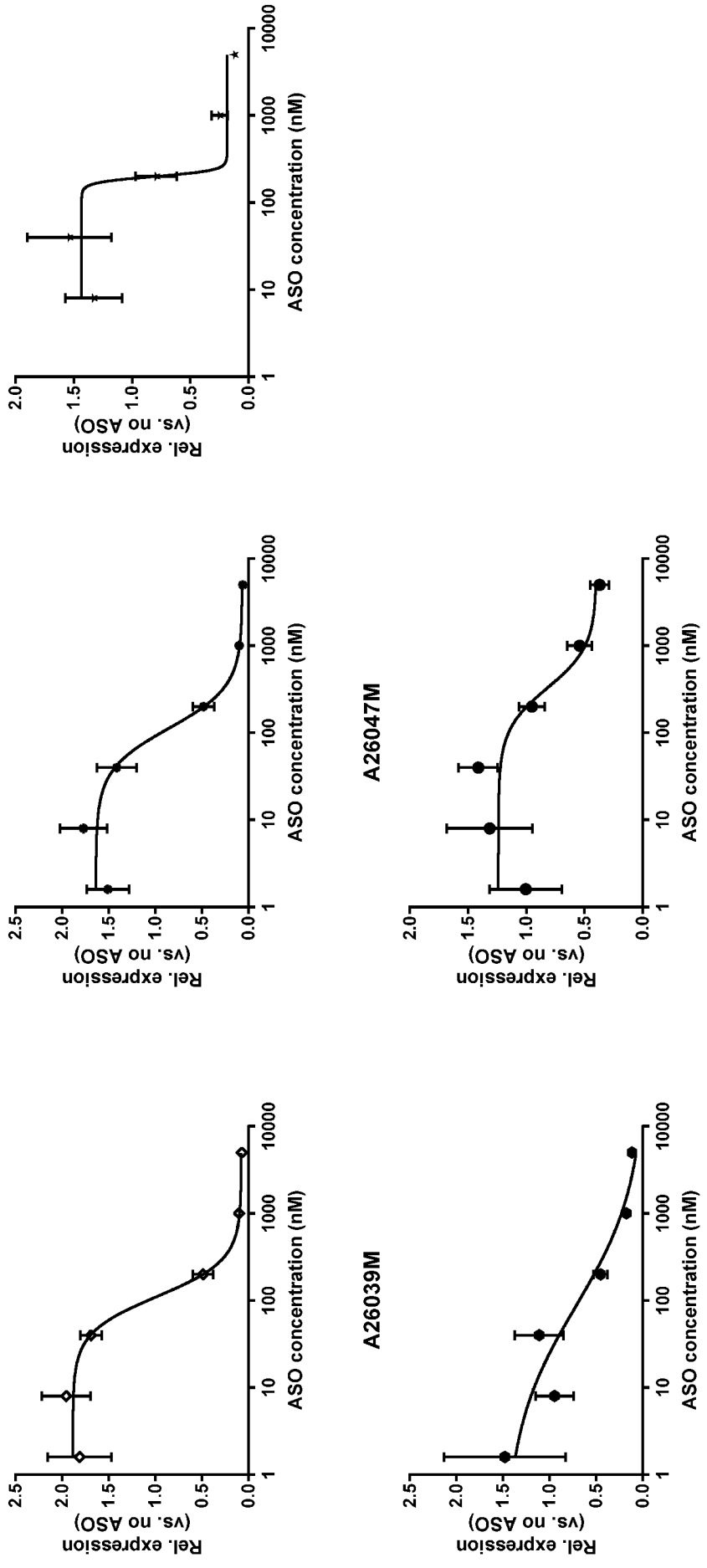
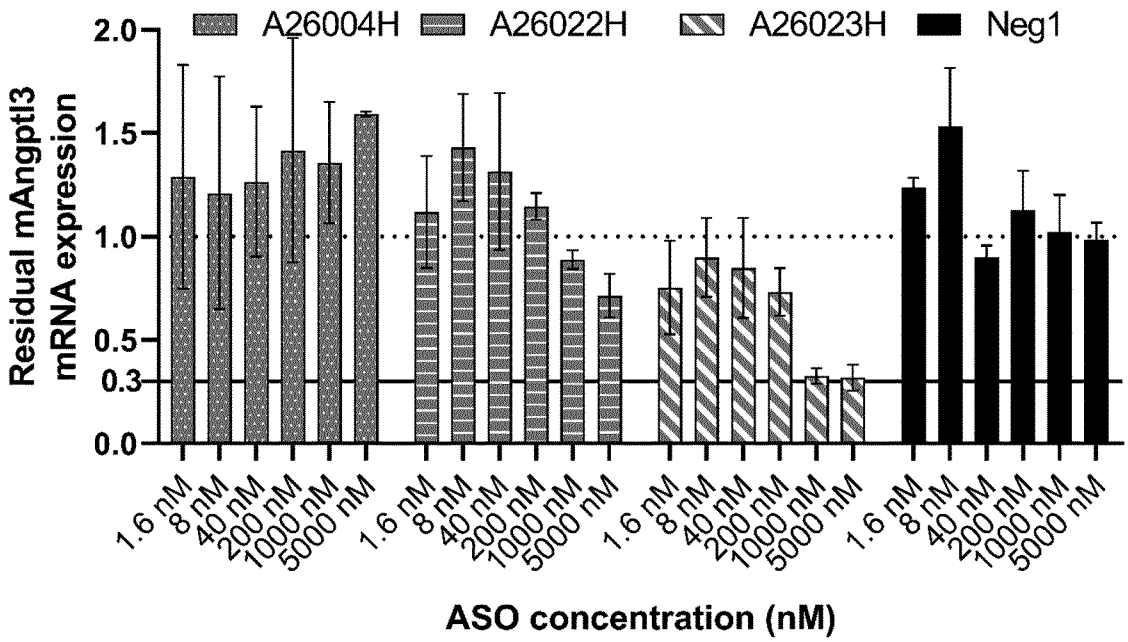


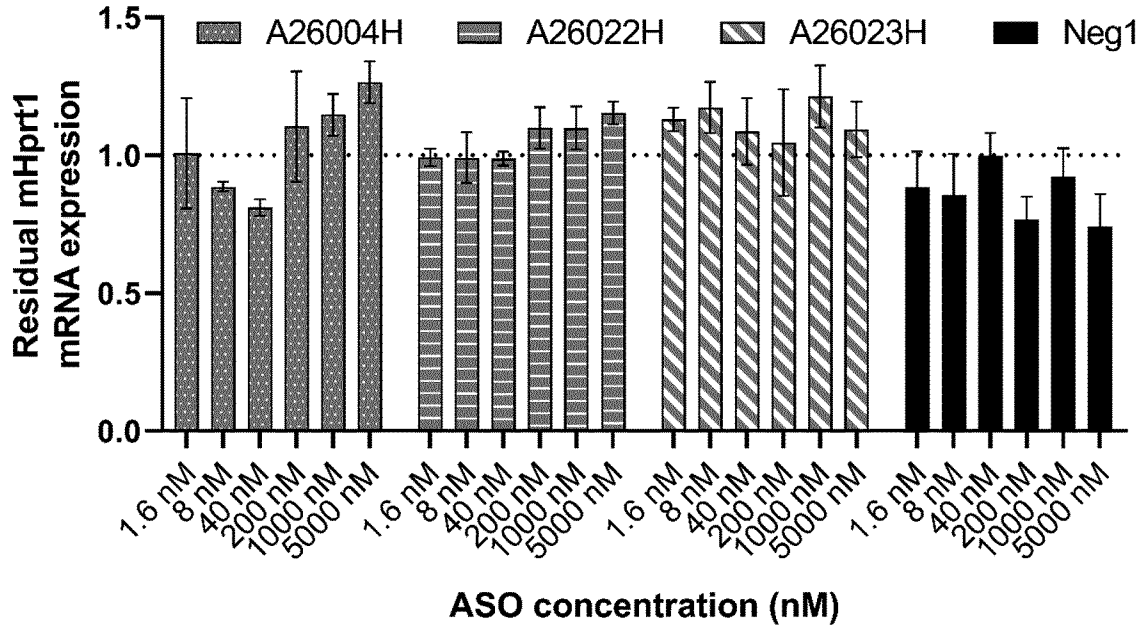
Fig. 7 (continued): IC_{50} determination of selected mouse ANGPTL3-specific antisense oligonucleotides

Fig. 8A and 8B: Analysis of dose-dependent inhibition of human ANGPTL3-specific ASOs in mouse hepatocytes

A)



B)



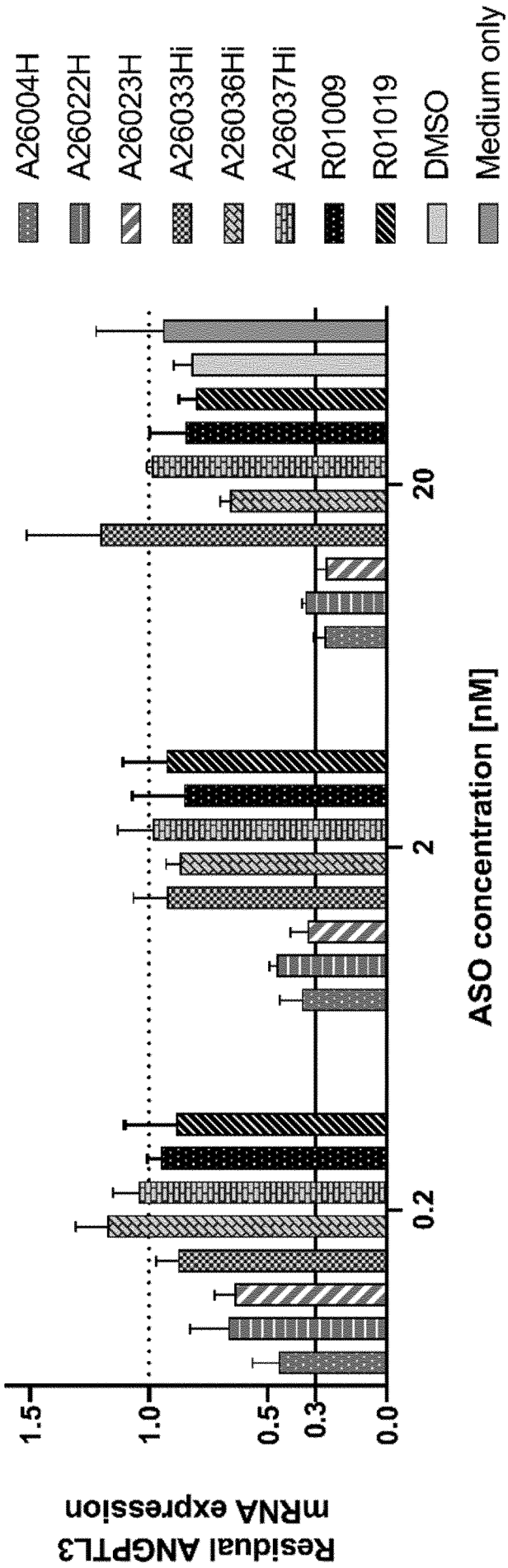


Fig. 9A: Analysis of dose-dependent inhibition of human ANGPTL3-specific ASOs in cynomolgus hepatocytes

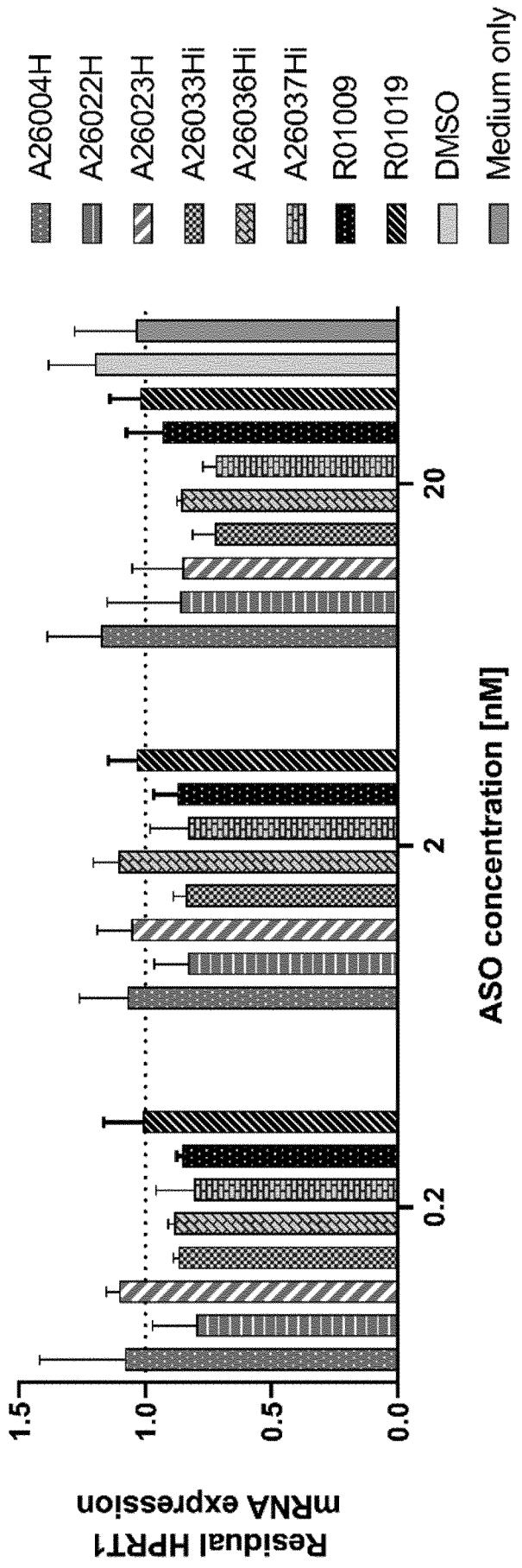


Fig. 9B: Analysis of dose-dependent inhibition of human ANGPTL3-specific ASOs in cynomolgus hepatocytes