

NLRP3 oligonucleotide for use in preventing and/or treating an inflammatory disease

The present invention refers to an oligonucleotide hybridizing with a nucleic acid sequence of NLR family pyrin domain containing 3 (NLRP3) and a pharmaceutical composition comprising such oligonucleotide together with a pharmaceutically acceptable carrier, excipient and/or dilutant to inhibit the expression of NLRP3 for example for use in
5 preventing and/or treating an inflammatory disease.

Technical background

NLR family pyrin domain containing 3 (NLRP3) is primarily expressed in cells of the
10 innate immune system like macrophages and dendritic cells. Upon activation, it forms a proteolytic complex with Apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1, the NLRP3 inflammasome, which cleaves pro-IL-1 β and pro-IL-18 to the mature forms of these pro-inflammatory cytokines. In addition, NLRP3 activation leads to a form of cell death called pyroptosis by cleaving gasdermin D into its pore-forming
15 active form (Anders, H.-J., and Muruve, D. A. (2011), J Am Soc Nephrol 22, 1007–18; Masood, H., Che, R., and Zhang, A. (2015), Kidney Dis (Basel) 1, 187–93).

Activation of the NLRP3-caspase-1-IL-1 β /IL-18 axis requires two signals: Signal 1 is derived from pattern recognition receptors, e.g., toll like receptor (TLR) 4 recognizing
20 lipopolysaccharide (LPS) and increases the transcription and translation of pro-IL-1 β , pro-IL-18 and NLRP3 through nuclear factor “kappa-light-chain-enhancer” of activated B-cells (NF κ B). Signal 2 is then required for activation of caspase-1 via NLRP3. Various danger associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) can act as signal 2, i.e., ATP or uric acid that are released by necroptotic cells or
25 reactive oxygen species (ROS) as a result of cellular stress (Sutterwala, F. S., Haasken, S., and Cassel, S. L. (2014), Ann N Y Acad Sci 1319, 82–95).

Dysregulation of the NLRP3 inflammasome by activating mutations in the NLRP3 gene is the underlying cause of cryopyrin-associated periodic syndromes (CAPS): Muckle–Wells

syndrome (MWS), familial cold autoinflammatory syndrome (FCAS), and chronic infantile neurologic cutaneous and articular syndrome (NOMID) (Kuemmerle-Deschner, J. B. (2015), *Semin Immunopathol* 37, 377–85). Moreover, NLRP3 activation and its downstream effects are involved in various inflammatory diseases of the kidney, arthritis, neuroinflammation and metabolic disorders. Blocking IL-1 β signalling with antibodies, i.e., Canakinumab, is an efficient treatment for many of the above mentioned diseases. However, NLRP3 has inflammasome-independent effects, presumably through enhancing SMAD signalling downstream of transforming growth factor beta (TGF β) via inducing mitochondrial ROS production (Bracey, N. A. et al., (2014), *Biol Chem* 289, 19571–84; Wang, W. et al., (2013), *J Immunol* 190, 1239–49). Thus, targeting NLRP3 instead of IL-1 β and/or IL-18 is a superior approach to treat NLRP3-mediated diseases.

Oligonucleotides of the present invention are very successful in the inhibition of the expression and activity of NLRP3, respectively. 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 of tissue,
- (ii) the blocking of multiple functions and activities, respectively, of a target by prevention of its expression,
- (iii) the combination of oligonucleotides with each other or an antibody, a polypeptide or a small molecule, and
- (iv) the inhibition of intracellular targets which are not accessible for an antibody or inhibitable via a small molecule.

Summary

The present invention refers to an oligonucleotide comprising or consisting of a sequence selected from the group consisting of SEQ ID NO.59, SEQ ID NO.133, SEQ ID NO.297 and SEQ ID NO.335 comprising at least one modified nucleotide, hybridizing with a nucleic acid sequence of a NLR family pyrin domain containing 3 (NLRP3) of SEQ ID NO.1 and/or SEQ ID NO.2 (human), SEQ ID NO.3 and/or SEQ ID NO.4 (mouse) resulting in a reduction of the level of NLRP3, NLRP3 mRNA, NLRP3 pre-mRNA or a combination thereof. The reduction of the level of NLRP3, NLRP3 mRNA, NLRP3 pre-mRNA or a combination thereof is 30 to 99 % compared to an untreated control.

The modified nucleotide is for example selected from the group consisting of a bridged nucleic acid such as LNA, cET, ENA, a 2'Fluoro modified nucleotide, a 2'O-Methyl modified nucleotide, a 2 O-Methoxy modified nucleotide, a FANA and a combination thereof.

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The oligonucleotide of the present invention, wherein the oligonucleotide comprises for example a modification or consists of a sequence selected from the group consisting of

- +G*+T*+A*A*T*G*T*C*A*A*C*G*G*A*+T*+C (A31109Hi; SEQ ID NO. 59),
 +G*+T*+A*A*T*G*T*C*A*A*C*G*G*A*+T*+C (A31055Hi, SEQ ID NO.59),
 10 +G*+T*A*A*T*G*T*C*A*A*C*G*G*A*+T*+C (A31110Hi, SEQ ID NO.59),
 +G*+T*+A*A*T*G*T*C*A*A*C*G*G*A*+T*+C (A31111Hi, SEQ ID NO.59),
 +G*T*+A*A*T*G*T*C*A*A*C*G*G*A*+T*+C (A31112Hi, SEQ ID NO.59),
 +G*+T*+A*A*T*G*T*C*A*A*C*G*G*A*+T*+C (A31368Hi, SEQ ID NO.59),
 +G*+T*+A*A*T*+G*T*C*A*A*C*G*G*A*+T*+C (A31369Hi, SEQ ID NO.59),
 15 +G*+T*+A*A*T*G*+T*C*A*A*C*G*G*A*+T*+C (A31370Hi, SEQ ID NO.59),
 +G*+T*+A*A*T*G*T*C*A*A*C*+G*G*A*+T*+C (A31371Hi, SEQ ID NO.59),
 +G*+T*+A*A*T*G*T*C*A*A*+C*G*G*A*+T*+C (A31372Hi, SEQ ID NO.59),
 +G*+T*+A*A*T*G*T*C*A*A*C*G*+G*A*+T*+C (A31373Hi, SEQ ID NO.59),
 +G*+A*C*T*G*T*C*A*C*G*T*C*T*C*+G*+G*+C (A31149H, SEQ ID NO.133),
 20 +C*+A*+T*A*G*T*T*C*T*C*T*G*C*A*A*C*+A*+G*+G (A31314Hi, SEQ ID NO.297),
 +T*+A*+G*T*A*T*C*A*C*T*G*T*A*T*G*T*+C*+C*+A (A31352Hi, SEQ ID NO.335)
 and a combination thereof, wherein + indicates an LNA nucleotide and * indicates a phosphorothioate (PTO) linkage between the nucleotides.

- 25 The oligonucleotide comprises for example the modification or consists of the sequence selected from the group consisting of +G*+T*+A*A*T*G*T*C*A*A*C*G*G*A*+T*+C (A31109Hi; SEQ ID NO. 59), +G*+A*C*T*G*T*C*A*C*G*T*C*T*C*+G*+G*+C (A31149H, SEQ ID NO.133), +C*+A*+T*A*G*T*T*C*T*C*T*G*C*A*A*C*+A*+G*+G (A31314Hi, SEQ ID NO.297), +T*+A*+G*T*A*T*C*A*C*T*G*T*A*T*G*T*+C*+C*+A (A31352Hi, SEQ ID NO.335) and a combination thereof, wherein + indicates an LNA nucleotide and * indicates a phosphorothioate (PTO) linkage between the nucleotides.
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Further, the oligonucleotide hybridizes for example with at least one exon or intron of SEQ ID NO.1 and/or with the mRNA of SEQ ID NO.2.

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The oligonucleotide inhibits the expression of NLRP3, NLRP3 mRNA, NLRP3 pre-mRNA or a combination thereof for example at a nanomolar or micromolar concentration.

5 The present invention further refers to a pharmaceutical composition comprising an oligonucleotide of the present invention and a pharmaceutically acceptable carrier, excipient, dilutant or a combination thereof. The pharmaceutical composition further comprises for example another active agent selected from the group consisting of an oligonucleotide, an antibody, a small molecule, a polypeptide, a lipid, a sugar and a combination thereof. The oligonucleotide and the other active agent inhibit for example
10 the same target or a different target. The other active agent for example modulates, e.g., inhibits or stimulates the target selected from the group consisting of NLRP3, CD39, CD73, IL-18, IL-1 receptor, IL-1R accessory protein, IL-18, IL-18 receptor, ASC, NLRC4, AIM2, Caspase-1, RIPK3, Gasdermin D, MLKL, TLR4, Caspase-8, P2X7, NFκB, RORγt, TGF-β, IL-21, IL-17, IL-22, IL-23, IL-6, TNF-α, CCR6, CCL20, STAT3, MMP-1, MMP-8,
15 ADAMTS-5, HMG-CoA, Myd-88, HMGB-1, ROS, TAK-1, Chop, FPR1, LIMCH1, caspase inhibitor and a combination thereof.

The oligonucleotide of the present invention or the pharmaceutical composition of the present invention is for example for use in a method of preventing and/or treating a
20 disorder, where an NLRP3 imbalance is involved.

The disorder is for example selected from the group consisting of a hyperproliferative disorder such as cancer, an inflammatory or autoimmune disorder, neurodegenerative disease, a neurological disorder, cardiovascular, metabolic disorder, renal disorder, liver
25 disorder, lung disorder, skin disorder, ocular disorder, disorder of the gastro-intestinal tract, joint inflammation, organ transplantation, fibrotic disorder and a combination thereof.

Further, the disorder is for example selected from the group consisting of Alzheimer's
30 disease, multiple sclerosis, autoimmune encephalitis, stroke, traumatic brain injury, atherosclerosis, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, hypertension, myocardial infarction, acute kidney injury, ischemia reperfusion injury, chronic kidney diseases, crystal-induced nephropathies, glomerulonephritis, silicosis, asthma, allergic airway inflammation, inflammatory bowel disease, colitis ulcerosa,
35 osteoarthritis, rheumatoid arthritis, juvenile idiopathic arthritis, transplantation of

kidney, lung, liver and/or heart, fibrotic disorder of kidney, lung, liver and/or heart, hyperinflammation following influenza infection, graft-versus-host disease, interstitial cystitis, uveitis, sinusitis, periodontal disease, optic neuritis, myelodysplastic syndrome, cryopyrin-associated periodic syndromes (CAPS) including familial cold
5 autoinflammatory syndrome (FCAS), the Muckle–Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disease (NOMID), gout, obesity-induced inflammation, insulin resistance, type 1 and type 2 diabetes, contact hypersensitivity and a combination thereof, and/ or a cancer selected from the group consisting of breast cancer, lung cancer, malignant melanoma, lymphoma, skin cancer, bone cancer, prostate cancer, liver cancer,
10 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
15 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, polycythemia vera,
20 adenocarcinoma, anaplastic astrocytoma, glioblastoma multiforma, leukemia, epidermoid carcinoma and a combination thereof.

The oligonucleotide or the pharmaceutical composition of the present invention is for example administered locally or systemically.

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Further, the present invention refers to a kit comprising an oligonucleotide and/or a pharmaceutical composition of the present invention and an instruction manual. The oligonucleotide and/or the pharmaceutical composition of the kit is for example for use in preventing and/or treating a disease caused by an imbalanced NLRP3 expression.

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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
35 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.

5 Description of figures

Fig. 1 is a schematic presentation of the signal cascade of the NLRP3 activation (see McAllister et al., Osteoarthritis and Cartilage, 2018). NLRP3 is part of the NLRP3 inflammasome comprising NLRP3, adaptor protein ASC and (pro-) caspase-1. Two signals are necessary for the activation of NLRP3: signal 1 induces transcription and translation of pro-IL-1 β , pro-IL-18 and NLRP3 via NF κ B. Signal 2 leads to the activation of the proteolytic activity of the inflammasome complex. Activated caspase-1 induces cleaving of pro-IL-1 β and pro-IL-18 to mature IL-1 β and IL-18 which are secreted into the extracellular space. NLRP3 induced Caspase-1 activity results additionally in a specific form of cell death which is pyroptosis.

Fig. 2 shows a single concentration efficacy screen in human THP-1 cells in a first screening round for human NLRP3-specific antisense oligonucleotides (ASOs) after three days treatment.

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Fig. 3 depicts a single concentration efficacy screen in human U87MG cells in a first screening round for human NLRP3-specific ASOs after three days treatment.

Fig. 4 depicts a single concentration efficacy screen in human PBMC-derived macrophages of selected human NLRP3-specific ASOs after six days treatment.

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Fig. 5 depicts a single concentration efficacy screen of human NLRP3-specific ASOs in human U87MG cells in a second screening round for human NLRP3-specific ASOs after three days treatment.

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Fig. 6 shows a single concentration efficacy screen of human NLRP3-specific ASOs in human PBMC-derived macrophages in a second screening round for human NLRP3-specific ASOs after six days treatment.

Fig. 7 depicts concentration-dependent NLRP3 mRNA knockdown by selected human NLRP3-specific ASOs from the first and second screening round in human PBMC-derived macrophages after six days treatment.

- 5 **Fig. 8** shows a single concentration efficacy screen in human THP-1-derived macrophages in a third screening round for human NLRP3-specific ASOs after three days treatment.

Fig. 9 shows a single concentration efficacy screen in human PBMC-derived macrophages in a third screening round for human NLRP3-specific ASOs after six days treatment

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Fig. 10 shows a single concentration efficacy screen in human THP-1-derived macrophages in a fourth screening round for human NLRP3-specific ASOs after three days treatment.

- 15 **Fig. 11** shows a single concentration efficacy screen in human PBMC-derived macrophages in a fourth screening round for human NLRP3-specific ASOs after six days treatment.

Fig. 12 depicts concentration-dependent NLRP3 mRNA knockdown by selected human NLRP3-specific ASOs from the first, second, third and fourth screening round in human THP-1-derived macrophages after three days treatment.

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Fig. 13 shows the efficacy of selected human NLRP3-specific ASOs in human microglial HMC3 cells after three days treatment.

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Fig. 14 shows a single concentration screen in human THP-1-derived macrophages in a fifth screening round after three days treatment with human NLRP3-specific ASOs.

- 30 **Fig. 15** shows a single concentration screen in human PBMC-derived macrophages in a fifth screening round after six days treatment with human NLRP3-specific ASOs.

Fig. 16 depicts functional assays investigating selected human NLRP3-specific ASOs in differentiated human THP-1-derived macrophages after six days treatment. **Fig. 16A** shows residual NLRP3 mRNA expression in ASO-treated THP-1-derived macrophages compared to mock-treated cells. **Fig. 16B** shows the inhibitory effects of the ASOs on

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caspase-1 activity. **Fig. 16C** shows IL-1 β protein expression of pro-IL-1 β and mature- IL-1 β in cell lysates and cell culture supernatants of ASO-treated THP-1-derived macrophages analyzed by Western Blot.

5 **Fig. 17A** and **17B** show single concentration efficacy screens in mouse 4T1 cells in a first screening round for mouse NLRP3-specific ASOs after three days treatment. **Fig 17A** depicts residual NLRP3 mRNA expression in ASO-treated 4T1 cells compared to mock-treated cells after three days treatment. **Fig 17B** depicts cell viability of 4T1 cells after three days ASO treatment assessed by cell titer blue assay.

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Fig. 18 depicts a single concentration efficacy screen in mouse Raw246.7 cells in a first screening round for mouse NLRP3-specific ASOs after three days treatment

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Fig. 19A and **19 B** show single concentration screens in mouse Raw246.7 cells in a second screening round for mouse NLRP3-specific ASOs after three days treatment.

Fig. 20 shows a single concentration screen in mouse 4T1 cells for selected mouse NLRP3-specific from the second screening round after three days treatment.

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Fig. 21 depicts concentration-dependent NLRP3 mRNA knockdown by selected NLRP3-specific ASOs from the first and second screening round in mouse Raw246.7 cells after three days treatment.

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Fig. 22 depicts functional assays investigating the mouse NLRP3-specific ASOs A31087Mi (SEQ ID NO. 419) in mouse bone marrow derived macrophages (BMDM) after six days treatment. **Fig. 22 A** depicts residual NLRP3 mRNA expression in ASO-treated BMDM compared to mock-treated cells. **Fig. 22 B** shows NLRP3 protein expression and IL-1 β protein expression of pro-IL-1 β and mature-IL-1 β in cell lysates and cell culture supernatants of ASO-treated BMDM cells analyzed by Western Blot.

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Fig. 23 shows the efficacy of selected mouse NLRP3-specific ASOs in mouse Raw246.7 cells after three days treatment.

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Fig. 24 shows the efficacy of selected mouse NLRP3-specific antisense oligonucleotides in mouse microglial IMG cells after three days treatment.

Detailed description

The present invention provides for the first time human and murine oligonucleotides which hybridize with mRNA sequences of NLR family pyrin domain containing 3 (NLRP3) and inhibit the expression and activity, respectively, of NLRP3 of human SEQ ID NO.1 (GRCh38_Ch1: 247412861-247452403, in particular GRCh38.p12_Ch1: 247412861-247452403, comprising 39543 nucleotides) and/or human SEQ ID NO. 2 (RefSeq ID NM_004895.4), murine SEQ ID NO.3 (GRmCh38_Ch11: 59539030-59569495, in particular GRmCh38.p4_Ch11: 59539030-59569495, comprising 30466 nucleotides), and/or SEQ ID NO. 4 (RefSeq ID NM_145827). Thus, the oligonucleotides of the present invention represent an interesting and highly efficient tool for use in a method of preventing and/or treating a disorder, where the NLRP3 expression and activity, respectively, is imbalanced, e.g., increased or decreased for example in an inflammatory disease.

In the following, the features of the present invention will be described in more detail. These features 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 features 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.

10 Oligonucleotides of the present invention are for example antisense oligonucleotides (ASO) consisting of or comprising 10 to 25 nucleotides, 12 to 20 nucleotides, 11 to 15 nucleotides, 13 to 18 nucleotides, or 14 to 17 nucleotides. The oligonucleotides for example consist of or comprise 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 25 nucleotides. The oligonucleotides of the present invention comprise at least one nucleotide which is modified. The modified
15 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, a 2 O-Methoxy modified nucleotide, a FANA or a combination thereof. The oligonucleotide of the present invention comprises nucleotides having for example one or more, two or more, three or more, four or more, five or more or six or more, for example six of the same
20 or different modifications. Further, the oligonucleotide of the present invention comprises a phosphate backbone, optionally a modified phosphate backbone, wherein the phosphate is for example a phosphorothioate or methylphosphonate or a combination thereof, or where sulfur, amines or hydrocarbons are substituted for the bridging of non-bridging atoms in the phosphodiester bond. Modified nucleotide backbones include for example
25 further chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and/or chiral phosphonate, phosphinate, phosphoramidate including 3'-amino phosphoramidate and/or aminoalkylphosphoramidate, thionoalkylphosphonate, thionoalkylphosphotriester, and borano phosphate having 3'-5'-linkage and/or 2'-5'-
30 linkages.

The oligonucleotide of the present invention comprises 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 1, 2, 3, 4, 5, or 6 modified
35 nucleotides, or a modified nucleotide is combined with one or more unmodified nucleotides.

The following Tables 1 and 2 present embodiments of oligonucleotides comprising modified nucleotides for example LNA which are indicated by (+) and phosphorothioate (PTO) indicated by (*). The oligonucleotides consisting of or comprising the sequences of Tables 1 and 2 (mRNA (Antisense) sequence 5'-3'), respectively, may comprise any other modified nucleotide and any other combination of modified and unmodified nucleotides. Some oligonucleotides are exon spanning, i.e., the oligonucleotide hybridizes with one or more different exons. Oligonucleotides of Table 1 hybridize with human NLRP3 mRNA:

SEQ ID NO.	Antisense Sequence 5'-3'	Name of the LNA-modified ASO	Antisense Sequence 5'-3' with PTO (*) and LNA (+)	Position on pre-mRNA (GRCh38, Chr1: 247412861-247452403; SEQ ID NO.1)
5	ACGATGCCATCTTGACC	A31001H	+A**C**G*A*T*G*C*A*T*C*T*T*A**C**C	5280
6	ACGGTGAACAACCACTT	A31002H	+A**C**G*G*T*G*A*A*C*A*A*C*C*A**C**T**T	5298
7	TACGGTGAACAACCAC	A31003H	+T**A**C*G*G*T*G*A*A*C*A*A*C**C**A**C	5300
8	TCGATCCAGGAGTGTGT	A31004H	+T**C**G*A*T*C*C*A*G*G*A*G*T*G**T**G**T	5394
9	ACACGGCACACGGATGA	A31005H	+A**C**A*C*G*G*C*A*C*A*C*G*G*A**T**G**A	5861
10	TCGGTCCACACTAAGAT	A31006H	+T**C**G*G*T*C*C*A*C*A*C*T*A**G**A**T	5890
11	CTTAGGCTTCGGTCCAC	A31007H	+C**T**T*A*G*G*C*T*T*C*G*G*T*C**C**A**C	5898
12	ATCATTAGCGTGGCTAG	A31008H	+A**T**C*A*T*T*A*G*C*G*T*G*G*C**T**A**G	6094
13	GCAGCGAAGATCCACAC	A31009H	+G**C**A*G*C*G*A*A*G*A*T*C*C*A**C**A**C	6148
14	TTGATCGCAGCGAAGA	A31010H	+T**T**G*A*T*C*G*C*A*G*C*G*A**A**G**A	6155
15	TGTTGATCGCAGCGAAG	A31011H	+T**G**T*T*G*A*T*C*G*C*A*G*C*G**A**A**G	6156
16	ACGTGCATTATCTGAAC	A31012H	+A**C**G*T*G*C*A*T*T*A*T*C*T*G**A**A**C	10370
17	TTCGAAACACGTGCAT	A31013H	+T**T**C*G*A*A*A*C*A*C*G*T*G**C**A**T	10379
18	ATTCTCGAAAGGTACTC	A31014H	+A**T**T*C*T*C*G*A*A*G*G*T*A**C**T**C	10450
19	CGTGTGTAGCGTTTGT	A31015H	+C**G**T*G*T*G*T*A*G*C*G*T*T**G**T**T	11076
20	CGTGTGTAGCGTTTGT	A31016H	+C**G**T*G*T*G*T*A*G*C*G*T*T**T**G**T	11077
21	GCAGTCGTGTGTAGCGT	A31017H	+G**C**A*G*T*C*G*T*G*T*G*T*A*G**C**G**T	11081
22	CTCTCACACGTCTTGGT	A31018H	+C**T**C*T*C*A*C*A*G*T*C*T*T*G**G**T	11160
23	AGAGCGGTCCTATGTGC	A31019H	+A**G**A*G*C*G*G*T*C*C*T*A*T*G**T**G**C	11522
24	AGAGCGGTCCTATGTG	A31020H	+A**G**A*G*C*G*G*T*C*C*T*A*T*G**T**G**G	11523
25	AGAAGACGTACACCGCG	A31021H	+A**G**A*A*G*A*C*G*T*A*C*A*A*C**C**G**G	11903
26	AACGTTTCGTCCTTCCTT	A31022H	+A**A**C*G*T*T*C*G*T*C*T*T*T*C**C**T**T	12205
27	GGAAGCTTCAAACGACT	A31023H	+G**G*A*A*G*C*T*T*C*A*A*A*C*G**A**C**T	12228
28	GGAAGCTTCAAACGACT	A31024H	+G**G**A*C*T*G*T*C*A*C*G*T*C**T**C**G	12249

29	TCGAATTTGCCATAGTT	A31025H	+T*+C*+G*A*A*T*T*T*G*C*A*T*A*+G*+T*+T	12273
30	TCGAATTTGCCATAGT	A31026H	+T*+C*+G*A*A*T*T*T*G*C*A*T*+A*+G*+T	12274
31	CTCGTACAAAACAGTAGA	A31027H	+C*+T*+C*G*T*A*C*A*A*C*A*G*T*+A*+G*+A	12475
32	AGGTCCAATTCAGTTAG	A31028H	+A*+G*+G*+T*C*C*A*A*T*T*C*A*G*T*+T*+A*+G	16795
33	AGCGTTTCACACAACAC	A31029H	+A*+G*+C*G*T*T*T*C*A*C*A*A*+C*+A*+C	16846
34	ATCTCCGAATGTTACAG	A31030H	+A*+T*+C*T*C*C*G*A*A*T*G*T*T*A*+C*+A*+G	16875
35	GTCGAAGCAGCACTCAT	A31031H	+G*+T*C*G*A*A*G*C*A*G*C*A*C*T*+C*+A*+T	21266
36	GGACCAAGGAGATGTCTG	A31032H	+G*+G*+A*C*C*A*A*G*G*A*G*A*T*G*+T*+C*+G	21279
37	ATTCGGAAGTCACCGAG	A31033H	+A*+T*+T*C*C*G*A*A*G*T*C*A*C*C*+G*+A*+G	21343
38	TCACATAAAATTGCGAC	A31034H	+T*+C*+A*C*A*T*A*A*A*A*T*T*G*C*+G*+A*+C	23225
39	AGACTGACGTAAGGCCA	A31035H	+A*+G*+A*C*T*G*A*C*G*T*A*A*G*G*C*+C*+A	31124
40	GTAAAGGTGCGTGAGAT	A31036H	+G*+T*+A*A*A*G*G*T*G*C*G*T*G*A*+G*+A*+T	31180
41	GTGTGACGTGAGGTTG	A31037H	+G*+T*+G*T*G*A*C*G*T*G*A*G*G*+T*+T*+G	31803
42	CAGTGTGACGTGAGGTT	A31038H	+C*+A*+G*T*G*T*G*A*C*G*T*G*A*G*+G*+T*+T	31804
43	CTAACGCACTTTTGTCT	A31039H	+C*+T*+A*A*C*G*C*A*C*T*T*T*T*+G*+T*+C	35572
44	CTCAAAGACGACGGTCA	A31040H	+C*+T*+C*A*A*A*G*A*C*G*A*C*G*G*+T*+C*+A	35616
45	CGGAGAACACTGGCGTC	A31041H	+C*+G*+G*A*G*A*A*C*A*C*T*G*G*C*G*+T*+C	35665
46	ATGGATCGCAGCTCTCT	A31042H	+A*+T*+G*G*A*T*C*G*C*A*G*C*T*C*T*+C*+T	35710
47	CGACACTCCACCGGAAG	A31043H	+C*+G*+A*C*A*C*T*C*C*A*C*G*G*+A*+A*+G	35754
48	TCTCCGACACTCCACCG	A31044H	+T*+C*+T*C*C*G*A*C*A*C*T*C*C*A*+C*+C*+G	35758
49	CGTCGGCAAGCTCTCTT	A31045H	+C*+G*+T*C*G*G*C*A*A*G*C*T*C*T*+C*+T*+T	35774
50	CGTCGGCAAGCTCTCT	A31046H	+C*+G*+T*C*G*G*C*A*A*G*C*T*C*+T*+C*+T	35775
51	AAGGCATCGTCGGCAAG	A31047H	+A*+A*+G*G*C*A*T*C*G*T*C*G*G*C*+A*+A*+G	35781
52	CTAACTGAGGCGCTGTG	A31048H	+C*+T*+A*A*C*T*G*A*G*G*C*G*C*T*+G*+T*+G	35879
53	TAGCACTACCGTGAGAG	A31049Hi	+T*+A*+G*C*A*C*T*A*C*G*T*G*A*+G*+A*+G	7906
54	CGTGCCTAATCTAGTG	A31050Hi	+C*+G*+T*G*C*C*T*A*A*T*C*T*+A*+G*+T*+G	9849
55	ACGACCAGTCTATTTTCG	A31051Hi	+A*+C*G*A*C*C*A*G*T*C*T*A*T*T*+T*+C*+G	9863
56	GCTTAATCACCGCCGCT	A31052Hi	+G*+C*+T*T*A*A*T*C*A*C*G*C*C*+G*+C*+T	10682
57	TCGGAAGCGAGTGGTAA	A31053Hi	+T*+C*+G*G*A*A*G*C*G*A*G*T*G*G*+T*+A*+A	10894
58	CATCGGAAGCGAGTGGT	A31054Hi	+C*+A*+T*C*G*G*A*A*G*C*G*A*G*T*+G*+G*+T	10896
59	GTAATGTCAACGGATC	A31055Hi	+G*+T*+A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C	13706
60	CCGTAGAAAACGTACC	A31056Hi	+C*+C*+G*T*A*G*A*A*A*C*C*G*T*A*+C*+C	14070
61	CCGTAAGGTGAACCTTC	A31057Hi	+C*+C*+G*T*A*A*G*G*T*G*A*A*C*C*T*+T*+C	14184
62	GACGTATCAGGCGATGG	A31058Hi	+G*+A*+C*G*T*A*T*C*A*G*G*C*G*A*+T*+G*+G	14401
63	TGACGTATCAGGCGAT	A31059Hi	+T*+G*+A*C*G*T*A*T*C*A*G*G*C*+G*+A*+T	14403
64	TCGACGAGAGCATG	A31060Hi	+T*+C*+G*G*A*C*G*G*A*G*A*G*C*+A*+T*+G	16512
65	CTAGAGTGGCGGACTTT	A31061Hi	+C*+T*+A*G*A*G*T*G*G*C*G*G*A*C*+T*+T*+T	18501
66	GAGTTACACTAAGGCCG	A31062Hi	+G*+A*+G*T*T*A*C*A*C*T*A*A*G*G*+C*+C*+G	19318
67	CCGGCCGTAAACAGTG	A31063Hi	+C*+C*G*G*C*C*G*T*A*A*A*C*A*+G*+T*+G	20629
68	TCCGTTGAAGGACGT	A31064Hi	+T*+C*C*G*T*T*G*A*A*G*G*A*+C*+G*+T	22478
69	TCGAGTCTAGCTTATC	A31065Hi	+T*+C*+G*A*G*T*C*T*A*G*C*T*T*+A*+T*+C	22594

70	TCGCCGTCACTCCAGTT	A31066Hi	+T*+C*+G*C*C*G*T*C*A*C*T*C*C*A*+G*T*+T	24910
71	GAGGCACTCGCCAAACG	A31067Hi	+G*+A*+G*G*C*A*C*T*C*G*C*C*A*A*+C*+G	24945
72	GGACCTGTACGACCTAT	A31068Hi	+G*+G*+A*C*C*T*G*T*A*C*G*A*C*C*+T*+A*+T	25310
73	TAGCGCAGGTGATTGC	A31069Hi	+T*+A*+G*C*G*C*A*G*G*T*G*A*T*T*+G*+C	29495
74	GGTGGCGTAATTCCTAG	A31070Hi	+G*+G*+T*G*G*C*G*T*A*A*T*T*C*C*+T*+A*+G	29545
75	TCTACGAAGTTCGGTT	A31071Hi	+T*+C*+T*A*C*G*A*A*G*T*T*C*G*+G*+T*+T	32254
76	TAGACGAGTTCTACGAA	A31072Hi	+T*+A*+G*A*C*G*A*G*T*T*C*T*A*C*+G*+A*+A	32262
77	ATGATGCGAGTTACGTT	A31073Hi	+A*+T*+G*A*T*G*C*G*A*G*T*T*A*C*+G*+T*+T	33476
78	TGATGCGAGTTACGT	A31074Hi	+T*+G*+A*T*G*C*G*A*G*T*T*A*+C*+G*+T	33477
79	CCTAATGTACGGTACTT	A31075Hi	+C*+C*+T*A*A*T*G*T*A*C*G*G*T*A*+C*+T*+T	34555
80	GCTCGTCGTGTCTTCG	A31076Hi	+G*+C*+T*C*G*T*C*G*T*G*T*C*C*T*+T*+C*+G	35277
81	GCTCGTCGTGTCTT	A31077Hi	+G*+C*+T*C*G*T*C*G*T*G*T*C*+C*+T*+T	35279
82	AGCCGGTGCTCGTCGTG	A31078Hi	+A*+G*+C*C*G*G*T*G*C*T*C*G*T*C*+G*+T*+G	35284
83	ATGGCCGCGAGACTCCG	A31079Hi	+A*+T*+G*G*C*C*G*C*G*A*G*A*C*T*C*+C*+G	35330
84	CGCCGCCGAGACCAAGA	A31080Hi	+C*+G*+C*C*G*C*C*G*A*G*A*C*C*A*+A*+G*+A	35346
29	TCGAATTTGCCATAGTT	A31081H	+T*+C*G*A*A*T*T*T*G*C*A*A*T*A*+G*+T*+T	12273
29	TCGAATTTGCCATAGTT	A31082H	+T*+C*+G*A*A*T*T*T*G*C*C*A*A*T*A*+G*+T*+T	12273
29	TCGAATTTGCCATAGTT	A31083H	+T*C*+G*A*A*T*T*T*G*C*C*A*A*T*A*+G*+T*+T	12273
29	TCGAATTTGCCATAGTT	A31084H	+T*+C*+G*A*A*T*T*T*G*C*C*A*A*T*A*+G*+T*+T	12273
30	TCGAATTTGCCATAGT	A31085H	+T*+C*G*A*A*T*T*T*G*C*C*A*A*T*+A*+G*+T	12274
30	TCGAATTTGCCATAGT	A31086H	+T*+C*+G*A*A*T*T*T*G*C*C*A*A*T*A*+G*+T	12274
30	TCGAATTTGCCATAGT	A31087H	+T*+C*+G*A*A*T*T*T*G*C*C*A*A*T*+A*+G*+T	12274
30	TCGAATTTGCCATAGT	A31088H	+T*C*+G*A*A*T*T*T*G*C*C*A*A*T*+A*+G*+T	12274
85	TTTGAATTTGCCATAGT	A31089H	+T*+T*+C*G*A*A*T*T*T*G*C*C*A*A*T*+A*+G*+T	12274
85	TTTGAATTTGCCATAGT	A31090H	+T*+T*C*G*A*A*T*T*T*G*C*C*A*A*T*+A*+G*+T	12274
85	TTTGAATTTGCCATAGT	A31091H	+T*+T*+C*G*A*A*T*T*T*G*C*C*A*A*T*A*+G*+T	12274
86	TTTGAATTTGCCATAG	A31092H	+T*+T*+T*C*G*A*A*T*T*T*G*C*C*A*A*T*+A*+G	12275
86	TTTGAATTTGCCATAG	A31093H	+T*+T*+T*+C*G*A*A*T*T*T*G*C*C*A*A*T*+A*+G	12275
86	TTTGAATTTGCCATAG	A31094H	+T*+T*+T*C*G*A*A*T*T*T*G*C*C*A*A*T*+A*+G	12275
87	TTGATCGCAGCGAAGAT	A31095H	+T*+T*+G*A*T*C*G*C*A*G*C*G*A*A*+G*+A*+T	6154
88	TCGAAAGGTACTCCAGT	A31096H	+T*+C*+G*A*A*A*G*G*T*A*C*T*C*C*+A*+G*+T	10446
89	GCTTCTCAGTACTTTC	A31097H	+G*+C*+T*T*C*T*C*A*C*G*T*A*C*T*+T*+T*+C	11005
90	TGTAGCGTTTGTGAGG	A31098H	+T*+G*+T*A*G*C*G*T*T*G*T*T*G*+A*+G*+G	11072
91	CAGTCGTGTGTAGCGTT	A31099H	+C*+A*+G*T*C*G*T*G*T*G*T*A*G*C*+G*+T*+T	11080
92	ACGCAGTCGTGTGTAGC	A31100H	+A*+C*+G*C*A*G*T*C*G*T*G*T*G*T*+A*+G*+C	11083
93	TGAGACGCAGTCGTGTG	A31101H	+T*+G*+A*G*A*C*G*C*A*G*T*C*G*T*+G*+T*+G	11087
94	TGATGAGACGCAGTCGT	A31102H	+T*+G*+A*T*G*A*G*A*C*G*C*A*G*T*+C*+G*+T	11090
95	GTGCAGAGCGGTCTAT	A31103H	+G*+T*+G*C*A*G*A*G*C*G*T*C*C*+T*+A*+T	11526
96	ATCTCGTACAAACAGTA	A31104H	+A*+T*+C*T*C*G*T*A*C*A*A*A*C*A*+G*+T*+A	12477
97	CTCCGAATGTTACAGCC	A31105H	+C*+T*+C*C*G*A*A*T*G*T*T*A*C*A*+G*+C*+C	16873
98	CATAAAATTGCGACTCC	A31106H	+C*+A*+T*A*A*A*A*T*T*G*C*G*A*C*+T*+C*+C	23222

99	AAGGCTCAAAGACGACG	A31107H	+A*+A*+G*G*C*T*C*A*A*G*A*C*G*+A*+C*+G	35620
100	GCATCGTCGGCAAGCTC	A31108H	+G*+C*+A*T*C*G*T*C*G*G*C*A*A*G*+C*+T*+C	35778
59	GTAATGTCAACGGATC	A31109Hi	+G*+T*+A*A*T*G*T*C*A*A*C*G*G*A*+T*+C	13706
59	GTAATGTCAACGGATC	A31110Hi	+G*+T*A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C	13706
59	GTAATGTCAACGGATC	A31111Hi	+G*+T*+A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C	13706
59	GTAATGTCAACGGATC	A31112Hi	+G*T*+A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C	13706
101	GGTAATGTCAACGGATC	A31113Hi	+G*+G*+T*A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C	13706
101	GGTAATGTCAACGGATC	A31114Hi	+G*+G*+T*A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C	13706
101	GGTAATGTCAACGGATC	A31115Hi	+G*+G*T*A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C	13706
101	GGTAATGTCAACGGATC	A31116Hi	+G*+G*+T*+A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C	13706
102	GGTAATGTCAACGGAT	A31117Hi	+G*+G*+T*A*A*T*G*T*C*A*A*C*G*+G*+A*+T	13707
102	GGTAATGTCAACGGAT	A31118Hi	+G*+G*+T*+A*A*T*G*T*C*A*A*C*G*+G*+A*+T	13707
103	ACGCTTAATCACCGCCG	A31119Hi	+A*+C*+G*C*T*T*A*A*T*C*A*A*C*G*+C*+C*+G	10684
104	CGAGTGGTAACCGGAA	A31120Hi	+C*+G*+A*G*T*G*G*T*A*A*C*G*+G*+A*+A	10888
105	TGTCATCGGAAGCGAGT	A31121Hi	+T*+G*+T*C*A*T*C*G*G*A*A*G*C*G*+A*+G*+T	10899
106	ACGTATCAGGCGATGG	A31122Hi	+A*+C*+G*T*A*A*T*C*A*G*G*C*G*A*+T*+G*+G	14401
107	GACGTATCAGGCGATG	A31123Hi	+G*+A*+C*G*T*A*A*T*C*A*G*G*C*G*+A*+T*+G	14402
108	CCTAGAGTGGCGGACTT	A31124Hi	+C*+C*+T*A*G*A*G*T*G*G*C*G*G*A*+C*+T*+T	18502
109	AGACGAGTTCTACGA	A31125Hi	+A*+G*+A*C*G*A*G*T*T*C*T*A*+C*+G*+A	32263
110	GATGCGAGTTACGTTT	A31126Hi	+G*+A*+T*G*C*G*A*G*T*T*A*C*G*+T*+T*+T	33475
111	GTGATATGATGCGAGT	A31127Hi	+G*+T*+G*A*A*T*A*T*G*A*A*T*G*C*G*+A*+G*+T	33482
112	TTCTCCTGTTGGCTCGA	A31128H	+T*+T*C*T*C*C*T*G*T*T*G*G*C*T*+C*+G*+A	5407
113	ACCAGGCAGTGAACACG	A31129H	+A*+C*C*A*G*G*C*A*G*T*G*A*A*C*+A*+C*+G	5873
114	TTAGGCTTCGGTCCACA	A31130H	+T*+T*+A*G*G*C*T*T*C*G*G*T*C*C*+A*+C*+A	5897
115	GGTCCTTAGGCTTCGGT	A31131H	+G*+G*T*C*C*T*T*A*G*G*C*T*T*C*+G*+G*+T	5902
116	TCGATCATTAGCGTGGC	A31132H	+T*+C*G*A*A*T*C*A*A*T*T*A*G*C*G*T*+G*+G*+C	6097
117	CGAAGATCCACACGGCC	A31133H	+C*+G*+A*A*G*A*A*T*C*C*A*A*C*A*C*G*+C*+C	6144
118	TCCTTGATGAGACGCAG	A31134H	+T*+C*C*T*T*G*A*A*T*G*A*G*A*C*G*+C*+A*+G	11094
119	TTGCCGATGGCCAGAAG	A31135H	+T*+T*+G*C*G*A*A*T*G*G*C*C*A*G*+A*+A*+G	11142
120	TCTTGGTCTTGCCGATG	A31136H	+T*+C*+T*T*G*G*T*C*T*T*G*C*G*+A*+T*+G	11150
121	CACACGTCTTGGTCTTG	A31137H	+C*+A*+C*A*C*G*T*C*T*T*G*G*T*C*+T*+T*+G	11156
122	GCTCTCACACGTCTTGG	A31138H	+G*C*+T*C*T*C*A*A*C*A*C*G*T*C*T*+T*+G*+G	11161
123	GCAGCTCATCGAAGCCG	A31139H	+G*C*+A*A*C*T*C*A*A*T*C*G*A*A*G*+C*+C*+G	11489
124	GCTCGTCAAAGGCACCT	A31140H	+G*+C*+T*C*G*T*C*A*A*G*G*C*A*+C*C*+T	11507
125	CAGAGCGGTCTATGTG	A31141H	+C*A*+G*A*G*C*G*T*C*C*T*A*A*T*+G*+T*+G	11523
126	CGGCCCTCTGCCAGTCA	A31142H	+C*+G*+G*C*C*T*T*C*T*G*C*C*A*G*+T*+C*+A	11543
127	CACATGCCGAGGATGGT	A31143H	+C*+A*C*A*T*G*G*C*G*A*G*G*A*T*+G*+G*+T	11671
128	TTGGCCTCGGAGAAACC	A31144H	+T*+T*+G*G*C*C*T*C*G*G*A*G*A*A*+A*C*+C	11697
129	GTGAAGAGGACCTCGTT	A31145H	+G*+T*+G*A*A*G*A*G*G*A*A*C*C*T*C*+G*T*+T	11787
130	GAAGACGTACACCGCG	A31146H	+G*+A*+A*G*A*A*C*G*T*A*A*C*A*C*+G*C*+G	11903
131	AGGAAGAAGACGTACAC	A31147H	+A*+G*+G*A*A*G*A*A*G*A*A*C*G*T*A*+C*+A*+C	11907

132	GCTGTAGAACTTCTCGC	A31148H	+G*C*+T*G*T*A*G*A*A*C*T*T*C*T*+C*+G*+C	12124
133	GACTGTACGTCTCGGC	A31149H	+G*+A*C*T*G*T*C*A*C*G*T*C*T*C*+G*+G*+C	12247
134	TGCACGAAGTCTCTC	A31150H	+T*+G*+C*A*C*G*A*A*G*T*C*O*T*C*+C*+T*+C	12498
135	TCTCCGAATGTTACAGC	A31151H	+T*+C*+T*C*C*G*A*A*T*G*T*T*A*C*+A*+G*+C	16874
136	TCATGCGAGAGGCCACA	A31152H	+T*+C*+A*T*G*C*G*A*G*A*G*G*C*A*+C*+A	21253
137	CACTCATGCGAGAGGCC	A31153H	+C*+A*+C*T*C*A*T*G*C*G*A*G*A*G*G*+C*+C	21256
138	AGAAGTCTGATTCCGAA	A31154H	+A*+G*+A*A*G*T*C*T*G*A*T*T*T*C*+G*+A*+A	21352
139	GGTGCCTGAGATTCTGA	A31155H	+G*+G*+T*G*C*G*T*G*A*G*A*T*T*C*+T*+G*+A	31175
140	CTCGCAGGTAAAGGTGC	A31156H	+C*+T*+C*G*C*A*G*G*T*A*A*A*G*G*+T*+G*+C	31187
141	CCTGAAGCTTGCAGTCG	A31157H	+C*+C*+T*G*A*A*G*C*T*T*G*C*A*G*+T*+C*+G	31259
142	CGTGAGGTTGCAGTTGT	A31158H	+C*+G*+T*G*A*G*G*T*T*G*C*A*G*T*+T*+G*+T	31796
143	GTGTGACGTGAGGTTGC	A31159H	+G*+T*G*T*G*A*C*G*T*G*A*G*G*T*+T*+G*+C	31802
144	CAGGCTCAGCTTTCGCA	A31160H	+C*+A*+G*G*C*T*C*A*G*C*T*T*T*C*+G*+C*+A	31862
145	ATCGCAGCTCTCTCCAC	A31161H	+A*+T*C*G*C*A*G*C*T*C*T*C*T*C*+C*+A*+C	35706
146	TGGCCTGGATGGATCGC	A31162H	+T*+G*+G*C*C*T*G*G*A*T*G*G*A*T*C*+G*+C	35718
147	GCAAGCTCTCTTCTCCG	A31163H	+G*+C*+A*A*G*C*T*C*T*C*T*T*C*T*+C*+C*+G	35769
148	GAAGGCATCGTCGCAA	A31164H	+G*+A*+A*G*G*C*A*T*C*G*T*C*G*G*+C*+A*+A	35782
149	AGGAAGGCATCGTCGGC	A31165H	+A*+G*+G*A*A*G*G*C*A*T*C*G*T*C*+G*+G*+C	35784
150	TGAGGCGCTGTGATGAC	A31166H	+T*+G*A*G*G*C*G*C*T*G*T*G*A*T*+G*+A*+C	35874
151	ACGTGTTCTTAGGATA	A31167Hi	+A*+C*+G*T*G*T*T*C*T*T*A*G*G*+A*+T*+A	7195
152	GCACTACCGTGAGAGG	A31168Hi	+G*C*+A*C*T*A*C*C*G*T*G*A*G*+A*+G*+G	7905
153	CACGGTTCTTCAAGGAG	A31169Hi	+C*+A*+C*G*G*T*T*C*T*T*C*A*A*G*+G*+A*+G	8158
154	AGACGGTGTAGTGTT	A31170Hi	+A*G*+A*C*G*G*T*G*T*A*G*T*G*+G*+T*+T	8871
155	GTCAC TAGATAGCATA	A31171Hi	+G*+T*+C*A*C*T*A*G*A*T*A*G*C*+A*+T*+A	9654
156	TATTCGTGCCTAATC	A31172Hi	+T*+A*+T*T*T*C*G*T*G*C*C*T*A*+A*+T*+C	9854
157	AGAACGACCAGTCTAT	A31173Hi	+A*+G*+A*A*C*G*A*C*C*A*G*T*C*+T*+A*+T	9867
158	GAACCTATAGTATGGC	A31174Hi	+G*+A*+A*C*C*T*A*T*A*G*T*A*T*+G*G*+C	10573
159	TAATCACC GCCGCTGG	A31175Hi	+T*+A*+A*T*C*A*C*C*G*C*G*C*G*+T*G*+G	10680
160	GAAACGCTTAATCACC	A31176Hi	+G*+A*+A*A*C*G*C*T*T*A*A*T*C*+A*+C*+C	10688
161	TGTGTAGCTACCAGCC	A31177Hi	+T*+G*+T*G*T*A*G*C*T*A*C*C*A*+G*+C*+C	10767
162	CCGAGAATCTCTGGTG	A31178Hi	+C*+C*G*A*G*A*A*T*C*T*C*T*G*G*+T*+G	10826
163	CGAGTGGTAACCGGAAT	A31179Hi	+C*+G*+A*G*T*G*G*T*A*A*C*G*G*+A*+A*+T	10887
164	TGTCATCGGAAGCGAG	A31180Hi	+T*+G*+T*C*A*T*C*G*G*A*A*G*C*+G*+A*+G	10900
165	CCACCTGAGATGCGCTC	A31181Hi	+C*+C*+A*C*C*T*G*A*G*A*T*G*C*G*+C*T*+C	10928
166	GTCAACGGATCAAGAA	A31182Hi	+G*+T*+C*A*A*C*G*G*A*T*C*A*A*+G*+A*+A	13701
167	CCTTCATGGCCAAACCA	A31183Hi	+C*+C*+T*T*C*A*T*G*G*C*C*A*A*A*+C*+C*+A	13748
168	GCACTTGTTCGGAC	A31184Hi	+G*+C*+A*C*T*T*G*T*T*T*C*G*+G*+A*+C	13843
169	CGCTGGTGAACAGGTTTC	A31185Hi	+C*+G*C*T*G*G*T*G*A*A*C*A*G*G*T*+T*+C	13926
170	GCCGTAGAAACCGTAC	A31186Hi	+G*+C*+C*G*T*A*G*A*A*A*C*C*G*+T*+A*+C	14071
171	ACTGTTTGCTGCCGTA	A31187Hi	+A*+C*+T*G*T*T*G*C*T*G*C*C*G*+T*+A	14081
172	AGGCACCATCGACCAGG	A31188Hi	+A*+G*+G*C*A*C*C*A*T*C*G*A*C*C*+A*+G*+G	14266

173	TATGAACTTGCACTCAC	A31189Hi	+T*+A*+T*G*A*A*C*T*T*G*C*A*C*T*+C*+A*+C	16921
174	CTAGAGTGGCGGACTT	A31190Hi	+C*+T*+A*C*A*G*T*G*G*C*G*A*+C*+T*+T	18502
175	ACTGCGTGTCCAGGCAA	A31191Hi	+A*+C*T*G*C*G*T*G*T*C*C*A*G*G*C*+A*+A	18654
176	CATTCACTGCGTGCCTT	A31192Hi	+C*+A*+T*T*C*A*G*T*G*C*G*T*G*C*+T*+T*+T	18775
177	TAAGCCACCGAACAGC	A31193Hi	+T*+A*+A*G*C*C*A*C*C*G*A*A*C*+A*+G*+C	18893
178	TGTTTGACGATTTTCATC	A31194Hi	+T*+G*+T*T*T*G*A*C*G*A*T*T*T*C*+A*+T*+C	20418
179	AGAGAGCTCCGGAATA	A31195Hi	+A*+G*+A*G*A*G*C*T*C*C*G*G*A*+A*+T*+A	20719
				20845
				20887
				21055
				21097
180	GGACACACCTGACAAGA	A31196Hi	+G*+G*+A*C*A*C*A*C*T*G*A*C*A*+A*+G*+A	20984
181	CGCCTCGCTGGCAGAA	A31197Hi	+C*+G*+C*C*T*C*G*C*T*G*G*C*A*+G*+A*+A	21442
182	CCGTTGAAGGACGTTT	A31198Hi	+C*+C*+G*T*T*G*A*A*G*G*A*C*G*+T*+T*+T	22476
183	ATCCGTTGAAGGACGT	A31199Hi	+A*+T*+C*C*G*T*T*G*A*A*G*G*A*+C*+G*+T	22478
184	TTGTAGTGTGTACTAG	A31200Hi	+T*+T*+G*T*A*G*T*G*T*G*T*A*C*+T*+A*+G	22544
185	TGTGAGTGTCTTGGAAG	A31201Hi	+T*+G*+T*G*A*G*T*G*T*C*T*T*G*G*+A*+A*+G	23557
186	AATATTCTAAGGCGAT	A31202Hi	+A*+A*+T*A*T*T*C*T*A*G*G*C*+G*+A*+T	24672
187	CGCCGTCCTCCAGTT	A31203Hi	+C*+G*+C*C*G*T*T*C*A*C*T*C*A*+G*+T*+T	24910
188	CCTGTACGACCTATAA	A31204Hi	+C*+C*+T*G*T*A*C*G*A*C*C*T*A*+T*+A*+A	25308
189	ACATGGATGACGACTT	A31205Hi	+A*+C*+A*T*G*G*A*T*G*A*C*G*A*+C*+T*+T	28147
190	ACGGATATCTTGCCAT	A31206Hi	+A*+C*+G*G*A*T*A*T*C*T*T*G*C*+C*+A*+T	28750
191	CAACAGACGGTGCAGT	A31207Hi	+C*+A*+A*C*A*G*A*C*G*G*T*G*C*+A*+G*+T	28958
192	GGTGTCTACGAGGCAGG	A31208Hi	+G*+G*+T*G*T*C*T*A*C*G*A*G*G*C*+A*+G*+G	29430
193	ATGCATCAATCTCGAT	A31209Hi	+A*+T*+G*C*A*T*C*A*A*T*T*C*T*C*+G*+A*+T	29648
194	GAACGTCCATTGTGAC	A31210Hi	+G*+A*+A*C*G*T*T*C*A*T*T*G*T*+G*+A*+C	30615
195	AGCCTTGTATGCTGGTA	A31211Hi	+A*+G*+C*C*T*T*G*T*A*T*G*C*T*G*+G*+T*+A	31637
196	GGACTAACTAGCTTCA	A31212Hi	+G*+G*+A*C*T*A*A*C*T*A*G*C*T*+T*+C*+A	31657
197	CCTTGGCCATGATCGCA	A31213Hi	+C*+C*+T*T*G*G*C*C*A*T*G*A*T*C*+G*+C*+A	32216
198	TCGGTTCCTTGTGTAG	A31214Hi	+T*+C*+G*G*T*T*C*C*T*G*T*G*T*G*+T*+A*+G	32244
199	AGACGAGTTCTACGAA	A31215Hi	+A*+G*+A*C*G*A*G*T*T*C*T*A*C*+G*+A*+A	32262
200	GTGACCATCGACTGAA	A31216Hi	+G*+T*+G*A*C*C*A*T*C*G*A*C*T*+G*+A*+A	33199
201	AGTGATATGATGCGAG	A31217Hi	+A*+G*+T*G*A*T*A*T*G*A*T*G*C*+G*+A*+G	33483
202	GATAAGAAGACGATGT	A31218Hi	+G*+A*+T*A*A*G*A*A*G*A*C*G*A*+T*+G*+T	33506
203	AGCGGAATCTTCGGAA	A31219Hi	+A*+G*+C*G*G*A*A*T*C*T*T*C*G*+G*+A*+A	33588
204	TGCCTCTCACATCTTCG	A31220Hi	+T*+G*+C*C*T*C*T*A*C*A*T*C*T*+T*+C*+G	34727
205	GCTCGTCGTGTCTTTC	A31221Hi	+G*+C*+T*C*G*T*T*C*G*T*G*T*C*+T*+T*+C	35278
206	TGCTCGTCGTGTCTTTC	A31222Hi	+T*+G*+C*T*C*G*T*T*C*G*T*G*T*C*+C*+T*+T	35279
207	CGCCGCCGAGACCAAG	A31223Hi	+C*+G*+C*C*G*C*C*G*A*G*A*C*C*+A*+A*+G	35347
208	GCTCCGCTCCTCGCCG	A31224Hi	+G*+C*+T*C*G*C*G*T*C*T*C*G*+C*+C*+G	35370
209	ACCAGGCAGTGAACACGG	A31225H	+A*+C*+C*A*G*G*C*A*G*T*G*A*A*C*A*+C*+G*+G	5872

210	GGTGCTTGCCATCTTCAT	A31226H	+G*+G*+T*G*C*T*T*G*C*A*T*C*T*T*+C*+A*+T	5935
211	GATAGTCCTCTAAGTGCAT	A31227H	+G*+A*+T*A*G*T*C*C*T*C*T*A*A*G*T*G*+C*+A*+T	6013
212	TAGTCCTCTAAGTGCATC	A31228H	+T*+A*+G*T*C*C*T*C*T*A*A*G*T*G*C*+A*+T*+C	6012
213	AGCGTGGCTAGATCCACAT	A31229H	+A*+G*+C*G*T*G*G*C*T*A*G*A*T*C*C*A*+C*+A*+T	6086
214	GCTAGATCCACATGGTCT	A31230H	+G*+C*+T*A*G*A*T*C*C*A*C*A*T*G*G*+T*+C*+T	6081
215	CATTAGCGTGGCTAGATC	A31231H	+C*+A*+T*T*A*G*C*G*T*G*G*C*T*A*G*+A*+T*+C	6091
216	ACACGTGCATTATCTGAAC	A31232H	+A*+C*+A*C*G*T*G*C*A*T*T*A*T*C*T*G*+A*+A*+C	10370
217	TCTCGAAAGGTACTCCAG	A31233H	+T*+C*+T*C*G*A*A*A*G*G*T*A*C*T*C*+C*+A*+G	10447
218	AATCTGCTTCTCACGTAC	A31234H	+A*+A*+T*C*T*G*C*T*T*C*T*C*A*C*G*+T*+A*+C	11009
219	GCGTTTGTGTGAGGCTCACA	A31235H	+G*+C*+G*T*T*T*G*T*T*G*A*G*G*C*T*C*+A*+C*+A	11066
220	GTGTAGCGTTTGTGAGGC	A31236H	+G*+T*+G*T*A*G*C*G*T*T*G*T*T*G*A*+G*+G*+C	11071
221	CGTGTGTAGCGTTTGTGTA	A31237H	+C*+G*+T*G*T*G*T*A*G*C*G*T*T*G*T*+T*+G*+A	11074
222	GCACTCGTGTGTAGCGTTT	A31238H	+G*+C*+A*C*T*C*G*T*G*T*G*T*A*G*C*G*+T*+T*+T	11079
223	TGTAGCGTTTGTGAGGC	A31239H	+T*+G*+T*A*G*C*G*T*T*G*T*T*G*A*+G*+G*+C	11071
224	AGTCGTGTGTAGCGTTTG	A31240H	+A*+G*+T*C*G*T*G*T*G*T*A*G*C*G*+T*+T*+G	11078
225	GCAGTCGTGTGTAGCGTT	A31241H	+G*+C*+A*G*T*C*G*T*G*T*G*A*G*C*+G*+T*+T	11080
226	CTCCGGTGCTCCTTGATG	A31242H	+C*+T*+C*C*G*G*T*G*C*T*C*C*T*T*G*+A*+T*+G	11102
227	GTCTTGGTCTTGCCGATGG	A31243H	+G*+T*+C*T*T*G*G*T*T*G*C*G*A*+T*+G*+G	11149
228	TAGTCAAACCTGTCTTGGT	A31244H	+T*+A*+G*T*C*A*A*A*C*C*T*G*T*C*T*T*+G*+G*+T	11329
229	TAGAACAGATAGTCAAACC	A31245H	+T*+A*+G*A*A*C*A*G*A*T*A*G*T*C*A*A*+A*+C*+C	11338
230	CGACAGTGGATATAGAACA	A31246H	+C*+G*+A*C*A*G*T*G*G*A*T*A*T*A*G*A*+A*+C*+A	11350
231	AGAACAGATAGTCAAACC	A31247H	+A*+G*+A*A*C*A*G*A*T*A*G*T*C*A*A*+A*+C*+C	11338
232	CGACAGTGGATATAGAAC	A31248H	+C*+G*+A*C*A*G*T*G*G*A*T*A*T*A*G*+A*+A*+C	11351
233	TCGTCAAAGGCACCTTGCA	A31249H	+T*+C*+G*T*C*A*A*A*G*G*C*A*C*C*T*T*+G*+C*+A	11503
234	CAGTGCAGAGCGGTCCTAT	A31250H	+C*+A*+G*T*G*C*A*G*A*G*C*G*T*C*C*+T*+A*+T	11526
235	TCGTCAAAGGCACCTTGC	A31251H	+T*+C*+G*T*C*A*A*A*G*G*C*A*C*C*T*+T*+G*+C	11504
236	CGGCCTTCTGCCAGTCAGT	A31252H	+C*+G*+G*C*C*T*T*C*T*G*C*C*A*G*T*C*+A*+G*+T	11541
237	AGAGCGGTCTATGTGCT	A31253H	+A*+G*+A*G*C*G*T*C*C*T*A*T*G*T*+G*+C*+T	11521
238	GGTCTCGTGGTGATGAGCA	A31254H	+G*+G*+T*C*T*C*G*T*G*G*A*T*G*A*+G*+C*+A	11620
239	CGAGGATGGTCCAGCAAGT	A31255H	+C*+G*+A*G*G*A*T*G*G*T*C*C*A*G*C*A*+A*+G*+T	11662
240	AGGATCTCCACATGCCGA	A31256H	+A*+G*+G*A*T*C*T*C*A*C*A*T*G*C*+C*+G*+A	11678
241	CACGATCCAGCAGACCAGG	A31257H	+C*+A*+C*G*A*T*C*C*A*G*C*A*G*A*C*C*+A*+G*+G	11819
242	GACACATCCGCCTTCTGCA	A31258H	+G*+A*+C*A*C*A*T*C*C*G*C*C*T*T*C*T*+G*+C*+A	12064
243	GCAGACACATCCGCCTTC	A31259H	+G*+C*+A*G*A*C*A*T*C*C*G*C*C*+T*+T*+C	12068
244	TACATGGCGGCAAAGAACT	A31260H	+T*+A*+C*A*T*G*G*C*G*G*C*A*A*G*A*+A*+C*+T	12163
245	GTACATGGCGGCAAAGAA	A31261H	+G*+T*+A*C*A*T*G*G*C*G*G*C*A*A*+G*+A*+A	12165
246	GGTAGTACATGGCGGCAA	A31262H	+G*+G*+T*A*G*T*A*C*A*T*G*G*C*G*G*+C*+A*+A	12169
247	TCGAATTTGCCATAGTTT	A31263H	+T*+C*+G*A*A*T*T*T*G*C*C*A*T*A*G*+T*+T*+T	12272
248	CTCGTACAAACAGTAGAAC	A31264H	+C*+T*+C*G*T*A*C*A*A*A*C*A*G*T*A*G*+A*+A*+C	12473
249	TCGTACAAACAGTAGAAC	A31265H	+T*+C*+G*T*A*C*A*A*A*C*A*G*T*A*G*+A*+A*+C	12473
250	TTGCACGAAGTCCTCCTC	A31266H	+T*+T*+G*C*A*C*G*A*G*T*C*C*T*C*+C*+T*+C	12498

251	GCATGTTATGGAGAAACC	A31267H	+G*+C*+A*T*G*T*T*A*T*G*G*A*G*A*A*+A*+C*+C	12628
252	GCGTTTCACACAACACTCT	A31268H	+G*+C*+G*T*T*T*C*A*C*A*A*A*C*A*C*+T*+C*+T	16843
253	CACAATCTCCGAATGTAC	A31269H	+C*+A*+C*A*A*T*C*T*C*C*G*A*A*T*G*T*+T*+A*+C	16877
254	GTGGAAGCAGCACTCATGC	A31271H	+G*+T*+C*G*A*A*G*C*A*G*C*A*C*T*C*A*+T*+G*+C	21264
255	GACCAAGGAGATGTGGAAG	A31272H	+G*+A*+C*C*A*A*G*G*A*G*A*T*G*T*C*G*+A*+A*+G	21276
256	TGTCGAAGCAGCACTCAT	A31273H	+T*+G*+T*C*G*A*A*G*C*A*G*C*A*C*T*+C*+A*+T	21266
257	GGACCAAGGAGATGTGGA	A31274H	+G*+G*+A*C*C*A*A*G*G*A*G*A*T*G*T*+C*+G*+A	21278
258	TTCTTCAGATTGCACAAC	A31275H	+T*+T*+C*T*T*C*A*G*A*T*T*G*C*A*C*+A*+A*+C	21390
259	GCGTGAGATTCTGATTAGT	A31276H	+G*+C*+G*T*G*A*G*A*T*T*C*T*G*A*T*T*+A*+G*+T	31170
260	AGGTGCGTGAGATTCTGAT	A31277H	+A*+G*+G*T*G*C*G*T*G*A*G*A*T*T*C*T*+G*+A*+T	31174
261	GTAAAGGTGCGTGAGATTTC	A31278H	+G*+T*+A*A*A*G*G*T*G*C*G*T*G*A*G*A*+T*+T*+C	31178
262	TTGTCTCCGAGAGTGTGTC	A31279H	+T*+T*+G*T*C*T*C*C*G*A*G*A*G*T*G*T*+T*+G*+C	31204
263	CGTGAGATTCTGATTAGT	A31280H	+C*+G*+T*G*A*G*A*T*T*C*T*G*A*T*T*+A*+G*+T	31170
264	GTGAGGTTGCAGTTGTCTA	A31281H	+G*+T*+G*A*G*G*T*G*C*A*G*T*G*T*+C*+T*+A	31793
265	TGACGTGAGGTTGCAGTTG	A31282H	+T*+G*+A*C*G*T*G*A*G*G*T*G*C*A*G*+T*+T*+G	31797
266	CCTCGCAGGTAAGGTGTC	A31283H	+C*+C*+T*C*G*C*A*G*G*T*A*A*A*G*G*+T*+G*+C	31187
267	TTGTCTCCGAGAGTGTG	A31284H	+T*+T*+G*T*C*T*C*C*G*A*G*A*G*T*G*+T*+T*+G	31205
268	TTTCGCAGGCTCTGGCTGG	A31285H	+T*+T*+T*C*G*C*A*G*G*C*T*C*T*G*G*C*+T*+G*+G	31850
269	ACGTGAGGTTGCAGTTGT	A31286H	+A*+C*+G*T*G*A*G*G*T*G*C*A*G*T*+T*+G*+T	31796
270	TCTTGAAGTGTTCCTAACG	A31287H	+T*+C*+T*T*G*A*A*G*G*T*G*T*T*C*T*A*+A*+C*+G	35583
271	TCTTGAAGTGTTCCTAAC	A31288H	+T*+C*+T*T*G*A*A*G*G*T*G*T*T*T*C*T*+A*+A*+C	35584
272	TACATGAGGTACCAAGA	A31289H	+T*+A*+C*A*T*G*A*G*G*T*C*A*C*C*A*+A*+G*+A	35906
273	TGCACAATGTGAGTGTACC	A31290Hi	+T*+G*+C*A*C*A*A*T*G*T*G*A*G*T*G*T*+A*+C*+C	6630
274	CACCTTCTCATAGCACTAC	A31291Hi	+C*+A*+C*C*T*T*C*T*T*A*T*A*G*C*A*C*+T*+A*+C	7914
275	TGAGTATATAAGTACTGAC	A31292Hi	+T*+G*+A*G*T*A*T*A*A*G*T*A*C*T*+G*+A*+C	8621
276	AGTACTTCTACAGTTGTGA	A31293Hi	+A*+G*+T*A*C*T*T*T*C*T*A*C*A*G*T*T*G*+T*+G*+A	8664
277	AGACAGATAAATTCAGGTG	A31294Hi	+A*+G*+A*C*A*G*A*T*A*A*C*T*T*C*A*G*+G*+T*+G	9563
278	TATAGGAGAAATTCAGGTT	A31295Hi	+T*+A*+T*A*G*G*A*G*A*A*A*T*T*C*A*G*+G*+T*+T	9619
279	AAGGCATTCTATAGGAGA	A31296Hi	+A*+A*+G*G*C*A*T*T*C*T*A*T*A*G*G*+A*+G*+A	9629
280	AACAACCTCTGCTGTCCAC	A31297Hi	+A*+A*+C*A*A*C*T*T*C*T*G*C*T*G*T*C*+C*+A*+C	10160
281	ACATGGCTCTGAGTTGCAG	A31298Hi	+A*+C*+A*T*G*G*C*T*T*G*A*G*T*T*G*+C*+A*+G	10236
282	ATTGAAACCTGGCCTAATA	A31299Hi	+A*+T*+T*G*A*A*A*C*C*T*G*G*C*C*T*A*+A*+T*+A	10297
283	AATCTCTGGTGTCAAGTTT	A31300Hi	+A*+A*+T*C*T*T*G*G*T*G*T*T*A*G*+T*+T*+T	10818
284	AGAAGAAGCTGGCGAGGA	A31301Hi	+A*+G*+A*G*A*A*G*C*T*G*G*C*G*A*+G*+G*+A	12776
285	GACTGACTCTTAGGCACTA	A31302Hi	+G*+A*+C*T*G*A*C*T*T*T*A*G*G*C*A*+C*+T*+A	13059
286	CAGACTTGGCCACTAGCTT	A31303Hi	+C*+A*+G*A*C*T*T*G*G*C*A*C*T*A*G*+C*+T*+T	13473
287	TCATGCGACAGACTTGCG	A31304Hi	+T*+C*+A*T*G*G*A*C*A*G*A*C*T*T*+G*+G*+C	13482
288	GACTCAACCACTTCATTG	A31305Hi	+G*+A*+C*T*C*A*A*C*C*A*C*T*T*C*A*+T*+T*+G	13494
289	CAGTCTTCATGGCCAAAC	A31306Hi	+C*+A*+G*T*C*C*T*T*C*A*T*G*G*C*C*A*+A*+A*+C	13750
290	GTGCTTTGGATACTCTGC	A31307Hi	+G*+T*+G*C*T*T*T*G*G*A*T*A*C*T*T*C*+T*+G*+C	13900
291	TTCAAATGCAAAGTCTAGC	A31308Hi	+T*+T*+C*A*A*A*T*G*C*A*A*G*T*C*T*+A*+G*+C	15571

292	TCACCTGTAGCTCTCATT	A31309Hi	+T*+C*+A*C*C*T*G*T*A*G*C*T*C*T*C*+A*+T*+T	15588
293	GGTCCTGAAGATCTTTCTC	A31310Hi	+G*+G*+T*C*C*T*G*A*A*G*A*T*C*T*T*+C*+T*+C	16964
294	ACAAAGCATTTCTGACCAGG	A31311Hi	+A*+C*+A*A*A*G*C*A*T*T*C*T*G*A*C*C*+A*+G*+G	18120
295	TTGATAAGACTGGTGACA	A31312Hi	+T*+T*+G*A*T*A*A*G*A*C*T*G*G*T*G*+A*+C*+A	18559
296	GGTGACTATTCATGTGAG	A31313Hi	+G*+G*+T*G*A*C*T*A*T*T*C*A*T*G*T*+G*+A*+G	23568
297	CATAGTTCTCTGCAACAGG	A31314Hi	+C*+A*+T*A*G*T*T*C*T*C*T*G*C*A*A*C*+A*+G*+G	23845
298	AGTGATGATTATTAGTGG	A31315Hi	+A*+G*+T*G*C*A*T*G*A*T*T*A*T*T*A*G*+T*+G*+G	23964
299	CTGAGTTCCACTACCTTCT	A31316Hi	+C*+T*+G*A*G*T*T*C*C*A*C*T*A*C*C*T*+T*+C*+T	24162
300	CGTTTGTCTACACTGAGT	A31317Hi	+C*+G*+T*T*T*G*T*C*T*A*C*A*C*T*G*+A*+G*+T	24175
301	GAATGCTGATCTGGCTGGT	A31318Hi	+G*+A*+A*T*G*C*T*G*A*T*C*T*G*G*C*T*+G*+G*+T	24450
302	TCCTCCGGCTCCTTCACAG	A31319Hi	+T*+C*+T*T*C*C*G*C*T*C*C*T*T*C*A*+C*+A*+G	25010
303	GTTCCATCTGCTTTGCCTG	A31320Hi	+G*+T*+T*C*C*A*T*C*T*G*C*T*T*G*C*+C*+T*+G	25035
304	TACACCAAGCATGTGTTAC	A31321Hi	+T*+A*+C*A*C*C*A*A*G*C*A*T*G*T*G*T*+T*+A*+C	25951
305	ACTATTGTAAATGGCTCTC	A31322Hi	+A*+C*+T*A*T*T*G*T*A*A*A*T*G*G*C*T*+C*+T*+C	26028
306	CACAAAGCTCTGATACTC	A31323Hi	+C*+A*+C*A*A*A*G*C*T*C*T*G*A*T*A*+C*+T*+C	26214
307	GACTTGTGTTCTTTACACAG	A31324Hi	+G*+A*+C*T*T*G*T*G*T*T*C*T*T*T*C*A*+C*+A*+G	26674
308	ATTAATGCCACTTATAGAT	A31325Hi	+A*+T*+T*A*A*T*G*C*C*A*C*T*T*A*T*A*+G*+A*+T	27090
309	ATAGGCTATACCTAACAC	A31326Hi	+A*+T*+A*G*G*C*T*A*T*A*C*C*T*A*A*+C*+A*+C	27163
				27199
				27235
				27253
310	ACATAGGCTATACCTAAC	A31327Hi	+A*+C*+A*T*A*G*G*C*T*A*T*A*C*C*T*+A*+A*+C	27237
				27255
311	GCCAAGCTAAGATGTCAC	A31328Hi	+G*+C*+C*A*A*G*C*T*A*A*G*A*T*G*T*+C*+A*+C	27640
312	CAGTGATGTCCACCTATA	A31329Hi	+C*+A*+G*T*G*A*T*G*T*C*C*A*C*C*T*+A*+T*+A	27890
313	GTAGGAGCTTCTTAGATG	A31330Hi	+G*+T*+A*G*G*A*G*C*T*T*C*T*T*A*G*+A*+T*+G	28024
314	GATATCTTGCCATGTACT	A31331Hi	+G*+A*+T*A*T*C*T*T*G*C*C*A*T*G*T*+A*+C*+T	28745
315	CAGGCTTTACCACTATTTT	A31332Hi	+C*+A*+G*G*C*T*T*A*C*C*A*C*T*A*T*+T*+T*+C	29041
316	TGAGTGGCTTCTACTAAG	A31333Hi	+T*+G*+A*G*T*G*G*C*T*T*C*T*A*C*T*+A*+A*+G	29223
317	TTTAAGCTCTTTGGTGTC	A31334Hi	+T*+T*+T*A*A*G*C*T*C*T*T*G*G*T*+G*+T*+C	29441
318	TTTGATGTACTATTCTTGA	A31335Hi	+T*+T*+T*G*A*T*G*T*A*C*T*A*T*T*C*T*+T*+G*+A	29617
319	GTTTCTCTCAAGGCCACAG	A31336Hi	+G*+T*+T*T*C*T*C*T*A*A*G*G*C*C*A*+C*+A*+G	29800
320	TGACCTTCTAAGCTGTAG	A31337Hi	+T*+G*+A*C*C*T*T*C*T*A*A*G*C*T*G*+T*+A*+G	30601
321	CCGTAACACTGCAGACAC	A31338Hi	+C*+C*+G*T*A*A*A*C*A*C*T*G*C*A*G*A*+C*+A*+C	30732
322	ACACCTCACTTCTAAGATA	A31339Hi	+A*+C*+A*C*C*T*C*A*C*T*T*C*T*A*A*G*+A*+T*+A	31371
323	GAAGTGGAATGTGTAGTTT	A31340Hi	+G*+A*+A*G*T*G*G*A*A*T*G*T*G*T*A*G*+T*+T*+T	32126
324	CCTTAAAGCCAAGCAGTGA	A31341Hi	+C*+C*+T*T*A*A*A*G*C*C*A*A*G*C*A*G*+T*+G*+A	32843
325	ACGTTTGAGAGGTTTATT	A31342Hi	+A*+C*+G*T*T*T*G*A*G*A*G*G*T*T*+A*+T*+T	33123
326	AAAGAGACAGTGACGACA	A31343Hi	+A*+A*+A*G*A*G*A*A*G*T*G*A*C*G*+A*+C*+A	33252
327	GAGTTTGTGCTCTGATT	A31344Hi	+G*+A*+G*T*T*T*G*T*G*C*T*C*T*G*+A*+T*+T	33530
328	GGAGAGGCTGGATTATGTG	A31345Hi	+G*+G*+A*G*A*G*G*C*T*G*G*A*T*T*A*T*+G*+T*+G	33566

329	GATGACAGTGTCTTGCAA	A31346Hi	+G*+A*+T*G*A*C*A*G*T*G*T*T*C*T*T*G*+C*+A*+A	33654
330	GATCATTAATATGGACAGA	A31347Hi	+G*+A*+T*C*A*T*T*A*A*T*A*T*G*G*A*C*+A*+G*+A	33941
331	GCTTGTATCGCAGCCTGA	A31348Hi	+G*+C*+T*T*G*T*T*A*T*C*G*C*A*G*C*C*+T*+G*+A	34241
332	CAGCTTGTATCGCAGCC	A31349Hi	+C*+A*+G*C*T*T*G*T*T*A*T*C*G*C*A*+G*+C*+C	34244
333	ACCAAAATCATCAGAGACT	A31350Hi	+A*+C*+C*A*A*A*T*T*C*A*T*C*A*G*A*G*+A*+C*+T	34331
334	CTCCTCTTTAATAAAGTTT	A31351Hi	+C*+T*+C*C*T*C*T*T*T*A*A*T*A*A*A*G*+T*+T*+T	34695
335	TAGTATCACTGTATGTCCA	A31352Hi	+T*+A*+G*T*A*T*C*A*C*T*G*T*A*T*G*T*+C*+C*+A	34843
336	GGTCATGTCAACTTGTTC	A31353Hi	+G*+G*+T*C*A*T*G*T*C*A*A*C*T*T*G*T*+T*+T*+C	35001
337	TCAGAGTCGTTAACCTTA	A31354Hi	+T*+C*+A*G*A*G*T*C*G*T*T*A*A*C*C*+T*+T*+A	35479
29	TCGAATTTGCCATAGTT	A31355H	+T*+C*+G*A*+A*T*T*T*G*C*C*A*T*A*+G*+T*+T	12273
29	TCGAATTTGCCATAGTT	A31356H	+T*+C*+G*A*A*+T*T*T*G*C*C*A*T*A*+G*+T*+T	12273
29	TCGAATTTGCCATAGTT	A31357H	+T*+C*+G*A*A*T*+T*T*G*C*C*A*T*A*+G*+T*+T	12273
29	TCGAATTTGCCATAGTT	A31358H	+T*+C*+G*A*A*T*T*T*G*C*C*A*T*+A*+G*+T*+T	12273
29	TCGAATTTGCCATAGTT	A31359H	+T*+C*+G*A*A*T*T*T*G*C*C*A*+T*A*+G*+T*+T	12273
29	TCGAATTTGCCATAGTT	A31360H	+T*+C*+G*A*A*T*T*T*G*C*C*+A*T*A*+G*+T*+T	12273
29	TCGAATTTGCCATAGTT	A31361H	+T*+C*+G*A*A*T*T*T*G*+C*C*A*T*A*+G*+T*+T	12273
30	TCGAATTTGCCATAGT	A31362H	+T*+C*+G*A*+A*T*T*T*G*C*C*A*T*+A*+G*+T	12274
30	TCGAATTTGCCATAGT	A31363H	+T*+C*+G*A*A*+T*T*T*G*C*C*A*T*+A*+G*+T	12274
30	TCGAATTTGCCATAGT	A31364H	+T*+C*+G*A*A*T*+T*T*G*C*C*A*T*+A*+G*+T	12274
30	TCGAATTTGCCATAGT	A31365H	+T*+C*+G*A*A*T*T*T*G*C*C*A*+T*+A*+G*+T	12274
30	TCGAATTTGCCATAGT	A31366H	+T*+C*+G*A*A*T*T*T*G*C*C*+A*T*+A*+G*+T	12274
30	TCGAATTTGCCATAGT	A31367H	+T*+C*+G*+A*A*T*T*T*G*C*C*A*+T*+A*+G*+T	12274
59	GTAATGTCAACGGATC	A31368Hi	+G*+T*+A*A*+T*G*T*C*A*A*C*G*G*+A*+T*+C	13706
59	GTAATGTCAACGGATC	A31369Hi	+G*+T*+A*A*T*+G*T*C*A*A*C*G*G*+A*+T*+C	13706
59	GTAATGTCAACGGATC	A31370Hi	+G*+T*+A*A*T*G*+T*C*A*A*C*G*G*+A*+T*+C	13706
59	GTAATGTCAACGGATC	A31371Hi	+G*+T*+A*A*T*G*T*C*A*A*C*+G*G*+A*+T*+C	13706
59	GTAATGTCAACGGATC	A31372Hi	+G*+T*+A*A*T*G*T*C*A*A*+C*G*G*+A*+T*+C	13706
59	GTAATGTCAACGGATC	A31373Hi	+G*+T*+A*+A*T*G*T*C*A*A*C*G*+G*+A*+T*+C	13706
SEQ ID NO.	Antisense Sequence 5'-3'	Name of the LNA-modified ASO	Antisense Sequence 5'-3' with PTO (*) and LNA (+)	Position on mRNA (NM_00489 5.4; SEQ ID NO.2)
338	CCACAATCTCCGAATGTTA	A31270H**	+C*+C*+A*C*A*A*T*C*T*C*G*A*A*T*G*+T*+T*+A	16878
339	CGTTTAGGCTATGTACTT	Neg1	+C*+G*+T*T*T*A*G*G*C*T*A*T*G*T*A*+C*+T*+T	Control
340	TACGCGCGGTTGTTTA	R01002	+T*+A*+C*G*C*G*C*G*T*T*G*T*+T*+T*+A	Control
341	GATCATTCGCGGACAAC	R01011	+G*+A*+T*C*A*T*T*T*C*G*C*G*G*A*C*+A*+A*+C	Control

Table 1: List of human NLRP3-specific ASOs hybridizing with human NLRP3 for example of SEQ ID NO.1; Neg1, R01002 and R01011 are antisense oligonucleotides representing a negative control which is not hybridizing with NLRP3 of SEQ ID NO.1 or

SEQ ID NO.2. An “H” after the ASO ID indicates a human NLRP3-specific sequence that binds to an exonic region of the pre-mRNA and a “Hi” after the ASO ID indicates a human NLRP3-specific sequence that binds to an intronic region of the pre-mRNA. ** = exon spanning oligo, position depicted in Table 1 indicates positions on mRNA of SEQ ID NO. 5 2 (RefSeq ID NM_004895.4) for exon spanning oligonucleotides.

The following Table 2 shows oligonucleotides hybridizing with murine NLRP3 mRNA:

SEQ ID NO.	Antisense Sequence 5'-3'	Name of the LNA-modified ASO	Antisense Sequence 5'-3' with PTO (*) and LNA (+)	Position on pre-mRNA (GRCmCh38, Chr11: 59539030-59569495; SEQ ID NO.3)
342	CTTGATCCAGACGTAT	A31001M	+C*+T*+T*G*A*T*C*(*A*G*A*C*G*+T*+A*+T	3807
343	AACGGACACTCGTCATC	A31002M	+A*+A*C*G*G*A*C*A*C*T*C*G*T*C*+A*+T*+C	4083
344	TTGCAACGGACACTCGT	A31003M	+T*+T*+G*C*A*A*C*G*G*A*C*A*C*T*+C*+G*+T	4087
345	CTCGCCTGTTGATCGCA	A31004M	+C*+T*+C*G*C*C*T*G*T*T*G*A*T*C*+G*+C*+A	4305
346	CTCGCCTGTTGATCGC	A31005M	+C*+T*+C*G*C*C*T*G*T*T*G*A*T*+C*+G*+C	4306
347	CTCGCCTGTTGATCG	A31006M	+C*+T*+C*G*C*C*T*G*T*T*G*A*+T*+C*+G	4307
348	TCTCGCCTGTTGATCG	A31007M	+T*+C*+T*C*G*C*C*T*G*T*T*G*A*+T*+C*+G	4307
349	AGACGCGCGTTCCTGT	A31008M	+A*+G*A*C*G*C*G*C*G*T*T*C*T*+G*+T	9015
350	CACCTAGACGCGCGTTC	A31009M	+C*+A*C*C*T*A*G*A*C*G*C*G*C*G*T*+T*+C	9019
351	CGTGTAGCGACTGTTGA	A31010M	+C*+G*+T*G*T*A*G*C*G*A*C*T*G*T*+T*+G*+A	9048
352	CGTGTAGCGACTGTTG	A31011M	+C*+G*+T*G*T*A*G*C*G*A*C*T*G*+T*+T*+G	9049
353	GCGTGTAGCGACTGTTG	A31012M	+G*+C*+G*T*G*T*A*G*C*G*A*C*T*G*+T*+T*+G	9049
354	GCGTGTAGCGACTGTT	A31013M	+G*+C*+G*T*G*T*A*G*C*G*A*C*T*+G*+T*+T	9050
355	TGCGTGTAGCGACTGTT	A31014M	+T*+G*+C*G*T*G*T*A*G*C*G*A*C*T*+G*+T*+T	9050
356	GGAGCTGCGTGTAGCGA	A31015M	+G*+G*A*G*C*T*G*C*G*T*G*T*A*G*C*+G*+A	9055
357	TAGTCCGCCGATGGT	A31016M	+T*+A*+G*T*C*C*G*G*G*C*G*A*T*+G*+G*+T	9122
358	TTAGTCCGCCGATGGT	A31017M	+T*+T*+A*G*T*C*C*G*G*C*G*A*T*+G*+G*+T	9122
359	ACCGGCTCGTCGTTAT	A31018M	+A*+C*C*G*G*C*C*T*C*G*T*C*G*T*+A*+T	9599
360	CTACCGGCTCGTCGTT	A31019M	+C*+T*+A*C*G*G*C*C*T*C*G*T*C*G*+T*+T	9601
361	CTACCGGCTCGTCGT	A31020M	+C*+T*A*C*G*G*C*C*T*C*G*T*C*+G*+T	9602
362	AAGGCTACCGGCTCGT	A31021M	+A*+A*G*G*C*T*A*C*G*G*C*C*T*+C*+G*+T	9605
363	TAGACGGCCGTAGTGGT	A31022M	+T*+A*+G*A*C*G*G*C*G*T*A*G*T*G*+G*+T	9869
364	TAGACGGCCGTAGTGG	A31023M	+T*+A*+G*A*C*G*G*C*G*T*A*G*+T*+G*+G	9870
365	GTAGACGGCCGTAGTGG	A31024M	+G*+T*+A*G*A*C*G*G*C*G*T*A*G*+T*+G*+G	9870
366	GTAGACGGCCGTAGTG	A31025M	+G*+T*+A*G*A*C*G*G*C*G*T*A*+G*+T*+G	9871
367	ACGTAGACGGCCGTAG	A31026M	+A*+C*+G*T*A*G*A*C*G*G*C*G*+T*+A*+G	9873
368	AGAAGACGTAGACGGC	A31027M	+A*+G*+A*A*G*A*C*G*T*A*G*A*+G*+G*+C	9878
369	AGCGGAGACGTCACTCT	A31028M	+A*+G*+C*G*G*A*G*A*C*G*T*A*G*T*+C*+T	10044

370	GGACCTTCACGTCTCGG	A31029M	+G*+G*+A*C*C*T*T*T*C*A*C*G*T*C*T*+C*+G*+G	10228
371	TAGTAGGACCTTCACGT	A31030M	+T*+A*+G*T*A*G*G*A*C*C*T*T*C*A*+C*+G*+T	10233
372	TAGTAGGACCTTCACG	A31031M	+T*+A*+G*T*A*G*G*A*C*C*T*T*C*A*+C*+G	10234
373	CTAGTAGGACCTTCACG	A31032M	+C*+T*+A*G*T*A*G*G*A*C*C*T*T*C*A*+C*+G	10234
374	ACAACACGCGGATGTGA	A31033M	+A*+C*+A*A*C*A*C*G*C*G*A*T*G*+T*+G*+A	19371
375	GCACGTTTTGTTTCACG	A31034M	+G*+C*+A*C*G*T*T*T*G*T*T*T*C*+A*C*+G	27157
376	ACGTTATCGATGTGAGG	A31035M	+A*+C*+G*T*T*A*T*C*G*A*T*G*T*G*+A*+G*+G	27405
377	ACGTTATCGATGTGAG	A31036M	+A*+C*+G*T*T*A*T*C*G*A*T*G*T*+G*+A*+G	27406
378	TTCACGTTATCGATGT	A31037M	+T*+T*+C*A*C*G*T*T*A*T*C*G*A*+T*+G*+T	27409
379	TTTCACGTTATCGATGT	A31038M	+T*+T*+T*C*A*C*G*T*T*A*T*C*G*A*+T*+G*+T	27409
380	GTTTCACGTTATCGAT	A31039M	+G*+T*+T*T*C*A*C*G*T*T*A*T*C*+G*+A*+T	27411
381	AGTTTCACGTTATCGAT	A31040M	+A*+G*+T*T*T*C*A*C*G*T*T*A*T*C*+G*+A*+T	27411
351	CGTGTAGCGACTGTTGA	A31041M	+C*+G*+T*G*T*A*G*C*G*A*C*T*G*+T*+T*+G*+A	9048
351	CGTGTAGCGACTGTTGA	A31042M	+C*+G*+T*+G*T*A*G*C*G*A*C*T*G*T*+T*+G*+A	9048
351	CGTGTAGCGACTGTTGA	A31043M	+C*+G*T*G*T*A*G*C*G*A*C*T*G*T*+T*+G*+A	9048
351	CGTGTAGCGACTGTTGA	A31044M	+C*+G*+T*G*T*A*G*C*G*A*C*T*G*T*+T*+G*+A	9048
375	GCACGTTTTGTTTCACG	A31045M	+G*+C*+A*+C*G*T*T*T*T*G*T*T*T*C*+A*C*+G	27157
375	GCACGTTTTGTTTCACG	A31046M	+G*+C*+A*C*G*T*T*T*T*G*T*T*T*+C*+A*C*+G	27157
375	GCACGTTTTGTTTCACG	A31047M	+G*C*+A*+C*G*T*T*T*T*G*T*T*T*C*A*+C*+G	27157
376	ACGTTATCGATGTGAGG	A31048M	+A*+C*+G*T*T*A*T*C*G*A*T*G*T*+G*+A*+G*+G	27405
376	ACGTTATCGATGTGAGG	A31049M	+A*+C*G*T*T*A*T*C*G*A*T*G*T*+G*+A*+G*+G	27405
382	ACGTGTACCTTACTGTA	A31050Mi	+A*+C*+G*T*G*T*A*C*C*T*T*A*C*T*+G*+T*+A	4411
383	CTACCTGCACGAGGAAT	A31051Mi	+C*+T*+A*C*C*T*G*C*A*C*G*A*G*G*+A*+A*+T	4618
384	GAACAATTACGGCTGC	A31052Mi	+G*+A*+A*+C*A*A*T*T*A*C*G*G*C*+T*+G*+C	5367
385	CCACATAGGTGCCGTAG	A31053Mi	+C*+C*+A*C*A*T*A*G*G*T*G*C*C*G*+T*+A*+G	5517
386	GCACGGATAGTAATAGG	A31054Mi	+G*+C*+A*+C*G*G*A*T*A*G*T*A*A*T*+A*+G*+G	6279
387	CTTACTATGACCGGCC	A31055Mi	+C*+T*+T*A*C*T*A*T*G*A*C*C*G*G*+C*+C	6355
388	TACTCTTACTATGACCG	A31056Mi	+T*+A*+C*T*C*T*T*A*C*T*A*T*G*A*+C*+C*+G	6358
389	GTGCAACCGCGAACCT	A31057Mi	+G*+T*+G*C*A*A*C*C*G*C*G*A*A*C*+C*+T	6509
390	CGTGCAACCGCGAACCT	A31058Mi	+C*+G*T*G*C*A*A*C*C*G*C*G*A*A*+C*+C*+T	6509
391	ACATTCTGTGCAACCGC	A31059Mi	+A*+C*+A*T*T*C*G*T*G*C*A*A*C*+C*+G*+C	6515
392	AACATTCTGTGCAACCGC	A31060Mi	+A*A*+C*A*A*T*T*C*G*T*G*C*A*A*C*+C*+G*+C	6515
393	CTCAGTTGGATGTACGT	A31061Mi	+C*+T*+C*A*G*T*T*G*G*A*T*G*T*A*+C*+G*+T	7132
394	CTCAGTTGGATGTACG	A31062Mi	+C*+T*+C*A*G*T*T*G*G*A*T*G*T*+A*+C*+G	7133
395	CACTGCTCGAGTGTATC	A31063Mi	+C*+A*C*T*G*C*T*C*G*A*G*T*G*T*+A*+T*+C	8170
396	TGGTATGACCGGACAGA	A31064Mi	+T*+G*+G*T*A*T*G*A*C*C*G*G*A*C*+A*+G*+A	8490
397	ATAGAGCGAGCTGTCTG	A31065Mi	+A*+T*+A*G*A*G*C*G*A*G*C*T*G*+T*+C*+G	8756
398	TATAGAGCGAGCTGTCTG	A31066Mi	+T*+A*+T*A*G*A*G*C*G*A*G*C*T*G*+T*+C*+G	8756
399	AGGATTTACGAACAAC	A31067Mi	+A*+G*+G*A*T*T*T*A*C*G*A*A*C*+A*+A*+C	8836
400	TAAGCTGCTAGCGGACC	A31068Mi	+T*+A*+A*G*C*T*G*C*T*A*G*C*G*G*A*+C*+C	8885
401	GCATGGAAGCGATATTC	A31069Mi	+G*+C*+A*T*G*G*A*A*G*C*G*A*T*A*+T*+T*+C	11021

				13044
402	TCTCAGAACGACGGAAT	A31070Mi	+T*+C*+T*C*A*G*A*A*C*G*A*C*G*G*+A*+A*+T	11341
403	GAGCATGTTTGCCGATA	A31071Mi	+G*+A*+G*C*A*T*G*T*T*T*G*C*G*+A*+T*+A	12045
404	CCGTTGGACTGGTATCA	A31072Mi	+C*+C*+G*T*T*G*G*A*C*T*G*G*T*A*+T*+C*+A	13244
405	TTCACGTATCAGCACA	A31073Mi	+T*+T*+C*A*C*G*T*A*T*C*A*G*C*+A*+C*+A	13672
406	GTGAACGTAATGGCAGG	A31074Mi	+G*+T*+G*A*A*C*G*T*A*A*T*G*G*C*+A*+G*+G	13906
407	TTAAAGACCGGTGTGCC	A31075Mi	+T*+T*+A*A*A*G*A*C*G*G*T*G*T*G*+C*+C	14147
408	ACCTCGAGTTTGGTTCA	A31076Mi	+A*+C*+C*T*C*G*A*G*T*T*T*G*G*T*+C*+A	14378
409	GACCGGTGATCTCATAA	A31077Mi	+G*+A*+C*C*G*G*T*G*A*T*C*T*C*A*+T*+A*+A	15826
410	GAACGACCGGTGATC	A31078Mi	+G*+A*+A*C*T*G*A*C*G*G*T*G*A*+T*+C	15832
411	CCGTGCGTTAGGAGAAT	A31079Mi	+C*+C*+G*T*C*G*T*T*A*G*G*A*G*+A*+A*+T	15893
412	TCTTCCGTGCGTTAG	A31080Mi	+T*+C*+T*T*G*T*C*G*T*G*C*G*T*+T*+A*+G	15899
413	TGAATGCACCAAGCTCG	A31081Mi	+T*+G*+A*A*T*G*C*A*C*C*A*A*G*C*+T*+C*+G	16071
414	CCGACCATATAATGCTA	A31082Mi	+C*+C*+G*+A*C*C*A*T*A*T*A*A*T*G*+C*T*+A	16351
415	AGCGTTCAGTAAGGACC	A31083Mi	+A*+G*+C*G*T*T*C*A*G*T*A*A*G*G*A*+C*+C	17288
416	TACGTTCTACTAGAGC	A31084Mi	+T*+A*+C*G*T*T*C*C*T*A*C*T*A*G*+A*+G*+C	17943
417	ACGGTGCATAGCTGGC	A31085Mi	+A*+C*+G*G*T*T*G*C*A*T*A*G*C*T*G*+G*+C	18058
418	TGTTACTGAGTACGACC	A31086Mi	+T*+G*+T*T*A*C*T*G*A*G*T*A*C*G*A*+C*+C	20304
419	AGCAATTTGGTAACGTC	A31087Mi	+A*+G*+C*A*A*T*T*T*G*G*T*A*A*C*+G*+T*+C	20517
420	CCGTGGCAGCACCTTAA	A31088Mi	+C*+C*+G*T*G*G*C*A*G*C*A*C*C*T*+T*+A*+A	20545
421	AACTGAGCGTAGTGAAG	A31089Mi	+A*+A*+C*T*G*A*G*C*G*T*A*G*T*G*+A*+A*+G	22910
422	TAACTTGCGGTATTATC	A31090Mi	+T*+A*+A*C*T*T*G*C*G*G*T*A*T*T*+A*+T*+C	23548
423	GTCTAACTTGC GGATAT	A31091Mi	+G*+T*C*T*A*A*C*T*T*G*G*G*G*+T*+A*+T	23552
424	CAATCCTCGTTGTTCC	A31092Mi	+C*+A*+A*T*C*C*T*C*G*T*T*G*T*+T*+C*+C	23744
425	TACGTGAGCAATCCTCG	A31093Mi	+T*+A*+C*G*T*T*C*A*G*C*A*A*T*C*C*+T*+C*+G	23751
426	AAGTTTGCTGTCCGGTA	A31094Mi	+A*+A*+G*T*T*T*G*C*T*G*T*C*G*+G*+T*+A	23854
427	CGAGAAGCGATGCTTAG	A31095Mi	+C*+G*+A*+G*A*A*G*C*G*A*T*G*C*T*+T*+A*+G	24285
428	GCTTGATCCAGACGAGAG	A31096Mi	+G*+C*+T*T*G*G*A*T*C*C*A*C*G*+A*G*+A*+G	25786
429	CACTGCGAGCTGCATC	A31097Mi	+C*+A*C*T*G*C*G*A*G*C*T*G*C*+A*+T*+C	25939
430	CGCATATACAAAGTCAC	A31098M	+C*+G*+C*A*T*A*T*A*C*A*A*A*G*T*+C*+A*+C	3705
431	CTTGATCCAGACGTATG	A31099M	+C*+T*+T*G*A*T*C*C*A*G*A*C*G*T*+A*+T*+G	3806
432	TTGATCCAGACGTATG	A31100M	+T*+T*+G*A*T*C*C*A*G*A*C*G*T*+A*+T*+G	3806
433	GCTTGATCCAGACGTAT	A31101M	+G*+C*+T*T*G*A*T*C*C*A*G*A*C*G*+T*+A*+T	3807
434	CGGACACTCGTCATCTT	A31102M	+C*+G*+G*A*C*A*T*C*G*T*C*A*T*+C*+T*+T	4081
435	AGCTCGTTGGAGAAATA	A31103M	+A*+G*+C*T*C*G*T*T*G*G*A*G*A*A*+A*+T*+A	9710
436	CTGTAGAATCTCTCGCA	A31104M	+C*+T*+G*T*A*G*A*A*T*C*T*C*T*C*+G*+C*+A	10097
437	ATAGTACATAGCAGCGA	A31105M	+A*+T*+A*G*T*A*C*A*T*A*G*C*A*G*+C*+G*+A	10143
438	TAGTACATAGCAGCGA	A31106M	+T*+A*+G*T*A*C*A*T*A*G*C*A*G*+C*+G*+A	10143
439	CAAACCTGCCGTAATTT	A31107M	+C*+A*+A*A*C*T*T*G*C*G*T*A*A*+T*+T*+T	10252
440	TAGAACAGTTCCAGTTG	A31108M	+T*+A*+G*A*A*C*A*G*T*T*C*C*A*G*+T*+T*+G	10442
441	TGCTTCGTAGATAGAGG	A31109M	+T*+G*+C*T*T*C*G*T*A*G*A*T*A*G*+A*+G*+G	26089

442	CATTGCTTCGTAGATAG	A31110M	+C*+A*+T*T*G*C*T*T*C*G*T*A*G*A*+T*+A*+G	26092
443	CGCCAAGATCATTGTTG	A31111M	+C*+G*+C*C*A*A*G*A*T*C*A*T*G*+T*+T*+G	26906
444	GCACAGATCGCCAAGAT	A31112M	+G*+C*+A*C*A*G*A*T*C*G*C*A*A*+G*+A*+T	26914
445	CGTTTTGTTTACGATT	A31113M	+C*+G*+T*T*T*T*G*T*T*T*C*A*C*G*+A*+T*+T	27154
375	GCACGTTTGTTCACG	A31114M	+G*+C*+A*C*G*T*T*T*G*T*T*T*C*+A*+C*+G	27157
446	GAAATCTCGAAGACTAT	A31115M	+G*+A*+A*A*T*C*T*C*G*A*A*G*A*C*+T*+A*+T	27211
447	CGTTATCGATGTGAGGA	A31116M	+C*+G*+T*T*A*T*C*G*A*T*G*T*G*A*+G*+G*+A	27404
448	GCAGTTTCACGTATCG	A31117M	+G*+C*+A*G*T*T*T*C*A*C*G*T*T*A*+T*+C*+G	27413
343	AACGGACACTCGTCATC	A31118M	+A*A*+A*+C*G*G*A*C*A*T*C*G*T*C*+A*+T*+C	4083
449	GAGCCAGCTTGCAACG	A31119M	+G*+A*+G*C*C*A*G*C*T*T*G*C*A*+A*+C*+G	4096
450	GTCTCGCCTGTTGATCG	A31120MR	+G*+T*+C*T*C*G*C*C*T*G*T*T*G*A*+T*+C*+G	4307
451	GTCTCGCCTGTTGATC	A31121MR	+G*+T*+C*T*C*G*C*C*T*G*T*T*G*A*+T*+C	4308
452	GTCGTCTGTACATCTTA	A31122M	+G*+T*+C*G*T*T*C*T*G*T*A*C*A*T*C*+T*+T*+A	8968
453	AGCGACTGTTGAGGTCC	A31123M	+A*+G*+C*G*A*C*T*G*T*T*G*A*G*G*+T*+C*+C	9043
454	AGTTGGAGCTGCGTGTA	A31124M	+A*+G*+T*T*G*G*A*G*C*T*G*C*G*T*+G*+T*+A	9059
455	GTCCAACATAATCTTCC	A31125MR	+G*+T*+C*C*A*A*C*A*T*A*A*T*C*T*+T*+C*+C	9267
456	GTCGTATGAGCAGAGA	A31126M	+G*+T*+C*G*T*T*A*T*G*A*G*C*A*G*+A*+G*+A	9590
359	ACCGGCTCGTCGTTAT	A31127M	+A*+C*+C*G*G*C*C*T*C*G*T*C*G*T*+T*+A*+T	9599
457	TACCGGCCCTCGTCGTT	A31128M	+T*+A*+C*C*G*G*C*C*T*C*G*T*C*+G*+T*+T	9601
458	ACGTAGACGGCCGTAGT	A31129M	+A*+C*+G*T*A*G*A*C*G*G*C*C*G*T*+A*+G*+T	9872
459	CCGTGCTCCGCAGATC	A31130M	+C*+C*+G*T*G*C*T*T*C*G*C*A*G*+A*+T*+C	10019
460	GCCGTGCTTCCGCAGAT	A31131M	+G*+C*+C*G*T*G*C*T*T*C*G*C*A*+G*+A*+T	10020
369	AGCGGAGACGTCACTCT	A31132M	+A*+G*+C*G*G*A*G*A*C*G*T*C*A*G*+T*+C*+T	10044
461	CTCTCGCAGTCCACTTC	A31133M	+C*+T*+C*T*C*G*C*A*G*T*C*C*A*C*+T*+T*+C	10088
462	ATCTCTCGCAGTCCACT	A31134M	+A*+T*+C*T*C*T*C*G*A*G*T*C*C*+A*+C*+T	10090
463	GAACCAATGCGAGATCC	A31135MR	+G*+A*+A*C*C*A*A*T*G*C*G*A*G*A*+T*+C*+C	19390
464	AGTAAGGCCGAATTC	A31137MR	+A*+G*+T*A*A*G*G*C*C*G*G*A*+T*+T*+C	26017
465	GGACTCGTGTCTGACCA	A31138M	+G*+G*+A*C*T*C*G*T*G*T*C*T*G*A*+C*+C*+A	3686
466	CGCGTTCCTGTCTTGA	A31139M	+C*+G*+C*G*T*T*C*T*G*T*C*T*C*+T*+G*+A	9009
467	TAGACGCGCTTCTGT	A31140M	+T*+A*+G*A*C*G*C*G*C*G*T*T*C*+T*+G*+T	9015
468	TAGACGCGCTTCTGT	A31141M	+T*+A*+G*A*C*G*C*G*C*G*T*T*C*+C*+T*+G	9016
469	CTCACCTAGACGCGCG	A31142M	+C*+T*+C*A*C*C*T*A*G*A*C*G*C*+G*+C*+G	9022
470	CTCTCACCTAGACGCG	A31143M	+C*+T*+C*T*C*A*C*C*T*A*G*A*C*+G*+C*+G	9024
471	GCTGCGTGTAGCGACTG	A31144M	+G*+C*+T*G*C*G*T*G*T*A*G*C*G*A*+C*+T*+G	9052
472	CGGCCGATGGTCAGGAG	A31145M	+C*+G*+G*C*C*G*A*T*G*G*T*C*A*G*+G*+A*+G	9116
473	CATTTTAGTCCGGCCGA	A31146M	+C*+A*+T*T*T*T*A*G*T*C*G*G*C*+C*+G*+A	9126
474	GCCTCGTCGTTATGAG	A31147M	+G*+C*+C*T*C*G*T*C*G*T*T*A*T*+G*+A*+G	9596
475	CAAGGCTACCGGCTCG	A31148M	+C*+A*+A*G*G*C*T*A*C*C*G*G*C*+T*+C*+G	9606
476	CGGCCGTAGTGGTCTT	A31149M	+C*+G*+G*C*C*G*T*A*G*T*G*G*T*+C*+T*+T	9866
477	AGACGTAGACGGCCG	A31150M	+A*+G*+A*C*G*T*A*G*A*C*G*G*+C*+C*+G	9876
478	AGAAGACGTAGACGGCC	A31151M	+A*+G*+A*A*G*A*C*G*T*A*G*A*C*G*+G*+C*+C	9877

479	GCAGGCCGTGCTTCCGC	A31152M	+G*+C*+A*G*G*C*G*T*G*C*T*T*C*+C*+G*+C	10024
480	TCGGTTCAGAAGATCTG	A31153M	+T*+C*+G*G*T*T*C*A*G*A*A*G*A*T*+C*+T*+G	10215
481	GGTTGGTGCTTAGACTT	A31154M	+G*+G*+T*T*G*G*T*G*C*T*T*A*G*A*+C*+T*+T	12790
482	TGTCGAAGCAGCATTGA	A31155M	+T*+G*+T*C*G*A*A*G*C*A*G*C*A*T*+T*+G*+A	16743
483	CGCTTCTAAGGCACGTT	A31156M	+C*+G*+C*T*T*C*T*A*A*G*G*C*A*G*+G*+T*+T	27167
484	TCACGTTATCGATGTGA	A31157M	+T*+C*+A*C*G*T*T*A*T*C*G*A*T*G*+T*+G*+A	27407
SEQ ID NO.	Antisense Sequence 5'-3'	Name of the LNA-modified ASO	Antisense Sequence 5'-3' with PTO (*) and LNA (+)	Position on mRNA (NM_145827; SEQ ID NO.4)
485	GCCGGAATTCACCAACC	A31136MR**	+G*+C*+C*G*G*A*A*T*T*C*A*C*C*A*+A*+C*+C	26010
486	TTATGTCCGGTTATTTT	S5	+T*+T*+A*T*G*T*C*G*G*T*T*A*T*+T*+T*+C	Control

Table 2: List of mouse NLRP3-specific ASOs hybridizing with mouse NLRP3 for example of SEQ ID NO. 3; S5 is an antisense oligonucleotide representing a negative control which is not hybridizing with NLRP3 of SEQ ID NO. 3 or SEQ ID NO. 4. An “M” after the ASO ID indicates a mouse NLRP3-specific sequence that binds to an exonic region of the pre-mRNA and a “Mi” after the ASO ID indicates a mouse NLRP3-specific sequence that binds to an intronic region of the pre-mRNA. ** = exon spanning oligo, position depicted in Table 2 indicates positions on mRNA of SEQ ID NO. 4 (RefSeq ID NM_145827) for exon spanning oligonucleotides. An “R” after the ASO ID indicates a mouse NLRP3-specific with cross-reactivity to rat.

The oligonucleotides of the present invention hybridize for example with mRNA and/or pre-mRNA of human NLRP3 of SEQ ID NO. 2 and SEQ ID NO 1, respectively, and/or of mouse NLRP3 of SEQ ID NO.4 and SEQ ID NO 3, respectively. Such oligonucleotides are called NLRP3 antisense oligonucleotides. Oligonucleotides of the present invention, which are for example antisense oligonucleotides, are shown in Tables 1 and 2. The present invention further refers to oligonucleotides such as antisense oligonucleotides having 80 to 99 %, 85 to 98 %, 90 to 95 or 93 % sequence homology to an oligonucleotide of Table 1 and/or Table 2. Such oligonucleotides still show an inhibitory activity of 70 to 100 %, 80 to 90 %, 70 %, 75 % 80 %, 85 %, 90 %, 95 % or 100 % compared to the oligonucleotide having the nucleic acid sequence shown in Table 1 or 2.

Each nucleotide of the sequence can be modified, wherein ASOs of the present invention preferably comprise a core of 6 to 8 unmodified nucleotides. ASOs of the present invention comprise for example one or more modified nucleotides, e.g., 1, 2, 3, 4 or 5 nucleotides at the 5'- and/or 3'-end of the oligonucleotide, i.e., on the 5'- and/or 3'-side of

the core. The 5'- and 3'-end are modified identically or differently. If the 5'- and 3'-ends are modified identically the nucleotides are modified at the same positions counted from the 5'- and 3'-end (in each case starting the counting with 1 from the end), respectively, having the same modification for example LNA-modification. If the 5'- and 3'-ends are modified differently the position of the modified nucleotide and/or the type of modification at the 5'- and 3'-ends differ; the type of nucleotide modification is the same (e.g., LNA) or different. Modified nucleotides such as LNA-modified nucleotides need not to follow in a row, but may be separated by one or more unmodified nucleotides. In the following some modification patterns at the 5'- and 3'-end of the ASOs of the present invention are described, wherein an unmodified nucleotide is indicated by “_” and the figure refers to the number of modified nucleotides such as LNA-modified nucleotides in a row. The modified nucleotide(s) is/are at any position of the 5'- and/or 3'-end of the ASO as shown in the following **Table 3**:

LNA modification at the 5'- side of the core	LNA modification at the 3'- side of the core	Abbreviation
3	3	3+3
3	2	3+2
2	3	2+3
1_1	3	1_1+3
1_1	2	1_1+2
1_1	1_1	1_1+1_1
3	1_1	3+1_1
2	1_1	2+1_1
2	2	2+2
4	3	4+3
4	2	4+2
4	1_1	4+1_1
2_1	3	2_1+3

2_1	1_1	2_1+1_1
2_1	2	2_1+2
3	4	3+4
2	4	2+4
1_1	4	1_1+4
3	1_2	3+1_2
1_1	1_2	1_1+1_2
2	1_2	2+1_2

Typical modification patterns of each ASO of the present invention, comprising for example LNA-modified nucleotides, are shown for example in the following **Table 4** which indicates specific positions of the LNA modifications at the 5'- and 3'-end of each

5 ASO:

Position of the modification at the 5'-end (counted from the 5'-end starting with 1)	Position of the modification at the 3'-end (counted from the 3'-end starting with 1)	Abbreviation
nucleotides 1 to 5	nucleotides 1 to 5	5+5
nucleotides 1 to 4	nucleotides 1 to 4	4+4
nucleotides 1 to 3	nucleotides 1 to 3	3+3
nucleotides 1 and 2	nucleotides 1 and 2	2+2
nucleotide 1	nucleotide 1	1+1
nucleotides 1 to 5	nucleotides 1 to 4	5+4
nucleotides 1 to 4	nucleotides 1 to 3	4+3
nucleotides 1 to 3	nucleotides 1 and 2	3+2
nucleotides 1 and 2	nucleotide 1	2+1
nucleotide 1	nucleotides 1 to 5	1+5
nucleotides 1 to 5	nucleotides 1 to 3	5+3
nucleotides 1 to 4	nucleotides 1 and 2	4+2

nucleotides 1 to 3	nucleotide 1	3+1
nucleotides 1 and 2	nucleotides 1 to 5	2+5
nucleotide 1	nucleotides 1 to 4	1+4
nucleotides 1 to 5	nucleotides 1 and 2	5+2
nucleotides 1 to 4	nucleotide 1	4+1
nucleotides 1 to 3	nucleotides 1 to 5	3+5
nucleotides 1 and 2	nucleotides 1 to 4	2+4
nucleotide 1	nucleotides 1 to 3	1+3
nucleotides 1 to 5	nucleotide 1	5+1
nucleotides 1 to 4	nucleotides 1 to 5	4+5
nucleotides 1 to 3	nucleotides 1 to 4	3+4
nucleotides 1 and 2	nucleotides 1 to 3	2+3
nucleotide 1	nucleotides 1 and 2	1+2
nucleotides 1 and 2	nucleotides 1 and 3	2+1 ₁
nucleotides 1 and 3	nucleotides 1 to 3	1 ₁ +3
nucleotides 1 and 3	nucleotides 1 and 3	1 ₁ +1 ₁
nucleotides 1 to 3	nucleotides 1 and 3	3+1 ₁
nucleotides 1 to 4	nucleotides 1 and 3	4+1 ₁
nucleotides 1, 2 and 4	nucleotides 1 to 3	2 ₁ +3
nucleotides 1, 2 and 4	nucleotides 1 and 3	2 ₁ +1 ₁
nucleotides 1, 2 and 4	nucleotides 1 and 2	2 ₁ +2
nucleotides 1 and 3	nucleotides 1 to 4	1 ₁ +4
nucleotides 1 to 3	nucleotides 1,2 and 4	3+1 ₂
nucleotides 1 and 3	nucleotides 1, 2 and 4	1 ₁ +1 ₂
nucleotides 1 and 2	nucleotides 1, 2 and 4	2+1 ₂

An oligonucleotide of the present invention further or alternatively hybridizes for example with the NLRP3 nucleic acid sequence of SEQ ID NO.1 and SEQ ID NO.2 (human) or SEQ ID NO.3 and SEQ ID NO.4 (murine) in a hybridizing active area. A hybridizing active area

is an area on the NLRP3 pre-mRNA of SEQ ID NO.1 (human) or SEQ ID NO.3 (mouse), wherein binding of an oligonucleotide most likely leads to potent knockdown of the NLRP3 expression. An oligonucleotide of the present invention hybridizes for example within these positions or overlaps with a terminal position.

5

The human NLRP3 oligonucleotides of the present invention hybridize for example with hybridizing active areas of human NLRP3 pre-mRNA for example of SEQ ID NO.1. Hybridizing active areas of SEQ ID NO.1 are listed for example in the following **Table 5** as well as the oligonucleotides hybridizing in these areas:

10

Region of SEQ ID NO.1	First position on SEQ ID NO.1	SEQ ID NO.
5280-5779		
A31001H	5280	5
A31002H	5298	6
A31003H	5300	7
A31004H	5394	8
A31128H	5407	112
5780-6279		
A31005H	5861	9
A31006H	5890	10
A31007H	5898	11
A31008H	6094	12
A31009H	6148	13
A31010H	6155	14
A31011H	6156	15
A31095H	6154	87
A31129H	5873	113
A31130H	5897	114
A31131H	5902	115
A31132H	6097	116
A31133H	6144	117
A31225H	5872	209
A31226H	5935	210
A31227H	6013	211
A31228H	6012	212
A31229H	6086	213
A31230H	6081	214
A31231H	6091	215
6280-6779		
A31290Hi	6630	273

6780-7279		
A31167Hi	7195	151
7780-8279		
A31049Hi	7906	53
A31168Hi	7905	152
A31169Hi	8158	153
A31291Hi	7914	274
8280-8779		
A31292Hi	8621	275
A31293Hi	8664	276
8780-9279		
A31170Hi	8871	154
9280-9779		
A31171Hi	9654	155
A31294Hi	9563	277
A31295Hi	9619	278
A31296Hi	9629	279
9780-10279		
A31050Hi	9849	54
A31051Hi	9863	55
A31172Hi	9854	156
A31173Hi	9867	157
A31297Hi	10160	280
A31298Hi	10236	281
10280-10779		
A31012H	10370	16
A31013H	10379	17
A31014H	10450	18
A31052Hi	10682	56
A31096H	10446	88
A31119Hi	10684	103
A31174Hi	10573	158
A31175Hi	10680	159
A31176Hi	10688	160
A31177Hi	10767	161
A31232H	10370	216
A31233H	10447	217
A31299Hi	10297	282
10780-11279		
A31015H	11076	19
A31016H	11077	20
A31017H	11081	21
A31018H	11160	22
A31053Hi	10894	57

A31054Hi	10896	58
A31097H	11005	89
A31098H	11072	90
A31099H	11080	91
A31100H	11083	92
A31101H	11087	93
A31102H	11090	94
A31120Hi	10888	104
A31121Hi	10899	105
A31134H	11094	118
A31135H	11142	119
A31136H	11150	120
A31137H	11156	121
A31138H	11161	122
A31178Hi	10826	162
A31179Hi	10887	163
A31180Hi	10900	164
A31181Hi	10928	165
A31234H	11009	218
A31235H	11066	219
A31236H	11071	220
A31237H	11074	221
A31238H	11079	222
A31239H	11071	223
A31240H	11078	224
A31241H	11080	225
A31242H	11102	226
A31243H	11149	227
A31300Hi	10818	283

11280-11779

A31019H	11522	23
A31020H	11523	24
A31103H	11526	95
A31139H	11489	123
A31140H	11507	124
A31141H	11523	125
A31142H	11543	126
A31143H	11671	127
A31144H	11697	128
A31244H	11329	228
A31245H	11338	229
A31246H	11350	230
A31247H	11338	231
A31248H	11351	232

A31249H	11503	233
A31250H	11526	234
A31251H	11504	235
A31252H	11541	236
A31253H	11521	237
A31254H	11620	238
A31255H	11662	239
A31256H	11678	240
11780-12279		
A31021H	11903	25
A31022H	12205	26
A31023H	12228	27
A31024H	12249	28
A31025H	12273	29
A31026H	12274	30
A31081H	12273	29
A31082H	12273	29
A31083H	12273	29
A31084H	12273	29
A31085H	12274	30
A31086H	12274	30
A31087H	12274	30
A31088H	12274	30
A31089H	12274	85
A31090H	12274	85
A31091H	12274	85
A31092H	12275	86
A31093H	12275	86
A31094H	12275	86
A31145H	11787	129
A31146H	11903	130
A31147H	11907	131
A31148H	12124	132
A31149H	12247	133
A31257H	11819	241
A31258H	12064	242
A31259H	12068	243
A31260H	12163	244
A31261H	12165	245
A31262H	12169	246
A31263H	12272	247
A31355H	12273	29
A31356H	12273	29
A31357H	12273	29

A31358H	12273	29
A31359H	12273	29
A31360H	12273	29
A31361H	12274	29
A31362H	12274	30
A31363H	12274	30
A31364H	12274	30
A31365H	12274	30
A31366H	12274	30
A31367H	12274	30
12280-12779		
A31027H	12475	31
A31104H	12477	96
A31150H	12498	134
A31264H	12473	248
A31265H	12473	249
A31266H	12498	250
A31267H	12628	251
A31301Hi	12776	284
12780-13279		
A31302Hi	13059	285
13280-13779		
A31055Hi	13706	59
A31109Hi	13706	59
A31110Hi	13706	59
A31111Hi	13706	59
A31112Hi	13706	59
A31113Hi	13706	101
A31114Hi	13706	101
A31115Hi	13706	101
A31116Hi	13706	101
A31117Hi	13707	102
A31118Hi	13707	102
A31182Hi	13701	166
A31183Hi	13748	167
A31303Hi	13473	286
A31304Hi	13482	287
A31305Hi	13494	288
A31306Hi	13750	289
A31368Hi	13706	59
A31369Hi	13706	59
A31370Hi	13706	59
A31371Hi	13706	59
A31372Hi	13706	59

A31373Hi	13706	59
13780-14279		
A31056Hi	14070	60
A31057Hi	14184	61
A31184Hi	13843	168
A31185Hi	13926	169
A31186Hi	14071	170
A31187Hi	14081	171
A31188Hi	14266	172
A31307Hi	13900	290
14280-14779		
A31058Hi	14401	62
A31059Hi	14403	63
A31122Hi	14401	106
A31123Hi	14402	107
15280-15779		
A31308Hi	15571	291
A31309Hi	15588	292
16280-16779		
A31060Hi	16512	64
16780-17279		
A31028H	16795	32
A31029H	16846	33
A31030H	16875	34
A31105H	16873	97
A31151H	16874	135
A31189Hi	16921	173
A31268H	16843	252
A31269H	16877	253
A31310Hi	16964	293
17780-18279		
A31311Hi	18120	294
18280-18779		
A31061Hi	18501	65
A31124Hi	18502	108
A31190Hi	18502	174
A31191Hi	18654	175
A31192Hi	18775	176
A31312Hi	18559	295
18780-19279		
A31193Hi	18893	177
19280-19779		
A31062Hi	19318	66
20280-20779		

A31063Hi	20629	67
A31194Hi	20418	178
A31195Hi	20719	179
20780-21279		
A31031H	21266	35
A31032H	21279	36
A31152H	21253	136
A31153H	21256	137
A31196Hi	20984	180
A31271H	21264	254
A31272H	21276	255
A31273H	21266	256
A31274H	21278	257
21280-21779		
A31033H	21343	37
A31154H	21352	138
A31197Hi	21442	181
A31275H	21390	258
22280-22779		
A31064Hi	22478	68
A31065Hi	22594	69
A31198Hi	22476	182
A31199Hi	22478	183
A31200Hi	22544	184
22780-23279		
A31034H	23225	38
A31106H	23222	98
23280-23779		
A31201Hi	23557	185
A31313Hi	23568	296
23780-24279		
A31314Hi	23845	297
A31315Hi	23964	298
A31316Hi	24162	299
A31317Hi	24175	300
24280-24779		
A31202Hi	24672	186
A31318Hi	24450	301
24780-25279		
A31066Hi	24910	70
A31067Hi	24945	71
A31203Hi	24910	187
A31319Hi	25010	302
A31320Hi	25035	303

25280-25779		
A31068Hi	25310	72
A31204Hi	25308	188
25780-26279		
A31321Hi	25951	304
A31322Hi	26028	305
A31323Hi	26214	306
26280-26779		
A31324Hi	26674	307
26780-27279		
A31325Hi	27090	308
A31326Hi	27163	309
A31327Hi	27237	310
27280-27779		
A31328Hi	27640	311
27780-28279		
A31205Hi	28147	189
A31329Hi	27890	312
A31330Hi	28024	313
28280-28779		
A31206Hi	28750	190
A31331Hi	28745	314
28780-29279		
A31207Hi	28958	191
A31332Hi	29041	315
A31333Hi	29223	316
29280-29779		
A31069Hi	29495	73
A31070Hi	29545	74
A31208Hi	29430	192
A31209Hi	29648	193
A31334Hi	29441	317
A31335Hi	29617	318
29780-30279		
A31336Hi	29800	319
30280-30779		
A31210Hi	30615	194
A31337Hi	30601	320
A31338Hi	30732	321
30780-31279		
A31035H	31124	39
A31036H	31180	40
A31155H	31175	139
A31156H	31187	140

A31157H	31259	141
A31276H	31170	259
A31277H	31174	260
A31278H	31178	261
A31279H	31204	262
A31280H	31170	263
A31283H	31187	266
A31284H	31205	267
31280-31779		
A31211Hi	31637	195
A31212Hi	31657	196
A31339Hi	31371	322
31780-32279		
A31037H	31803	41
A31038H	31804	42
A31071Hi	32254	75
A31072Hi	32262	76
A31125Hi	32263	109
A31158H	31796	142
A31159H	31802	143
A31160H	31862	144
A31213Hi	32216	197
A31214Hi	32244	198
A31215Hi	32262	199
A31281H	31793	264
A31282H	31797	265
A31285H	31850	268
A31286H	31796	269
A31340Hi	32126	323
32780-33279		
A31216Hi	33199	200
A31341Hi	32843	324
A31342Hi	33123	325
A31343Hi	33252	326
33280-33779		
A31073Hi	33476	77
A31074Hi	33477	78
A31126Hi	33475	110
A31127Hi	33482	111
A31217Hi	33483	201
A31218Hi	33506	202
A31219Hi	33588	203
A31344Hi	33530	327
A31345Hi	33566	328

A31346Hi	33654	329
33780-34279		
A31347Hi	33941	330
A31348Hi	34241	331
A31349Hi	34244	332
34280-34779		
A31075Hi	34555	79
A31220Hi	34727	204
A31350Hi	34331	333
A31351Hi	34695	334
34780-35279		
A31076Hi	35277	80
A31077Hi	35279	81
A31221Hi	35278	205
A31222Hi	35279	206
A31352Hi	34843	335
A31353Hi	35001	336
35280-35779		
A31039H	35572	43
A31040H	35616	44
A31041H	35665	45
A31042H	35710	46
A31043H	35754	47
A31044H	35758	48
A31045H	35774	49
A31046H	35775	50
A31078Hi	35284	82
A31079Hi	35330	83
A31080Hi	35346	84
A31107H	35620	99
A31108H	35778	100
A31161H	35706	145
A31162H	35718	146
A31163H	35769	147
A31223Hi	35347	207
A31224Hi	35370	208
A31287H	35583	270
A31288H	35584	271
A31354Hi	35479	337
35780-36279		
A31047H	35781	51
A31048H	35879	52
A31164H	35782	148
A31165H	35784	149
A31166H	35874	150
A31289H	35906	272

Table 5 shows some hybridizing active regions of SEQ ID NO.1 and human NLRP3 antisense oligonucleotides hybridizing in these regions.

- 5 The mouse NLRP3 oligonucleotides of the present invention hybridize with hybridizing active regions of NLRP3 pre-mRNA for example of SEQ ID NO.3 Hybridizing active areas of SEQ ID NO.3 are for example listed in the following **Table 6** as well as the oligonucleotides hybridizing in these areas:

Region of SEQ ID NO.3	First position on SEQ ID NO.3	SEQ ID NO.
3686-4185		
A31001M	3807	342
A31002M	4083	343
A31003M	4087	344
A31098M	3705	430
A31099M	3806	431
A31100M	3806	432
A31101M	3807	433
A31102M	4081	434
A31118M	4083	343
A31119M	4096	449
A31138M	3686	465
4186-4685		
A31004M	4305	345
A31005M	4306	346
A31006M	4307	347
A31007M	4307	348
A31050Mi	4411	382
A31051Mi	4618	383
A31120MR	4307	450
A31121MR	4308	451
5186-5685		
A31052Mi	5367	384
A31053Mi	5517	385
6186-6685		
A31054Mi	6279	386
A31055Mi	6355	387
A31056Mi	6358	388
A31057Mi	6509	389
A31058Mi	6509	390
A31059Mi	6515	391

A31060Mi	6515	392
6686-7185		
A31061Mi	7132	393
A31062Mi	7133	394
7686-8185		
A31063Mi	8170	395
8186-8685		
A31064Mi	8490	396
8686-9185		
A31008M	9015	349
A31009M	9019	350
A31010M	9048	351
A31011M	9049	352
A31012M	9049	353
A31013M	9050	354
A31014M	9050	355
A31015M	9055	356
A31016M	9122	357
A31017M	9122	358
A31041M	9048	351
A31042M	9048	351
A31043M	9048	351
A31044M	9048	351
A31065Mi	8756	397
A31066Mi	8756	398
A31067Mi	8836	399
A31068Mi	8885	400
A31122M	8968	452
A31123M	9043	453
A31124M	9059	454
A31139M	9009	466
A31140M	9015	467
A31141M	9016	468
A31142M	9022	469
A31143M	9024	470
A31144M	9052	471
A31145M	9116	472
A31146M	9126	473
9186-9685		
A31018M	9599	359
A31019M	9601	360
A31020M	9602	361
A31021M	9605	362
A31125MR	9267	455

A31126M	9590	456
A31127M	9599	359
A31128M	9601	457
A31147M	9596	474
A31148M	9606	475
9686-10185		
A31022M	9869	363
A31023M	9870	364
A31024M	9870	365
A31025M	9871	366
A31026M	9873	367
A31027M	9878	368
A31028M	10044	369
A31103M	9710	435
A31104M	10097	436
A31105M	10143	437
A31106M	10143	438
A31129M	9872	458
A31130M	10019	459
A31131M	10020	460
A31132M	10044	369
A31133M	10088	461
A31134M	10090	462
A31149M	9866	476
A31150M	9876	477
A31151M	9877	478
A31152M	10024	479
10186-10685		
A31029M	10228	370
A31030M	10233	371
A31031M	10234	372
A31032M	10234	373
A31107M	10252	439
A31108M	10442	440
A31153M	10215	480
10686-11185		
A31069Mi	11021	401
11186-11685		
A31070Mi	11341	402
11686-12185		
A31071Mi	12045	403
12686-13185		
A31154M	12790	481
13186-13685		

A31072Mi	13244	404
A31073Mi	13672	405
13686-14185		
A31074Mi	13906	406
A31075Mi	14147	407
14186-14685		
A31076Mi	14378	408
15686-16185		
A31077Mi	15826	409
A31078Mi	15832	410
A31079Mi	15893	411
A31080Mi	15899	412
A31081Mi	16071	413
16186-16685		
A31082Mi	16351	414
16686-17185		
A31155M	16743	482
17186-17685		
A31083Mi	17288	415
17686-18185		
A31084Mi	17943	416
A31085Mi	18058	417
19186-19685		
A31033M	19371	374
A31135MR	19390	463
20186-20685		
A31086Mi	20304	418
A31087Mi	20517	419
A31088Mi	20545	420
22686-23185		
A31089Mi	22910	421
23186-23685		
A31090Mi	23548	422
A31091Mi	23552	423
23686-24185		
A31092Mi	23744	424
A31093Mi	23751	425
A31094Mi	23854	426
24186-24685		
A31095Mi	24285	427
25686-26185		
A31096Mi	25786	428
A31097Mi	25939	429
A31109M	26089	441

A31110M	26092	442
A31137MR	26017	464
26686-27185		
A31034M	27157	375
A31045M	27157	375
A31046M	27157	375
A31047M	27157	375
A31111M	26906	443
A31112M	26914	444
A31113M	27154	445
A31114M	27157	375
A31156M	27167	483
27186-27685		
A31035M	27405	376
A31036M	27406	377
A31037M	27409	378
A31038M	27409	379
A31039M	27411	380
A31040M	27411	381
A31048M	27405	376
A31049M	27405	376
A31115M	27211	446
A31116M	27404	447
A31117M	27413	448
A31157M	27407	484

Table 6 shows some hybridizing active regions of SEQ ID NO. 3 and mouse NLRP3 antisense oligonucleotides hybridizing in these regions.

- 5 The oligonucleotide of the present invention inhibits for example at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of NLRP3 such as the, e.g., human or murine, **NLRP3** expression compared to an untreated control. The oligonucleotide is for example administered via gymnotic delivery i.e., without a transfection reagent. The oligonucleotides of the present
- 10 invention inhibit **NLRP3 expression** for example in a cell, tissue, organ, or a subject. The oligonucleotide of the present invention inhibits the expression of **NLRP3** at a nanomolar or micromolar concentration for example 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 μ M.

15

The oligonucleotide of the present invention is used for example in a concentration of 1, 3, 5, 9, 10, 15, 27, 30, 40, 50, 75, 82, 100, 250, 300, 500, or 740 nM, or 1, 2.2, 3, 5, 6.6 or 10 μ M.

5 An oligonucleotide of the present invention hybridizes for example with at least one exon and/or intron of SEQ ID NO.1 and optionally with the mRNA of SEQ ID NO.2. The oligonucleotide hybridizes for example with 2, 3, 4 or 5 exons and/or with 2, 3, 4 or 5 introns.

10 In addition, the present invention refers to a pharmaceutical composition comprising an oligonucleotide of the present invention and a pharmaceutically acceptable carrier, excipient and/or dilutant. The pharmaceutical composition further comprises for example another active agent for example selected from the group consisting of an oligonucleotide, an antibody, a small molecule, a polypeptide, a lipid, sugar and a combination thereof.

15

The oligonucleotide or the pharmaceutical composition of the present invention is for example for use in a method of preventing and/or treating a disorder. The disorder is for example characterized by an NLRP3 imbalance, i.e., the NLRP3 level is increased in comparison to the level in a normal, healthy cell, tissue, organ or subject. The NLRP3
20 level can be measured by any standard method such as immunohistochemistry, western blot, quantitative real time PCR, HHPLC, FPLC or QuantiGene assay known to a person skilled in the art.

A disorder treatable by an oligonucleotide of the present invention or a pharmaceutical
25 composition comprising such oligonucleotide is for example selected from the group consisting of an inflammatory or autoimmune disorder, a neurological disorder, cardiovascular or metabolic disorder, renal disorder, liver disorder lung disorder, skin disorder, ocular disorder, disorder of the gastro-intestinal tract, joint inflammation, organ transplantation, fibrotic disorder and a combination thereof. Furthermore, the disorder is
30 a hyperproliferative disorder such as a cancer. A cancer is for example selected from the group consisting of 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,
35 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,
5 leiomyomater tumor, cervical dysplasia, retinoblastoma, soft tissue sarcoma, malignant carcinoid, topical skin lesion, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic sarcoma, malignant hypercalcemia, renal cell tumor, polycythemia vera, adenocarcinoma, anaplastic astrocytoma, glioblastoma multiforma, leukemia, epidermoid carcinoma and a combination thereof.

10

In more detail such disorder is for example selected from the group consisting of Alzheimer's disease, multiple sclerosis, autoimmune encephalitis, stroke, traumatic brain injury, atherosclerosis, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, hypertension, myocardial infarction, acute kidney injury, ischemia reperfusion injury,
15 chronic kidney diseases, crystal-induced nephropathies, glomerulonephritis, silicosis, asthma, allergic airway inflammation, inflammatory bowel disease, colitis ulcerosa, osteoarthritis, rheumatoid arthritis, juvenile idiopathic arthritis, transplantation of kidney, lung, liver and/or heart, fibrotic disorder of kidney, lung, liver and/or heart, hyperinflammation following influenza infection, graft-versus-host disease, interstitial
20 cystitis, uveitis, sinusitis, periodontal disease, myelodysplastic syndrome, cryopyrin-associated periodic syndromes, gout, obesity-induced inflammation, insulin resistance, type 1 and type 2 diabetes, contact hypersensitivity, psoriasis, alopecia and a combination thereof.

25 An 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, intraventricular, transdermal, rectal, intraarticular, intraocular, intravitreal, subconjunctival, retro bulbar, intra nasal, intracameral, intratracheal, intrapleural, per
30 inhalation, intraurethral and/or intra vesical. In addition, an oligonucleotide or a pharmaceutical composition of the present invention is for example used in an *ex vivo* treatment of a transplant.

35 According to the present invention, one or more oligonucleotides of the present invention can be administered together, at the same time point for example in a pharmaceutical

composition or separately, or on staggered intervals. Alternatively, one or more oligonucleotides of the present invention are for example administered together with another active agent such as another oligonucleotide (i.e., not being part of the present invention), an antibody, a small molecule, a polypeptide, a statin, a vaccine, an adjuvant, a chemotherapeutic agent, a cytotoxic agent, an allergen, an antibiotic, a siRNA molecule, a TLR antagonist, an activated cell, a cell therapy product, a peptide, a polypeptide, a protein, a gene therapy vector, and/or a co-stimulatory molecule (e.g., a cytokine, a chemokine, a protein ligand, a trans-activating factor, a peptide or peptide comprising modified amino acid and/or a therapeutic mRNA), at the same time point for example in a pharmaceutical composition or separately, or on staggered intervals. The other active agent interacts or inhibits the same target and/or a different target than the oligonucleotide of the present invention, wherein interacting means that the active agent has an indirect effect on the target. Another active agent interacts or inhibits for example a target selected from the group consisting of NLRP3, CD39, CD73, IL-1 β , IL-1 receptor, IL-1R accessory protein, IL-18, IL-18 receptor, ASC, NLRC4, AIM2, Caspase-1, RIPK3, Gasdermin D, MLKL, TLR4, Caspase-8, P2X7, NF κ B, ROR γ t, TGF- β , IL-21, IL-17, IL-22, IL-23, IL-6, TNF- α , CCR6, CCL20, STAT3, MMP-1, MMP-8, ADAMTS-5, HMG-CoA, Myd-88, HMGB-1, ROS, TAK-1, Chop, FPR1, LIMCH1, caspase inhibitor and a combination thereof.

20

Further, one or more of the oligonucleotides of the present invention are connected, e.g., via a chemical bond for example based on a linker. The chemical bond such as a linker is for example cleavable or non-cleavable.

The present invention further refers to a kit comprising an oligonucleotide and/or a pharmaceutical composition of the present invention and an instruction manual. The oligonucleotide and/or the pharmaceutical composition of the kit is for example for use in preventing and/or treating a disease caused by an imbalanced NLRP3 expression. Diseases based on such imbalanced NLRP3 expression are mentioned above. Alternatively, the kit is for example a diagnostic kit for the determination of the NLRP3 level in a sample. The sample is for example blood, saliva, urine, liquor or smear.

A subject of the present invention is for example a mammalian, a bird or a fish, wherein the mammalian is for example a human, horse, cow, cat, dog, or rabbit.

35

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
5 cells endogenously expressing NLRP3 either in an unstimulated state or after stimulation with for example LPS, 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 IC₅₀ values than endogenous systems which are closer to therapeutically relevant *in vivo* systems. Further, in the following experiments no
10 transfecting agent is used, i.e., gymnotic delivery is performed. Transfecting agents are known to increase the activity of an oligonucleotide which influences the IC₅₀ value (see for example Zhang et al., Gene Therapy, 2011, 18, 326-333; Stanton et al., Nucleic Acid Therapeutics, Vol. 22, No. 5, 2012). As artificial systems using a transfecting agent are hard or impossible to translate into therapeutic approaches and no transfection
15 formulation has been approved so far for oligonucleotides, the following experiments are performed without any transfecting agent.

Example 1: Design of mouse and human NLRP3-specific antisense oligonucleotides (ASOs)

20 For the design of human ASOs with specificity for exonic regions within the human NLRP3 gene the NLRP3 mRNA of SEQ ID NO.2 (RefSeq ID NM_004895.4) was used. For ASOs with specificity for intronic regions within the human NLRP3 gene the NLRP3 pre-mRNA of SEQ ID NO.1 (GRCh38, Chr1: 247412861-247452403) as annotated in FASTA
25 format (visible range) downloaded from https://www.ncbi.nlm.nih.gov/nuccore/NM_004895.4 was used. An “H” after the ASO ID indicates a human NLRP3-specific sequence that binds to an exonic region of the pre-mRNA and a “Hi” after the ASO ID indicates a human NLRP3-specific sequence that binds to an intronic region of the pre-mRNA. 16, 17, 18 and 19 mers were designed according to
30 in house criteria.

For the design of mouse ASOs with specificity for exonic regions within the mouse NLRP3 gene the NLRP3 mRNA of SEQ ID NO.4 (RefSeq ID NM_145827) was used. For ASOs with specificity for intronic regions within the mouse NLRP3 gene the NLRP3 pre-mRNA of SEQ ID NO.3 (GRmCh38, Chr11: 59539030-59569495) as annotated in FASTA format
35 (visible range) downloaded from https://www.ncbi.nlm.nih.gov/nuccore/NM_145827 was

used. An “M” after the ASO ID indicates a mouse NLRP3-specific sequence that binds to an exonic region of the pre-mRNA and a “Mi” after the ASO ID indicates a mouse NLRP3-specific sequence that binds to an intronic region of the pre-mRNA. 15, 16 and 17 mers were designed according to in house criteria.

5

Neg1 (SEQ ID NO.339; described in WO2014154843 A1), S5 (SEQ ID NO.486), R01002 (SEQ ID NO.340) and R01011 (SEQ ID NO.341) were used as non-targeting control oligonucleotides in some experiments (Table 1 and Table 2).

10 **Example 2: First screening - single concentration efficacy screen for human NLRP3 antisense oligonucleotides in THP-1 cells**

Knockdown efficacy of human NLRP3-specific ASOs were tested in human THP-1 cells (human monocytic cell line derived from an acute monocytic leukemia patient). The cells were treated with the respective human NLRP3-specific ASO or control oligonucleotide Neg1 (SEQ ID NO.339) at a concentration of 10 μ M. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in Fig. 2.

Treatment of THP-1 cells with the A31058Hi (SEQ ID NO.62), A31026H (SEQ ID NO.30) and A31065Hi (SEQ ID NO.69) ASOs resulted in a target inhibition of >40 % (represented by a residual NLRP3 mRNA expression of <0.6 as compared to mock-treated cells) (Fig. 2).

Example 3: First screening - single concentration efficacy screen for human NLRP3 antisense oligonucleotides in U87MG cells

30

Knockdown efficacy of human NLRP3-specific ASOs were tested in human U87MG cells (human primary glioblastoma cell line). The cells were treated with the respective human NLRP3-specific ASO or control oligonucleotide Neg1 at a concentration of 10 μ M. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, cells were lysed. Human

35

HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in Fig. 3.

5

Treatment of U87-MG cells with the A31059Hi (SEQ ID NO.63), A31026H (SEQ ID NO.30), A31001H (SEQ ID NO.5) and A31025H (SEQ ID NO.29) ASOs resulted in a target inhibition of >40 % (represented by a residual NLRP3 mRNA expression of <0.6 as compared to mock-treated cells) (Fig. 3).

10

Example 4: First screening - single concentration efficacy screen for human NLRP3 antisense oligonucleotides in PBMC-derived macrophages

Knockdown efficacy of selected human NLRP3-specific ASOs from the first screening round were tested in human peripheral blood mononuclear cell (PBMC)-derived macrophages. PBMC were seeded in 96-well flat bottom plate and after two hours, non-adherent cells were washed away and the adherent cells (predominantly monocytes) were cultured in the presence of 20 ng/ml hGM-CSF and 50 ng/ml hIL-4. Cells were treated with the respective human NLRP3-specific ASO or control oligonucleotide Neg1 at a concentration of 10 μ M. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, the supernatant and loose cells were removed and replaced by fresh medium containing 20 ng/ml hGM-CSF, 50 ng/ml hIL-4 and the respective human NLRP3-specific ASO or control oligonucleotide Neg1 at a concentration of 10 μ M. On day 6, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in Fig. 4.

Treatment of PBMC-derived macrophages with the A31025H (SEQ ID NO.29), A31026H (SEQ ID NO.30), A31059Hi (SEQ ID NO.63), A31065Hi (SEQ ID NO.69) and A31055Hi (SEQ ID NO.59) ASOs resulted in a target inhibition of >70 % (represented by a residual NLRP3 mRNA expression of <0.3 as compared to mock-treated cells) (Fig. 4).

35

Example 5: Second screening - single concentration efficacy screen of NLRP3-specific ASOs in U87MG cells

Knockdown efficacy of human NLRP3-specific ASOs was tested in human U87MG cells in a second screening round. The cells were treated with the respective human NLRP3-specific ASO or control oligonucleotide Neg1 (SEQ ID NO.339) at a concentration of 10 μ M. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in Fig. 5.

Treatment of U87-MG cells with the A31111Hi (SEQ ID NO.59), A31110Hi (SEQ ID NO.59), A31025H (SEQ ID NO.29), A31026H (SEQ ID NO.30), A31092H (SEQ ID NO.86), A31085H (SEQ ID NO.30), A31091H (SEQ ID NO.85) and A31083H (SEQ ID NO.29) ASOs resulted in a target inhibition of >66 % (represented by a residual NLRP3 mRNA expression of <0.34 as compared to mock-treated cells) (Fig. 5).

Example 6: Second screening - single concentration efficacy screen of human NLRP3-specific ASOs in PBMC-derived macrophages

Knockdown efficacy of selected human NLRP3-specific ASOs from the second screening round were tested in PBMC-derived macrophages. Plastic adherent PBMC were treated with 10 μ M of the respective human NLRP3-specific ASO or control oligonucleotide Neg1 (SEQ ID NO.339) in the presence of 20 ng/ml hGM-CSF and 50 ng/ml hIL-4. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in Fig. 6.

Treatment of PBMC-derived macrophages with the A31111Hi (SEQ ID NO.59), A31109Hi (SEQ ID NO.59), A31085H (SEQ ID NO.30), A31086H (SEQ ID NO.30), A31084H (SEQ

ID NO.29), A31092H (SEQ ID NO.86), A31089H (SEQ ID NO.85) and A31026H (SEQ ID NO.30) ASOs resulted in a target inhibition of at least >66 % (represented by a residual NLRP3 mRNA expression of <0.34 as compared to mock-treated cells) (Fig. 6).

5 Example 7: Investigation of the concentration-dependent target knockdown by selected human NLRP3-specific ASOs in human PBMC-derived macrophages

The concentration-dependent knockdown of NLRP3 mRNA expression by selected human NLRP3-specific ASOs from the first and second screening round was investigated on mRNA level in human PBMC-derived macrophages and the respective IC₅₀ values were calculated. Plastic adherent PBMC were cultured in the presence of 20 ng/ml hGM-CSF and 50 ng/ml hIL-4 and treated for three days with the respective ASO at the following concentrations: 10000 nM, 3333 nM, 1111 nM, 370 nM, 123 nM, 41 nM and 14 nM. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides. After three days, the cell culture supernatant and loose cells were removed and replaced by fresh medium containing 40 ng/ml hGMCSF and 100 ng/ml hIL-4 and antisense oligonucleotides at a final concentration ranging from 14 nM to 10 µM. On day six, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in Fig. 7 and Table 7. The half maximal inhibitory concentration (IC₅₀) values of the concentration response curve for selected candidates are indicated in the Table 8. All ASOs concentration-dependently inhibited the expression of NLRP3 mRNA.

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ASO ID	Concentration	Residual NLRP3 mRNA expression compared to mock-treated cells (set as 1)	ASO ID	Concentration	Residual NLRP3 mRNA expression compared to mock-treated cells (set as 1)
A31025H (SEQ ID NO.29)	10 µM	0.18	A31089H (SEQ ID NO.85)	10 µM	0.17
	3.3 µM	0.22		3.3 µM	0.32
	1.1 µM	0.29		1.1 µM	0.45
	370 nM	0.47		370 nM	0.50
	123 nM	1.05		123 nM	0.59

	41 nM	1.14		41 nM	0.67
	14 nM	0.96		14 nM	0.76
A31026H (SEQ ID NO. 30)	10 μ M	0.11	A31092H (SEQ ID NO.86)	10 μ M	0.29
	3.3 μ M	0.31		3.3 μ M	0.43
	1.1 μ M	0.42		1.1 μ M	0.60
	370 nM	0.73		370 nM	0.59
	123 nM	1.37		123 nM	0.96
	41 nM	1.47		41 nM	0.93
	14 nM	1.22		14 nM	0.77
A31055Hi (SEQ ID NO.59)	10 μ M	0.17	A31109Hi (SEQ ID NO.59)	10 μ M	0.45
	3.3 μ M	0.47		3.3 μ M	0.52
	1.1 μ M	0.87		1.1 μ M	0.80
	370 nM	1.12		370 nM	0.69
	123 nM	1.08		123 nM	0.71
	41 nM	1.65		41 nM	1.04
	14 nM	1.17		14 nM	1.27
A31084H (SEQ ID NO.29)	10 μ M	0.20	A31110Hi (SEQ ID NO.59)	10 μ M	0.30
	3.3 μ M	0.25		3.3 μ M	0.64
	1.1 μ M	0.33		1.1 μ M	1.01
	370 nM	0.54		370 nM	0.73
	123 nM	0.95		123 nM	0.99
	41 nM	1.06		41 nM	1.09
	14 nM	1.39		14 nM	1.39
A31085H (SEQ ID NO.30)	10 μ M	0.26	A31111Hi (SEQ ID NO.59)	10 μ M	0.25
	3.3 μ M	0.32		3.3 μ M	0.54
	1.1 μ M	0.57		1.1 μ M	0.86
	370 nM	0.79		370 nM	0.87
	123 nM	1.04		123 nM	1.03
	41 nM	1.44		41 nM	1.12
	14 nM	1.53		14 nM	1.52
A31086H (SEQ ID NO.30)	10 μ M	0.17			
	3.3 μ M	0.22			
	1.1 μ M	0.39			
	370 nM	0.59			
	123 nM	1.02			
	41 nM	1.14			
	14 nM	1.01			

Table 7: Concentration-dependent inhibition of NRLP3 mRNA expression in PBMC-derived macrophages by selected NLRP3-specific ASOs after six days treatment.

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ASO ID	IC ₅₀ values (nM)	R squared
A31025H	248.50	0.86
A31026H	281.00	0.82
A31055Hi	2190.00	0.71
A31084H	100.90	0.76
A31085H	154.40	0.85
A31086H	298.00	0.89
A31092H	949.90	0.55

10 **Table 8:** Half maximal inhibitory concentration (IC₅₀) values and R values of selected human NLRP3-specific ASOs after six days treatment.

Example 8: Third screening - single concentration efficacy screen for human NLRP3-specific antisense oligonucleotides in THP-1-derived macrophages

15 Knockdown efficacy of human NLRP3-specific ASOs were tested in human THP-1-derived macrophages in a third screening round. THP-1 cells were differentiated into macrophages by addition of 10 nM PMA. The respective NLRP3-specific ASOs or control oligonucleotides (Neg1 (SEQ ID NO.339), R01002 (SEQ ID NO.340) and R01011 (SEQ ID NO.341)) were added at 5 μ M final concentration. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control).
 20 After three days treatment, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 8**.
 25 Treatment of THP-1-derived macrophages with the A31025H (SEQ ID NO.29), A31111Hi (SEQ ID NO.59), A31109Hi (SEQ ID NO.59), A31195Hi (SEQ ID NO.179), A31112Hi (SEQ ID NO.59), A31203Hi (SEQ ID NO.187), A31194Hi (SEQ ID NO.178), A31187Hi (SEQ ID NO.171) and A31055Hi (SEQ ID NO.59) ASOs resulted in a target inhibition of >66 % (represented by a residual NLRP3 mRNA expression of <0.34 as compared to mock-treated cells) (**Fig. 8**).
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Example 9: Third screening - single concentration efficacy screen of human NLRP3-specific ASOs in human PBMC-derived macrophages

35 Knockdown efficacy of human NLRP3-specific ASOs was tested in PBMC-derived macrophages in a third screening round. Adherent PBMC cells were cultured in the

presence of 20 ng/ml hGM-CSF and 50 ng/ml hIL-4. Cells were treated with the respective human NLRP3-specific ASO or control oligonucleotides (Neg1 (SEQ ID NO.339), R01002 (SEQ ID NO.340) and R01011 (SEQ ID NO.341)) at a concentration of 5 μ M. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides.

5 After three days treatment, the supernatant and loose cells were removed and replaced by fresh medium containing 20 ng/ml hGM-CSF, 50 ng/ml hIL-4 and the respective human NLRP3-specific ASO or control oligonucleotide at a concentration of 5 μ M. On day six, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were

10 normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 9**.

Treatment of PBMC-derived macrophages with the A31151H (SEQ ID NO.135), A31026H (SEQ ID NO.30), A31146H (SEQ ID NO.130), A31135H (SEQ ID NO.119), A31149H (SEQ

15 ID NO.133), A31213Hi (SEQ ID NO.197), A31203Hi (SEQ ID NO.187), A31150H (SEQ ID NO.134) and A31025H (SEQ ID NO.29) ASOs resulted in a target inhibition of >67 % (represented by a residual NLRP3 mRNA expression of <0.33 as compared to mock-treated cells (untreated control)) (**Fig. 9**).

20 **Example 10: Fourth screening - single concentration efficacy screen for human NLRP3-specific antisense oligonucleotides in THP-1-derived macrophages**

Knockdown efficacy of human NLRP3-specific ASOs was tested in human THP-1-derived macrophages in a fourth screening round. THP-1 cells were differentiated into

25 macrophages by addition of 10 nM PMA. The respective NLRP3-specific ASOs or control oligonucleotides (Neg1 (SEQ ID NO.339), R01002 (SEQ ID NO.340) and R01011 (SEQ ID NO.341)) were added at 5 μ M final concentration. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, cells were lysed. Human HPRT1 and human NLRP3 mRNA

30 expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 10**.

Treatment of THP-1-derived macrophages with the A31352Hi (SEQ ID NO.335),

35 A31055Hi (SEQ ID NO.59), A31314Hi (SEQ ID NO.297), A31317Hi (SEQ ID NO.300),

A31332Hi (SEQ ID NO.315), A31324Hi (SEQ ID NO.307), A31304Hi (SEQ ID NO.287) and A31302Hi (SEQ ID NO.285) ASOs resulted in a target inhibition of >67 % (represented by a residual NLRP3 mRNA expression of <0.33 as compared to mock-treated cells) (Fig. 10).

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Example 11: Fourth screening - single concentration efficacy screen of human NLRP3-specific ASOs in PBMC-derived macrophages

Knockdown efficacy of human NLRP3-specific ASOs was tested in PBMC-derived macrophages in a fourth screening round. Adherent PBMC cells were cultured in the presence of 20 ng/ml hGM-CSF and 50 ng/ml hIL-4. Cells were treated with the respective human NLRP3-specific ASO or control oligonucleotides (Neg1 (SEQ ID NO.339), R01002 (SEQ ID NO.340) and R01011 (SEQ ID NO.341)) at a concentration of 5 μ M. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, the supernatant and loose cells were removed and replaced by fresh medium containing 20 ng/ml hGM-CSF, 50 ng/ml hIL-4 and the respective human NLRP3-specific ASO or control oligonucleotide at a concentration of 5 μ M. On day six, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in Fig. 11.

Treatment of PBMC-derived macrophages with the A31302Hi (SEQ ID NO.285), A31352Hi (SEQ ID NO.335), A31026H (SEQ ID NO.30), A31307Hi (SEQ ID NO.290), A31055Hi (SEQ ID NO.59), A31304Hi (SEQ ID NO.287) and A31303Hi (SEQ ID NO.286) ASOs resulted in a target inhibition of >80 % (represented by a residual NLRP3 mRNA expression of <0.2 as compared to mock-treated cells) (Fig. 11).

Example 12: Investigation of the concentration-dependent target knockdown by selected human NLRP3-specific ASOs in human THP-1-derived macrophages

The concentration-dependent knockdown of NLRP3 mRNA expression by selected human NLRP3-specific ASOs from the first, second, third and fourth screening round was investigated on mRNA level in human THP-1-derived macrophages and the respective

IC₅₀ values were calculated. THP-1 cells differentiated to macrophages in the presence of 10 nM PMA were treated for three days with the respective ASO at the following concentrations: 10000 nM, 3333 nM, 1111 nM, 370 nM, 123 nM, 41 nM and 14 nM. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides. After three days, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 12** and **Table 9**. The half maximal inhibitory concentration (IC₅₀) values of the dose response curve for selected candidates are indicated in the **Table 10**. All ASOs dose-dependently inhibited the expression of NLRP3 mRNA.

ASO ID	Concentration	Residual NLRP3 mRNA expression compared to mock-treated cells (set as 1)	ASO ID	Concentration	Residual NLRP3 mRNA expression compared to mock-treated cells (set as 1)
A31055Hi (SEQ ID NO.59)	14 nM	0.82	A31314Hi (SEQ ID NO.297)	14 nM	0.97
	41 nM	0.86		41 nM	0.89
	123 nM	0.81		123 nM	0.80
	370 nM	0.62		370 nM	0.59
	1.1 µM	0.54		1.1 µM	0.46
	3.3 µM	0.37		3.3 µM	0.37
	10 µM	0.31		10 µM	0.23
A31109Hi (SEQ ID NO.59)	14 nM	0.84	A31317Hi (SEQ ID NO.300)	14 nM	0.85
	41 nM	0.93		41 nM	0.86
	123 nM	0.71		123 nM	0.84
	370 nM	0.58		370 nM	0.70
	1.1 µM	0.39		1.1 µM	0.58
	3.3 µM	0.32		3.3 µM	0.45
	10 µM	0.25		10 µM	0.37
A31111Hi (SEQ ID NO.59)	14 nM	0.66	A31324Hi (SEQ ID NO.307)	14 nM	1.00
	41 nM	0.91		41 nM	0.91
	123 nM	0.71		123 nM	0.88
	370 nM	0.59		370 nM	0.73
	1.1 µM	0.46		1.1 µM	0.65
	3.3 µM	0.43		3.3 µM	0.58
	10 µM	0.32		10 µM	0.48
A31149H	14 nM	-	A31332Hi	14 nM	0.93

(SEQ ID NO.133)	41 nM	0.80	(SEQ ID NO.315)	41 nM	0.86
	123 nM	0.76		123 nM	0.82
	370 nM	0.57		370 nM	0.70
	1.1 μ M	0.51		1.1 μ M	0.58
	3.3 μ M	0.40		3.3 μ M	0.44
	10 μ M	0.30		10 μ M	0.27
A31203Hi (SEQ ID NO.187)	14 nM	1.05	A31334Hi (SEQ ID NO.317)	14 nM	1.09
	41 nM	0.95		41 nM	1.03
	123 nM	0.82		123 nM	0.93
	370 nM	0.69		370 nM	0.85
	1.1 μ M	0.54		1.1 μ M	0.67
	3.3 μ M	0.38		3.3 μ M	0.60
A31243H (SEQ ID NO.227)	10 μ M	0.29	A31352Hi (SEQ ID NO.335)	10 μ M	0.48
	14 nM	0.98		14 nM	0.95
	41 nM	0.94		41 nM	0.90
	123 nM	0.74		123 nM	0.61
	370 nM	0.65		370 nM	0.44
	1.1 μ M	0.51		1.1 μ M	0.28
A31292Hi (SEQ ID NO.275)	3.3 μ M	0.37	A31353Hi (SEQ ID NO.336)	3.3 μ M	0.21
	10 μ M	0.30		10 μ M	0.19
	14 nM	1.01		14 nM	1.03
	41 nM	0.86		41 nM	0.94
	123 nM	0.71		123 nM	0.82
	370 nM	0.70		370 nM	0.70
A31302Hi (SEQ ID NO.285)	1.1 μ M	0.63	A31331Hi (SEQ ID NO.314)	1.1 μ M	0.57
	3.3 μ M	0.51		3.3 μ M	0.41
	10 μ M	0.40		10 μ M	0.35
	14 nM	0.89		14 nM	-
	41 nM	0.87		41 nM	1.01
	123 nM	0.86		123 nM	0.95
A31304Hi (SEQ ID NO.287)	370 nM	0.73		370 nM	0.88
	1.1 μ M	0.59		1.1 μ M	0.74
	3.3 μ M	0.48		3.3 μ M	0.59
	10 μ M	0.35		10 μ M	0.47
	14 nM	0.93			
	41 nM	0.90			
	123 nM	0.76			
	370 nM	0.62			
	1.1 μ M	0.48			
	3.3 μ M	0.41			
	10 μ M	0.31			

Table 9: Concentration-dependent inhibition of NLRP3 mRNA expression in THP-1-derived macrophages by selected NLRP3-specific ASOs after three days treatment.

	ASO	IC ₅₀ (nM)	R squared
5	A31352Hi (SEQ ID NO.335)	163,3	0,97
	A31314Hi (SEQ ID NO.297)	439,9	0,98
	A31109Hi (SEQ ID NO.59)	297,3	0,94
	A31332Hi (SEQ ID NO.315)	4925	0,98
10	A31203Hi (SEQ ID NO.187)	589,7	0,96
	A31149H (SEQ ID NO.133)	604,6	0,90
	A31243H (SEQ ID NO.227)	411	0,94
	A31304Hi (SEQ ID NO.287)	342,7	0,97
15	A31055Hi (SEQ ID NO.59)	1449	0,88
	A31111Hi (SEQ ID NO.59)	-	0,70
	A31302Hi (SEQ ID NO.285)	-	0,92
	A31353Hi (SEQ ID NO.336)	538	0,96
20	A31317Hi (SEQ ID NO.300)	2972	0,88
	A31292Hi (SEQ ID NO.275)	599,7	0,87
	A31331Hi (SEQ ID NO.314)	1595	0,88
25	A31334Hi (SEQ ID NO.317)	798,1	0,92
	A31324Hi (SEQ ID NO.307)	515,3	0,95

Table 10: Half maximal inhibitory concentration (IC₅₀) values and R values of selected human NLRP3-specific ASOs after three days treatment.

Example 13: Efficacy of selected human NLRP3 antisense oligonucleotides in human microglial HMC3 cells

Knockdown efficacy of human NLRP3-specific ASOs selected from previous screening rounds were tested in human HMC3 cells (human microglial clone 3 cell line). The cells

were treated with the respective human NLRP3-specific ASO or control oligonucleotides R01002 (SEQ ID NO.340), R01011 (SEQ ID NO.341) or Neg1 (SEQ ID NO.339) at a concentration of 5 μ M. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 13**.

Treatment of HMC3 cells with the A31352Hi (SEQ ID NO.335), A31026H (SEQ ID NO.30), A31055Hi (SEQ ID NO.59) and A31025H (SEQ ID NO.29) ASOs resulted in a target inhibition of >40 % (represented by a residual NLRP3 mRNA expression of <0.6 as compared to mock-treated cells) (**Fig. 13**).

Example 14: Fifth screening - single concentration efficacy screen for human NLRP3-specific antisense oligonucleotides in THP-1-derived macrophages

Knockdown efficacy of human NLRP3-specific ASOs was tested in human THP-1-derived macrophages in a fifth screening round. THP-1 cells were differentiated into macrophages by addition of 10 nM PMA. The respective NLRP3-specific ASOs or control oligonucleotides (Neg1 (SEQ ID NO.339), R01002 (SEQ ID NO.340) and R01011 (SEQ ID NO.341)) were added at 5 μ M final concentration. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides. After three days treatment, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 14**.

Treatment of THP-1-derived macrophages with the A31358H (SEQ ID NO.29), A31359H (SEQ ID NO.29) and A31026H (SEQ ID NO.30) ASOs resulted in a target inhibition of >60 % (represented by a residual NLRP3 mRNA expression of <0.4 as compared to mock-treated cells) (**Fig. 14**).

Example 15: Fifth screening - single concentration efficacy screen of human NLRP3-specific ASOs in PBMC-derived macrophages

Knockdown efficacy of human NLRP3-specific ASOs was tested in PBMC-derived macrophages in a fifth screening round. Adherent PBMCs were cultured in the presence of 20 ng/ml hGM-CSF and 50 ng/ml hIL-4. Cells were treated with the respective human NLRP3-specific ASO or control oligonucleotides (Neg1 (SEQ ID NO.339), R01002 (SEQ ID NO.340) and R01011 (SEQ ID NO.341)) at a concentration of 5 μ M. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, the supernatant and loose cells were removed and replaced by fresh medium containing 20 ng/ml hGM-CSF, 50 ng/ml hIL-4 and the respective human NLRP3-specific ASO or control oligonucleotide at a concentration of 5 μ M. On day six, cells were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 15**.

Treatment of PBMC-derived macrophages with the A31365H (SEQ ID NO.30), A31371Hi (SEQ ID NO.59) and A31372Hi (SEQ ID NO.59) ASOs resulted in a target inhibition of >60 % (represented by a residual NLRP3 mRNA expression of <0.4 as compared to mock-treated cells) (**Fig. 15**).

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Example 16: NLRP3 mRNA knockdown prevents cleavage of pro-IL-1 β and secretion of mature IL-1 β in human THP-1-derived macrophages

In order to further investigate the target knockdown efficacy of selected human NLRP3-specific ASOs at the mRNA level, THP-1 cells were treated with the NLRP3-specific ASOs A31109Hi (SEQ ID NO.59), A31149H (SEQ ID NO. 133), A31314Hi (SEQ ID NO.297) or A31352Hi (SEQ ID NO.335) or the control oligonucleotide Neg1 (SEQ ID NO.339), R01002 (SEQ ID NO.340) or R01011 (SEQ ID NO.341), respectively, at a concentration of 5 μ M. Mock-treated cells (no antisense oligonucleotide) were cultured without the addition of oligonucleotides (untreated control). After three days treatment, medium was replaced by fresh medium containing 5 nM Phorbol 12-myristate 13-acetate (PMA) and the respective human NLRP3-specific ASO or control oligonucleotide at a concentration of 5 μ M. After six days treatment, cell culture supernatant was replaced by serum free medium containing 10 μ g/ml LPS for 4h (signal 1) and 5 mM ATP for 30 min (signal 2) in order to induce IL-1 β production and activate the inflammasome complex. Afterwards the cells

were lysed. Human HPRT1 and human NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in Fig. 16A. Treatment of THP-1-derived
5 macrophages with the selected NLRP3-specific ASOs resulted in a target inhibition of >58 % (represented by a residual NLRP3 mRNA expression of <0.42 as compared to mock-treated cells) (**Fig. 16A**).

In order to further investigate the effect of selected human NLRP3-specific ASOs in the
10 inflammasome activation, Caspase-1 activity was assessed by Caspase-Glo® 1 Inflammasome Assay in cell lysate of THP-1-derived macrophages after six days ASO treatment. Data are shown in **Fig. 16B** as mean and SD. ASO treatment with the selected NLRP3-specific ASOs resulted in a caspase-1 activity inhibition of >65 % (represented by a caspase activity <0.35 as compared to mock treated cells set as 1) (**Fig. 16B**).

15 As part of the inflammasome complex, NLRP3 is required for cleavage of pro-IL-1 β into its mature and secreted form. As a proof-of-concept it was aimed to demonstrate that IL-1 β secretion by THP-1-derived macrophages can be inhibited through treatment with NLRP3 specific antisense oligonucleotides. Pro-IL1 β and mature IL-1 β protein expression
20 was analyzed in cell lysates and cell culture supernatants of THP-1-derived macrophages by western blot after six days ASO treatment (**Fig. 16C**). Mature IL-1 β was only present in the supernatants of either mock-treated cells or cells treated with the control oligonucleotides, but not in cells treated with NLRP3-specific A31109Hi (SEQ ID NO.59), A31149H (SEQ ID NO. 133), A31314Hi (SEQ ID NO.297) or A31352Hi (SEQ ID NO.335)
25 (**Fig. 16C**). These results demonstrated that inhibition of NLRP3 on mRNA level with antisense oligonucleotides effectively prevented the cleavage of pro-IL-1 β in THP-1-derived macrophages.

Example 17: First screening - single concentration efficacy screen for mouse 30 NLRP3-specific antisense oligonucleotides in 4T1 cells

Knockdown efficacy of mouse NLRP3-specific ASOs was tested in mouse 4T1 cells (breast cancer cell line derived from the mammary gland tissue of a mouse). The cells were treated with the respective mouse NLRP3-specific ASO or control oligonucleotide Neg1 (SEQ ID
35 NO.339) at a concentration of 10 μ M. As unstimulated 4T1 cells did not express NLRP3 to

a sufficient extent, LPS was added to the cell culture at a final concentration of 1 µg/ml. After three days treatment, cells were lysed. Mouse HPRT1 and mouse NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 17A**.

Treatment of 4T1 cells with the A31034M (SEQ ID NO.375), A31040M (SEQ ID NO.381) and A31035M (SEQ ID NO.376) ASOs resulted in a target inhibition of ≥ 80 % (represented by a residual NLRP3 mRNA expression of < 0.2 as compared to mock-treated cells) (**Fig. 17A**).

Cell viability of 4T1 cells after three days ASO treatment was also determined by Cell Titer Blue assay. Absolute Fluorescence Intensity (FI) normalized to mock-treated cells ("no antisense oligonucleotides" set as 1) is shown in **Fig. 17B**. Treatment of 4T1 cells with mouse NLRP3-specific ASOs for three days has no effect on cell viability (**Fig. 17B**).

Example 18: First screening - single concentration efficacy screen for mouse NLRP3-specific antisense oligonucleotides in Raw246.7 cells

Knockdown efficacy of mouse NLRP3-specific ASOs was tested in mouse Raw246.7 cells (murine macrophage from blood). The cells were treated with the respective mouse NLRP3-specific ASO or control oligonucleotide Neg1 (SEQ ID NO.339) at a concentration of 10 µM. After three days treatment, cells were lysed. Mouse HPRT1 and mouse NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 18**.

Treatment of Raw246.7 cells with the A31010M (SEQ ID NO.351), A31007M (SEQ ID NO.348), A31011M (SEQ ID NO.352) and A31008 (SEQ ID NO.349) ASOs resulted in a target inhibition of > 70 % (represented by a residual NLRP3 mRNA expression of < 0.3 as compared to mock-treated cells) (**Fig. 18**).

Example 20: Second screening - single concentration efficacy screen for mouse NLRP3-specific antisense oligonucleotides in Raw246.7 cells

Knockdown efficacy of mouse NLRP3-specific ASOs was tested in mouse Raw246.7 cells in a second screening round. Raw246.7 cells were treated with the respective mouse NLRP3-specific ASO or control oligonucleotide Neg1 (SEQ ID NO.339) at a concentration of 10 μ M. After three days treatment, cells were lysed. Mouse HPRT1 and mouse NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-treated cells (set as 1) is shown as mean and SD in **Fig. 19A** and **Fig. 19B**

Treatment of Raw246.7 cells with A31010M (SEQ ID NO. 351), A31042M (SEQ ID NO. 351), A31041M (SEQ ID NO. 351), A31044M (SEQ ID NO. 351), A31034M (SEQ ID NO. 375), A31035M (SEQ ID NO. 376), A31045M (SEQ ID NO. 375) and A31043M (SEQ ID NO. 351) ASOs had a knockdown efficacy of >90% (represented by a residual NLRP3 mRNA expression of < 0.1 as compared to mock treated cells) in Raw246.7 cells after three days treatment (**Fig. 19A**). Among the antisense oligonucleotides that target the introns of the NLRP3 pre-mRNA, 18 caused a target knockdown of >80% (represented by a residual NLRP3 mRNA expression of <0.2) with two antisense oligonucleotides (A31052Mi (SEQ ID NO.384) and A31071Mi (SEQ ID NO.403)) inhibiting target mRNA expression by >90% (**Fig. 19B**)

Example 20: Second screening - single concentration efficacy screen for mouse NLRP3-specific antisense oligonucleotides in 4T1 cells

Knockdown efficacy of the most potent antisense oligonucleotides in Raw246.7 cells from the second screening round were further screened in 4T1 cells. 4T1 cells were treated with the respective antisense oligonucleotides or control oligonucleotide (Neg1, SEQ ID NO.339) at a final concentration of 10 μ M. To induce NLRP3 mRNA expression, cells were simultaneously treated with 1 μ g/ml LPS. After three days, cells were lysed and mouse HPRT1 as well as mouse NLRP3 mRNA expression was measured using the QuantiGene RNA Singleplex assay. NLRP3 mRNA expression values were normalized to expression of the housekeeping gene HPRT1. Residual NLRP3-mRNA expression relative to mock-treated cells ("no antisense oligonucleotides" set as 1) is shown as mean and SD as shown in **Fig. 20**.

Treatment of 4T1 cells with the A31094Mi (SEQ ID NO.426), A31087Mi (SEQ ID NO.419), A31056Mi (SEQ ID NO.388), A31084Mi (SEQ ID NO.416) and A31080Mi (SEQ ID NO.412) ASOs resulted in a target inhibition of >85 % (represented by a residual NLRP3 mRNA expression of <0.15 as compared to mock-treated cells) (**Fig. 20**).

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Example 21: Concentration response curve of selected mouse NLRP3-specific antisense oligonucleotides in Raw246.7 cells

Selected antisense oligonucleotides from the first and second screening rounds which showed no signs of cellular toxicity *in vitro* were selected for IC₅₀ determination. Raw246.7 cells were treated with the respective mouse NLRP3-specific ASO at the following concentrations: 10000 nM, 5000 nM, 2500 nM, 1250 nM, 325 nM, 313 nM and 156 nM. After three days, cells were lysed and mouse HPRT1 as well as mouse NLRP3 mRNA expression was measured using the QuantiGene RNA Singleplex assay (**Fig. 21**). NLRP3 mRNA expression values were normalized to expression of the housekeeping gene HPRT1. Residual NLRP3 mRNA expression relative to mock-treated cells (“no antisense oligonucleotide” set as 1) is shown as mean and SD in **Fig. 21** and **Table 11**. The half maximal inhibitory concentration (IC₅₀) values of the concentration response curve for selected candidates are indicated in the **Table 12**. All ASOs concentration-dependently inhibited the expression of NLRP3 mRNA with two candidates having an IC₅₀ value in nanomolar range.

ASO ID	Concentration	Residual NLRP3 mRNA expression compared to mock-treated cells (set as 1)	ASO ID	Concentration	Residual NLRP3 mRNA expression compared to mock-treated cells (set as 1)
A31010M (SEQ ID NO.351)	10 µM	0.20	A31084Mi (SEQ ID NO.416)	10 µM	0.21
	5 µM	0.32		5 µM	0.22
	2.5 µM	0.36		2.5 µM	0.44
	1.25 µM	0.51		1.25 µM	0.65
	625 nM	0.68		625 nM	0.92
	313 nM	0.67		313 nM	1.11
	156 nM	0.73		157 nM	1.12
A31034M	10 µM	0.26		10 µM	0.15
	5 µM	0.40		5 µM	0.20

(SEQ ID NO.375)	2.5 μ M	0.36	A31087Mi (SEQ ID NO.419)	2.5 μ M	0.25
	1.25 μ M	0.56		1.25 μ M	0.67
	625 nM	1.12		625 nM	0.94
	313 nM	1.07		313 nM	0.97
	157 nM	0.82		157 nM	1.33
A31041Mi (SEQ ID NO.351)	10 μ M	0.24	A31094Mi (SEQ ID NO.426)	10 μ M	0.22
	5 μ M	0.39		5 μ M	0.33
	2.5 μ M	0.38		2.5 μ M	0.46
	1.25 μ M	0.99		1.25 μ M	0.78
	625 nM	1.27		625 nM	0.94
	313 nM	1.17		313 nM	0.95
	157 nM	1.05		157 nM	0.95
A31045M (SEQ ID NO.375)	10 μ M	0.36	A31117M (SEQ ID NO.448)	10 μ M	0.28
	5 μ M	0.36		5 μ M	0.28
	2.5 μ M	0.49		2.5 μ M	0.40
	1.25 μ M	0.54		1.25 μ M	0.65
	625 nM	0.85		625 nM	0.85
	313 nM	0.98		313 nM	0.78
	157 nM	1.26		157 nM	0.87
A31052Mi (SEQ ID NO.384)	10 μ M	0.08			
	5 μ M	0.27			
	2.5 μ M	0.64			
	1.25 μ M	0.73			
	625 nM	0.93			
	313 nM	0.93			
	157 nM	1.03			

Table 11: Concentration-dependent inhibition of NRLP3 mRNA expression in Raw246.7 cells by selected NLRP3-specific ASOs after 3 days treatment.

Antisense oligonucleotide	IC ₅₀ [nM]	R squared
A31010M (SEQ ID NO.351)	560.9	0.76
A31034M (SEQ ID NO.375)	~ 1206	0.75
A31041M (SEQ ID NO.351)	1721	0.79
A31045M (SEQ ID NO.375)	851.7	0.83
A31052Mi (SEQ ID NO.384)	4039	0.89
A31084Mi (SEQ ID NO.416)	1446	0.93
A31087Mi (SEQ ID NO.419)	1327	0.85
A31094Mi (SEQ ID NO.426)	1824	0.94

A31117M (SEQ ID NO.448)	1498	0.90
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Table 12: Half maximal inhibitory concentration (IC₅₀) values and R values of selected mouse NLRP3-specific ASOs after three days treatment.

5 Example 22: Murine NLRP3-specific antisense oligonucleotides prevent cleavage of pro-IL-1 β in mouse bone marrow derived macrophages (BMDM)

Knockdown efficacy of the selected mouse NLRP3-specific ASOs A31087Mi (SEQ ID NO.419) was further screened in bone marrow derived macrophages (BMDM). For that,
 10 murine bone marrow cells were differentiated into BMDM for seven days in the presence of 50 ng/ml M-CSF. During the last four days of the BMDM differentiation protocol, cells were treated with the respective mouse NLRP3-specific ASOs A31087Mi or control oligonucleotide (Neg1 (SEQ ID NO.339) or R01002 (SEQ ID NO.340) at a concentration of 10 μ M. After seven days treatment, cell culture supernatant was replaced by serum free
 15 medium containing 200 ng/ml LPS for 4h (signal 1) and 5 mM ATP for 30 min (signal 2) in order to induce IL-1 β production and activate the inflammasome complex. Afterwards the cells were lysed. Mouse HPRT1 and mouse NLRP3 mRNA expression was measured using the QuantiGene Singleplex assay (ThermoFisher) and the NLRP3 expression values were normalized to HPRT1 values. Residual NLRP3 mRNA expression relative to mock-
 20 treated cells (set as 1) is shown as mean and SD in **Fig. 22A**. Treatment of BMDM with the selected NLRP3-specific ASOs resulted in a target inhibition of ≥ 94 % (represented by a residual NLRP3 mRNA expression of ≤ 0.06 as compared to mock-treated cells) (**Fig. 22A**).

25 As part of the inflammasome complex, NLRP3 is required for cleavage of pro-IL-1 β into its mature and secreted form. As a proof-of-concept it was aimed to demonstrate that IL-1 β secretion by primary immune cells can be inhibited through treatment with NLRP3 specific antisense oligonucleotides. NLRP3, pro-IL1 β and mature IL-1 β protein expression was analyzed by Western Blot in cell lysates and cell culture supernatants of BMDM after
 30 six days ASO treatment (**Fig. 22B**). NLRP3 could be detected in the cell supernatant and cell lysates of mock-treated cells and cells treated with the control oligonucleotides, but not in cells treated with NLRP3-specific ASO A31087 Mi (SEQ ID NO. 419) (**Fig. 22B**). As the transcription and translation of pro-IL-1 β is independent of NLRP3, pro-IL-1 β could be detected in the cell lysates under all treatment conditions (cells treated with NLRP3-

specific ASOs, control oligonucleotides or mock-treated cells (no ASO) (**Fig. 22B**). In contrast, mature IL-1 β was only present in the supernatants of either mock-treated cells (no ASO, untreated control) or cells that were treated with the control oligonucleotides, but not in cells treated with NLRP3-specific ASOs A31087Mi (SEQ ID NO. 419) (**Fig. 22B**). These results demonstrate that inhibition of NLRP3 on mRNA level with antisense oligonucleotides effectively prevents the cleavage of pro-IL-1 β in BMM.

Example 23: Efficacy of selected mouse NLRP3 antisense oligonucleotides in Raw246.7 cells

Knockdown efficacy of selected mouse NLRP3-specific ASOs was further analyzed in Raw246.7 cells. Raw246.7 cells were treated with the respective antisense oligonucleotides or control oligo (Neg1) at a final concentration of 5 μ M. After three days, cells were lysed and mouse HPRT1 as well as mouse NLRP3 mRNA expression was measured using the QuantiGene RNA Singleplex assay. NLRP3 mRNA expression values were normalized to the expression of the housekeeping gene HPRT1. Residual NLRP3-mRNA expression relative to mock-treated cells ("no antisense oligonucleotides" set as 1, untreated control) is shown as mean and SD as shown in **Fig. 23**.

Treatment of Raw246.7 cells with the A31087Mi (SEQ ID NO.419), A31084Mi (SEQ ID NO.416), A31061Mi (SEQ ID NO.393), A31035M (SEQ ID NO.376), A31010M (SEQ ID NO.351) and A31041M (SEQ ID NO.351) ASOs resulted in a target inhibition of ≥ 85 % (represented by a residual NLRP3 mRNA expression of ≤ 0.15 as compared to mock-treated cells) (**Fig. 23**).

Example 24: Efficacy of selected mouse NLRP3 antisense oligonucleotides in mouse microglial IMG cells

Knockdown efficacy of selected mouse NLRP3-specific ASOs was further assessed in IMG cells (microglial cell line isolated from the brains of adult mice). IMG cells were treated with the respective antisense oligonucleotides or control oligo (Neg1) at a final concentration of 5 μ M. After three days, cells were lysed and mouse HPRT1 as well as mouse NLRP3 mRNA expression was measured using the QuantiGene RNA Singleplex assay. NLRP3 mRNA expression values were normalized to expression of the

housekeeping gene HPRT1. Residual NLRP3-mRNA expression relative to mock-treated cells ("no ASO" set as 1, untreated control) is shown as mean and SD as shown in **Fig. 2**. Treatment of IMG cells with the A31087Mi (SEQ ID NO.419), A31084Mi (SEQ ID NO.416) and A31034M (SEQ ID NO.375) ASOs resulted in a target inhibition of ≥ 66 % (represented by a residual NLRP3 mRNA expression of ≤ 0.34 as compared to mock-treated cells) (**Fig. 24**).

Claims

- 1 Oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO.59, SEQ ID NO. 335, SEQ ID NO.297, and SEQ ID NO. 133 comprising at least one modified nucleotide, hybridizing with a nucleic acid sequence of a NLR family pyrin domain containing 3 (NLRP3) of human SEQ ID NO.1 and/or human SEQ ID NO.2 resulting in a reduction of the level of NLRP3, NLRP3 mRNA, NLRP3 pre-mRNA or a combination thereof.
2. The oligonucleotide of claim 1, 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, a 2 O-Methoxy modified nucleotide, a FANA and a combination thereof.
3. The oligonucleotide of claim 1 or 2, wherein the reduction of the level of NLRP3, NLRP3 mRNA, NLRP3 pre-mRNA or a combination thereof is 30 to 99 % compared to an untreated control.
4. The oligonucleotide of any one of claims 1 to 3, wherein the oligonucleotide comprises a modification selected from the group consisting of
- +G*+T*+A*A*T*G*T*C*A*A*C*G*G*A*+T*+C (A31109Hi; SEQ ID NO. 59),
- +G*+T*+A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C (A31055Hi, SEQ ID NO.59),
- +G*+T*A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C (A31110Hi, SEQ ID NO.59),
- +G*+T*+A*+A*T*G*T*C*A*A*C*G*G*+A*+T*+C (A31111Hi, SEQ ID NO.59),
- +G*T*+A*A*T*G*T*C*A*A*C*G*G*+A*+T*+C (A31112Hi, SEQ ID NO.59),
- +G*+T*+A*A*+T*G*T*C*A*A*C*G*G*+A*+T*+C (A31368Hi, SEQ ID NO.59),
- +G*+T*+A*A*T*+G*T*C*A*A*C*G*G*+A*+T*+C (A31369Hi, SEQ ID NO.59),
- +G*+T*+A*A*T*G*+T*C*A*A*C*G*G*+A*+T*+C (A31370Hi, SEQ ID NO.59),
- +G*+T*+A*A*T*G*T*C*A*A*C*+G*G*+A*+T*+C (A31371Hi, SEQ ID NO.59),
- +G*+T*+A*A*T*G*T*C*A*A*+C*G*G*+A*+T*+C (A31372Hi, SEQ ID NO.59),
- +G*+T*+A*+A*T*G*T*C*A*A*C*G*G*+A*+T*+C (A31373Hi, SEQ ID NO.59),
- +G*+A*C*T*G*T*C*A*C*G*T*C*T*C*+G*+G*+C (A31149H, SEQ ID NO.133),
- +C*+A*+T*A*G*T*T*C*T*C*T*G*C*A*A*C*+A*+G*+G (A31314Hi, SEQ ID NO.297),
- +T*+A*+G*T*A*T*C*A*C*T*G*T*A*T*G*T*+C*+C*+A (A31352Hi, SEQ ID NO.335),

and a combination thereof, wherein + indicates an LNA nucleotide and * indicates a phosphorothioate (PTO) linkage between the nucleotides.

5. The oligonucleotide of any one of claims 1 to 4, wherein the oligonucleotide comprises
 5 the modification selected from the group consisting of
 +G*+T*+A*A*T*G*T*C*A*A*C*G*G*A*+T*+C (A31109Hi; SEQ ID NO. 59),
 +G*+A*C*T*G*T*C*A*C*G*T*C*T*C*+G*+G*+C (A31149H, SEQ ID NO.133),
 +C*+A*+T*A*G*T*T*C*T*C*T*G*C*A*A*C*+A*+G*+G (A31314Hi, SEQ ID NO.297),
 +T*+A*+G*T*A*T*C*A*C*T*G*T*A*T*G*T*+C*+C*+A (A31352Hi, SEQ ID NO.335)
 10 and a combination thereof, wherein + indicates an LNA nucleotide and * indicates a
 phosphorothioate (PTO) linkage between the nucleotides.

6. The oligonucleotide of any one of claims 1 to 5, wherein the oligonucleotide hybridizes
 15 with at least one exon or intron of SEQ ID NO.1 and/or with the mRNA of SEQ ID NO.2.

7. The oligonucleotide of any one of claims 1 to 6, wherein the oligonucleotide inhibits the
 expression of NLRP3, NLRP3 mRNA, NLRP3 pre-mRNA or a combination thereof at a
 nanomolar or micromolar concentration.

20 8. Pharmaceutical composition comprising an oligonucleotide of any one of claims 1 to 7
 and a pharmaceutically acceptable carrier, excipient, dilutant or a combination thereof.

9. The pharmaceutical composition of claim 8, further comprising another active agent
 selected from the group consisting of an oligonucleotide, an antibody, a small molecule, a
 25 polypeptide, a lipid, a sugar and a combination thereof.

10. The pharmaceutical composition of claim 9, wherein the oligonucleotide and the other
 active agent inhibit the same target or a different target.

30 11. The pharmaceutical composition of claim 10, wherein the other active agent modulates
 the target selected from the group consisting of NLRP3, CD39, CD73, IL-18, IL-1 receptor,
 IL-1R accessory protein, IL-18, IL-18 receptor, ASC, NLRC4, AIM2, Caspase-1, RIPK3,
 Gasdermin D, MLKL, TLR4, Caspase-8, P2X7, NFkB, RORyt, TGF-β, IL-21, IL-17, IL-22,
 IL-23, IL-6, TNF-α, CCR6, CCL20, STAT3, MMP-1, MMP-8, ADAMTS-5, HMG-CoA,

Myd-88, HMGB-1, ROS, TAK-1, Chop, FPR1, LIMCH1, caspase inhibitor and a combination thereof.

12. The oligonucleotide of any one of claims 1 to 7 or the pharmaceutical composition of
5 any one of claims 8 to 11 for use in a method of preventing and/or treating a disorder, where
an NLRP3 imbalance is involved.

13. The oligonucleotide or the pharmaceutical composition for use according to claim 12,
wherein the disorder is selected from the group consisting of a hyperproliferative disorder
10 such as cancer, an inflammatory or autoimmune disorder, neurodegenerative disease, a
neurological disorder, cardiovascular, metabolic disorder, renal disorder, liver disorder,
lung disorder, skin disorder, ocular disorder, disorder of the gastro-intestinal tract, joint
inflammation, organ transplantation, fibrotic disorder and a combination thereof.

14. The oligonucleotide or the pharmaceutical composition for use according to claim 12 or
15 13, wherein the disorder is selected from the group consisting of Alzheimer's disease,
multiple sclerosis, autoimmune encephalitis, stroke, traumatic brain injury,
atherosclerosis, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis,
hypertension, myocardial infarction, acute kidney injury, ischemia reperfusion injury,
20 chronic kidney diseases, crystal-induced nephropathies, glomerulonephritis, silicosis,
asthma, allergic airway inflammation, inflammatory bowel disease, colitis ulcerosa,
osteoarthritis, rheumatoid arthritis, juvenile idiopathic arthritis, transplantation of
kidney, lung, liver and/or heart, fibrotic disorder of kidney, lung, liver and/or heart,
hyperinflammation following influenza infection, graft-versus-host disease, interstitial
25 cystitis, uveitis, sinusitis, periodontal disease, optic neuritis, myelodysplastic syndrome,
cryopyrin-associated periodic syndromes (CAPS) including familial cold
autoinflammatory syndrome (FCAS), the Muckle-Wells syndrome (MWS) and neonatal-
onset multisystem inflammatory disease (NOMID), gout, obesity-induced inflammation,
insulin resistance, type 1 and type 2 diabetes, contact hypersensitivity and a combination
30 thereof, and/or a cancer selected from the group consisting of 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, osteosarcoma, Ewing's
35 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
- 5 sarcoma, malignant carcinoid, topical skin lesion, rhabdomyosarcoma, Kaposi's sarcoma, osteogenic sarcoma, malignant hypercalcemia, renal cell tumor, polycythemia vera, adenocarcinoma, anaplastic astrocytoma, glioblastoma multiforma, leukemia, epidermoid carcinoma and a combination thereof.
- 10 15. The oligonucleotide or the pharmaceutical composition for use according to any one of claims 12 to 13, wherein the oligonucleotide or the composition is administered locally or systemically.