

EXTRACELLULAR VESICLE-NLRP3 ANTAGONIST

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of U.S. Provisional Application No. 63/150,453, filed February 17, 2021, which is herein incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED
ELECTRONICALLY VIA EFS-WEB

[0002] The content of the electronically submitted sequence listing (Name: 4000_124PC01_SEQLISTING_ST25.txt; Size: 302,477 Bytes; and Date of Creation: February 17, 2022) submitted in this application is incorporated herein by reference in its entirety.

FIELD OF DISCLOSURE

[0003] The present disclosure relates to methods of treating peripheral neuropathy in a subject in need thereof by administering to the subject extracellular vesicles (EVs), *e.g.*, exosomes, comprising an NLRP3 antagonist. In some aspects, the NLRP3 antagonist comprises an antisense oligonucleotide (ASO). In certain aspects of the disclosure, the extracellular vesicle further comprises a scaffold protein.

BACKGROUND

[0004] Exosomes are small extracellular vesicles that are naturally produced by every eukaryotic cell. Exosomes comprise a membrane that encloses an internal space (*i.e.*, lumen). As drug delivery vehicles, EVs, *e.g.*, exosomes, offer many advantages over traditional drug delivery methods as a new treatment modality in many therapeutic areas. In particular, exosomes have intrinsically low immunogenicity, even when administered to a different species.

[0005] Antisense oligonucleotides have emerged as a powerful means of regulating target gene expression *in vitro* or *in vivo*. However, there remains a need to improve the stability and targeting of ASOs *in vivo*.

[0006] Accordingly, new and more effective engineered-EVs (*e.g.*, exosomes), particularly those that can be used to deliver therapeutic agents that can reduce the expression of a gene associated with a disease (*e.g.*, cancer), are necessary to better enable therapeutic use and other applications of EV-based technologies.

SUMMARY OF DISCLOSURE

[0007] Some aspects of the present disclosure are directed to a method of treating peripheral neuropathy in a subject in need thereof, comprising administering to the subject an extracellular vesicle comprising an exogenous NLRP3 antagonist.

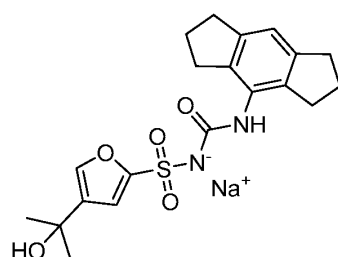
[0008] Some aspects of the present disclosure are directed to a method of reducing, ameliorating, or treating one or more symptoms of a peripheral neuropathy in a subject in need thereof, comprising administering to the subject an extracellular vesicle comprising an exogenous NLRP3 antagonist.

[0009] In some aspects, the exogenous NLRP3 antagonist is a chemical compound, an siRNA, an shRNA, an antisense oligonucleotide, a protein, or any combination thereof.

[0010] In some aspects, the extracellular vesicle targets a cell selected from the group consisting of a macrophage, a myeloid-derived suppressor cell (MDSC), a monocyte, a basophil, a neutrophil, an eosinophil, and any combination thereof.

[0011] In some aspects, the extracellular vesicle comprising the ASO or the ASO induces M2 macrophage polarization in the subject. In some aspects, the extracellular vesicle comprising the ASO or the ASO reduces myeloid inflammation in a nerve, meningeal myeloid inflammation, nerve sheath inflammation, or any combination thereof. In some aspects, the extracellular vesicle comprising the ASO reduces myeloid inflammation in a sheath. In some aspects, the extracellular vesicle comprising the ASO reduces macrophage influx in one or more of a root, nerve, and/or muscle. In some aspects, the extracellular vesicle comprising the ASO reduces macrophage phagocytosis in one or more of a root, nerve, and/or muscle.

[0012] In some aspects, the exogenous NLRP3 antagonist is a small molecule. In some aspects, the small molecule is selected from the group consisting of MCC950, Tanilast, Oridonin, CY-09, Bay 11-7082, Parthenolide, 3,4-methylenedioxy- β -nitrostyrene (MNB), β -hydroxybutyrate (BHB), dimethyl sulfoxide (DMSO), type I interferon, and any combination thereof. In some aspects, the exogenous NLRP3 antagonist comprises the formula (I):



[0013] (I).

[0014] In some aspects, the exogenous NLRP3 antagonist comprises MCC950.

[0015] In some aspects, the exogenous NLRP3 antagonist comprises an antisense oligonucleotide (ASO). In some aspects, the ASO comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length that is complementary to a nucleic acid sequence within a *NLRP3* transcript. In some aspects, the contiguous nucleotide sequence is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% complementary to the nucleic acid sequence within the *NLRP3* transcript.

[0016] In some aspects, the ASO is capable of reducing NLRP3 protein expression in a human cell (*e.g.*, an immune cell), wherein the human cell expresses the NLRP3 protein. In some aspects, the NLRP3 protein expression is reduced by at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to NLRP3 protein expression in a human cell that is not exposed to the ASO. In some aspects, the ASO is capable of reducing a level of *NLRP3* mRNA in a human cell (*e.g.*, an immune cell), wherein the human cell expresses the *NLRP3* mRNA. In some aspects, the level of *NLRP3* mRNA is reduced by at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to the level of the *NLRP3* mRNA in a human cell that is not exposed to the ASO.

[0017] In some aspects, the ASO is a gapmer, a mixmer, or a totalmer. In some aspects, the ASO comprises one or more nucleoside analogs. In some aspects, one or more of the nucleoside analogs comprises a 2'-O-alkyl-RNA; 2'-O-methyl RNA (2'-OMe); 2'-alkoxy-RNA; 2'-O-methoxyethyl-RNA (2'-MOE); 2'-amino-DNA; 2'-fluoro-RNA; 2'-fluoro-DNA; arabino nucleic acid (ANA); 2'-fluoro-ANA; or bicyclic nucleoside analog. In some aspects, one or more of the nucleoside analogs is a sugar modified nucleoside. In some aspects, the sugar-modified nucleoside is an affinity enhancing 2' sugar modified nucleoside. In some aspects, one or more of the nucleoside analogs comprises a nucleoside comprising a bicyclic sugar. In some aspects, one or more of the nucleoside analogs comprises an LNA. In some aspects, one or more of the nucleotide analogs is selected from the group consisting of constrained ethyl nucleoside (cEt), 2',4'-constrained 2'-O-methoxyethyl (cMOE), α -L-LNA, β -D-LNA, 2'-O,4'-C-ethylene-bridged nucleic acids (ENA), amino-LNA, oxy-LNA, thio-LNA, and any combination thereof. In some aspects, the ASO comprises one or more 5'-methyl-cytosine nucleobases.

[0018] In some aspects, the contiguous nucleotide sequence is complementary to a nucleic acid sequence within (i) a 5' untranslated region (UTR); (ii) a coding region; or (iii) a 3' UTR of the *NLRP3* transcript. In some aspects, the contiguous nucleotide sequence is complementary to a nucleic acid sequence comprising (i) nucleotides 1 – 534 of SEQ ID NO: 3; (ii) nucleotides 448 – 2193 of SEQ ID NO: 3; (iii) nucleotides 2125 – 3036 of SEQ ID NO: 3; (iv) nucleotides 2987 – 3990 of SEQ ID NO: 3; (v) 3996 – 4456 of SEQ ID NO: 3, (vi) nucleotides 106 – 334 of SEQ ID NO: 3; (vii) nucleotides 648 – 2113 of SEQ ID NO: 3; (viii) nucleotides 2225 – 2956 of SEQ ID NO: 3; (ix) nucleotides 2987 – 3810 of SEQ ID NO: 3; (x) 3996 – 4376 of SEQ ID NO: 3; (xi) nucleotides 156 – 284 of SEQ ID NO: 3; (xii) nucleotides 698 – 2063 of SEQ ID NO: 3; (xiii) nucleotides 2275 – 2906 of SEQ ID NO: 3; (xiv) nucleotides 3037 – 3760 of SEQ ID NO: 3; (xv) 4046 – 4326 of SEQ ID NO: 3; (xvi) nucleotides 196 – 244 of SEQ ID NO: 3; (xvii) nucleotides 738 – 2003 of SEQ ID NO: 3; (xviii) nucleotides 2315 – 2866 of SEQ ID NO: 3; (xix) nucleotides 3077 – 3720 of SEQ ID NO: 3; or (xx) 4086 – 4286 of SEQ ID NO: 3.

[0019] In some aspects, the contiguous nucleotide sequence is complementary to a nucleic acid sequence within (i) nucleotides 206 – 234 of SEQ ID NO: 3; (ii) nucleotides 748-2013 of SEQ ID NO: 3; (iii) nucleotides 2325 – 2856 of SEQ ID NO: 3; (iv) nucleotides 3087 – 3710 of SEQ ID NO: 3; or (v) 4096 – 4276 of SEQ ID NO: 3.

[0020] In some aspects, the contiguous nucleotide sequence comprises a nucleotide sequence complementary to a sequence selected from the sequences in FIGs. 1A and 1B. In some aspects, the continuous nucleotide sequence is fully complementary to a nucleotide sequence within the *NLRP3* transcript. In some aspects, the ASO comprises a nucleotide sequence selected from SEQ ID NOs: 101-200, with one or two mismatches. In some aspects, the ASO comprises a nucleotide sequence selected from SEQ ID NOs: 101-200. In some aspects, the ASO is from 14 to 20 nucleotides in length.

[0021] In some aspects, the contiguous nucleotide sequence comprises one or more modified internucleoside linkages. In some aspects, the one or more modified internucleoside linkages is a phosphorothioate linkage. In some aspects, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% of internucleoside linkages are modified. In some aspects, each of the internucleoside linkages in the ASO is a phosphorothioate linkage.

[0022] In some aspects, the extracellular vesicle comprises an anchoring moiety. In some aspects, the *NLRP3* antagonist is linked to the anchoring moiety.

[0023] In some aspects, the extracellular vesicle comprises an exogenous targeting moiety. In some aspects, the exogenous targeting moiety comprises a peptide, an antibody or an antigen-

binding fragment thereof, a chemical compound, an RNA aptamer, or any combination thereof. In some aspects, the exogenous targeting moiety comprises a peptide.

[0024] In some aspects, the exogenous targeting moiety comprises a microprotein, a designed ankyrin repeat protein (darpin), an anticalin, an adnectin, an aptamer, a peptide mimetic molecule, a natural ligand for a receptor, a camelid nanobody, or any combination thereof. In some aspects, the exogenous targeting moiety comprises a full-length antibody, a single domain antibody, a heavy chain only antibody (VHH), a single chain antibody, a shark heavy chain only antibody (VNAR), an scFv, a Fv, a Fab, a Fab', a F(ab')₂, or any combination thereof. In some aspects, the antibody is a single chain antibody.

[0025] In some aspects, the exogenous targeting moiety targets the exosome to the liver, heart, lungs, brain, kidneys, central nervous system, peripheral nervous system, muscle, bone, joint, skin, intestine, bladder, pancreas, lymph nodes, spleen, blood, bone marrow, or any combination thereof.

[0026] In some aspects, the EV comprises a scaffold moiety linking the exogenous targeting moiety to the EV. In some aspects, the anchoring moiety and/or the scaffold moiety is a Scaffold X or a Scaffold Y. In some aspects, the exogenous NLRP3 antagonist is linked to the anchoring moiety and/or the scaffold moiety on the exterior surface of the EV. In some aspects, the exogenous NLRP3 antagonist is linked to the anchoring moiety and/or the scaffold moiety on the luminal surface of the EV. In some aspects, the anchoring moiety comprises sterol, GM1, a lipid, a vitamin, a small molecule, a peptide, or a combination thereof. In some aspects, the anchoring moiety comprises cholesterol. In some aspects, the anchoring moiety comprises a phospholipid, a lysophospholipid, a fatty acid, a vitamin (e.g., vitamin D and/or vitamin E), or any combination thereof.

[0027] In some aspects, the exogenous NLRP3 antagonist is linked to the anchoring moiety and/or the scaffold moiety by a linker. In some aspects, the exogenous NLRP3 antagonist is linked to the EV by a linker. In some aspects, the linker is a polypeptide. In some aspects, the linker is a non-polypeptide moiety. In some aspects, the linker comprise ethylene glycol. In some aspects, the linker comprises HEG, TEG, PEG, or any combination thereof. In some aspects, the linker comprises acrylic phosphoramidite (e.g., ACRYDITE™), adenylation, azide (NHS Ester), digoxigenin (NHS Ester), cholesterol-TEG, I-LINKER™, an amino modifier (e.g., amino modifier C6, amino modifier C12, amino modifier C6 dT, or Uni-Link™ amino modifier), alkyne, 5' Hexynyl, 5-Octadiynyl dU, biotinylation (e.g., biotin, biotin (Azide), biotin dT, biotin-TEG, dual

biotin, PC biotin, or desthiobiotin), thiol modification (thiol modifier C3 S-S, dithiol or thiol modifier C6 S-S), or any combination thereof.

[0028] In some aspects, the linker is a cleavable linker. In some aspects, the linker comprises valine-alanine-p-aminobenzylcarbamate or valine-citrulline-p-aminobenzylcarbamate. In some aspects, the linker comprises (i) a maleimide moiety and (ii) valine-alanine-p-aminobenzylcarbamate or valine-citrulline-p-aminobenzylcarbamate.

[0029] In some aspects, the EV is an exosome.

[0030] In some aspects, the peripheral neuropathy is associated with diabetes, a trauma, an autoimmune disorder, a kidney disorder, a liver disorder, hypothyroidism, a vascular disorder, an abnormal vitamin level, alcohol use, or any combination thereof. In some aspects, the peripheral neuropathy comprises chemotherapy-induced peripheral neuropathy (CIPN).

[0031] In some aspects, the subject was previously administered a chemotherapy. In some aspects, the chemotherapy comprises a platinum derivative, a vinca alkaloid, bortezomib, a taxane, or any combination thereof. In some aspects, the chemotherapy comprises cisplatin, carboplatin, oxaliplatin, docetaxel, vincristine, paclitaxel, gemcitabine, or any combination thereof.

[0032] In some aspects, the extracellular vesicle reduces the severity or occurrence of one or more symptom in the subject selected from tingling, pain, burning, numbness, sensitivity to hot, sensitivity to cold, difficulty with fine motor skills, and any combination thereof.

BRIEF DESCRIPTION OF FIGURES

[0033] FIG. 1 is a table listing various ASO sequences that target the *NLPR3* transcript. The table includes the following information (from left to right): (i) description, (ii) the ASO sequence without any particular design or chemical structure, (iii) SEQ ID number designated for the ASO sequence only, (iv) the length of the ASO in number of nucleotides (NT), (ii) the target start and end positions on the *NLPR3* transcript sequence (SEQ ID NO: 3). The ASOs are from 5' to 3'. The symbols in the chemical structures are as follows: Nb means LNA; dN means DNA; 5MdC means 5-Methyl-dC; Nm means MOE; and s means phosphorothioate.

[0034] FIG. 2 is an image of a mouse injected intrathecally with exosomes, showing the distribution of the exosomes throughout the CNS, as labelled.

[0035] FIGs. 3A-3B are graphical representations illustrating pain, as measured using the Von Frey test, in mice administered a negative control (sham; no cisplatin); cisplatin and PBS; cisplatin and MCC950 (days 15-20); cisplatin and control exosomes (no ASO; day 15); cisplatin and ASONLRP3 (free ASO; day 15); cisplatin and exoASONLRP3 (day 5); cisplatin and

exoASONLRP3 (day 15); or cisplatin and egabapentin (days 8 and 21). FIG. 3A shows the pain levels as measure over time, and FIG. 3B shows pain levels for each group at day 21.

DETAILED DESCRIPTION OF DISCLOSURE

[0036] Some aspects of the present disclosure are directed to a method of treating a peripheral neuropathy in a subject in need thereof, comprising administering to the subject an extracellular vesicle (EV), *e.g.*, an exosome, comprising an NLRP3 antagonist. Some aspects of the present disclosure are directed to a method of reducing, ameliorating, or treating one or more symptoms of a peripheral neuropathy in a subject in need thereof, comprising administering to the subject an extracellular vesicle comprising an exogenous NLRP3 antagonist. In some aspect, the NLRP3 antagonist comprises an antisense oligonucleotide (ASO). In some aspects, the ASO comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length that is complementary to a nucleic acid sequence within a *NLRP3* transcript.

I. Definitions

[0037] In order that the present description can be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

[0038] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0039] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0040] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0041] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure

is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0042] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0043] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" can modify a numerical value above and below the stated value by a variance of, *e.g.*, 10 percent, up or down (higher or lower). For example, if it is stated that "the ASO reduces expression of NLRP3 protein in a cell following administration of the ASO by at least about 60%," it is implied that the NLRP3 levels are reduced by a range of 50% to 70%.

[0044] The term "peripheral neuropathy," as used herein, refers to a disease or condition characterized by damage, inflammation, injury, or disease of a nerve that carries messages to and from the brain and spinal cord from and to the rest of the body. In certain aspects, the peripheral neuropathy comprises inflammation of or surrounding a peripheral nerve. In some aspects, the peripheral neuropathy is associated with diabetes, a trauma, an autoimmune disorder, a kidney disorder, a liver disorder, hypothyroidism, a vascular disorder, an abnormal vitamin level, alcohol use, or any combination thereof. In some aspects, the peripheral neuropathy is a chemotherapy-induced peripheral neuropathy.

[0045] The term "chemotherapy-induced peripheral neuropathy" or "CIPN," as used herein, refers to a neuropathy arising in subject administered one or more chemotherapy. CIPN is one of the most common side effects cause by antineoplastic agents, occurring in 19% to more than 85% of patients administered a chemotherapy. The highest prevalence of CIPN is in patients administered platinum-based drugs (70% to 100%), taxanes (11% to 87%), thalidomide and its analogues (20% to 60%), and ixabepilone (60% to 65%). CIPN presents with varying intensity and

duration, and symptoms can range from acute, transient thermal sensations to permanent changes in peripheral nerves accompanied by chronic pain and irreversible nerve damage. CIPN is a predominantly sensory neuropathy that may be accompanied by motor and autonomic changes. Pain and sensory abnormalities may persist for months or even years after the cessation of chemotherapy, resulting in some cancer-free patients that suffer from debilitating neuropathy induced by the previous cancer treatment. Symptoms usually present first in the feet and hands and commonly present as a typical “glove and stocking” neuropathy with the most distal parts of the limbs exhibiting the greatest deficits. Symptoms of CIPN include, but are not limited to, numbness, tingling, altered touch sensation, impaired vibration, paresthesias, dysesthesias induced by touch and warm or cool temperatures, painful sensations (including spontaneous burning, shooting or electric shock-like pain), mechanical or thermal allodynia or hyperalgesia, and a loss of sensory perception (in severe cases). *See, e.g., Zajackowska et al., Int. J. Mol. Sci. 20(6):1451 (March 2019), which is incorporated by reference herein in its entirety.*

[0046] The term "antisense oligonucleotide" (ASO) refers to an oligomer or polymer of nucleosides, such as naturally-occurring nucleosides or modified forms thereof, that are covalently linked to each other through internucleotide linkages. The ASO useful for the disclosure includes at least one non-naturally occurring nucleoside. An ASO is at least partially complementary to a target nucleic acid, such that the ASO hybridizes to the target nucleic acid sequence.

[0047] The term "nucleic acids" or "nucleotides" is intended to encompass plural nucleic acids. In some aspects, the term "nucleic acids" or "nucleotides" refers to a target sequence, *e.g.*, pre-mRNAs, mRNAs, or DNAs *in vivo* or *in vitro*. When the term refers to the nucleic acids or nucleotides in a target sequence, the nucleic acids or nucleotides can be naturally occurring sequences within a cell. In other aspects, "nucleic acids" or "nucleotides" refer to a sequence in the ASOs of the disclosure. When the term refers to a sequence in the ASOs, the nucleic acids or nucleotides can be non-naturally occurring, *i.e.*, chemically synthesized, enzymatically produced, recombinantly produced, or any combination thereof. In some aspects, the nucleic acids or nucleotides in the ASOs are produced synthetically or recombinantly, but are not a naturally occurring sequence or a fragment thereof. In some aspects, the nucleic acids or nucleotides in the ASOs are not naturally occurring because they contain at least one nucleoside analog that is not naturally occurring in nature.

[0048] The term "nucleotide" as used herein, refers to a glycoside comprising a sugar moiety, a base moiety and a covalently linked group (linkage group), such as a phosphate or phosphorothioate internucleotide linkage group, and covers both naturally occurring nucleotides,

such as DNA or RNA, and non-naturally occurring nucleotides comprising modified sugar and/or base moieties, which are also referred to as "nucleotide analogs" herein. Herein, a single nucleotide can be referred to as a monomer or unit. In certain aspects, the term "nucleotide analogs" refers to nucleotides having modified sugar moieties. Non-limiting examples of the nucleotides having modified sugar moieties (*e.g.*, LNA) are disclosed elsewhere herein. In other aspects, the term "nucleotide analogs" refers to nucleotides having modified nucleobase moieties. The nucleotides having modified nucleobase moieties include, but are not limited to, 5-methyl-cytosine, isocytosine, pseudoisocytosine, 5-bromouracil, 5-propynyluracil, 6-aminopurine, 2-aminopurine, inosine, diaminopurine, and 2-chloro-6-aminopurine. In some aspects, the terms "nucleotide", "unit" and "monomer" are used interchangeably. It will be recognized that when referring to a sequence of nucleotides or monomers, what is referred to is the sequence of bases, such as A, T, G, C or U, and analogs thereof.

[0049] The term "nucleoside" as used herein is used to refer to a glycoside comprising a sugar moiety and a base moiety, and can therefore be used when referring to the nucleotide units, which are covalently linked by the internucleotide linkages between the nucleotides of the ASO. In the field of biotechnology, the term "nucleotide" is often used to refer to a nucleic acid monomer or unit. In the context of an ASO, the term "nucleotide" can refer to the base alone, *i.e.*, a nucleobase sequence comprising cytosine (DNA and RNA), guanine (DNA and RNA), adenine (DNA and RNA), thymine (DNA) and uracil (RNA), in which the presence of the sugar backbone and internucleotide linkages are implicit. Likewise, particularly in the case of oligonucleotides where one or more of the internucleotide linkage groups are modified, the term "nucleotide" can refer to a "nucleoside." For example the term "nucleotide" can be used, even when specifying the presence or nature of the linkages between the nucleosides.

[0050] The term "nucleotide length" as used herein means the total number of the nucleotides (monomers) in a given sequence. For example, the sequence of ASO-NLRP3-206 (SEQ ID NO: 101) has 20 nucleotides; thus the nucleotide length of the sequence is 20. The term "nucleotide length" is therefore used herein interchangeably with "nucleotide number."

[0051] As one of ordinary skill in the art would recognize, the 5' terminal nucleotide of an oligonucleotide does not comprise a 5' internucleotide linkage group, although it can comprise a 5' terminal group.

[0052] The compounds described herein can contain several asymmetric centers and can be present in the form of optically pure enantiomers, mixtures of enantiomers such as, for example, racemates, mixtures of diastereoisomers, diastereoisomeric racemates or mixtures of

diastereoisomeric racemates. In some aspects, the asymmetric center can be an asymmetric carbon atom. The term "asymmetric carbon atom" means a carbon atom with four different substituents. According to the Cahn-Ingold-Prelog Convention an asymmetric carbon atom can be of the "R" or "S" configuration.

[0053] As used herein, the term "bicyclic sugar" refers to a modified sugar moiety comprising a 4 to 7 membered ring comprising a bridge connecting two atoms of the 4 to 7 membered ring to form a second ring, resulting in a bicyclic structure. In some aspects, the bridge connects the C2' and C4' of the ribose sugar ring of a nucleoside (*i.e.*, 2'-4' bridge), as observed in LNA nucleosides.

[0054] As used herein, a "coding region" or "coding sequence" is a portion of polynucleotide which consists of codons translatable into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is typically not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, untranslated regions ("UTRs"), and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide.

[0055] The term "non-coding region" as used herein means a nucleotide sequence that is not a coding region. Examples of non-coding regions include, but are not limited to, promoters, ribosome binding sites, transcriptional terminators, introns, untranslated regions ("UTRs"), non-coding exons and the like. Some of the exons can be wholly or part of the 5' untranslated region (5' UTR) or the 3' untranslated region (3' UTR) of each transcript. The untranslated regions are important for efficient translation of the transcript and for controlling the rate of translation and half-life of the transcript.

[0056] The term "region" when used in the context of a nucleotide sequence refers to a section of that sequence. For example, the phrase "region within a nucleotide sequence" or "region within the complement of a nucleotide sequence" refers to a sequence shorter than the nucleotide sequence, but longer than at least 10 nucleotides located within the particular nucleotide sequence or the complement of the nucleotides sequence, respectively. The term "sub-sequence" or "subsequence" can also refer to a region of a nucleotide sequence.

[0057] The term "downstream," when referring to a nucleotide sequence, means that a nucleic acid or a nucleotide sequence is located 3' to a reference nucleotide sequence. In certain aspects, downstream nucleotide sequences relate to sequences that follow the starting point of

transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[0058] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence.

[0059] As used herein, the term "regulatory region" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding region, and which influence the transcription, RNA processing, stability, or translation of the associated coding region. Regulatory regions can include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites, UTRs, and stem-loop structures. If a coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0060] The term "transcript" as used herein can refer to a primary transcript that is synthesized by transcription of DNA and becomes a messenger RNA (mRNA) after processing, *i.e.*, a precursor messenger RNA (pre-mRNA), and the processed mRNA itself. The term "transcript" can be interchangeably used with "pre-mRNA" and "mRNA." After DNA strands are transcribed to primary transcripts, the newly synthesized primary transcripts are modified in several ways to be converted to their mature, functional forms to produce different proteins and RNAs, such as mRNA, tRNA, rRNA, lncRNA, miRNA and others. Thus, the term "transcript" can include exons, introns, 5' UTRs, and 3' UTRs.

[0061] The term "expression" as used herein refers to a process by which a polynucleotide produces a gene product, for example, a RNA or a polypeptide. It includes, without limitation, transcription of the polynucleotide into messenger RNA (mRNA) and the translation of an mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation or splicing, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0062] The terms "identical" or percent "identity" in the context of two or more nucleic acids refer to two or more sequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid

substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software are known in the art that can be used to obtain alignments of amino acid or nucleotide sequences.

[0063] One such non-limiting example of a sequence alignment algorithm is the algorithm described in Karlin *et al.*, 1990, *Proc. Natl. Acad. Sci.*, 87:2264-2268, as modified in Karlin *et al.*, 1993, *Proc. Natl. Acad. Sci.*, 90:5873-5877, and incorporated into the NBLAST and XBLAST programs (Altschul *et al.*, 1991, *Nucleic Acids Res.*, 25:3389-3402). In certain aspects, Gapped BLAST can be used as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. BLAST-2, WU-BLAST-2 (Altschul *et al.*, 1996, *Methods in Enzymology*, 266:460-480), ALIGN, ALIGN-2 (Genentech, South San Francisco, California) or Megalign (DNASTAR) are additional publicly available software programs that can be used to align sequences. In certain aspects, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (*e.g.*, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 90 and a length weight of 1, 2, 3, 4, 5, or 6). In certain alternative aspects, the GAP program in the GCG software package, which incorporates the algorithm of Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) can be used to determine the percent identity between two amino acid sequences (*e.g.*, using either a BLOSUM 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5). Alternatively, in certain aspects, the percent identity between nucleotide or amino acid sequences is determined using the algorithm of Myers and Miller (CABIOS, 4:11-17 (1989)). For example, the percent identity can be determined using the ALIGN program (version 2.0) and using a PAM120 with residue table, a gap length penalty of 12 and a gap penalty of 4. One skilled in the art can determine appropriate parameters for maximal alignment by particular alignment software. In certain aspects, the default parameters of the alignment software are used.

[0064] In certain aspects, the percentage identity "X" of a first nucleotide sequence to a second nucleotide sequence is calculated as $100 \times (Y/Z)$, where Y is the number of amino acid residues scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be higher than the percent identity of the second sequence to the first sequence.

[0065] Different regions within a single polynucleotide target sequence that align with a polynucleotide reference sequence can each have their own percent sequence identity. It is noted

that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

[0066] As used herein, the terms "homologous" and "homology" are interchangeable with the terms "identity" and "identical."

[0067] The term "naturally occurring variant thereof" refers to variants of the NLRP3 polypeptide sequence or *NLRP3* nucleic acid sequence (*e.g.*, transcript) which exist naturally within the defined taxonomic group, such as mammalian, such as mouse, monkey, and human. Typically, when referring to "naturally occurring variants" of a polynucleotide the term also can encompass any allelic variant of the *NLRP3*-encoding genomic DNA which is found at Chromosomal position 1q44 at 247,416,156-247,449,108 (*i.e.*, nucleotides 247,416,156-247,449,108 of GenBank Accession No. NC_000001.11) by chromosomal translocation or duplication, and the RNA, such as mRNA derived therefrom. "Naturally occurring variants" can also include variants derived from alternative splicing of the *NLRP3* mRNA. When referenced to a specific polypeptide sequence, *e.g.*, the term also includes naturally occurring forms of the protein, which can therefore be processed, *e.g.*, by co- or post-translational modifications, such as signal peptide cleavage, proteolytic cleavage, glycosylation, *etc.*

[0068] In determining the degree of "complementarity" between the ASOs of the disclosure (or regions thereof) and the target region of the nucleic acid which encodes mammalian NLRP3 (*e.g.*, the *NLRP3* gene), such as those disclosed herein, the degree of "complementarity" (also, "homology" or "identity") is expressed as the percentage identity (or percentage homology) between the sequence of the ASO (or region thereof) and the sequence of the target region (or the reverse complement of the target region) that best aligns therewith. The percentage is calculated by counting the number of aligned bases that are identical between the two sequences, dividing by the total number of contiguous monomers in the ASO, and multiplying by 100. In such a comparison, if gaps exist, it is preferable that such gaps are merely mismatches rather than areas where the number of monomers within the gap differs between the ASO of the disclosure and the target region.

[0069] The term "complement" as used herein indicates a sequence that is complementary to a reference sequence. It is well known that complementarity is the base principle of DNA replication and transcription as it is a property shared between two DNA or RNA sequences, such that when they are aligned antiparallel to each other, the nucleotide bases at each position in the sequences will be complementary, much like looking in the mirror and seeing the reverse of things.

Therefore, for example, the complement of a sequence of 5'"ATGC"3' can be written as 3'"TACG"5' or 5'"GCAT"3'. The terms "reverse complement", "reverse complementary", and "reverse complementarity" as used herein are interchangeable with the terms "complement", "complementary", and "complementarity." In some aspects, the term "complementary" refers to 100% match or complementarity (*i.e.*, fully complementary) to a contiguous nucleic acid sequence within a *NLRP3* transcript. In some aspects, the term "complementary" refers to at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% match or complementarity to a contiguous nucleic acid sequence within a *NLRP3* transcript.

[0070] The terms "corresponding to" and "corresponds to," when referencing two separate nucleic acid or nucleotide sequences can be used to clarify regions of the sequences that correspond or are similar to each other based on homology and/or functionality, although the nucleotides of the specific sequences can be numbered differently. For example, different isoforms of a gene transcript can have similar or conserved portions of nucleotide sequences whose numbering can differ in the respective isoforms based on alternative splicing and/or other modifications. In addition, it is recognized that different numbering systems can be employed when characterizing a nucleic acid or nucleotide sequence (*e.g.*, a gene transcript and whether to begin numbering the sequence from the translation start codon or to include the 5'UTR). Further, it is recognized that the nucleic acid or nucleotide sequence of different variants of a gene or gene transcript can vary. As used herein, however, the regions of the variants that share nucleic acid or nucleotide sequence homology and/or functionality are deemed to "correspond" to one another. For example, a nucleotide sequence of a *NLRP3* transcript corresponding to nucleotides X to Y of SEQ ID NO: 1 ("reference sequence") refers to an *NLRP3* transcript sequence (*e.g.*, *NLRP3* pre-mRNA or mRNA) that has an identical sequence or a similar sequence to nucleotides X to Y of SEQ ID NO: 1, wherein X is the start site and Y is the end site (as shown in FIG. 1). A person of ordinary skill in the art can identify the corresponding X and Y residues in the *NLRP3* transcript sequence by aligning the *NLRP3* transcript sequence with SEQ ID NO: 1.

[0071] The terms "corresponding nucleotide analog" and "corresponding nucleotide" are intended to indicate that the nucleobase in the nucleotide analog and the naturally occurring nucleotide have the same pairing, or hybridizing, ability. For example, when the 2-deoxyribose unit of the nucleotide is linked to an adenine, the "corresponding nucleotide analog" contains a pentose unit (different from 2-deoxyribose) linked to an adenine.

[0072] The annotation of ASO chemistry is as follows Beta-D-oxy LNA nucleotides are designated by OxyB where B designates a nucleotide base such as thymine (T), uridine (U), cytosine (C), 5-methylcytosine (MC), adenine (A) or guanine (G), and thus include OxyA, OxyT, OxyMC, OxyC and OxyG. DNA nucleotides are designated by DNAb, where the lower case b designates a nucleotide base such as thymine (T), uridine (U), cytosine (C), 5-methylcytosine (Mc), adenine (A) or guanine (G), and thus include DNAA, DNAt, DNA and DNAg. The letter M before C or c indicates 5-methylcytosine. The letter "s" indicates a phosphorothioate internucleotide linkage.

[0073] The term "ASO Number" or "ASO No." as used herein refers to a unique number given to a nucleotide sequence having the detailed chemical structure of the components, *e.g.*, nucleosides (*e.g.*, DNA), nucleoside analogs (*e.g.*, beta-D-oxy-LNA), nucleobase (*e.g.*, A, T, G, C, U, or MC), and backbone structure (*e.g.*, phosphorothioate or phosphorodiester). For example, ASO-NLRP3-206 can refer to NLRP3-206 (SEQ ID NO: 101).

[0074] "Potency" is normally expressed as an IC₅₀ or EC₅₀ value, in μ M, nM or pM unless otherwise stated. Potency can also be expressed in terms of percent inhibition. IC₅₀ is the median inhibitory concentration of a therapeutic molecule. EC₅₀ is the median effective concentration of a therapeutic molecule relative to a vehicle or control (*e.g.*, saline). In functional assays, IC₅₀ is the concentration of a therapeutic molecule that reduces a biological response, *e.g.*, transcription of mRNA or protein expression, by 50% of the biological response that is achieved by the therapeutic molecule. In functional assays, EC₅₀ is the concentration of a therapeutic molecule that produces 50% of the biological response, *e.g.*, transcription of mRNA or protein expression. IC₅₀ or EC₅₀ can be calculated by any number of means known in the art.

[0075] As used herein, the term "inhibiting," *e.g.*, the expression of *NLRP3* gene transcript and/or NLRP3 protein refers to the ASO reducing the expression of the *NLRP3* gene transcript and/or NLRP3 protein in a cell or a tissue. In some aspects, the term "inhibiting" refers to complete inhibition (100% inhibition or non-detectable level) of *NLRP3* gene transcript or NLRP3 protein. In other aspects, the term "inhibiting" refers to at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or at least 99% inhibition of *NLRP3* gene transcript and/or NLRP3 protein expression in a cell or a tissue.

[0076] As used herein, the term "extracellular vesicle" or "EV" refers to a cell-derived vesicle comprising a membrane that encloses an internal space. Extracellular vesicles comprise all membrane-bound vesicles (*e.g.*, exosomes, nanovesicles) that have a smaller diameter than the cell

from which they are derived. In some aspects, extracellular vesicles range in diameter from 20 nm to 1000 nm, and can comprise various macromolecular payload either within the internal space (*i.e.*, lumen), displayed on the external surface of the extracellular vesicle, and/or spanning the membrane. In some aspects, the payload can comprise nucleic acids, proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. In certain aspects, an extracellular vehicle comprises a scaffold moiety. By way of example and without limitation, extracellular vesicles include apoptotic bodies, fragments of cells, vesicles derived from cells by direct or indirect manipulation (*e.g.*, by serial extrusion or treatment with alkaline solutions), vesiculated organelles, and vesicles produced by living cells (*e.g.*, by direct plasma membrane budding or fusion of the late endosome with the plasma membrane). Extracellular vesicles can be derived from a living or dead organism, explanted tissues or organs, prokaryotic or eukaryotic cells, and/or cultured cells. In some aspects, the extracellular vesicles are produced by cells that express one or more transgene products.

[0077] As used herein, the term "exosome" refers to an extracellular vesicle with a diameter between 20-300 nm (*e.g.*, between 40-200 nm). Exosomes comprise a membrane that encloses an internal space (*i.e.*, lumen), and, in some aspects, can be generated from a cell (*e.g.*, producer cell) by direct plasma membrane budding or by fusion of the late endosome with the plasma membrane. In certain aspects, an exosome comprises a scaffold moiety. As described *infra*, exosome can be derived from a producer cell, and isolated from the producer cell based on its size, density, biochemical parameters, or a combination thereof. In some aspects, the EVs, *e.g.*, exosomes, of the present disclosure are produced by cells that express one or more transgene products.

[0078] As used herein, the term "nanovesicle" refers to an extracellular vesicle with a diameter between 20-250 nm (*e.g.*, between 30-150 nm) and is generated from a cell (*e.g.*, producer cell) by direct or indirect manipulation such that the nanovesicle would not be produced by the cell without the manipulation. Appropriate manipulations of the cell to produce the nanovesicles include but are not limited to serial extrusion, treatment with alkaline solutions, sonication, or combinations thereof. In some aspects, production of nanovesicles can result in the destruction of the producer cell. In some aspects, population of nanovesicles described herein are substantially free of vesicles that are derived from cells by way of direct budding from the plasma membrane or fusion of the late endosome with the plasma membrane. In certain aspects, a nanovesicle comprises a scaffold moiety. Nanovesicles, once derived from a producer cell, can be isolated from the producer cell based on its size, density, biochemical parameters, or a combination thereof.

[0079] As used herein the term "surface-engineered EVs, *e.g.*, exosomes" (*e.g.*, Scaffold X-engineered EVs, *e.g.*, exosomes) refers to an EV, *e.g.*, exosome, with the membrane or the surface of the EV, *e.g.*, exosome, modified in its composition so that the surface of the engineered EV, *e.g.*, exosome, is different from that of the EV, *e.g.*, exosome, prior to the modification or of the naturally occurring EV, *e.g.*, exosome. The engineering can be on the surface of the EV, *e.g.*, exosome, or in the membrane of the EV, *e.g.*, exosome, so that the surface of the EV, *e.g.*, exosome, is changed. For example, the membrane is modified in its composition of a protein, a lipid, a small molecule, a carbohydrate, *etc.* The composition can be changed by a chemical, a physical, or a biological method or by being produced from a cell previously or concurrently modified by a chemical, a physical, or a biological method. Specifically, the composition can be changed by a genetic engineering or by being produced from a cell previously modified by genetic engineering. In some aspects, a surface-engineered EV, *e.g.*, exosome, comprises an exogenous protein (*i.e.*, a protein that the EV, *e.g.*, exosome, does not naturally express) or a fragment or variant thereof that can be exposed to the surface of the EV, *e.g.*, exosome, or can be an anchoring point (attachment) for a moiety exposed on the surface of the EV, *e.g.*, exosome. In other aspects, a surface-engineered EV, *e.g.*, exosome, comprises a higher expression (*e.g.*, higher number) of a natural exosome protein (*e.g.*, Scaffold X) or a fragment or variant thereof that can be exposed to the surface of the EV, *e.g.*, exosome, or can be an anchoring point (attachment) for a moiety exposed on the surface of the EV, *e.g.*, exosome.

[0080] As used herein the term "lumen-engineered exosome" (*e.g.*, Scaffold Y-engineered exosome) refers to an EV, *e.g.*, exosome, with the membrane or the lumen of the EV, *e.g.*, exosome, modified in its composition so that the lumen of the engineered EV, *e.g.*, exosome, is different from that of the EV, *e.g.*, exosome, prior to the modification or of the naturally occurring EV, *e.g.*, exosome. The engineering can be directly in the lumen or in the membrane of the EV, *e.g.*, exosome so that the lumen of the EV, *e.g.*, exosome is changed. For example, the membrane is modified in its composition of a protein, a lipid, a small molecule, a carbohydrate, *etc.* so that the lumen of the EV, *e.g.*, exosome is modified. The composition can be changed by a chemical, a physical, or a biological method or by being produced from a cell previously modified by a chemical, a physical, or a biological method. Specifically, the composition can be changed by a genetic engineering or by being produced from a cell previously modified by genetic engineering. In some aspects, a lumen-engineered exosome comprises an exogenous protein (*i.e.*, a protein that the EV, *e.g.*, exosome does not naturally express) or a fragment or variant thereof that can be exposed in the lumen of the EV, *e.g.*, exosome or can be an anchoring point (attachment) for a moiety exposed on

the inner layer of the EV, *e.g.*, exosome. In other aspects, a lumen-engineered EV, *e.g.*, exosome, comprises a higher expression of a natural exosome protein (*e.g.*, Scaffold X or Scaffold Y) or a fragment or variant thereof that can be exposed to the lumen of the exosome or can be an anchoring point (attachment) for a moiety exposed in the lumen of the exosome.

[0081] The term "modified," when used in the context of EVs, *e.g.*, exosomes described herein, refers to an alteration or engineering of an EV, *e.g.*, exosome and/or its producer cell, such that the modified EV, *e.g.*, exosome is different from a naturally-occurring EV, *e.g.*, exosome. In some aspects, a modified EV, *e.g.*, exosome described herein comprises a membrane that differs in composition of a protein, a lipid, a small molecular, a carbohydrate, *etc.* compared to the membrane of a naturally-occurring EV, *e.g.*, exosome (*e.g.*, membrane comprises higher density or number of natural exosome proteins and/or membrane comprises proteins that are not naturally found in exosomes (*e.g.*, an ASO). In certain aspects, such modifications to the membrane changes the exterior surface of the EV, *e.g.*, exosome (*e.g.*, surface-engineered EVs, *e.g.*, exosomes described herein). In certain aspects, such modifications to the membrane changes the lumen of the EV, *e.g.*, exosome (*e.g.*, lumen-engineered EVs, *e.g.*, exosomes described herein).

[0082] As used herein, the term "scaffold moiety" refers to a molecule that can be used to anchor a payload or any other compound of interest (*e.g.*, an ASO) to the EV, *e.g.*, exosome either on the luminal surface or on the exterior surface of the EV, *e.g.*, exosome. In certain aspects, a scaffold moiety comprises a synthetic molecule. In some aspects, a scaffold moiety comprises a non-polypeptide moiety. In other aspects, a scaffold moiety comprises a lipid, carbohydrate, or protein that naturally exists in the EV, *e.g.*, exosome. In some aspects, a scaffold moiety comprises a lipid, carbohydrate, or protein that does not naturally exist in the EV, *e.g.*, exosome. In certain aspects, a scaffold moiety is Scaffold X. In some aspects, a scaffold moiety is Scaffold Y. In further aspects, a scaffold moiety comprises both Scaffold X and Scaffold Y. Non-limiting examples of other scaffold moieties that can be used with the present disclosure include: aminopeptidase N (CD13); Neprilysin, AKA membrane metalloendopeptidase (MME); ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1); Neuropilin-1 (NRP1); CD9, CD63, CD81, PDGFR, GPI anchor proteins, lactadherin (MFGE8), LAMP2, and LAMP2B.

[0083] As used herein, the term "Scaffold X" refers to exosome proteins that have recently been identified on the surface of exosomes. *See, e.g.*, U.S. Pat. No. 10,195,290, which is incorporated herein by reference in its entirety. Non-limiting examples of Scaffold X proteins include: prostaglandin F2 receptor negative regulator ("the PTGFRN protein"); basigin ("the BSG protein"); immunoglobulin superfamily member 2 ("the IGSF2 protein"); immunoglobulin

superfamily member 3 ("the IGSF3 protein"); immunoglobulin superfamily member 8 ("the IGSF8 protein"); integrin beta-1 ("the ITGB1 protein"); integrin alpha-4 ("the ITGA4 protein"); 4F2 cell-surface antigen heavy chain ("the SLC3A2 protein"); a class of ATP transporter proteins ("the ATP1A1 protein," "the ATP1A2 protein," "the ATP1A3 protein," "the ATP1A4 protein," "the ATP1B3 protein," "the ATP2B1 protein," "the ATP2B2 protein," "the ATP2B3 protein," "the ATP2B protein"); and a functional fragment thereof. In some aspects, a Scaffold X protein can be a whole protein or a fragment thereof (*e.g.*, functional fragment, *e.g.*, the smallest fragment that is capable of anchoring another moiety on the exterior surface or on the luminal surface of the EV, *e.g.*, exosome). In some aspects, a Scaffold X can anchor a moiety (*e.g.*, an ASO) to the external surface or the luminal surface of the exosome.

[0084] As used herein, the term "Scaffold Y" refers to exosome proteins that were newly identified within the lumen of exosomes. *See, e.g.*, International Publ. No. WO/2019/099942, which is incorporated herein by reference in its entirety. Non-limiting examples of Scaffold Y proteins include: myristoylated alanine rich Protein Kinase C substrate ("the MARCKS protein"); myristoylated alanine rich Protein Kinase C substrate like 1 ("the MARCKSL1 protein"); and brain acid soluble protein 1 ("the BASP1 protein"). In some aspects, a Scaffold Y protein can be a whole protein or a fragment thereof (*e.g.*, functional fragment, *e.g.*, the smallest fragment that is capable of anchoring a moiety to the luminal surface of the exosome). In some aspects, a Scaffold Y can anchor a moiety (*e.g.*, an ASO) to the luminal surface of the EV, *e.g.*, exosome. In some aspects, a Scaffold Y can anchor a moiety (*e.g.*, an ASO) to the exterior surface of the EV, *e.g.*, exosome.

[0085] As used herein, the term "fragment" of a protein (*e.g.*, therapeutic protein, Scaffold X, or Scaffold Y) refers to an amino acid sequence of a protein that is shorter than the naturally-occurring sequence, N- and/or C-terminally deleted or any part of the protein deleted in comparison to the naturally occurring protein. As used herein, the term "functional fragment" refers to a protein fragment that retains protein function. Accordingly, in some aspects, a functional fragment of a Scaffold X protein retains the ability to anchor a moiety on the luminal surface or on the exterior surface of the EV, *e.g.*, exosome. Similarly, in certain aspects, a functional fragment of a Scaffold Y protein retains the ability to anchor a moiety on the luminal surface or exterior surface of the EV, *e.g.*, exosome. Whether a fragment is a functional fragment can be assessed by any art known methods to determine the protein content of EVs, *e.g.*, exosomes including Western Blots, FACS analysis and fusions of the fragments with autofluorescent proteins like, *e.g.*, GFP. In certain aspects, a functional fragment of a Scaffold X protein retains at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or at least about 100% of the

ability, *e.g.*, an ability to anchor a moiety, of the naturally occurring Scaffold X protein. In some aspects, a functional fragment of a Scaffold Y protein retains at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or at least about 100% of the ability, *e.g.*, an ability to anchor another molecule, of the naturally occurring Scaffold Y protein.

[0086] As used herein, the term "variant" of a molecule (*e.g.*, functional molecule, antigen, Scaffold X and/or Scaffold Y) refers to a molecule that shares certain structural and functional identities with another molecule upon comparison by a method known in the art. For example, a variant of a protein can include a substitution, insertion, deletion, frameshift or rearrangement in another protein.

[0087] In some aspects, a variant of a Scaffold X comprises a variant having at least about 70% identity to the full-length, mature PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, SLC3A2, or ATP transporter proteins or a fragment (*e.g.*, functional fragment) of the PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, SLC3A2, or ATP transporter proteins. In some aspects, variants or variants of fragments of PTGFRN share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with PTGFRN according to SEQ ID NO: 301 or with a functional fragment thereof. In some aspects variants or variants of fragments of BSG share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with BSG according to SEQ ID NO: 303 or with a functional fragment thereof. In some aspects variants or variants of fragments of IGSF2 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with IGSF2 according to SEQ ID NO: 308 or with a functional fragment thereof. In some aspects variants or variants of fragments of IGSF3 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with IGSF3 according to SEQ ID NO: 309 or with a functional fragment thereof. In some aspects variants or variants of fragments of IGSF8 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with IGSF8 according to SEQ ID NO: 304 or with a functional fragment thereof. In some aspects variants or variants of fragments of ITGB1 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%

sequence identity with ITGB1 according to SEQ ID NO: 305 or with a functional fragment thereof. In some aspects variants or variants of fragments of ITGA4 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ITGA4 according to SEQ ID NO: 306 or with a functional fragment thereof. In some aspects variants or variants of fragments of SLC3A2 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with SLC3A2 according to SEQ ID NO: 307 or with a functional fragment thereof. In some aspects variants or variants of fragments of ATP1A1 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ATP1A1 according to SEQ ID NO: 310 or with a functional fragment thereof. In some aspects variants or variants of fragments of ATP1A2 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ATP1A2 according to SEQ ID NO: 311 or with a functional fragment thereof. In some aspects variants or variants of fragments of ATP1A3 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ATP1A3 according to SEQ ID NO: 312 or with a functional fragment thereof. In some aspects variants or variants of fragments of ATP1A4 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ATP1A4 according to SEQ ID NO: 313 or with a functional fragment thereof. In some aspects variants or variants of fragments of ATP1B3 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ATP1B3 according to SEQ ID NO: 314 or with a functional fragment thereof. In some aspects variants or variants of fragments of ATP2B1 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ATP2B1 according to SEQ ID NO: 315 or with a functional fragment thereof. In some aspects variants or variants of fragments of ATP2B2 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ATP2B2 according to SEQ ID NO: 316 or with a functional fragment

thereof. In some aspects variants or variants of fragments of ATP2B3 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ATP2B3 according to SEQ ID NO: 317 or with a functional fragment thereof. In some aspects variants or variants of fragments of ATP2B4 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with ATP2B4 according to SEQ ID NO: 318 or with a functional fragment thereof. In some aspects, the variant or variant of a fragment of Scaffold X protein disclosed herein retains the ability to be specifically targeted to EVs, *e.g.*, exosomes. In some aspects, the Scaffold X includes one or more mutations, for example, conservative amino acid substitutions.

[0088] In some aspects, a variant of a Scaffold Y comprises a variant having at least 70% identity to MARCKS, MARCKSL1, BASP1, or a fragment of MARCKS, MARCKSL1, or BASP1. In some aspects variants or variants of fragments of MARCKS share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with MARCKS according to SEQ ID NO: 401 or with a functional fragment thereof. In some aspects variants or variants of fragments of MARCKSL1 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with MARCKSL1 according to SEQ ID NO: 402 or with a functional fragment thereof. In some aspects variants or variants of fragments of BASP1 share at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity with BASP1 according to SEQ ID NO: 403 or with a functional fragment thereof. In some aspects, the variant or variant of a fragment of Scaffold Y protein retains the ability to be specifically targeted to the luminal surface of EVs, *e.g.*, exosomes. In some aspects, the Scaffold Y includes one or more mutations, *e.g.*, conservative amino acid substitutions.

[0089] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan),

beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the substitution is considered to be conservative. In another aspect, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0090] The term "percent sequence identity" or "percent identity" between two polynucleotide or polypeptide sequences refers to the number of identical matched positions shared by the sequences over a comparison window, taking into account additions or deletions (*i.e.*, gaps) that must be introduced for optimal alignment of the two sequences. A matched position is any position where an identical nucleotide or amino acid is presented in both the target and reference sequence. Gaps presented in the target sequence are not counted since gaps are not nucleotides or amino acids. Likewise, gaps presented in the reference sequence are not counted since target sequence nucleotides or amino acids are counted, not nucleotides or amino acids from the reference sequence.

[0091] The percentage of sequence identity is calculated by determining the number of positions at which the identical amino-acid residue or nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. The comparison of sequences and determination of percent sequence identity between two sequences may be accomplished using readily available software both for online use and for download. Suitable software programs are available from various sources, and for alignment of both protein and nucleotide sequences. One suitable program to determine percent sequence identity is *bl2seq*, part of the BLAST suite of programs available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast.ncbi.nlm.nih.gov). *Bl2seq* performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, *e.g.*, Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at www.ebi.ac.uk/Tools/psa.

[0092] Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16,

80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

[0093] One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. Sequence alignments can be derived from multiple sequence alignments. One suitable program to generate multiple sequence alignments is ClustalW2, available from www.clustal.org. Another suitable program is MUSCLE, available from www.drive5.com/muscle/. ClustalW2 and MUSCLE are alternatively available, *e.g.*, from the EBI.

[0094] It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data (*e.g.*, crystallographic protein structures), functional data (*e.g.*, location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at www.tcoffee.org, and alternatively available, *e.g.*, from the EBI. It will also be appreciated that the final alignment used to calculate percent sequence identity may be curated either automatically or manually.

[0095] The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In one aspect, the polynucleotide variants contain alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. In another aspect, nucleotide variants are produced by silent substitutions due to the degeneracy of the genetic code. In other aspects, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination. Polynucleotide variants can be produced for a variety of reasons, *e.g.*, to optimize codon expression for a particular host (change codons in the human mRNA to others, *e.g.*, a bacterial host such as *E. coli*).

[0096] Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present disclosure. Alternatively, non-naturally occurring variants can be produced by mutagenesis techniques or by direct synthesis.

[0097] Using known methods of protein engineering and recombinant DNA technology, variants can be generated to improve or alter the characteristics of the polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. Ron *et al.*, *J. Biol. Chem.* 268: 2984-2988 (1993),

incorporated herein by reference in its entirety, reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli *et al.*, *J. Biotechnology* 7:199-216 (1988), incorporated herein by reference in its entirety.)

[0098] Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (*J. Biol. Chem* 268:22105-22111 (1993), incorporated herein by reference in its entirety) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

[0099] As stated above, polypeptide variants include, *e.g.*, modified polypeptides. Modifications include, *e.g.*, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation (Mei *et al.*, *Blood* 116:270-79 (2010), which is incorporated herein by reference in its entirety), proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. In some aspects, Scaffold X and/or Scaffold Y is modified at any convenient location.

[0100] As used herein the term "linked to" or "conjugated to" are used interchangeably and refer to a covalent or non-covalent bond formed between a first moiety and a second moiety, *e.g.*, a cholesterol and an ASO or Scaffold X and an ASO, respectively, *e.g.*, a scaffold moiety expressed in or on the extracellular vesicle and an ASO, *e.g.*, Scaffold X (*e.g.*, a PTGFRN protein), respectively, in the luminal surface of or on the external surface of the extracellular vesicle.

[0101] The term "encapsulated", or grammatically different forms of the term (*e.g.*, encapsulation, or encapsulating) refers to a status or process of having a first moiety (*e.g.*, an ASO) inside a second moiety (*e.g.*, an EV, *e.g.*, exosome) without chemically or physically linking the two moieties. In some aspects, the term "encapsulated" can be used interchangeably with "in the lumen of." Non-limiting examples of encapsulating a first moiety (*e.g.*, an ASO) into a second moiety (*e.g.*, EVs, *e.g.*, exosomes) are disclosed elsewhere herein.

[0102] As used herein, the term "producer cell" refers to a cell used for generating an EV, *e.g.*, exosome. A producer cell can be a cell cultured *in vitro*, or a cell *in vivo*. A producer cell includes, but not limited to, a cell known to be effective in generating EVs, *e.g.*, exosomes, *e.g.*, HEK293 cells, Chinese hamster ovary (CHO) cells, mesenchymal stem cells (MSCs), BJ human foreskin fibroblast cells, fHDF fibroblast cells, AGE.HN[®] neuronal precursor cells, CAP[®] amniocyte cells, adipose mesenchymal stem cells, RPTEC/TERT1 cells. In certain aspects, a producer cell is not an antigen-presenting cell. In some aspects, a producer cell is not a dendritic cell, a B cell, a mast cell, a macrophage, a neutrophil, Kupffer-Browicz cell, cell derived from any of these cells, or any combination thereof. In some aspects, the EVs, *e.g.*, exosomes useful in the present disclosure do not carry an antigen on MHC class I or class II molecule exposed on the surface of the EV, *e.g.*, exosome, but instead can carry an antigen in the lumen of the EV, *e.g.*, exosome or on the surface of the EV, *e.g.*, exosome by attachment to Scaffold X and/or Scaffold Y.

[0103] As used herein, the terms "isolate," "isolated," and "isolating" or "purify," "purified," and "purifying" as well as "extracted" and "extracting" are used interchangeably and refer to the state of a preparation (*e.g.*, a plurality of known or unknown amount and/or concentration) of desired EVs, that have undergone one or more processes of purification, *e.g.*, a selection or an enrichment of the desired EV preparation. In some aspects, isolating or purifying as used herein is the process of removing, partially removing (*e.g.*, a fraction) of the EVs from a sample containing producer cells. In some aspects, an isolated EV composition has no detectable undesired activity or, alternatively, the level or amount of the undesired activity is at or below an acceptable level or amount. In other aspects, an isolated EV composition has an amount and/or concentration of desired EVs at or above an acceptable amount and/or concentration. In other aspects, the isolated EV composition is enriched as compared to the starting material (*e.g.*, producer cell preparations) from which the composition is obtained. This enrichment can be by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or greater than 99.9999% as compared to the starting material. In some aspects, isolated

EV preparations are substantially free of residual biological products. In some aspects, the isolated EV preparations are 100% free, 99% free, 98% free, 97% free, 96% free, 95% free, 94% free, 93% free, 92% free, 91% free, or 90% free of any contaminating biological matter. Residual biological products can include abiotic materials (including chemicals) or unwanted nucleic acids, proteins, lipids, or metabolites. Substantially free of residual biological products can also mean that the EV composition contains no detectable producer cells and that only EVs are detectable.

[0104] As used herein, the term "payload" refers to an agent that acts on a target (*e.g.*, a target cell) that is contacted with the EV. A non-limiting examples of payload that can be included on the EV, *e.g.*, exosome, is an ASO. Payloads that can be introduced into or onto an EV, *e.g.*, exosome, and/or a producer cell include agents such as, nucleotides (*e.g.*, nucleotides comprising a detectable moiety or a toxin or that disrupt transcription), nucleic acids (*e.g.*, DNA or mRNA molecules that encode a polypeptide such as an enzyme, or RNA molecules that have regulatory function such as miRNA, dsDNA, lncRNA, and siRNA), amino acids (*e.g.*, amino acids comprising a detectable moiety or a toxin or that disrupt translation), polypeptides (*e.g.*, enzymes), lipids, carbohydrates, and small molecules (*e.g.*, small molecule drugs and toxins). In certain aspects, a payload comprises an ASO. As used herein, the term "antibody" encompasses an immunoglobulin whether natural or partly or wholly synthetically produced, and fragments thereof. The term also covers any protein having a binding domain that is homologous to an immunoglobulin binding domain. "Antibody" further includes a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. As used herein, the term "antigen" refers to any agent that when introduced into a subject elicits an immune response (cellular or humoral) to itself. Use of the term antibody is meant to include whole antibodies, polyclonal, monoclonal and recombinant antibodies, fragments thereof, and further includes single-chain antibodies, humanized antibodies, murine antibodies, chimeric, mouse-human, mouse-primate, primate-human monoclonal antibodies, anti-idiotypic antibodies, antibody fragments, such as, *e.g.*, scFv, (scFv)₂, Fab, Fab', and F(ab')₂, F(ab1)₂, Fv, dAb, and Fd fragments, diabodies, and antibody-related polypeptides. Antibody includes bispecific antibodies and multispecific antibodies so long as they exhibit the desired biological activity or function.

[0105] The terms "individual," "subject," "host," and "patient," are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. The compositions and methods described herein are applicable to both human therapy and veterinary applications. In some aspects, the subject is a mammal, and in other aspects

the subject is a human. As used herein, a "mammalian subject" includes all mammals, including without limitation, humans, domestic animals (*e.g.*, dogs, cats and the like), farm animals (*e.g.*, cows, sheep, pigs, horses and the like) and laboratory animals (*e.g.*, monkey, rats, mice, rabbits, guinea pigs and the like).

[0106] The term "pharmaceutical composition" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the composition would be administered. Such composition can be sterile.

[0107] As used herein, the term "substantially free" means that the sample comprising EVs, *e.g.*, exosomes, comprise less than 10% of macromolecules by mass/volume (m/v) percentage concentration. Some fractions may contain less than 0.001%, less than 0.01%, less than 0.05%, less than 0.1%, less than 0.2%, less than 0.3%, less than 0.4%, less than 0.5%, less than 0.6%, less than 0.7%, less than 0.8%, less than 0.9%, less than 1%, less than 2%, less than 3%, less than 4%, less than 5%, less than 6%, less than 7%, less than 8%, less than 9%, or less than 10% (m/v) of macromolecules.

[0108] As used herein, the term "macromolecule" means nucleic acids, contaminant proteins, lipids, carbohydrates, metabolites, or a combination thereof.

[0109] As used herein, the term "conventional exosome protein" means a protein previously known to be enriched in exosomes, including but is not limited to CD9, CD63, CD81, PDGFR, GPI anchor proteins, lactadherin (MFGE8), LAMP2, and LAMP2B, a fragment thereof, or a peptide that binds thereto.

[0110] "Administering," as used herein, means to give a composition comprising an EV, *e.g.*, exosome, disclosed herein to a subject via a pharmaceutically acceptable route. Routes of administration can be intravenous, *e.g.*, intravenous injection and intravenous infusion. Additional routes of administration include, *e.g.*, subcutaneous, intramuscular, oral, nasal, and pulmonary administration. EVs, *e.g.*, exosomes can be administered as part of a pharmaceutical composition comprising at least one excipient.

[0111] An "effective amount" of, *e.g.*, an ASO or an extracellular vesicle as disclosed herein, is an amount sufficient to carry out a specifically stated purpose. An "effective amount" can be determined empirically and in a routine manner, in relation to the stated purpose.

[0112] "Treat," "treatment," or "treating," as used herein refers to, *e.g.*, the reduction in severity of a disease or condition; the reduction in the duration of a disease course; the amelioration or elimination of one or more symptoms associated with a disease or condition; the provision of

beneficial effects to a subject with a disease or condition, without necessarily curing the disease or condition. The term also includes prophylaxis or prevention of a disease or condition or its symptoms thereof. In one aspect, the "treating" or "treatment" includes inducing hematopoiesis in a subject in need thereof. In some aspects, the disease or condition is associated with a hematopoiesis or a deficiency thereof. In certain aspects, the disease or condition is a cancer. In some aspects, the treating enhances hematopoiesis in a subject having a cancer, wherein the enhanced hematopoiesis comprises increased proliferation and/or differentiation of one or more immune cell in the subject

[0113] "Prevent" or "preventing," as used herein, refers to decreasing or reducing the occurrence or severity of a particular outcome. In some aspects, preventing an outcome is achieved through prophylactic treatment. In some aspects, an EV, *e.g.*, an exosome, comprising an ASO, described herein, is administered to a subject prophylactically. In some aspects, the subject is at risk of developing cancer. In some aspects, the subject is at risk of developing a hematopoietic disorder.

II. Methods of the Disclosure

[0114] Some aspects of the present disclosure are directed to methods of preventing and/or treating a peripheral neuropathy in a subject in need thereof, comprising administering an EV comprising an NLRP3 antagonist disclosed herein. As described herein, the NLRP3 antagonist is an ASO. ASOs useful for the present disclosure can specifically hybridize to one or more regions of a NLRP3 transcript (*e.g.*, pre-mRNA or mRNA), resulting in reduction and/or inhibition of NLRP3 protein expression in a cell. Accordingly, EVs comprising such an ASO can be useful for preventing and/or treating a peripheral neuropathy associated with increased expression of a NLRP3 protein.

[0115] In some aspects, a peripheral neuropathy that can be treated with the present methods is characterized by increased inflammation. In some aspects, the peripheral neuropathy is associated with diabetes, a trauma, an autoimmune disorder, a kidney disorder, a liver disorder, hypothyroidism, a vascular disorder, an abnormal vitamin level, alcohol use, or any combination thereof. In some aspects, the peripheral neuropathy comprises inflammation of one or more peripheral nerve.

[0116] In some aspects, the the peripheral neuropathy comprises chemotherapy-induced peripheral neuropathy (CIPN). In some aspects, the subject was previously administered a chemotherapy. In some aspects, the subject was administered a chemotherapy at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 6 weeks,

at least about 8 weeks, at least about 10 weeks, at least about 12 weeks, at least about 16 weeks, at least about 20 weeks, at least about 24 weeks, or at least about 28 weeks prior to administering an EV comprising an NLRP3 antagonist disclosed herein. In some aspects, the subject is administered an EV comprising an NLRP3 antagonist disclosed herein during concurrent chemotherapy. In some aspects, the EV comprising an NLRP3 antagonist disclosed herein is administered on the same day as the chemotherapy. In some aspects, the EV comprising an NLRP3 antagonist disclosed herein and the chemotherapy are not administered on the same day. In some aspects, the chemotherapy comprises a platinum derivative, a vinca alkaloid, bortezomib, a taxane, or any combination thereof. In some aspects, the chemotherapy comprises cisplatin, carboplatin, oxaliplatin, docetaxel, vincristine, paclitaxel, gemcitabine, or any combination thereof. In certain aspects, the chemotherapy comprises cisplatin.

[0117] In some aspects, the EV comprising an NLRP3 antagonist disclosed herein reduces the severity and/or occurrence of one or more symptom of a peripheral neuropathy, *e.g.*, CIPN, in the subject. In some aspects, the EV comprising an NLRP3 antagonist disclosed herein the extracellular vesicle or the ASO induces M2 macrophage polarization in the subject. In some aspects, the EV comprising an NLRP3 antagonist disclosed herein reduces myeloid inflammation in a nerve, meningeal myeloid inflammation, nerve sheath inflammation, or any combination thereof. In some aspects, the EV comprising an NLRP3 antagonist disclosed herein reduces myeloid inflammation in a sheath. In some aspects, the EV comprising an NLRP3 antagonist disclosed herein reduces macrophage influx in one or more of a root, nerve, and/or muscle. In some aspects, the EV comprising an NLRP3 antagonist disclosed herein reduces macrophage phagocytosis in one or more of a root, nerve, and/or muscle. In some aspects, the extracellular vesicle reduces the severity or occurrence of one or more symptom in the subject selected from tingling, pain, burning, numbness, sensitivity to hot, sensitivity to cold, difficulty with fine motor skills, and any combination thereof. In some aspects, the extracellular vesicle reduces the severity or occurrence of pain.

[0118] In some aspects, the EVs are administered intravenously to the circulatory system of the subject. In some aspects, the EVs are infused in suitable liquid and administered into a vein of the subject. In some aspects, the EVs are administered intra-arterially to the circulatory system of the subject. In some aspects, the EVs are infused in suitable liquid and administered into an artery of the subject.

[0119] In some aspects, the EVs are administered to the subject by intrathecal administration. In some aspects, the EVs are administered by intrathecal administration, followed

by application of a mechanical convective force to the torso. See, e.g., Verma et al., Alzheimer's Dement. 12:e12030 (2020); which is incorporated by reference herein in its entirety). As such, certain aspects of the present disclosure are directed to methods of administering an EV, e.g., an exosome, to a subject in need thereof, comprising administering the EV, e.g., exosome, to the subject by intrathecal injection, followed by applying a mechanical convective force to the torso of the subject. In some aspects, the mechanical convective force is achieved using a high frequency chest wall or lumbothoracic oscillating respiratory clearance device (e.g., a Smart Vest or Smart Wrap, ELECTROMED INC, New Prague, MN, USA). In some aspects, the mechanical convective force, e.g., the oscillating vest, facilitates spread of the intrathecally dosed EVs, e.g., exosomes, further down the nerve thus allowing for better EV, e.g., exosome, delivery to nerves.

[0120] In some aspects, the intra- and trans-compartmental biodistribution of exosomes can be manipulated by exogenous extracorporeal forces acting upon a subject after compartmental delivery of exosomes. This includes the application of mechanical convection, for example by way of applying percussion, vibration, shaking, or massaging of a body compartment or the entire body. Following intrathecal dosing for example, the application of chest wall vibrations by several means including an oscillating mechanical jacket can spread the biodistribution of exosomes along the neuraxis or along cranial and spinal nerves, which can be helpful in the treatment of nerve disorders by drug carrying exosomes.

[0121] In some aspects, the application of external mechanical convective forces via an oscillating jacket or other similar means can be used to remove exosomes and other material from the cerebrospinal fluid of the intrathecal space and out to the peripheral circulation. This aspect can help remove endogenous toxic exosomes and other deleterious macromolecules such as beta-amyloid, tau, alpha-synuclein, TDP43, neurofilament and excessive cerebrospinal fluid from the intrathecal space to the periphery for elimination.

[0122] In some aspects, exosomes delivered via the intracerebroventricular route can be made to translocate throughout the neuraxis by simultaneously incorporating a lumbar puncture and allowing for ventriculo-lumbar perfusion wherein additional fluid is infused into the ventricles after exosome dosing, while allowing the existing neuraxial column of CSF to exit is the lumbar puncture. Ventriculo-lumbar perfusion can allow ICV dosed exosome to spread along the entire neuraxis and completely cover the subarachoid space in order to treat leptomeningeal inflammation and other diseases.

[0123] In some aspects, the application of external extracorporeal focused ultrasound, thermal energy (heat) or cold may be used to manipulate the compartmental pharmacokinetics and drug release properties of exosomes engineered to be sensitive to these phenomena.

[0124] In some aspects, the intracompartmental behavior and biodistribution of exosomes engineered to contain paramagnetic material can be manipulated by the external application of magnets or a magnetic field.

[0125] In some aspects, the EVs are administered via an injection into the spinal canal, or into the subarachnoid space so that it reaches the cerebrospinal fluid (CSF). In some aspects, the EVs are administered intratumorally into one or more tumors of the subject. In some aspects, the EVs are administered to the subject by intranasal administration. In some aspects, the EVs can be insufflated through the nose in a form of either topical administration or systemic administration. In certain aspects, the EVs are administered as nasal spray. In some aspects, the EVs are administered to the subject by intraperitoneal administration. In some aspects, the EVs are infused in suitable liquid and injected into the peritoneum of the subject. In some aspects, the intraperitoneal administration results in distribution of the EVs to the lymphatics. In some aspects, the intraperitoneal administration results in distribution of the EVs to the thymus, spleen, and/or bone marrow. In some aspects, the intraperitoneal administration results in distribution of the EVs to one or more lymph nodes. In some aspects, the intraperitoneal administration results in distribution of the EVs to one or more of the cervical lymph node, the inguinal lymph node, the mediastinal lymph node, or the sternal lymph node. In some aspects, the intraperitoneal administration results in distribution of the EVs to the pancreas. In some aspects, the EVs, e.g., exosomes, are administered to the subject by periocular administration. In some aspects, the s are injected into the periocular tissues. Periocular drug administration includes the routes of subconjunctival, anterior sub-Tenon's, posterior sub-Tenon's, and retrobulbar administration.

II.A. NLRP3 Antagonist

[0126] Certain aspects of the present disclosure are directed to methods of administering to a subject an EV comprising an exogenous NLRP3 antagonist. As used herein, an "NLRP3 antagonist" is a substance which inhibits or blocks the NLRP3 pathway. The NLRP3 antagonist can directly affect the activity and/or expression of NLRP3. Alternatively, the NLRP3 antagonist can indirectly affect the activity and/or expression NLRP3, including be affecting the activity and/or expression of another factor in the NLRP3 pathway. In some aspects, the NLRP3 antagonist is selected from a chemical compound, an siRNA, an shRNA, an antisense oligonucleotide, a

peptide (*e.g.*, a protein), and any combination thereof. In certain aspects, the NLRP3 antagonist is an ASO, *e.g.*, any ASO disclosed herein.

[0127] In some aspects, the NLRP3 Antagonist is an antisense oligonucleotide, a phosphorodiamidate Morpholino oligomer (PMO), or a peptide-conjugated phosphorodiamidate morpholino oligomer (PPMO).

II.A.1. Antisense Oligonucleotides (ASOs)

[0128] In some aspects, the present disclosure employs antisense oligonucleotides (ASOs) for use in treating a peripheral neuropathy in a subject in need thereof by modulating the function of nucleic acid molecules encoding mammalian NLRP3, such as the *NLRP3* nucleic acid, *e.g.*, *NLRP3* transcript, including *NLRP3* pre-mRNA, and *NLRP3* mRNA, or naturally occurring variants of such nucleic acid molecules encoding mammalian NLRP3. The term "ASO" in the context of the present disclosure, refers to a molecule formed by covalent linkage of two or more nucleotides (*i.e.*, an oligonucleotide).

[0129] The ASO comprises a contiguous nucleotide sequence of from about 10 to about 30, such as 10–20, 14–20, 16–20, or 15–25, nucleotides in length. In certain aspects, the ASO is 20 nucleotides in length. In certain aspects, the ASO is 18 nucleotides in length. In certain aspects, the ASO is 19 nucleotides in length. In certain aspects, the ASO is 17 nucleotides in length. In certain aspects, the ASO is 16 nucleotides in length. In certain aspects, the ASO is 15 nucleotides in length. In certain aspects, the ASO is 14 nucleotides in length. In certain aspects, the ASO is 13 nucleotides in length. In certain aspects, the ASO is 12 nucleotides in length. In certain aspects, the ASO is 11 nucleotides in length. In certain aspects, the ASO is 10 nucleotides in length.

[0130] In some aspects, the ASO comprises a contiguous nucleotide sequence of from about 10 to about 50 nucleotides in length, *e.g.*, about 10 to about 45, about 10 to about 40, about 10 or about 35, or about 10 to about 30. In certain aspects, the ASO is 21 nucleotides in length. In certain aspects, the ASO is 22 nucleotides in length. In certain aspects, the ASO is 23 nucleotides in length. In certain aspects, the ASO is 24 nucleotides in length. In certain aspects, the ASO is 25 nucleotides in length. In certain aspects, the ASO is 26 nucleotides in length. In certain aspects, the ASO is 27 nucleotides in length. In certain aspects, the ASO is 28 nucleotides in length. In certain aspects, the ASO is 29 nucleotides in length. In certain aspects, the ASO is 30 nucleotides in length. In certain aspects, the ASO is 31 nucleotides in length. In certain aspects, the ASO is 32 nucleotides in length. In certain aspects, the ASO is 33 nucleotides in length. In certain aspects, the ASO is 34 nucleotides in length. In certain aspects, the ASO is 35 nucleotides in length. In

certain aspects, the ASO is 36 nucleotides in length. In certain aspects, the ASO is 37 nucleotides in length. In certain aspects, the ASO is 38 nucleotides in length. In certain aspects, the ASO is 39 nucleotides in length. In certain aspects, the ASO is 40 nucleotides in length. In certain aspects, the ASO is 41 nucleotides in length. In certain aspects, the ASO is 42 nucleotides in length. In certain aspects, the ASO is 43 nucleotides in length. In certain aspects, the ASO is 44 nucleotides in length. In certain aspects, the ASO is 45 nucleotides in length. In certain aspects, the ASO is 46 nucleotides in length. In certain aspects, the ASO is 47 nucleotides in length. In certain aspects, the ASO is 48 nucleotides in length. In certain aspects, the ASO is 49 nucleotides in length. In certain aspects, the ASO is 50 nucleotides in length.

[0131] The terms "antisense ASO," "antisense oligonucleotide," and "oligomer" as used herein are interchangeable with the term "ASO."

[0132] A reference to a SEQ ID number includes a particular nucleobase sequence, but does not include any design or full chemical structure. Furthermore, the ASOs disclosed in the figures herein show a representative design, but are not limited to the specific design shown in the figures unless otherwise indicated. For example, when a claim (or this specification) refers to SEQ ID NO: 101, it includes the nucleotide sequence of SEQ ID NO: 101 only. The design of any ASO disclosed herein can be written as SEQ ID NO: XX, wherein each of the first nucleotide, the second nucleotide, the third nucleotide, the first nucleotide, the second nucleotide, and the Nth nucleotide from the 5' end is a modified nucleotide, *e.g.*, LNA, and each of the other nucleotides is a non-modified nucleotide (*e.g.*, DNA).

[0133] In various aspects, the ASO of the disclosure does not comprise RNA (units). In some aspects, the ASO comprises one or more DNA units. In one aspect, the ASO according to the disclosure is a linear molecule or is synthesized as a linear molecule. In some aspects, the ASO is a single stranded molecule, and does not comprise short regions of, for example, at least 3, 4 or 5 contiguous nucleotides, which are complementary to equivalent regions within the same ASO (*i.e.* duplexes) - in this regard, the ASO is not (essentially) double stranded. In some aspects, the ASO is essentially not double stranded. In some aspects, the ASO is not a siRNA. In various aspects, the ASO of the disclosure can consist entirely of the contiguous nucleotide region. Thus, in some aspects the ASO is not substantially self-complementary.

[0134] In other aspects, the present disclosure includes fragments of ASOs. For example, the disclosure includes at least one nucleotide, at least two contiguous nucleotides, at least three contiguous nucleotides, at least four contiguous nucleotides, at least five contiguous nucleotides, at least six contiguous nucleotides, at least seven contiguous nucleotides, at least eight contiguous

nucleotides, or at least nine contiguous nucleotides of the ASOs disclosed herein. Fragments of any of the sequences disclosed herein are contemplated as part of the disclosure.

II.A.1.a. The Target

[0135] Suitably the ASO of the disclosure is capable of down-regulating (*e.g.*, reducing or removing) expression of the *NLRP3* mRNA or NLRP3 protein. In this regard, the ASO of the disclosure can block formation and thus the activity of the NLRP3 inflammasome through the reduction in *NLRP3* mRNA levels, typically in a mammalian cell, such as a human cell, such as an immune cell (*e.g.*, a macrophage, a dendritic cell, a B cell, and/or a T cell). In particular, the present disclosure is directed to ASOs that target one or more regions of the *NLRP3* pre-mRNA (*e.g.*, intron regions, exon regions, and/or exon-intron junction regions). Unless indicated otherwise, the term "NLRP3," as used herein, can refer to NLRP3 from one or more species (*e.g.*, humans, non-human primates, dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, and bears).

[0136] NLRP3 (*NLRP3*) is also known as NLR family pyrin domain containing 3. Synonyms of NLRP3/NLRP3 are known and include *NLRP3*; *C1orf7*; *CIAS1*; *NALP3*; *PYPAF1*; nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing 3; cold-induced autoinflammatory syndrome 1 protein; cryopyrin; NACHT, LRR and PYD domains-containing protein 3; angiotensin/vasopressin receptor AII/AVP-like; caterpillar protein 1.1; CLR1.1; cold-induced autoinflammatory syndrome 1 protein; and PYRIN-containing APAF1-like protein 1. The sequence for the human NLRP3 gene can be found under publicly available GenBank Accession Number NC_000001.11:247416156-247449108. The human *NLRP3* gene is found at chromosome location 1q44 at 247,416,156-247,449,108.

[0137] The sequence for the human *NLRP3* pre-mRNA transcript (SEQ ID NO: 1) corresponds to the reverse complement of residues 247,416,156-247,449,108 of chromosome 1q44. The *NLRP3* mRNA sequence (GenBank Accession No. NM_001079821.2) is provided in SEQ ID NO: 3 (Table 1), except that the nucleotide "t" in SEQ ID NO: 3 is shown as "u" in the mRNA. The sequence for human NLRP3 protein can be found under publicly available Accession Numbers: Q96P20, (canonical sequence, SEQ ID NO: 1; Table 1), Q96P20-2 (SEQ ID NO: 4), Q96P20-3 (SEQ ID NO: 5), Q96P20-4 (SEQ ID NO: 6), Q96P20-5 (SEQ ID NO: 7), and Q96P20-6 (SEQ ID NO: 8), each of which is incorporated by reference herein in its entirety.

Table 1. NLRP3 mRNA and Protein Sequences

NLRP3 mRNA Sequence
GTAGATGAGGAACTGAAGTTGAGGAATAGTGAAGAGTTTGTCCAATGTCATAGCCCCGTAATCAACGGGACAAAA ATTTTCTTGCTGATGGGTCAAGATGGCATCGTGAAGTGGTTGTTACCGTAACTGTAATACAATCCTGTTTATGG ATTTGTTTGCATATTTTCCCTCCATAGGGAAACCTTCTTCCATGGCTCAGGACACACTCCTGGATCGAGCCAAC

AGGAGAACTTTCTGGTAAGCATTTGGCTAACTTTTTTTTTTTTGGATGGAGTCTTGCTGTGCTGCCTAGGCTGGA GTGCAGTGGCGTGATCTTGGCTCACTGCAGCCTCCACTTCCCGGGTTCAATCAATTCTCCTACCTCAACTTCTCTGA GTAGCTGGGATTACAGGCGCCCGCCACCACACCCGGCTCATTTTTGTACTTTTAGTAGAGACACAGTTTTGCCATG TTGGCCAGGCTGGTCTTGAATTCCTCAGCTCAGGTGATCTGCCTGCCTTGGCCTCTCAAAGTCTGGGATTACAGG CGTGAGCCACTGTGCCCCGGCTTGGCTAACTTTTCAAAATTAAAGATTTTGACTTGTTACAGTCATGTGACATTTT TTTCTTTCTGTTTGGCTGAGTTTTTGTATAATTTATATCTCTCAAAGTGGAGACTTTAAAAAGACTCATCCGTGTGC CGTGTTCACTGCCTGGTATCTTAGTGTGGACCGAAGCCTAAGGACCCTGAAAACAGCTGCAGATGAAGATGGCAAG CACCCGCTGCAAGCTGGCCAGGTACCTGGAGGACCTGGAGGATGTGGACTTGAAGAAATTTAAGATGCACTTAGAG GACTATCCTCCCCAGAAGGGCTGCATCCCCCTCCCGAGGGGTGAGACAGAGAAGGCAGACCATGTGGATCTAGCCA CGCTAATGATCGACTTCAATGGGGAGGAGAAGCGTGGGCCATGGCCGTGTGGATCTTCGCTGCGATCAACAGGAG AGACCTTTTATGAGAAAGCAAAAGAGATGAGCCGAAGTGGGGTTCAGATAATGCACGTGTTTTCGAATCCCCTGTG ATATGCCAGGAAGACAGCATTGAAGAGGAGTGGATGGGTTTTACTGGAGTACCTTTCGAGAATCTCTATTTGTAAAA TGAAGAAAGATTACCGTAAGAAGTACAGAAAGTACGTGAGAAGCAGATTCCAGTGCATTGAAGACAGGAATGCCCG TCTGGGTGAGAGTGTGAGCCTCAACAAACGCTACACACGACTGCGTCTCATCAAGGAGCACCCGAGCCAGCAGGAG AGGGAGCAGGAGCTTCTGGCCATCGGCAAGACCAAGCGTGTGAGAGCCCCGTGAGTCCCATTAAGATGGAGTTGC TGTTTTGACCCCGATGATGAGCATTCTGAGCCTGTGCACACCGTGGTGTTCAGGGGGCGGCAGGGATTGGGAAAAA AATCCTGGCCAGGAAGATGATGTTGGACTGGGCGTCGGGGACACTCTACCAAGACAGGTTTTGACTATCTGTTCTAT ATCCCATGTGGGAGGTGAGCCTTGTGACACAGAGGAGCCTGGGGGACCTGATCATGAGCTGCCCCGACCCAA ACCCACCCATCCACAAGATCGTGAGAAAAACCTCCAGAATCCTCTCCTCATGGACGGCTTCGATGAGCTGCAAGG TGCCTTTGACGAGCACATAGGACCGCTCTGCACTGACTGGCAGAAGGCCGAGCGGGGAGACATTCTCCTGAGCAGC CTCATCAGAAAGAAGCTGTTCCCGAGGCCTCTCTGCTCATCACACGAGACCTGTGGCCCTGGAGAACTGCAGC ACTTGCTGGACCATCCTCGGCATGTGGAGATCCTGGGTTTCTCCGAGGCCAAAAGGAAAGAGTACTTCTTCAAGTA CTTCTCTGATGAGGCCCCAAGCCAGGGCAGCCTTCACTGCTGATTCAAGGAGAACGAGGTCCTCTTACCATGTGCTTC ATCCCCCTGGTCTGCTGGATCGTGTGCACTGGACTGAAACAGCAGATGGAGAGTGGCAAGAGCCTTGGCCAGACAT CCAAGACCACCACCGCGGTGTACGTCTTCTTCTTCCAGTTTGTGTCAGCCCCGGGGAGGGAGCCAGGAGCACGG CCTCTGCGCCACCTCTGGGGCTCTGCTCTTTGGCTGCAGATGGAATCTGGAACCAGAAAACTCTGTTTGAGGAG TCCGACCTCAGGAATCATGGACTGCAGAAGGCGGATGTGTCTGCTTTCCTGAGGATGAACCTGTTCCAAAAGGAAG TGGACTGCGAGAAGTCTACAGCTTCATCCACATGACTTTCCAGGAGTTCTTTGCCGCCATGTACTACCTGCTGGA AGAGGAAAAGGAAGGAAGGACGAACGTTCCAGGGAGTCGTTTGAAGCTTCCAGCCGAGACGTGACAGTCTTCTG GAAAACATATGGCAAATTCGAAAAGGGGTATTTGATTTTTGTGTTGACGTTTCCCTCTTTGGCCTGGTAAACCAAGGAGA GGACCTCCTACTTGGAGAAGAAATTAAGTTGCAAGATCTCTCAGCAAATCAGGCTGGAGCTGCTGAAATGGATTGA AGTGAAAGCCAAAGCTAAAAAGCTGCAGATCCAGCCAGCCAGCTGGAATTGTTCTACTGTTTGTACGAGATGCAG GAGGAGGACTTCGTGCAAGGGCCATGGACTATTTCCCCAAGATTGAGATCAATCTCTCCACCAGAATGGACCACA TGGTTTTCTTCTTTTGCAATTGAGAACTGTCATCGGGTGGAGTCACTGTCCCTGGGGTTTTCTCCATAACATGCCCAA GGAGGAAGAGGAGGAGGAAAAGGAAGGCCGACACCTTGATATGGTGCAGTGTGCTCTCCCAAGCTCCTCTCATGCT GCCTGTTCTCATGGATTGGTGAACAGCCACCTCACTTCCAGTTTTTGCCGGGGCTCTTTTCACTTCTGAGCACA GCCAGAGTCTAACTGAATTGGACCTCAGTGACAATTTCTTGGGGGACCCAGGGATGAGAGTGTGTGTGAAACGCT CCAGCATCCTGGCTGTAACTTTCGAGATTGTGGTTGGGGCGCTGTGGCCTCTCGCATGAGTGTGCTTTCGACATC TCCTTGGTCTCTCAGCAGCAACCAGAAGCTGGTGGAGCTGGACCTGAGTGACAACGCCCTCGGTGACTTCGGAATCA GACTTCTGTGTGTGGGACTGAAGCACCTGTTGTGCAATCTGAAGAAGCTCTGGTTGGTCAGCTGCTGCCTCACATC AGCATGTTGTGAGGACTTGTGATCAGTATTGAGCACCAGCCATTCCCTGACCAGACTCTATGTGGGGGAGAATGCC TTGGGAGACTCAGGAGTGCAGATTTTATGTGAAAAAGCCAAAGAAATCCACAGTGTAACTGCAGAAACCGGGCTGG TGAATTCTGGCCTTACGTCACTGTGTTGTTGAGCTTTGTCTCGGTACTCAGCACTAATCAGAATCTCACGCACCT TTACCTGCGAGGCAACACTCTCGGAGACAAGGGGATCAAATACTCTGTGAGGAGCTCTTGACCCCCGACTGCAAG CTTCAGGTGTTGGAATTAGACAACCTGCAACCTCACGTCACTGCTGCTGGGATCTTTCCACACTTCTGACCTCCA GCCAGAGCCTGCGAAAGCTGAGCCTGGGCAACAATGACCTGGGCGACCTGGGGGTGATGATGTTCTGTGAAGTGTCT GAAACAGCAGAGCTGCCCTCTGCAGAACCTGGGGTGTGTGAAATGTATTTCAATTATGAGACAAAAAGTGCCTTA GAAACACTTCAAGAAGAAAAGCCTGAGCTGACCGTCTCTTTGAGCCTTCTTGGTAGGAGTGGAAACGGGGCTGCC AGACGCCAGTGTCTCCGGTCCCTCCAGCTGGGGGCCCTCAGGTGGAGAGAGCTGCGATCCATCCAGGCCAAGACC ACAGCTCTGTGATCCTTCCGGTGGAGTGTGCGAGAAGAGAGCTTGCCGACGATGCCTTCCGTGTCAGAGCTTGGGC ATCTCCTTTACGCCAGGGTGAGGAAGACACCAGGACAATGACAGCATCGGGTGTGTTGTGTCATCACAGCGCCTCAG TTAGAGGATGTTCTCTTGGTGACCTCATGTAATTAGCTCATTCAATAAAGCACTTTCTTTATTTTTCTCTCTCTCT GTCTAACTTTCTTTTTCTATCTTTTTCTTCTTTGTTCTGTTTACTTTTGTCTATATCATCATTTCCCGCTATCTT TCTATTAACAGCATAACACAGAACTAGTTGACTATATATTATGTTGAAATTTTATGGCAGCTATTTATTTATTT AAATTTTTTGTAAACAGTTTTGTTTTCTAATAAGAAAAATCCATGCTTTTTGTAGCTGGTTGAAAATTCAGGAATAT GTAAAACCTTTTTGGTATTTAATTAATGATTCCTTTCTTAATTTTAAAAA (SEQ ID NO: 3)
NLRP3 Protein Sequence
MKMASTRKCLARYLEDLEDVDLKKFKMHLEDYPPQKGCIPLRGQTEKADHVDLATLMIDFNGEKAWAMAVWIFA AINRRDLYEKAKRDEPKWGSNDARVSNPTVICQEDSIEBEWMGLLEBYLSRISICKMKKDYRKKYRKYVRSRQCIE DRNARLGESVSLNKRYTRLRLIKEHRSQQEREQELLAIGKTKTCESPVSPKIMELLFDPDEHSEPVHTVVFQGA

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GIGKTI LARKMMLDWASGTLYQDRFDYLFYIHCREVSLVTQ RSLGDLIMSCCPDNPPHKTIVRKPSRILFLMDGF
DELQGA FDEHIGPLCTDWQKAERGDILLSSLIRKLLPEASLLITTRPVALEKLQHLLDHPRHVEILGFSEAKRKE
YFFKYFSDEAQARAAFSLIQENEVLFTMCFIPLVCWIVCTGLKQQMESGKSLAQTSTTTAVYVFFLSSLLQPRGG
SQEHGLCAHLWGLCSLAADGIWNQKILFEESDLRNHGLQKADVSAFLRMNLFQKEVDCEKFYSFIHMTFQEFFAAM
YYLLEEEKEGRTNVP GSRLKLP SRDVTV LLENYGKFEKGYLIFVVRFLFGLVNQERTSYLEKKLSCKISQQIRLEL
LKWIEVKAKAKKLQIQPSQLELFYCLYEMQEEDFVQRAMDYFPKIBINLSTRMDH MVSSFCIENCHRVESLSLGLF
HNMPKEEEEEKEGRHLD MVQCVPSSSHAACSHGLVNSHLTSSFCRGLFSVLSTSQSLTELDLSDNSLGDPMRV
LCETLQHGPCNIRRLWLGRGCL SHECCFDISLVLSSNQKLVELDLS DNALGDFGIRLLCVGLKHL LCNLKKLWLVS
CCLTSACQDLASVLSTSHSLTRLYVGENALGDSGVAILCEKAKNPQC NLQKLGLVNSGLTSVCCSALSSVLSTNQ
NLTHLYLRGNTLGDKGIKLLCEGLLHPDCKLQVLELDN CNLTSHCCWDLSTLLTSSQSLRKLSLGNNDLGLGVMM
FCEVLKQQSCLLQNLGLSEMYFNYETKSALET LQEEKPELTVVFEPSW (SEQ ID NO: 2)
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[0138] An example of a target nucleic acid sequence of the ASOs is *NLRP3* pre-mRNA. SEQ ID NO: 1 represents a human *NLRP3* genomic sequence (*i.e.*, reverse complement of nucleotides 247,416,156-247,449,108 of chromosome 1q44). SEQ ID NO: 1 is identical to a *NLRP3* pre-mRNA sequence except that nucleotide "t" in SEQ ID NO: 1 is shown as "u" in pre-mRNA. In certain aspects, the "target nucleic acid" comprises an intron of a *NLRP3* protein-encoding nucleic acids or naturally occurring variants thereof, and RNA nucleic acids derived therefrom, *e.g.*, pre-mRNA. In other aspects, the target nucleic acid comprises an exon region of a *NLRP3* protein-encoding nucleic acids or naturally occurring variants thereof, and RNA nucleic acids derived therefrom, *e.g.*, pre-mRNA. In yet other aspects, the target nucleic acid comprises an exon-intron junction of a *NLRP3* protein-encoding nucleic acids or naturally occurring variants thereof, and RNA nucleic acids derived therefrom, *e.g.*, pre-mRNA. In some aspects, for example when used in research or diagnostics the "target nucleic acid" can be a cDNA or a synthetic oligonucleotide derived from the above DNA or RNA nucleic acid targets. The human *NLRP3* protein sequence encoded by the *NLRP3* pre-mRNA is shown as SEQ ID NO: 3. In other aspects, the target nucleic acid comprises an untranslated region of a *NLRP3* protein-encoding nucleic acids or naturally occurring variants thereof, *e.g.*, 5' UTR, 3' UTR, or both.

[0139] In some aspects, an ASO of the disclosure hybridizes to a region within the introns of a *NLRP3* transcript, *e.g.*, SEQ ID NO: 1. In certain aspects, an ASO of the disclosure hybridizes to a region within the exons of a *NLRP3* transcript, *e.g.*, SEQ ID NO: 1. In other aspects, an ASO of the disclosure hybridizes to a region within the exon-intron junction of a *NLRP3* transcript, *e.g.*, SEQ ID NO: 1. In some aspects, an ASO of the disclosure hybridizes to a region within a *NLRP3* transcript (*e.g.*, an intron, exon, or exon-intron junction), *e.g.*, SEQ ID NO: 1, wherein the ASO has a design according to formula: 5' A-B-C 3' as described elsewhere herein.

[0140] In some aspects, the ASO targets a mRNA encoding a particular isoform of *NLRP3* protein (*e.g.*, Isoform 1). In some aspects, the ASO targets all isoforms of *NLRP3* protein. In other

aspects, the ASO targets two isoforms (*e.g.*, Isoform 1 and Isoform 2, Isoform 3 and Isoform 4, and Isoform 5 and Isoform 6) of NLRP3 protein.

[0141] In some aspects, the ASO comprises a contiguous nucleotide sequence (*e.g.*, 10 to 30 nucleotides in length, *e.g.*, 20 nucleotides in length) that are complementary to a nucleic acid sequence within a *NLRP3* transcript, *e.g.*, a region corresponding to SEQ ID NO: 1. In some aspects, the target region corresponds to nucleotides 206-225 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-206; SEQ ID NO: 101). In some aspects, the target region corresponds to nucleotides 208-227 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-208; SEQ ID NO: 102). In some aspects, the target region corresponds to nucleotides 214-233 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-214; SEQ ID NO: 103). In some aspects, the target region corresponds to nucleotides 748-767 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-748; SEQ ID NO: 104). In some aspects, the target region corresponds to nucleotides 825-844 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-825; SEQ ID NO: 105). In some aspects, the target region corresponds to nucleotides 892-911 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-892; SEQ ID NO: 106). In some aspects, the target region corresponds to nucleotides 898-917 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-898; SEQ ID NO: 107). In some aspects, the target region corresponds to nucleotides 899-918 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-899; SEQ ID NO: 108). In some aspects, the target region corresponds to nucleotides 900-919 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-900; SEQ ID NO: 109). In some aspects, the target region corresponds to nucleotides 902-921 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-902; SEQ ID NO: 110). In some aspects, the target region corresponds to nucleotides 903-922 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-903; SEQ ID NO: 111). In some aspects, the target region corresponds to nucleotides 954-973 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-954; SEQ ID NO: 112). In some aspects, the target region corresponds to nucleotides 960-979 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-960; SEQ ID NO: 113). In some aspects, the target region corresponds to nucleotides 964-983 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-964; SEQ ID NO: 114). In some aspects, the target region corresponds to nucleotides 966-985 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-966; SEQ ID NO: 115). In some aspects, the target region corresponds to nucleotides 969-988 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-969; SEQ ID NO: 116). In some aspects, the target region corresponds to nucleotides 970-989 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-970; SEQ ID NO: 117). In some aspects, the target region corresponds to nucleotides 971-990 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-971; SEQ ID NO: 118). In some aspects, the target region corresponds to nucleotides 1016-1035 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1016; SEQ ID NO: 119). In some aspects, the target region corresponds to nucleotides 1021-1040 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1021; SEQ ID NO: 120). In some aspects, the target region corresponds to nucleotides 1028-1047 of SEQ ID NO: 3 (*e.g.*, ASO-

NLRP3-1028; SEQ ID NO: 121). In some aspects, the target region corresponds to nucleotides 1103-1122 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1103; SEQ ID NO: 122). In some aspects, the target region corresponds to nucleotides 1108-1127 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1108; SEQ ID NO: 123). In some aspects, the target region corresponds to nucleotides 1113-1132 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1113; SEQ ID NO: 124). In some aspects, the target region corresponds to nucleotides 1159-1178 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1159; SEQ ID NO: 125). In some aspects, the target region corresponds to nucleotides 1173-1192 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1173; SEQ ID NO: 126). In some aspects, the target region corresponds to nucleotides 1197-1216 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1197; SEQ ID NO: 127). In some aspects, the target region corresponds to nucleotides 1204-1223 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1204; SEQ ID NO: 128). In some aspects, the target region corresponds to nucleotides 1227-1246 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1227; SEQ ID NO: 129). In some aspects, the target region corresponds to nucleotides 1232-1251 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1232; SEQ ID NO: 130). In some aspects, the target region corresponds to nucleotides 1239-1258 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1239; SEQ ID NO: 131). In some aspects, the target region corresponds to nucleotides 1240-1259 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1240; SEQ ID NO: 132). In some aspects, the target region corresponds to nucleotides 1241-1260 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1241; SEQ ID NO: 133). In some aspects, the target region corresponds to nucleotides 1242-1261 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1242; SEQ ID NO: 134). In some aspects, the target region corresponds to nucleotides 1313-1332 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1313; SEQ ID NO: 135). In some aspects, the target region corresponds to nucleotides 1314-1333 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1314; SEQ ID NO: 136). In some aspects, the target region corresponds to nucleotides 1341-1360 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1341; SEQ ID NO: 137). In some aspects, the target region corresponds to nucleotides 1343-1362 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1343; SEQ ID NO: 138). In some aspects, the target region corresponds to nucleotides 1346-1365 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1346; SEQ ID NO: 139). In some aspects, the target region corresponds to nucleotides 1491-1510 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1491; SEQ ID NO: 140). In some aspects, the target region corresponds to nucleotides 1561-1580 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1561; SEQ ID NO: 141). In some aspects, the target region corresponds to nucleotides 1568-1587 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1568; SEQ ID NO: 142). In some aspects, the target region corresponds to nucleotides 1664-1683 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1664; SEQ ID NO: 143). In some aspects, the target region corresponds to nucleotides 1670-1689 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1670;

SEQ ID NO: 144). In some aspects, the target region corresponds to nucleotides 1676-1695 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1676; SEQ ID NO: 145). In some aspects, the target region corresponds to nucleotides 1678-1697 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1678; SEQ ID NO: 146). In some aspects, the target region corresponds to nucleotides 1680-1699 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1680; SEQ ID NO: 147). In some aspects, the target region corresponds to nucleotides 1681-1700 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1681; SEQ ID NO: 148). In some aspects, the target region corresponds to nucleotides 1682-1701 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1682; SEQ ID NO: 149). In some aspects, the target region corresponds to nucleotides 1688-1707 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1688; SEQ ID NO: 150). In some aspects, the target region corresponds to nucleotides 1693-1712 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1693; SEQ ID NO: 151). In some aspects, the target region corresponds to nucleotides 1704-1723 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1704; SEQ ID NO: 152). In some aspects, the target region corresponds to nucleotides 1718-1737 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1718; SEQ ID NO: 153). In some aspects, the target region corresponds to nucleotides 1720-1739 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1720; SEQ ID NO: 154). In some aspects, the target region corresponds to nucleotides 1723-1742 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1723; SEQ ID NO: 155). In some aspects, the target region corresponds to nucleotides 1837-1856 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1837; SEQ ID NO: 156). In some aspects, the target region corresponds to nucleotides 1932-1951 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1932; SEQ ID NO: 157). In some aspects, the target region corresponds to nucleotides 1993-2012 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-1993; SEQ ID NO: 158). In some aspects, the target region corresponds to nucleotides 2325-2344 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-2325; SEQ ID NO: 159). In some aspects, the target region corresponds to nucleotides 2432-2451 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-2432; SEQ ID NO: 160). In some aspects, the target region corresponds to nucleotides 2472-2491 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-2472; SEQ ID NO: 161). In some aspects, the target region corresponds to nucleotides 2543-2562 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-2543; SEQ ID NO: 162). In some aspects, the target region corresponds to nucleotides 2638-2657 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-2638; SEQ ID NO: 163). In some aspects, the target region corresponds to nucleotides 2639-2658 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-2639; SEQ ID NO: 164). In some aspects, the target region corresponds to nucleotides 2667-2686 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-2667; SEQ ID NO: 165). In some aspects, the target region corresponds to nucleotides 2672-2691 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-2672; SEQ ID NO: 166). In some aspects, the target region corresponds to nucleotides 2699-2718 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-2699; SEQ ID NO:

167). In some aspects, the target region corresponds to nucleotides 2750-2769 of SEQ ID NO: 3 (e.g., ASO-NLRP3-2750; SEQ ID NO: 168). In some aspects, the target region corresponds to nucleotides 2755-2774 of SEQ ID NO: 3 (e.g., ASO-NLRP3-2755; SEQ ID NO: 169). In some aspects, the target region corresponds to nucleotides 2760-2779 of SEQ ID NO: 3 (e.g., ASO-NLRP3-2760; SEQ ID NO: 170). In some aspects, the target region corresponds to nucleotides 2830-2849 of SEQ ID NO: 3 (e.g., ASO-NLRP3-2830; SEQ ID NO: 171). In some aspects, the target region corresponds to nucleotides 2836-2855 of SEQ ID NO: 3 (e.g., ASO-NLRP3-2836; SEQ ID NO: 172). In some aspects, the target region corresponds to nucleotides 3087-3106 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3087; SEQ ID NO: 173). In some aspects, the target region corresponds to nucleotides 3094-3113 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3094; SEQ ID NO: 174). In some aspects, the target region corresponds to nucleotides 3109-3128 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3109; SEQ ID NO: 175). In some aspects, the target region corresponds to nucleotides 3120-3139 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3120; SEQ ID NO: 176). In some aspects, the target region corresponds to nucleotides 3212-3231 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3212; SEQ ID NO: 177). In some aspects, the target region corresponds to nucleotides 3476-3495 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3476; SEQ ID NO: 178). In some aspects, the target region corresponds to nucleotides 3481-3500 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3481; SEQ ID NO: 179). In some aspects, the target region corresponds to nucleotides 3488-3507 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3488; SEQ ID NO: 180). In some aspects, the target region corresponds to nucleotides 3489-3508 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3489; SEQ ID NO: 181). In some aspects, the target region corresponds to nucleotides 3493-3512 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3493; SEQ ID NO: 182). In some aspects, the target region corresponds to nucleotides 3498-3517 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3498; SEQ ID NO: 183). In some aspects, the target region corresponds to nucleotides 3500-3519 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3500; SEQ ID NO: 184). In some aspects, the target region corresponds to nucleotides 3502-3521 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3502; SEQ ID NO: 185). In some aspects, the target region corresponds to nucleotides 3503-3522 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3503; SEQ ID NO: 186). In some aspects, the target region corresponds to nucleotides 3504-3523 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3504; SEQ ID NO: 187). In some aspects, the target region corresponds to nucleotides 3508-3527 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3508; SEQ ID NO: 188). In some aspects, the target region corresponds to nucleotides 3514-3533 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3514; SEQ ID NO: 189). In some aspects, the target region corresponds to nucleotides 3561-3580 of SEQ ID NO: 3 (e.g., ASO-NLRP3-3561; SEQ ID NO: 190). In some

aspects, the target region corresponds to nucleotides 3580-3599 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-3580; SEQ ID NO: 191). In some aspects, the target region corresponds to nucleotides 3585-3604 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-3585; SEQ ID NO: 192). In some aspects, the target region corresponds to nucleotides 3593-3612 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-3593; SEQ ID NO: 193). In some aspects, the target region corresponds to nucleotides 3598-3617 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-3598; SEQ ID NO: 194). In some aspects, the target region corresponds to nucleotides 3652-3671 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-3652; SEQ ID NO: 195). In some aspects, the target region corresponds to nucleotides 3676-3695 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-3676; SEQ ID NO: 196). In some aspects, the target region corresponds to nucleotides 3690-3709 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-3690; SEQ ID NO: 197). In some aspects, the target region corresponds to nucleotides 4096-4115 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-4096; SEQ ID NO: 198). In some aspects, the target region corresponds to nucleotides 4105-4124 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-4105; SEQ ID NO: 199). In some aspects, the target region corresponds to nucleotides 4256-4275 of SEQ ID NO: 3 (*e.g.*, ASO-NLRP3-4256; SEQ ID NO: 200).

[0142] In some aspects, the ASO of the present disclosure hybridizes to multiple target regions within the *NLRP3* transcript (*e.g.*, genomic sequence, SEQ ID NO: 1). In some aspects, the ASO hybridizes to two different target regions within the *NLRP3* transcript. In some aspects, the ASO hybridizes to three different target regions within the *NLRP3* transcript. The sequences of exemplary ASOs that hybridizes to multiple target regions, and the start/end sites of the different target regions are provided in FIG. 1. In some aspects, the ASOs that hybridizes to multiple regions within the *NLRP3* transcript (*e.g.*, genomic sequence, SEQ ID NO: 1) are more potent (*e.g.*, having lower EC₅₀) at reducing *NLRP3* expression compared to ASOs that hybridizes to a single region within the *NLRP3* transcript (*e.g.*, genomic sequence, SEQ ID NO: 1).

II.A.1.b. ASO Sequences

[0143] The ASOs of the disclosure comprise a contiguous nucleotide sequence which corresponds to the complement of a region of *NLRP3* transcript, *e.g.*, a nucleotide sequence corresponding to SEQ ID NO: 1 or SEQ ID NO: 3.

[0144] In certain aspects, the disclosure provides an ASO from 10 – 30, such as 10 – 15 nucleotides, 10 – 20 nucleotides, 10 – 25 nucleotides in length, or about 20 nucleotides in length, wherein the contiguous nucleotide sequence has at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to a region within the complement of a *NLRP3*

transcript, such as SEQ ID NO: 1 or SEQ ID NO: 3 or naturally occurring variant thereof. Thus, for example, the ASO hybridizes to a single stranded nucleic acid molecule having the sequence of SEQ ID NO: 1 or SEQ ID NO: 3 or a portion thereof.

[0145] The ASO can comprise a contiguous nucleotide sequence which is fully complementary (perfectly complementary) to the equivalent region of a nucleic acid which encodes a mammalian NLPR3 protein (*e.g.*, SEQ ID NO: 1 or SEQ ID NO: 3). The ASO can comprise a contiguous nucleotide sequence which is fully complementary (perfectly complementary) to a nucleic acid sequence, or a region within the sequence, corresponding to nucleotides X-Y of SEQ ID NO: 1 or SEQ ID NO: 3, wherein X and Y are the start site and the end site, respectively, as shown in FIG. 1.

[0146] The ASO can comprise a contiguous nucleotide sequence which is fully complementary (perfectly complementary) to the equivalent region of a mRNA which encodes a mammalian NLPR3 protein (*e.g.*, SEQ ID NO: 3). The ASO can comprise a contiguous nucleotide sequence which is fully complementary (perfectly complementary) to a mRNA sequence, or a region within the sequence, corresponding to nucleotides X-Y of SEQ ID NO: 3, wherein X and Y are the start site and the end site, respectively.

[0147] In some aspects, the nucleotide sequence of the ASOs of the disclosure or the contiguous nucleotide sequence has at least about 80% sequence identity to a sequence selected from SEQ ID NOs: 101 to 200 (*i.e.*, the sequences in FIG. 1), such as at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96% sequence identity, at least about 97% sequence identity, at least about 98% sequence identity, at least about 99% sequence identity, such as about 100% sequence identity (homologous). In some aspects, the ASO has a design described elsewhere herein or a chemical structure shown elsewhere herein (*e.g.*, FIG. 1).

[0148] In some aspects the ASO (or contiguous nucleotide portion thereof) is selected from, or comprises, one of the sequences selected from the group consisting of SEQ ID NOs: 101 to 200 or a region of at least 10 contiguous nucleotides thereof, wherein the ASO (or contiguous nucleotide portion thereof) can optionally comprise one, two, three, or four mismatches when compared to the corresponding *NLRP3* transcript.

[0149] In some aspects, the ASO comprises a sequence selected from the group consisting of SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116,

SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, or SEQ ID NO: 200.

[0150] In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 101. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 102. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 103. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 104. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 105. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 106. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 107. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 108. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 109. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 110. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 111. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 112. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 113. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 114. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 115. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 116. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 117. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 118. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 119. In some aspects, the ASO comprises the sequence as set

[illegible]

[illegible]

forth in SEQ ID NO: 197. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 198. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 199. In some aspects, the ASO comprises the sequence as set forth in SEQ ID NO: 200.

[0151] In some aspects, the ASOs of the disclosure bind to the target nucleic acid sequence (*e.g.*, *NLRP3* transcript) and are capable of inhibiting or reducing expression of the *NLRP3* transcript by at least 10% or 20% compared to the normal (*i.e.*, control) expression level in the cell, *e.g.*, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% compared to the normal expression level (*e.g.*, expression level in cells that have not been exposed to the ASO).

[0152] In some aspects, the ASOs of the disclosure are capable of reducing expression of *NLRP3* mRNA *in vitro* by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% in target cells when the cells are in contact with the ASO compared to cells that are not in contact with the ASO (*e.g.*, contact with saline).

[0153] In some aspects, the ASO can tolerate 1, 2, 3, or 4 (or more) mismatches, when hybridizing to the target sequence and still sufficiently bind to the target to show the desired effect, *i.e.*, down-regulation of the target mRNA and/or protein. Mismatches can, for example, be compensated by increased length of the ASO nucleotide sequence and/or an increased number of nucleotide analogs, which are disclosed elsewhere herein.

[0154] In some aspects, the ASO of the disclosure comprises no more than three mismatches when hybridizing to the target sequence. In other aspects, the contiguous nucleotide sequence comprises no more than two mismatches when hybridizing to the target sequence. In other aspects, the contiguous nucleotide sequence comprises no more than one mismatch when hybridizing to the target sequence.

II.A.1.c. ASO Length

[0155] The ASOs can comprise a contiguous nucleotide sequence of a total of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides in length. It should be understood that when a range is given for an ASO, or contiguous nucleotide sequence length, the range includes the lower and upper lengths provided in the range, for example from (or between) 10–30, includes both 10 and 30.

[0156] In some aspects, the ASOs comprise a contiguous nucleotide sequence of a total of about 14-20, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleotides in length. In certain aspects, the ASOs comprise a contiguous nucleotide sequence of a total of about 20 contiguous nucleotides in length. In certain aspects, ASOs of the present disclosure are 14 nucleotides in length. In certain aspects, ASOs of the present disclosure are 15 nucleotides in length. In certain aspects, ASOs of the present disclosure are 16 nucleotides in length. In certain aspects, ASOs of the present disclosure are 17 nucleotides in length. In certain aspects, ASOs of the present disclosure are 18 nucleotides in length. In certain aspects, ASOs of the present disclosure are 19 nucleotides in length.

II.A.1.d. Nucleosides and Nucleoside analogs

[0157] In one aspect of the disclosure, the ASOs comprise one or more non-naturally occurring nucleoside analogs. "Nucleoside analogs" as used herein are variants of natural nucleosides, such as DNA or RNA nucleosides, by virtue of modifications in the sugar and/or base moieties. Analogs could in principle be merely "silent" or "equivalent" to the natural nucleosides in the context of the oligonucleotide, *i.e.* have no functional effect on the way the oligonucleotide works to inhibit target gene expression. Such "equivalent" analogs can nevertheless be useful if, for example, they are easier or cheaper to manufacture, or are more stable to storage or manufacturing conditions, or represent a tag or label. In some aspects, however, the analogs will have a functional effect on the way in which the ASO works to inhibit expression; for example by producing increased binding affinity to the target and/or increased resistance to intracellular nucleases and/or increased ease of transport into the cell. Specific examples of nucleoside analogs are described by *e.g.* Freier & Altmann; *Nucl. Acid Res.*, 1997, 25, 4429-4443 and Uhlmann; *Curr. Opinion in Drug Development*, 2000, 3(2), 293-213, and in Scheme 1. The ASOs of the present disclosure can contain more than one, more than two, more than three, more than four, more than five, more than six, more than seven, more than eight, more than nine, more than 10, more than 11, more than 12, more than 13, more than 14, more than 15, more than 16, more than 18, more than 19, or more than 20 nucleoside analogs. In some aspects, the nucleoside analogs in the ASOs are the same. In other aspects, the nucleoside analogs in the ASOs are different. The nucleotide analogs in the ASOs can be any one of or combination of the following nucleoside analogs.

[0158] In some aspects, the nucleoside analog comprises a 2'-O-alkyl-RNA; 2'-O-methyl RNA (2'-OMe); 2'-alkoxy-RNA; 2'-O-methoxyethyl-RNA (2'-MOE); 2'-amino-DNA; 2'-fluoro-RNA; 2'-fluoro-DNA; arabino nucleic acid (ANA); 2'-fluoro-ANA; bicyclic nucleoside analog; or

any combination thereof. In some aspects, the nucleoside analog comprises a sugar modified nucleoside. In some aspects, the nucleoside analog comprises a nucleoside comprising a bicyclic sugar. In some aspects, the nucleoside analog comprises an LNA.

[0159] In some aspects, the nucleoside analog is selected from the group consisting of constrained ethyl nucleoside (cEt), 2',4'-constrained 2'-O-methoxyethyl (cMOE), α -L-LNA, β -D-LNA, 2'-O,4'-C-ethylene-bridged nucleic acids (ENA), amino-LNA, oxy-LNA, thio-LNA, and any combination thereof. In some aspects, the ASO comprises one or more 5'-methyl-cytosine nucleobases.

[0160] The term nucleobase includes the purine (*e.g.*, adenine and guanine) and pyrimidine (*e.g.*, uracil, thymine and cytosine) moiety present in nucleosides and nucleotides which form hydrogen bonds in nucleic acid hybridization. In the context of the present disclosure, the term nucleobase also encompasses modified nucleobases which may differ from naturally occurring nucleobases, but are functional during nucleic acid hybridization. In some aspects, the nucleobase moiety is modified by modifying or replacing the nucleobase. In this context, "nucleobase" refers to both naturally occurring nucleobases such as adenine, guanine, cytosine, thymidine, uracil, xanthine and hypoxanthine, as well as non-naturally occurring variants. Such variants are for example described in Hirao *et al.*, (2012) *Accounts of Chemical Research* vol 45 page 2055 and Bergstrom (2009) *Current Protocols in Nucleic Acid Chemistry* Suppl. 37 1.4.1.

[0161] In some aspects, the nucleobase moiety is modified by changing the purine or pyrimidine into a modified purine or pyrimidine, such as substituted purine or substituted pyrimidine, such as a nucleobase selected from isocytosine, pseudoisocytosine, 5-methyl-cytosine, 5-thiozolo-cytosine, 5-propynyl-cytosine, 5-propynyl-uracil, 5-bromouracil, 5-thiazolo-uracil, 2-thio-uracil, 2-thio-thymine, inosine, diaminopurine, 6-aminopurine, 2-aminopurine, 2,6-diaminopurine, and 2-chloro-6-aminopurine.

[0162] The nucleobase moieties may be indicated by the letter code for each corresponding nucleobase, *e.g.*, A, T, G, C, or U, wherein each letter may optionally include modified nucleobases of equivalent function. For example, in the exemplified oligonucleotides, the nucleobase moieties are selected from A, T, G, C, and 5-methyl-cytosine. Optionally, for LNA gapmers, 5-methyl-cytosine LNA nucleosides may be used.

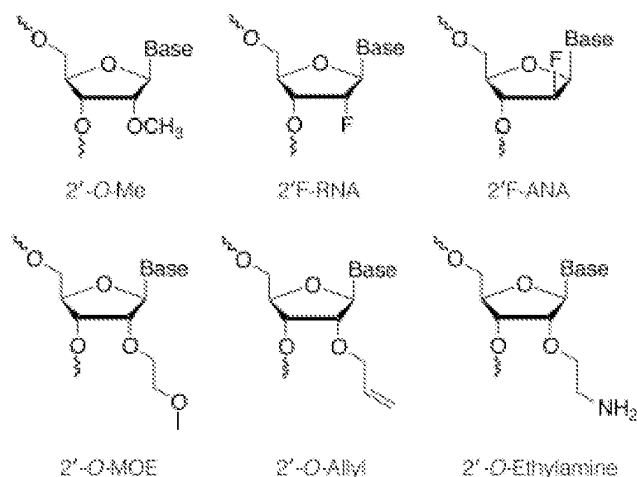
[0163] The ASO of the disclosure can comprise one or more nucleosides which have a modified sugar moiety, *i.e.* a modification of the sugar moiety when compared to the ribose sugar moiety found in DNA and RNA. Numerous nucleosides with modification of the ribose sugar

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

[0164] Such modifications include those where the ribose ring structure is modified, *e.g.* by replacement with a hexose ring (HNA), or a bicyclic ring, which typically have a biradical bridge between the C2' and C4' carbons on the ribose ring (LNA), or an unlinked ribose ring which typically lacks a bond between the C2' and C3' carbons (*e.g.*, UNA). Other sugar modified nucleosides include, for example, bicyclohexose nucleic acids (WO2011/017521) or tricyclic nucleic acids (WO2013/154798). Modified nucleosides also include nucleosides where the sugar moiety is replaced with a non-sugar moiety, for example in the case of peptide nucleic acids (PNA), or morpholino nucleic acids.

[0165] Sugar modifications also include modifications made via altering the substituent groups on the ribose ring to groups other than hydrogen, or the 2'-OH group naturally found in RNA nucleosides. Substituents may, for example be introduced at the 2', 3', 4', or 5' positions. Nucleosides with modified sugar moieties also include 2' modified nucleosides, such as 2' substituted nucleosides. Indeed, much focus has been spent on developing 2' substituted nucleosides, and numerous 2' substituted nucleosides have been found to have beneficial properties when incorporated into oligonucleotides, such as enhanced nucleoside resistance and enhanced affinity.

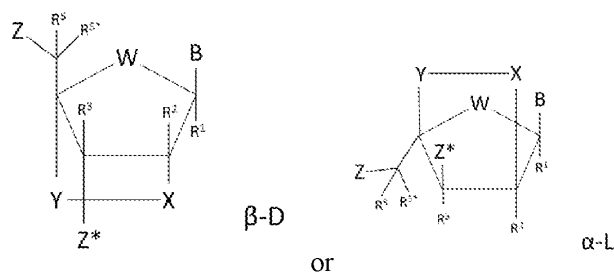
[0166] A 2' sugar modified nucleoside is a nucleoside which has a substituent other than H or -OH at the 2' position (2' substituted nucleoside) or comprises a 2' linked biradical, and includes 2' substituted nucleosides and LNA (2' – 4' biradical bridged) nucleosides. For example, the 2' modified sugar may provide enhanced binding affinity (*e.g.*, affinity enhancing 2' sugar modified nucleoside) and/or increased nuclease resistance to the oligonucleotide. Examples of 2' substituted modified nucleosides are 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-Fluoro-RNA, 2'-Fluro-DNA, arabino nucleic acids (ANA), and 2'-Fluoro-ANA nucleoside. For further examples, please *see, e.g.*, Freier & Altmann; *Nucl. Acid Res.*, 1997, 25, 4429-4443; Uhlmann, *Curr. Opinion in Drug Development*, 2000, 3(2), 293-213; and Deleavey and Damha, *Chemistry and Biology* 2012, 19, 937. Below are illustrations of some 2' substituted modified nucleosides.



[0167] LNA nucleosides are modified nucleosides which comprise a linker group (referred to as a biradical or a bridge) between C2' and C4' of the ribose sugar ring of a nucleoside (*i.e.*, 2'-4' bridge), which restricts or locks the conformation of the ribose ring. These nucleosides are also termed bridged nucleic acid or bicyclic nucleic acid (BNA) in the literature. The locking of the conformation of the ribose is associated with an enhanced affinity of hybridization (duplex stabilization) when the LNA is incorporated into an oligonucleotide for a complementary RNA or DNA molecule. This can be routinely determined by measuring the melting temperature of the oligonucleotide/complement duplex.

[0168] Non limiting, exemplary LNA nucleosides are disclosed in WO 99/014226, WO 00/66604, WO 98/039352, WO 2004/046160, WO 00/047599, WO 2007/134181, WO 2010/077578, WO 2010/036698, WO 2007/090071, WO 2009/006478, WO 2011/156202, WO 2008/154401, WO 2009/067647, WO 2008/150729, Morita *et al.*, *Bioorganic & Med.Chem. Lett.* 12, 73-76, Seth *et al.*, *J. Org. Chem.* 2010, Vol 75(5) pp. 1569-81, and Mitsuoka *et al.*, *Nucleic Acids Research* 2009, 37(4), 1225-1238.

[0169] In some aspects, the modified nucleoside or the LNA nucleosides of the ASO of the disclosure has a general structure of the formula I or II:



Formula I

Formula II

wherein

W is selected from -O-, -S-, -N(R^a)-, -C(R^aR^b)-, in particular -O-;

B is a nucleobase or a modified nucleobase moiety;

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

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

R¹, R², R³, R⁵ and R^{5*} are independently selected from hydrogen, halogen, alkyl, alkenyl, alkynyl, hydroxy, alkoxy, alkoxyalkyl, alkenyloxy, carboxyl, alkoxycarbonyl, alkylcarbonyl, formyl, azide, heterocycle and aryl; and

X, Y, R^a and R^b are as defined herein.

[0170] In some aspects, -X-Y-, R^a is hydrogen or alkyl, in particular hydrogen or methyl. In some aspects of -X-Y-, R^b is hydrogen or alkyl, in particular hydrogen or methyl. In other aspects of -X-Y-, one or both of R^a and R^b are hydrogen. In further aspects of -X-Y-, only one of R^a and R^b is hydrogen. In some aspects of -X-Y-, one of R^a and R^b is methyl and the other one is hydrogen. In certain aspects of -X-Y-, R^a and R^b are both methyl at the same time.

[0171] In some aspects, -X-, R^a is hydrogen or alkyl, in particular hydrogen or methyl. In some aspects of -X-, R^b is hydrogen or alkyl, in particular hydrogen or methyl. In other aspects of -X-, one or both of R^a and R^b are hydrogen. In certain aspects of -X-, only one of R^a and R^b is hydrogen. In certain aspects of -X-, one of R^a and R^b is methyl and the other one is hydrogen. In other aspects of -X-, R^a and R^b are both methyl at the same time.

[0172] In some aspects, -Y-, R^a is hydrogen or alkyl, in particular hydrogen or methyl. In certain aspects of -Y-, R^b is hydrogen or alkyl, in particular hydrogen or methyl. In other aspects of -Y-, one or both of R^a and R^b are hydrogen. In some aspects of -Y-, only one of R^a and R^b is hydrogen. In other aspects of -Y-, one of R^a and R^b is methyl and the other one is hydrogen. In some aspects of -Y-, R^a and R^b are both methyl at the same time.

[0173] In some aspects, R¹, R², R³, R⁵ and R^{5*} are independently selected from hydrogen and alkyl, in particular hydrogen and methyl.

[0174] In some aspects, R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time.

[0175] In some aspects, R¹, R², R³, are all hydrogen at the same time, one of R⁵ and R^{5*} is hydrogen and the other one is as defined above, in particular alkyl, more particularly methyl.

[0176] In some aspects, R¹, R², R³, are all hydrogen at the same time, one of R⁵ and R^{5*} is hydrogen and the other one is azide..

[0177] In some aspects, -X-Y- is -O-CH₂-, W is oxygen and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. Such LNA nucleosides are disclosed in WO 99/014226, WO 00/66604, WO 98/039352 and WO 2004/046160, which are all hereby incorporated by reference, and include what are commonly known in the art as beta-D-oxy LNA and alpha-L-oxy LNA nucleosides.

[0178] In some aspects, -X-Y- is -S-CH₂-, W is oxygen and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. Such thio LNA nucleosides are disclosed in WO 99/014226 and WO 2004/046160 which are hereby incorporated by reference.

[0179] In some aspects, -X-Y- is -NH-CH₂-, W is oxygen and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. Such amino LNA nucleosides are disclosed in WO 99/014226 and WO 2004/046160, which are hereby incorporated by reference.

[0180] In some aspects, -X-Y- is -O-CH₂CH₂- or -OCH₂CH₂CH₂-, W is oxygen, and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. Such LNA nucleosides are disclosed in WO 00/047599 and Morita *et al.*, *Bioorganic & Med.Chem. Lett.* 12, 73-76, which are hereby incorporated by reference, and include what are commonly known in the art as 2'-O-4'C-ethylene bridged nucleic acids (ENA).

[0181] In some aspects, -X-Y- is -O-CH₂-, W is oxygen, R¹, R², R³ are all hydrogen at the same time, one of R⁵ and R^{5*} is hydrogen and the other one is not hydrogen, such as alkyl, for example methyl. Such 5' substituted LNA nucleosides are disclosed in WO 2007/134181, which is hereby incorporated by reference.

[0182] In some aspects, -X-Y- is -O-CR^aR^b-, wherein one or both of R^a and R^b are not hydrogen, in particular alkyl such as methyl, W is oxygen, R¹, R², R³ are all hydrogen at the same time, one of R⁵ and R^{5*} is hydrogen and the other one is not hydrogen, in particular alkyl, for example methyl. Such bis modified LNA nucleosides are disclosed in WO 2010/077578, which is hereby incorporated by reference.

[0183] In some aspects, -X-Y- is -O-CH(CH₂-O-CH₃)- ("2' O-methoxyethyl bicyclic nucleic acid", Seth *et al.*, *J. Org. Chem.* 2010, Vol 75(5) pp. 1569-81).

[0184] In some aspects, -X-Y- is -O-CHR^a-, W is oxygen and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. Such 6'-substituted LNA nucleosides are disclosed in WO 2010/036698 and WO 2007/090071, which are both hereby incorporated by reference. In such 6'-substituted LNA nucleosides, R^a is in particular C1-C6 alkyl, such as methyl.

[0185] In some aspects, -X-Y- is -O-CH(CH₂-O-CH₃)-, W is oxygen and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. Such LNA nucleosides are also known in the art as cyclic MOEs (cMOE) and are disclosed in WO 2007/090071.

- [0186] In some aspects, -X-Y- is -O-CH(CH₃)-.
- [0187] In some aspects, -X-Y- is -O-CH₂-O-CH₂- (Seth *et al.*, *J. Org. Chem* 2010 op. cit.)
- [0188] In some aspects, -X-Y- is -O-CH(CH₃)-, W is oxygen and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. Such 6'-methyl LNA nucleosides are also known in the art as cET nucleosides, and may be either (S)-cET or (R)-cET diastereoisomers, as disclosed in WO 2007/090071 (beta-D) and WO 2010/036698 (alpha-L) which are both hereby incorporated by reference.
- [0189] In some aspects, -X-Y- is -O-CR^aR^b-, wherein neither R^a nor R^b is hydrogen, W is oxygen, and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. In certain aspects, R^a and R^b are both alkyl at the same time, in particular both methyl at the same time. Such 6'-di-substituted LNA nucleosides are disclosed in WO 2009/006478 which is hereby incorporated by reference.
- [0190] In some aspects, -X-Y- is -S-CHR^a-, W is oxygen, and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. Such 6'-substituted thio LNA nucleosides are disclosed in WO 2011/156202, which is hereby incorporated by reference. In certain aspects of such 6'-substituted thio LNA, R^a is alkyl, in particular methyl.
- [0191] In some aspects, -X-Y- is -C(=CH₂)C(R^aR^b)-, such as, W is oxygen, and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. Such vinyl carbo LNA nucleosides are disclosed in WO 2008/154401 and WO 2009/067647, which are both hereby incorporated by reference.
- [0192] In some aspects, -X-Y- is -N(OR^a)-CH₂-, W is oxygen and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. In some aspects, R^a is alkyl such as methyl. Such LNA nucleosides are also known as N substituted LNAs and are disclosed in WO 2008/150729, which is hereby incorporated by reference.
- [0193] In some aspects, -X-Y- is -O-NCH₃- (Seth *et al.*, *J. Org. Chem* 2010 op. cit.).
- [0194] In some aspects, -X-Y- is ON(R^a)-N(R^a)-O-, -NR^a-CR^aR^b-CR^aR^b-, or -NR^a-CR^aR^b-, W is oxygen, and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. In certain aspects, R^a is alkyl, such as methyl. (Seth *et al.*, *J. Org. Chem* 2010 op. cit.).
- [0195] In some aspects, R⁵ and R^{5*} are both hydrogen at the same time. In other aspects, one of R⁵ and R^{5*} is hydrogen and the other one is alkyl, such as methyl. In such aspects, R¹, R² and R³ can be in particular hydrogen and -X-Y- can be in particular -O-CH₂- or -O-CHC(R^a)₃-, such as -O-CH(CH₃)-.
- [0196] In some aspects, -X-Y- is -CR^aR^b-O-CR^aR^b-, such as -CH₂-O-CH₂-, W is oxygen and R¹, R², R³, R⁵ and R^{5*} are all hydrogen at the same time. In such aspects, R^a can be in particular alkyl such as methyl. Such LNA nucleosides are also known as conformationally restricted

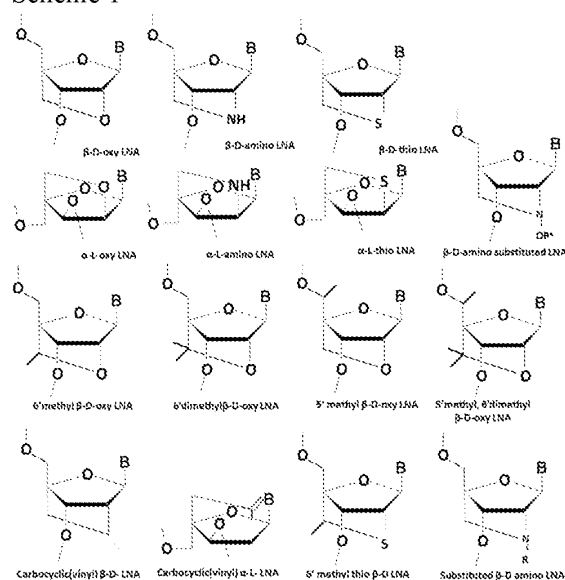
nucleotides (CRNs) and are disclosed in WO 2013/036868, which is hereby incorporated by reference.

[0197] In some aspects, $-X-Y-$ is $-O-CR^aR^b-O-CR^aR^b-$, such as $-O-CH_2-O-CH_2-$, W is oxygen and R^1, R^2, R^3, R^5 and R^{5*} are all hydrogen at the same time. In certain aspects, R^a can be in particular alkyl such as methyl. Such LNA nucleosides are also known as COC nucleotides and are disclosed in Mitsuoka *et al.*, *Nucleic Acids Research* 2009, 37(4), 1225-1238, which is hereby incorporated by reference.

[0198] It will be recognized that, unless specified, the LNA nucleosides may be in the beta-D or alpha-L stereoisomer.

[0199] Certain examples of LNA nucleosides are presented in Scheme 1.

Scheme 1



[0200] As illustrated elsewhere, in some aspects of the disclosure the LNA nucleosides in the oligonucleotides are beta-D-oxy-LNA nucleosides.

II.A.1.e. Nuclease mediated degradation

[0201] Nuclease mediated degradation refers to an oligonucleotide capable of mediating degradation of a complementary nucleotide sequence when forming a duplex with such a sequence.

[0202] In some aspects, the oligonucleotide may function via nuclease mediated degradation of the target nucleic acid, where the oligonucleotides of the disclosure are capable of recruiting a nuclease, particularly an endonuclease, preferably an endoribonuclease (RNase), such as RNase H. Examples of oligonucleotide designs which operate via nuclease mediated

mechanisms are oligonucleotides which typically comprise a region of at least 5 or 6 DNA nucleosides and are flanked on one side or both sides by affinity enhancing nucleosides, for example gapmers.

II.A.1.f. RNase H Activity and Recruitment

[0203] The RNase H activity of an antisense oligonucleotide refers to its ability to recruit RNase H when in a duplex with a complementary RNA molecule and induce degradation of the complementary RNA molecule. WO01/23613 provides *in vitro* methods for determining RNaseH activity, which may be used to determine the ability to recruit RNaseH. Typically, an oligonucleotide is deemed capable of recruiting RNase H if, when provided with a complementary target nucleic acid sequence, it has an initial rate, as measured in pmol/l/min, of at least 5%, such as at least 10% or more than 20% of the of the initial rate determined when using a oligonucleotide having the same base sequence as the modified oligonucleotide being tested, but containing only DNA monomers, with phosphorothioate linkages between all monomers in the oligonucleotide, and using the methodology provided by Example 91 - 95 of WO01/23613.

[0204] In some aspects, an oligonucleotide is deemed essentially incapable of recruiting RNaseH if, when provided with the complementary target nucleic acid, the RNaseH initial rate, as measured in pmol/l/min, is less than 20%, such as less than 10%, such as less than 5% of the initial rate determined when using a oligonucleotide having the same base sequence as the oligonucleotide being tested, but containing only DNA monomers, with no 2' substitutions, with phosphorothioate linkages between all monomers in the oligonucleotide, and using the methodology provided by Example 91 - 95 of WO01/23613.

II.A.1.g. ASO Design

[0205] The ASO of the disclosure can comprise a nucleotide sequence which comprises both nucleosides and nucleoside analogs, and can be in the form of a gapmer. Examples of configurations of a gapmer that can be used with the ASO of the disclosure are described in U.S. Patent Appl. Publ. No. 2012/0322851.

[0206] The term "gapmer" as used herein refers to an antisense oligonucleotide which comprises a region of RNase H recruiting oligonucleotides (gap) which is flanked 5' and 3' by one or more affinity enhancing modified nucleosides (flanks). The term "LNA gapmer" is a gapmer oligonucleotide wherein at least one of the affinity enhancing modified nucleosides is an LNA nucleoside. The term "mixed wing gapmer" refers to an LNA gapmer wherein the flank regions comprise at least one LNA nucleoside and at least one DNA nucleoside or non-LNA modified

nucleoside, such as at least one 2' substituted modified nucleoside, such as, for example, 2'-O-alkyl-RNA, 2'-O-methyl-RNA, 2'-alkoxy-RNA, 2'-O-methoxyethyl-RNA (MOE), 2'-amino-DNA, 2'-Fluoro-RNA, 2'-Fluro-DNA, arabino nucleic acid (ANA), and 2'-Fluoro-ANA nucleoside(s).

[0207] In some aspects, the ASO of the disclosure can be in the form of a mixmer. In some aspects, the ASO of the disclosure can be in the form of a totalmer. In some aspects, in addition to enhancing affinity of the ASO for the target region, some nucleoside analogs also mediate RNase (*e.g.*, RNaseH) binding and cleavage. Since α -L-LNA monomers recruit RNaseH activity to a certain extent, in some aspects, gap regions (*e.g.*, region B as referred to herein) of ASOs containing α -L-LNA monomers consist of fewer monomers recognizable and cleavable by the RNaseH, and more flexibility in the mixmer construction is introduced.

[0208] In some aspects, the ASO of the disclosure is a gapmer and comprises a contiguous stretch of nucleotides (*e.g.*, one or more DNA) which is capable of recruiting an RNase, such as RNaseH, referred to herein in as region B (B), wherein region B is flanked at both 5' and 3' by regions of nucleoside analogs 5' and 3' to the contiguous stretch of nucleotides of region B— these regions are referred to as regions A (A) and C (C), respectively. In some aspects, the nucleoside analogs are sugar modified nucleosides (*e.g.*, high affinity sugar modified nucleosides). In certain aspects, the sugar modified nucleosides of regions A and C enhance the affinity of the ASO for the target nucleic acid (*i.e.*, affinity enhancing 2' sugar modified nucleosides). In some aspects, the sugar modified nucleosides are 2' sugar modified nucleosides, such as high affinity 2' sugar modifications, such as LNA and/or 2'-MOE.

[0209] In a gapmer, the 5' and 3' most nucleosides of region B are DNA nucleosides, and are positioned adjacent to nucleoside analogs (*e.g.*, high affinity sugar modified nucleosides) of regions A and C, respectively. In some aspects, regions A and C can be further defined by having nucleoside analogs at the end most distant from region B (*i.e.*, at the 5' end of region A and at the 3' end of region C).

[0210] In some aspects, the ASOs of the present disclosure comprise a nucleotide sequence of formula (5' to 3') A-B-C, wherein: (A) (5' region or a first wing sequence) comprises at least one nucleoside analog (*e.g.*, 3-5 LNA units); (B) comprises at least four consecutive nucleosides (*e.g.*, 4-24 DNA units), which are capable of recruiting RNase (when formed in a duplex with a complementary RNA molecule, such as the pre-mRNA or mRNA target); and (C) (3' region or a second wing sequence) comprises at least one nucleoside analog (*e.g.*, 3-5 LNA units).

[0211] In some aspects, region A comprises 3-5 nucleoside analogs, such as LNA, region B consists of 6-24 (*e.g.*, 6, 7, 8, 9, 10, 11, 12, 13, or 14) DNA units, and region C consists of 3 or

4 nucleoside analogs, such as LNA. Such designs include (A-B-C) 3-14-3, 3-11-3, 3-12-3, 3-13-3, 4-9-4, 4-10-4, 4-11-4, 4-12-4, and 5-10-5. In some aspects, the ASO has a design of LLLD_nLLL, LLLLD_nLLLL, or LLLLLD_nLLLLL, wherein the L is a nucleoside analog, the D is DNA, and n can be any integer between 4 and 24. In some aspects, n can be any integer between 6 and 14. In some aspects, n can be any integer between 8 and 12. In some aspects, the ASO has a design of LLLMMD_nMMLLL, LLLMD_nMLLL, LLLLMMMD_nMMLLLL, LLLLMD_nMLLLL, LLLLLMMMD_nMMLLLLL, or LLLLLLMD_nMMLLLLL, wherein the D is DNA, n can be any integer between 3 and 15, the L is LNA, and the M is 2'MOE.

[0212] Further gapmer designs are disclosed in WO2004/046160, WO 2007/146511, and WO2008/113832, each of which is hereby incorporated by reference in its entirety.

II.A.1.H. Internucleotide Linkages

[0213] The monomers of the ASOs described herein are coupled together via linkage groups. Suitably, each monomer is linked to the 3' adjacent monomer via a linkage group.

[0214] The person having ordinary skill in the art would understand that, in the context of the present disclosure, the 5' monomer at the end of an ASO does not comprise a 5' linkage group, although it may or may not comprise a 5' terminal group.

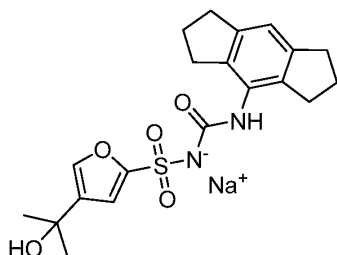
[0215] In some aspects, the contiguous nucleotide sequence comprises one or more modified internucleoside linkages. The terms "linkage group" or "internucleoside linkage" are intended to mean a group capable of covalently coupling together two nucleosides. Non-limiting examples include phosphate groups and phosphorothioate groups.

[0216] The nucleosides of the ASO of the disclosure or contiguous nucleosides sequence thereof are coupled together via linkage groups. Suitably, each nucleoside is linked to the 3' adjacent nucleoside via a linkage group.

[0217] In some aspects, the internucleoside linkage is modified from its normal phosphodiester to one that is more resistant to nuclease attack, such as phosphorothioate, which is cleavable by RNaseH, also allows that route of antisense inhibition in reducing the expression of the target gene. In some aspects, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of internucleoside linkages are modified.

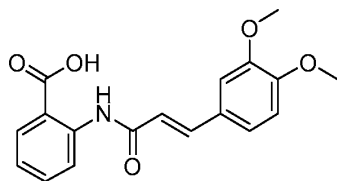
II.A.2. Small Molecule NLRP3 Inhibitors

[0218] In some aspects, the NLRP3 antagonist is a small molecule. In some aspects, the NLRP3 is selected from MCC950, Tanilast, Oridonin, CY-09, Bay 11-7082, Parthenolide, 3,4-methylenedioxy- β -nitrostyrene (MNB), β -hydroxybutyrate (BHB), dimethyl sulfoxide (DMSO), type I interferon, and any combination thereof (*see, e.g.*, Cell Death and Disease 10:128 (2019)). In some aspects, the NLRP3 antagonist comprises the formula:



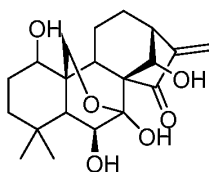
[0219] In some aspects, the NLRP3 antagonist comprises MCC950 (*see, e.g.*, Nat. Med. 21, 248 (2015)).

[0220] In some aspects, the NLRP3 antagonist comprises the formula:



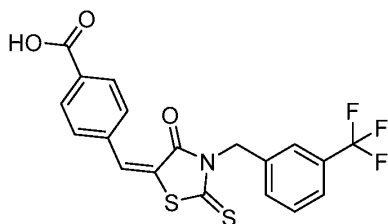
[0221] In some aspects, the NLRP3 antagonist comprises tanilast (*see, e.g.*, EMBO Mol. Med. 10, e8689 (2018)).

[0222] In some aspects, the NLRP3 antagonist comprises the formula:



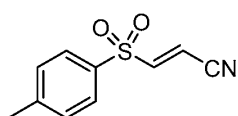
[0223] In some aspects, the NLRP3 antagonist comprises oridonin (*see, e.g.*, Nat. Commun. 9, 2550 (2018)).

[0224] In some aspects, the NLRP3 antagonist comprises the formula:



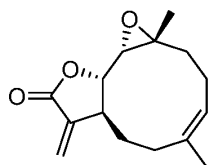
[0225] In some aspects, the NLRP3 antagonist comprises CY-09 (*see, e.g., J. Exp. Med.* 214, 3219–3238 (2017)).

[0226] In some aspects, the NLRP3 antagonist comprises the formula:



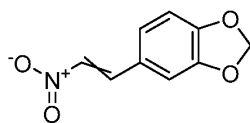
[0227] In some aspects, the antagonist of the NLRP3 pathway comprises Bay 11-7082 (*see, e.g., J. Biol. Chem.* 285, 9792–9802 (2010)).

[0228] In some aspects, the NLRP3 antagonist comprises the formula:



[0229] In some aspects, the antagonist of the NLRP3 pathway comprises parthenolide (*see, e.g., J Biol Chem.* 285:9792–9802 (2010)).

[0230] In some aspects, the NLRP3 antagonist comprises the formula:



[0231] In some aspects, the antagonist of the NLRP3 pathway comprises 3,4-methylenedioxy-β-nitrostyrene (MNB) (*see, e.g., J Biol Chem.* 289:1142–1150 (2014)).

III. Extracellular Vesicles, *e.g., Exosomes*

[0232] Some aspects of the present disclosure are directed to methods of treating a peripheral neuropathy in a subject in need thereof comprising administering to the subject an EV comprising an exogenous NLRP3 antagonist disclosed herein. As such, some aspects of the present

disclosure are directed to EVs, *e.g.*, exosomes, comprising an NLRP3 antagonist. In some aspects, the NLRP3 antagonist is a chemical compound, an siRNA, an shRNA, an ASO, a protein, or any combination thereof. The ASO can be any ASO described herein or a functional fragment thereof. In certain aspects, the ASO reduces the level of an *NLRP3* mRNA or an NLRP3 protein in a target cell. In some aspects, administration of the EV, *e.g.*, exosome, described herein reduces, blocks, or inhibits formation of the NLRP3 inflammasome in a target cell.

[0233] In some aspects, the EV, *e.g.*, the exosome, comprises at least one ASO. In some aspects, the EV, *e.g.*, the exosome, comprises at least two ASOs, *e.g.*, a first ASO comprising a first nucleotide sequence and a second ASO comprising a second nucleotide sequence. In some aspects, the EV, *e.g.*, the exosome, comprises at least three ASOs, at least four ASOs, at least five ASOs, at least six ASOs, or more than six ASOs. In some aspects, each of the first ASO, the second ASO, the third ASO, the fourth ASO, the fifth ASO, the sixth ASO, and/or the Nth ASO is different.

[0234] In some aspects, the EV, *e.g.* the exosome, comprises a first ASO and a second ASO, wherein the first ASO comprises a first nucleotide sequence that is complimentary to a first target sequence in a first transcript, and wherein the second ASO comprises a second nucleotide sequence that is complimentary to a second target sequence in the first transcript. In some aspects, the first target sequence does not overlap with the second target sequence. In some aspects, the first target sequence comprises at least one nucleotide that is within the 5'UTR of the transcript, and the second target sequence does not comprise a nucleotide that is within the 5'UTR. In some aspects, the first target sequence comprises at least one nucleotide that is within the 3'UTR of the transcript, and the second target sequence does not comprise a nucleotide that is within the 3'UTR. In some aspects, the first target sequence comprises at least one nucleotide that is within the 5'UTR of the transcript, and the second target sequence comprises at least one nucleotide that is within the 3'UTR.

[0235] In some aspects, the first ASO targets a sequence within an exon-intron junction, and the second ASO targets a sequence within an exon-intron junction. In some aspects, the first ASO targets a sequence within an exon-intron junction, and the second ASO targets a sequence within an exon. In some aspects, the first ASO targets a sequence within an exon-intron junction, and the second ASO targets a sequence within an intron. In some aspects, the first ASO targets a sequence within an exon, and the second ASO targets a sequence within an exon. In some aspects, the first ASO targets a sequence within an intron, and the second ASO targets a sequence within

an exon. In some aspects, the first ASO targets a sequence within an intron, and the second ASO targets a sequence within an intron.

[0236] In some aspects, the EV, *e.g.* the exosome, comprises a first ASO and a second ASO, wherein the first ASO comprises a first nucleotide sequence that is complimentary to a first target sequence in a first transcript, and wherein the second ASO comprises a second nucleotide sequence that is complimentary to a second target sequence in a second transcript, wherein the first transcript is not the product of the same gene as the second transcript.

[0237] In some aspects, the EV, *e.g.*, the exosome, targets an immune cell. In some aspects the immune cell is selected from a macrophage, a monocyte, a dendritic cell, a B cell, a T cell, and any combination thereof. In certain aspects, the EV, *e.g.*, the exosome, targets a myeloid lineage cell (*e.g.*, a neutrophil, myeloid-derived suppressor cell (MDSC, *e.g.*, a monocytic MDSC or a granulocytic MDSC), monocyte, macrophage, hematopoietic stem cell, basophil, neutrophil, or eosinophil), or any combination thereof. In certain aspects, the EV, *e.g.*, the exosome, targets a macrophage. In certain aspects, the EV, *e.g.*, the exosome, targets a dendritic cell. In certain aspects, the EV, *e.g.*, the exosome, targets a B cell. In certain aspects, the EV, *e.g.*, the exosome, targets a T cell.

[0238] In some aspects, the EV, *e.g.*, the exosome, reduces the expression of one or more gene that is upregulated by the NLRP3 inflammasome. In some aspects, the EV, *e.g.*, the exosome, reduces IL-1 beta expression in serum. In some aspects, the EV, *e.g.*, the exosome, reduces inflammation in a subject. In some aspects, the EV, *e.g.*, the exosome, treats chronic inflammation in a subject in need thereof. In some aspects, the EV, *e.g.*, the exosome, treats auto inflammation in a subject in need thereof.

[0239] In some aspects, the EV, *e.g.*, the exosome, treats a neuro-inflammatory disease in a subject in need thereof. In some aspects, the EV, *e.g.*, the exosome, treats an inflammatory neuropathy in a subject in need thereof. In some aspects, the EV, *e.g.*, the exosome, reduces myeloid inflammation in a nerve. In some aspects, the EV, *e.g.*, the exosome, reduces myeloid inflammation in a sheath. In some aspects, the EV, *e.g.*, the exosome, reduces macrophage influx in one or more of a root, nerve, and/or muscle. In some aspects, the EV, *e.g.*, the exosome, reduces macrophage phagocytosis in one or more of a root, nerve, and/or muscle. In some aspects, the EV, *e.g.*, the exosome, treats chemotherapy-induced peripheral neuropathy (CIPN) in a subject in need thereof.

[0240] As described *supra*, EVs, *e.g.*, exosomes, described herein are extracellular vesicles with a diameter between about 20-300 nm. The size of the EV, *e.g.*, exosome, described herein can be measured according to methods described, *infra*.

[0241] In some aspects, an EV, *e.g.*, exosome, of the present disclosure comprises a bi-lipid membrane ("EV, *e.g.*, exosome, membrane"), comprising an interior (luminal) surface and an exterior surface. In certain aspects, the interior (luminal) surface faces the inner core (*i.e.*, lumen) of the EV, *e.g.*, exosome. In certain aspects, the exterior surface can be in contact with the endosome, the multivesicular bodies, or the membrane/cytoplasm of a producer cell or a target cell

[0242] In some aspects, the EV, *e.g.*, exosome, membrane comprises lipids and fatty acids. In some aspects, the EV, *e.g.*, exosome, membrane comprises phospholipids, glycolipids, fatty acids, sphingolipids, phosphoglycerides, sterols, cholesterol, and phosphatidylserines.

[0243] In some aspects, the EV, *e.g.*, exosome, membrane comprises an inner leaflet and an outer leaflet. The composition of the inner and outer leaflet can be determined by transbilayer distribution assays known in the art, *see, e.g.*, Kuypers *et al.*, *Biochim Biophys Acta* 1985 819:170. In some aspects, the composition of the outer leaflet is between approximately 70-90% choline phospholipids, between approximately 0-15% acidic phospholipids, and between approximately 5-30% phosphatidylethanolamine. In some aspects, the composition of the inner leaflet is between approximately 15-40% choline phospholipids, between approximately 10-50% acidic phospholipids, and between approximately 30-60% phosphatidylethanolamine.

[0244] In some aspects, the EV, *e.g.*, exosome, membrane comprises one or more polysaccharide, such as glycan.

[0245] In some aspects, the EV, *e.g.*, exosome, of the present disclosure comprises an ASO, wherein the ASO is linked to the EV via a scaffold moiety, either on the exterior surface of the EV or on the luminal surface of the EV.

[0246] In some aspects, the EV, *e.g.*, exosome, comprising an ASO comprises an anchoring moiety, which optionally comprising a linker, between the ASO and the exosome membrane. Non-limiting examples of the linkers are disclosed elsewhere herein.

III.A. Anchoring moieties (AM)

[0247] One or more anchoring moieties (AMs) can be used to anchor an ASO to the EV of the present disclosure. In some aspects, the ASO is linked directly to the anchoring moiety or via a linker. In some aspects, the ASO can be attached to an anchoring moiety or linker combination via reaction between a "reactive group" (RG; *e.g.*, amine, thiol, hydroxy, carboxylic acid, or azide)

with a "reactive moiety" (RM; e.g., maleimide, succinate, NHS). Several potential synthetic routes are envisioned, for example:

[AM]-/Reactive moiety/ + /Reactive group/-[ASO]

[AM]-[Linker]_n-/Reactive moiety/ + /Reactive group/-[ASO]

[AM]-/Reactive moiety/ + /Reactive group/-[Linker]_n-[ASO]

[AM]-[Linker]_n-/Reactive moiety/ + /Reactive group/-[Linker]_n-[ASO]

[0248] The anchoring moiety can insert into the lipid bilayer of an EV, e.g., an exosome, allowing the loading of the exosome with an ASO. Currently, a predominant obstacle to the commercialization of exosomes as a delivery vehicle for polar ASOs, is highly inefficient loading. This obstacle can be overcome by modifying polar ASOs, prior to loading them into exosomes. Thus, as described herein, modification of ASOs facilitates their loading into exosomes.

[0249] The methods of loading exosomes with modified polar ASOs set forth herein significantly improve loading efficiency as compared to the loading efficiency previously reported for introducing unmodified ASOs into exosomes by, for example, electroporation or cationic lipid transfection.

[0250] In some aspects, the modifications increase the hydrophobicity of the an ASO by at least about 1, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, or at least about 10 fold relative to native (non-modified) ASO. In some aspects, the modifications increase the hydrophobicity of the ASO by at least about 1, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, or at least about 10 orders of magnitude relative to native (non-modified) ASO.

[0251] In some aspects, the modifications increase the hydrophobicity of the ASO by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, or at least about 1000% relative to native (non-modified) ASO, e.g., the corresponding unmodified ASO. Increases in hydrophobicity can be assessed using any suitable method. For example, hydrophobicity can be determined by measuring the percentage solubility in an organic solvent, such as octanol, as compared to solubility in an aqueous solvent, such as water.

[0252] In some aspect, an anchoring moiety can be chemically conjugated to an ASO to enhance its hydrophobic character. In exemplary aspects, the anchoring moiety is a sterol (e.g., cholesterol), GM1, a lipid, a vitamin, a small molecule, a peptide, or a combination thereof. In some aspects, the moiety is a lipid. In some aspects, the anchoring moiety is a sterol, e.g., cholesterol. Additional hydrophobic moieties include, for example, phospholipids, lysophospholipids, fatty acids, or vitamins (e.g., vitamin D or vitamin E).

[0253] In some aspects, the anchoring moiety is conjugated at the termini of the ASO either directly or via one or more linkers (i.e., "terminal modification"). In other aspects, the anchoring moiety is conjugated to other portions of the ASO.

[0254] In some aspects, the ASO can include a detectable label. Exemplary labels include fluorescent labels and/or radioactive labels. In some aspects, where ASOs are fluorescently labeled, the detectable label can be, for example, Cy3. Adding a detectable label to ASOs can be used as a way of labeling exosomes, and following their biodistribution. In other aspects, a detectable label can be attached to exosomes directly, for example, by way of labeling an exosomal lipid and/or an exosomal peptide.

[0255] The different components of an ASO (i.e., anchoring moieties, linkers and linker combinations, and ASOs) can be linked by amide, ester, ether, thioether, disulfide, phosphoramidate, phosphotriester, phosphorodithioate, methyl phosphonate, phosphodiester, or phosphorothioate linkages or, alternatively any or other linkage.

[0256] In some aspects, the different components of an ASO can be linker using bifunctional linkers (i.e., linkers containing two functional groups), such as N-succinimidyl-3-(2-pyridyldithio)propionate, N-4-maleimide butyric acid, S-(2-pyridyldithio)cysteamine, iodoacetoxysuccinimide, N-(4-maleimidebutyloxy) succinimide, N-[5-(3'-maleimide propylamide)-1-carboxypentyl]iminodiacetic acid, N-(5-aminopentyl)-iminodiacetic acid, and the like.

III.A.1. Anchoring moieties

[0257] Suitable anchoring moieties capable of anchoring an ASO to the surface of an EV, e.g., an exosome, comprise for example sterols (e.g., cholesterol), lipids, lysophospholipids, fatty acids, or fat-soluble vitamins, as described in detail below.

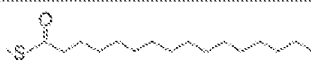
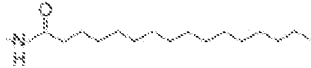
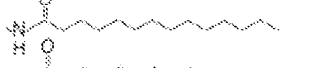
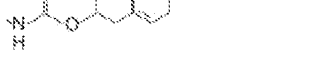
[0258] In some aspects, the anchoring moiety can be a lipid. A lipid anchoring moiety can be any lipid known in the art, e.g., palmitic acid or glycosylphosphatidylinositols. In some aspects,

the lipid, is a fatty acid, phosphatide, phospholipid (e.g., phosphatidyl choline, phosphatidyl serine, or phosphatidyl ethanolamine), or analogue thereof (e.g. phosphatidylcholine, lecithin, phosphatidylethanolamine, cephalin, or phosphatidylserine or analogue or portion thereof, such as a partially hydrolyzed portion thereof).

[0259] Generally, anchoring moieties are chemically attached. However, an anchoring moiety can be attached to an ASO enzymatically. In some aspects, it is possible to attach an anchoring moiety to an ASO via modification of cell culture conditions. For example, by using a culture medium where myristic acid is limiting, some other fatty acids including shorter-chain and unsaturated, can be attached to an N-terminal glycine. For example, in BK channels, myristate has been reported to be attached posttranslationally to internal serine/threonine or tyrosine residues via a hydroxyester linkage.

[0260] The anchoring moiety can be conjugated to an ASO directly or indirectly via a linker combination, at any chemically feasible location, e.g., at the 5' and/or 3' end of the ASO. In one aspect, the anchoring moiety is conjugated only to the 3' end of the ASO. In one aspect, the anchoring moiety is conjugated only to the 5' end of the ASO. In one aspect, the anchoring moiety is conjugated at a location which is not the 3' end or 5' end of the ASO.

[0261] Some types of membrane anchors that can be used to practice the methods of the present disclosure presented in the following table:

Modification	Modifying Group
S-Palmitoylation	
N-Palmitoylation	
N-Myristoylation	
O-Acylation	
Farnesylation	
Geranylgeranylation	
Cholesterol	

[0262] In some aspects, an anchoring moiety of the present disclosure can comprise two or more types of anchoring moieties disclosed herein. For example, in some aspects, an anchoring

moiety can comprise two lipids, e.g., a phospholipids and a fatty acid, or two phospholipids, or two fatty acids, or a lipid and a vitamin, or cholesterol and a vitamin, etc. which taken together have 6-80 carbon atoms (i.e., an equivalent carbon number (ECN) of 6-80).

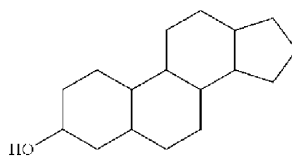
[0263] In some aspects, the combination of anchoring moieties, e.g., a combination of the lipids (e.g., fatty acids) has an ECN of 6-80, 8-80, 10-80, 12-80, 14-80, 16-80, 18-80, 20-80, 22-80, 24-80, 26-80, 28-80, 30-80, 4-76, 6-76, 8-76, 10-76, 12-76, 14-76, 16-76, 18-76, 20-76, 22-76, 24-76, 26-76, 28-76, 30-76, 6-72, 8-72, 10-72, 12-72, 14-72, 16-72, 18-72, 20-72, 22-72, 24-72, 26-72, 28-72, 30-72, 6-68, 8-68, 10-68, 12-68, 14-68, 16-68, 18-68, 20-68, 22-68, 24-68, 26-68, 28-68, 30-68, 6-64, 8-64, 10-64, 12-64, 14-64, 16-64, 18-64, 20-64, 22-64, 24-64, 26-64, 28-64, 30-64, 6-60, 8-60, 10-60, 12-56, 14-56, 16-56, 18-56, 20-56, 22-56, 24-56, 26-56, 28-56, 30-56, 6-52, 8-52, 10-52, 12-52, 14-52, 16-52, 18-52, 20-52, 22-52, 24-52, 26-52, 28-52, 30-52, 6-48, 8-48, 10-48, 12-48, 14-48, 16-48, 18-48, 20-48, 22-48, 24-48, 26-48, 28-48, 30-48, 6-44, 8-44, 10-44, 12-44, 14-44, 16-44, 18-44, 20-44, 22-44, 24-44, 26-44, 28-44, 30-44, 6-40, 8-40, 10-40, 12-40, 14-40, 16-40, 18-40, 20-40, 22-40, 24-40, 26-40, 28-40, 30-40, 6-36, 8-36, 10-36, 12-36, 14-36, 16-36, 18-36, 20-36, 22-36, 24-36, 26-36, 28-36, 30-36, 6-32, 8-32, 10-32, 12-32, 14-32, 16-32, 18-32, 20-32, 22-32, 24-32, 26-32, 28-32, or 30-32.

III.A.1.a. Cholesterol and other sterols

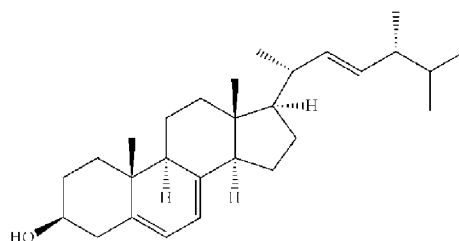
[0264] In some aspects, the anchoring moiety comprises a sterol, steroid, hopanoid, hydroxysteroid, secosteroid, or analog thereof with lipophilic properties. In some aspects, the anchoring moiety comprises a sterol, such as a phytosterol, mycosterol, or zoosterol. Exemplary zoosterols include cholesterol and 24S-hydroxycholesterol; exemplary phytosterols include ergosterol (mycosterol), campesterol, sitosterol, and stigmasterol. In some aspects, the sterol is selected from ergosterol, 7-dehydrocholesterol, cholesterol, 24S-hydroxycholesterol, lanosterol, cycloartenol, fucosterol, saringosterol, campesterol, β -sitosterol, sitostanol, coprostanol, avenasterol, or stigmasterol. Sterols may be found either as free sterols, acylated (sterol esters), alkylated (steryl alkyl ethers), sulfated (sterol sulfate), or linked to a glycoside moiety (steryl glycosides), which can be itself acylated (acylated sterol glycosides).

[0265] In some aspects, the anchoring moiety comprises a steroid. In some aspects, the steroid is selected from dihydrotestosterone, uvaol, hecigenin, diosgenin, progesterone, or cortisol.

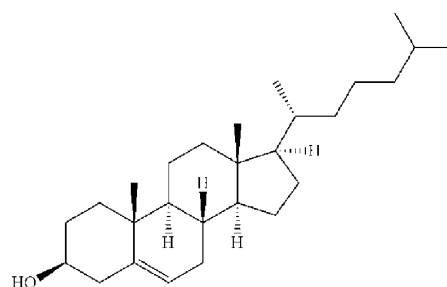
[0266] For example, sterols may be conjugated to the ASO directly or via a linker combination at the available —OH group of the sterol. Exemplary sterols have the general skeleton shown below:



[0267] As a further example, ergosterol has the structure below:

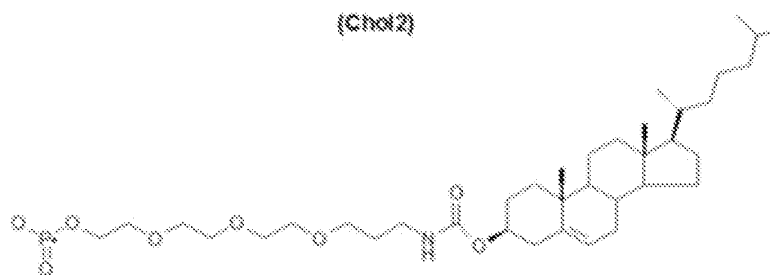


[0268] Cholesterol has the structure below:



[0269] Accordingly, in some embodiments, the free —OH group of a sterol or steroid is used to conjugate the ASO directly or via a linker combination, to the sterol (e.g., cholesterol) or steroid.

[0270] In some aspects, the ASO is conjugated to the EV by the following structure:



III.A.1.b. Fatty acids

[0271] In some aspects, the anchoring moiety is a fatty acid. In some aspects, the fatty acid is a short-chain, medium-chain, or long-chain fatty acid. In some aspects, the fatty acid is a saturated fatty acid. In some aspects, the fatty acid is an unsaturated fatty acid. In some aspects, the fatty acid is a monounsaturated fatty acid. In some aspects, the fatty acid is a polyunsaturated fatty acid, such as an ω -3 (omega-3) or ω -6 (omega-6) fatty acid.

[0272] In some aspects, the lipid, e.g., fatty acid, has a C₂-C₆₀ chain. In some embodiments, the lipid, e.g., fatty acid, has a C₂-C₂₈ chain. In some aspects, the fatty acid, has a C₂-C₄₀ chain. In some aspects, the fatty acid, has a C₂-C₁₂ or C₄-C₁₂ chain. In some aspects, the fatty acid, has a C₄-C₄₀ chain. In some aspects, the fatty acid, has a C₄-C₄₀, C₂-C₃₈, C₂-C₃₆, C₂-C₃₄, C₂-C₃₂, C₂-C₃₀, C₄-C₃₀, C₂-C₂₈, C₄-C₂₈, C₂-C₂₆, C₄-C₂₆, C₂-C₂₄, C₄-C₂₄, C₆-C₂₄, C₈-C₂₄, C₁₀-C₂₄, C₂-C₂₂, C₄-C₂₂, C₆-C₂₂, C₈-C₂₂, C₁₀-C₂₂, C₂-C₂₀, C₄-C₂₀, C₆-C₂₀, C₈-C₂₀, C₁₀-C₂₀, C₂-C₁₈, C₄-C₁₈, C₆-C₁₈, C₈-C₁₈, C₁₀-C₁₈, C₁₂-C₁₈, C₁₄-C₁₈, C₁₆-C₁₈, C₂-C₁₆, C₄-C₁₆, C₆-C₁₆, C₈-C₁₆, C₁₀-C₁₆, C₁₂-C₁₆, C₁₄-C₁₆, C₂-C₁₅, C₄-C₁₅, C₆-C₁₅, C₈-C₁₅, C₉-C₁₅, C₁₀-C₁₅, C₁₁-C₁₅, C₁₂-C₁₅, C₁₃-C₁₅, C₂-C₁₄, C₄-C₁₄, C₆-C₁₄, C₈-C₁₄, C₉-C₁₄, C₁₀-C₁₄, C₁₁-C₁₄, C₁₂-C₁₄, C₂-C₁₃, C₄-C₁₃, C₆-C₁₃, C₇-C₁₃, C₈-C₁₃, C₉-C₁₃, C₁₀-C₁₃, C₁₁-C₁₃, C₂-C₁₂, C₄-C₁₂, C₆-C₁₂, C₇-C₁₂, C₈-C₁₂, C₉-C₁₂, C₁₀-C₁₂, C₂-C₁₁, C₄-C₁₁, C₆-C₁₁, C₇-C₁₁, C₈-C₁₁, C₉-C₁₁, C₂-C₁₀, C₄-C₁₀, C₂-C₉, C₄-C₉, C₂-C₈, C₂-C₇, C₄-C₇, C₂-C₆, or C₄-C₆, chain. In some aspects, the fatty acid, has a C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₅, C₁₆, C₁₇, C₁₈, C₁₉, C₂₀, C₂₁, C₂₂, C₂₃, C₂₄, C₂₅, C₂₆, C₂₇, C₂₈, C₂₉, C₃₀, C₃₁, C₃₂, C₃₃, C₃₄, C₃₅, C₃₆, C₃₇, C₃₈, C₃₉, C₄₀, C₄₁, C₄₂, C₄₃, C₄₄, C₄₅, C₄₆, C₄₇, C₄₈, C₄₉, C₅₀, C₅₁, C₅₂, C₅₃, C₅₄, C₅₅, C₅₆, C₅₇, C₅₈, C₅₉, or C₆₀ chain.

[0273] In some aspects, the anchoring moiety comprises two fatty acids, each of which is independently selected from a fatty acid having a chain with any one of the foregoing ranges or numbers of carbon atoms. In some aspects, one of the fatty acids is independently a fatty acid with a C₆-C₂₁ chain and one is independently a fatty acid with a C₁₂-C₃₆ chain. In some embodiments, each fatty acid independently has a chain of 11, 12, 13, 14, 15, 16, or 17 carbon atoms.

[0274] Suitable fatty acids include saturated straight-chain fatty acids, saturated branched fatty acids, unsaturated fatty acids, hydroxy fatty acids, and polycarboxylic acids. In some aspects, such fatty acids have up to 32 carbon atoms.

[0275] Examples of useful saturated straight-chain fatty acids include those having an even number of carbon atoms, such as butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachic acid, behenic acid, lignoceric acid, hexacosanoic acid, octacosanoic acid, triacontanoic acid and n-dotriacontanoic acid, and those having an odd number of carbon atoms, such as propionic acid, n-valeric acid, enanthic acid, pelargonic acid,

hendecanoic acid, tridecanoic acid, pentadecanoic acid, heptadecanoic acid, nonadecanoic acid, heneicosanoic acid, tricosanoic acid, pentacosanoic acid, and heptacosanoic acid.

[0276] Examples of suitable saturated branched fatty acids include isobutyric acid, isocaproic acid, isocaprylic acid, isocapric acid, isolauric acid, 11-methyldodecanoic acid, isomyristic acid, 13-methyl-tetradecanoic acid, isopalmitic acid, 15-methyl-hexadecanoic acid, isostearic acid, 17-methyloctadecanoic acid, isoarachic acid, 19-methyl-eicosanoic acid, α -ethyl-hexanoic acid, α -hexyldecanoic acid, α -heptylundecanoic acid, 2-decyltetradecanoic acid, 2-undecyltetradecanoic acid, 2-decylpentadecanoic acid, 2-undecylpentadecanoic acid, and Fine oxocol 1800 acid (product of Nissan Chemical Industries, Ltd.). Suitable saturated odd-carbon branched fatty acids include anteiso fatty acids terminating with an isobutyl group, such as 6-methyl-octanoic acid, 8-methyl-decanoic acid, 10-methyl-dodecanoic acid, 12-methyl-tetradecanoic acid, 14-methyl-hexadecanoic acid, 16-methyl-octadecanoic acid, 18-methyl-eicosanoic acid, 20-methyl-docosanoic acid, 22-methyl-tetracosanoic acid, 24-methyl-hexacosanoic acid, and 26-methyloctacosanoic acid.

[0277] Examples of suitable unsaturated fatty acids include 4-decenoic acid, caproic acid, 4-dodecenoic acid, 5-dodecenoic acid, lauroleic acid, 4-tetradecenoic acid, 5-tetradecenoic acid, 9-tetradecenoic acid, palmitoleic acid, 6-octadecenoic acid, oleic acid, 9-octadecenoic acid, 11-octadecenoic acid, 9-eicosenoic acid, cis-11-eicosenoic acid, cetoleic acid, 13-docosenoic acid, 15-tetracosenoic acid, 17-hexacosenoic acid, 6,9,12,15-hexadecatetraenoic acid, linoleic acid, linolenic acid, α -eleostearic acid, β -eleostearic acid, punicic acid, 6,9,12,15-octadecatetraenoic acid, parinaric acid, 5,8,11,14-eicosatetraenoic acid, 5,8,11,14,17-eicosapentaenoic acid, 7,10,13,16,19-docosapentaenoic acid, 4,7,10,13,16,19-docosaheptaenoic acid, and the like.

[0278] Examples of suitable hydroxy fatty acids include α -hydroxylauric acid, α -hydroxymyristic acid, α -hydroxypalmitic acid, α -hydroxystearic acid, ω -hydroxylauric acid, α -hydroxyarachic acid, 9-hydroxy-12-octadecenoic acid, ricinoleic acid, α -hydroxybehenic acid, 9-hydroxy-trans-10,12-octadecadienic acid, kamolenic acid, ipurollic acid, 9,10-dihydroxystearic acid, 12-hydroxystearic acid and the like.

[0279] Examples of suitable polycarboxylic acids include oxalic acid, malonic acid, succinic acid, glutaric acid, adipic acid, pimelic acid, suberic acid, azelaic acid, sebacic acid, D,L-malic acid, and the like.

[0280] In some aspects, each fatty acid is independently selected from propionic acid, butyric acid, valeric acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid, capric acid, undecylic acid, lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric

acid, stearic acid, nonadecylic acid, arachidic acid, heneicosylic acid, behenic acid, tricosylic acid, lignoceric acid, pentacosylic acid, cerotic acid, heptacosylic acid, montanic acid, nonacosylic acid, melissic acid, henatriacontylic acid, lacceroic acid, psyllic acid, geddic acid, ceroplastic acid, hexatriacontylic acid, heptatriacontanoic acid, or octatriacontanoic acid.

[0281] In some aspects, each fatty acid is independently selected from α -linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid, linoleic acid, gamma-linoleic acid, dihomo-gamma-linoleic acid, arachidonic acid, docosatetraenoic acid, palmitoleic acid, vaccenic acid, paullinic acid, oleic acid, elaidic acid, gondoic acid, eurcic acid, nervonic acid, mead acid, adrenic acid, bosseopentaenoic acid, ozubondo acid, sardine acid, herring acid, docosahexaenoic acid, or tetracosanolpentaenoic acid, or another monounsaturated or polyunsaturated fatty acid.

[0282] In some aspects, one or both of the fatty acids is an essential fatty acid. In view of the beneficial health effects of certain essential fatty acids, the therapeutic benefits of disclosed therapeutic-loaded exosomes may be increased by including such fatty acids in the therapeutic agent. In some aspects, the essential fatty acid is an n-6 or n-3 essential fatty acid selected from the group consisting of linolenic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, adrenic acid, docosapentaenoic n-6 acid, alpha-linolenic acid, stearidonic acid, the 20:4n-3 acid, eicosapentaenoic acid, docosapentaenoic n-3 acid, or docosahexaenoic acid.

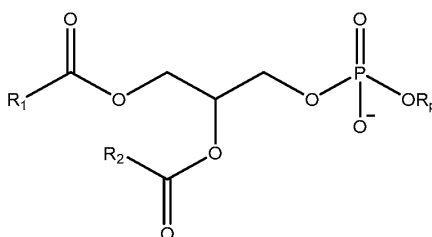
[0283] In some aspects, each fatty acid is independently selected from all-cis-7,10,13-hexadecatrienoic acid, α -linolenic acid, stearidonic acid, eicosatrienoic acid, eicosatetraenoic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid, docosahexaenoic acid (DHA), tetracosapentaenoic acid, tetracosahexaenoic acid, or lipoic acid. In other aspects, the fatty acid is selected from eicosapentaenoic acid, docosahexaenoic acid, or lipoic acid. Other examples of fatty acids include all-cis-7,10,13-hexadecatrienoic acid, α -linolenic acid (ALA or all-cis-9,12,15-octadecatrienoic acid), stearidonic acid (STD or all-cis-6,9,12,15-octadecatetraenoic acid), eicosatrienoic acid (ETE or all-cis-11,14,17-eicosatrienoic acid), eicosatetraenoic acid (ETA or all-cis-8,11,14,17-eicosatetraenoic acid), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA, clupanodonic acid or all-cis-7,10,13,16,19-docosapentaenoic acid), docosahexaenoic acid (DHA or all-cis-4,7,10,13,16,19-docosahexaenoic acid), tetracosapentaenoic acid (all-cis-9,12,15,18,21-docosahexaenoic acid), or tetracosahexaenoic acid (nisinic acid or all-cis-6,9,12,15,18,21-tetracosenoic acid). In some aspects, the fatty acid is a medium-chain fatty acid such as lipoic acid.

[0284] Fatty acid chains differ greatly in the length of their chains and may be categorized according to chain length, e.g. as short to very long. Short-chain fatty acids (SCFA) are fatty acids with chains of about five or less carbons (e.g. butyric acid). In some aspects, the fatty acid is a

SCFA. Medium-chain fatty acids (MCFA) include fatty acids with chains of about 6-12 carbons, which can form medium-chain triglycerides. In some aspects, the fatty acid is a MCFA. Long-chain fatty acids (LCFA) include fatty acids with chains of 13-21 carbons. In some aspects, the fatty acid is a LCFA. In some aspects, the fatty acid is a LCFA. Very long chain fatty acids (VLCFA) include fatty acids with chains of 22 or more carbons, such as 22-60, 22-50, or 22-40 carbons. In some aspects, the fatty acid is a VLCFA.

III.A.1.c. Phospholipids

[0285] In some aspects, the anchoring moiety comprises a phospholipid. Phospholipids are a class of lipids that are a major component of all cell membranes. They can form lipid bilayers because of their amphiphilic characteristic. The structure of the phospholipid molecule generally consists of two hydrophobic fatty acid "tails" and a hydrophilic "head" consisting of a phosphate group. For example, a phospholipid can be a lipid according to the following formula:



in which R_p represents a phospholipid moiety and R₁ and R₂ represent fatty acid moieties with or without unsaturation that may be the same or different.

[0286] A phospholipid moiety may be selected, for example, from the non-limiting group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2 lysophosphatidyl choline, and a sphingomyelin.

[0287] Particular phospholipids may facilitate fusion to a lipid bilayer, e.g., the lipid bilayer of an exosomal membrane. For example, a cationic phospholipid may interact with one or more negatively charged phospholipids of a membrane. Fusion of a phospholipid to a membrane may allow one or more elements of a lipid-containing composition to bind to the membrane or to pass through the membrane.

[0288] A fatty acid moiety may be selected, for example, from the non-limiting group consisting of lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanoic acid, arachidic acid,

arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid.

[0289] The phospholipids using as anchoring moieties in the present disclosure can be natural or non-natural phospholipids. Non-natural phospholipid species including natural species with modifications and substitutions including branching, oxidation, cyclization, and alkynes are also contemplated. For example, a phospholipid may be functionalized with or cross-linked to one or more alkynes (e.g., an alkenyl group in which one or more double bonds is replaced with a triple bond). Under appropriate reaction conditions, an alkyne group may undergo a copper-catalyzed cycloaddition upon exposure to an azide.

III.A.2. Linker combinations

[0290] In some aspects, an ASO is linked to a hydrophobic membrane anchoring moiety disclosed herein via a linker combination, which can comprise any combination of cleavable and/or non-cleavable linkers. The main function of a linker combination is to provide the optimal spacing between the anchoring moiety or moieties and the BAM target. For example, in the case of an ASO, the linker combination should reduce steric hindrances and position the ASO so it can interact with a target nucleic acid, e.g., a mRNA or a miRNA.

[0291] Linkers may be susceptible to cleavage ("cleavable linker") thereby facilitating release of the biologically active molecule. Thus, in some aspects, a linker combination disclosed herein can comprise a cleavable linker. Such cleavable linkers may be susceptible, for example, to acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide bond cleavage, at conditions under which the biologically active molecule remains active. Alternatively, linkers may be substantially resistant to cleavage ("non-cleavable linker"). In some aspects, the cleavable linker comprises a spacer. In some aspects the spacer is PEG.

[0292] In some aspects, a linker combination comprises at least 2, at least 3, at least 4, at least 5, or at least 6 or more different linkers disclosed herein. In some aspects, linkers in a linker combination can be linked by an ester linkage (e.g., phosphodiester or phosphorothioate ester).

[0293] In some aspects, the linker is direct bond between an anchoring moiety and a BAM, e.g., an ASO.

III.A.2.a. Non-cleavable linkers

[0294] In some aspects, the linker combination comprises a "non-cleavable linker." Non-cleavable linkers are any chemical moiety capable of linking two or more components of a modified biologically active molecule of the present disclosure (e.g., a biologically active molecule and an anchoring moiety; a biologically active molecule and a cleavable linker; an anchoring moiety and a cleavable linker) in a stable, covalent manner and does not fall off under the categories listed above for cleavable linkers. Thus, non-cleavable linkers are substantially resistant to acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage and disulfide bond cleavage.

[0295] Furthermore, non-cleavable refers to the ability of the chemical bond in the linker or adjoining to the linker to withstand cleavage induced by an acid, photolabile-cleaving agent, a peptidase, an esterase, or a chemical or physiological compound that cleaves a disulfide bond, at conditions under which a cyclic dinucleotide and/or the antibody does not lose its activity. In some aspects, the biologically active molecule is attached to the linker via another linker, e.g., a self-immolative linker.

[0296] In some aspects, the linker combination comprises a non-cleavable linker comprising, e.g., tetraethylene glycol (TEG), hexaethylene glycol (HEG), polyethylene glycol (PEG), succinimide, or any combination thereof. In some aspects, the non-cleavable linker comprises a spacer unit to link the biologically active molecule to the non-cleavable linker.

[0297] In some aspects, one or more non-cleavable linkers comprise smaller units (e.g., HEG, TEG, glycerol, C2 to C12 alkyl, and the like) linked together. In one aspect, the linkage is an ester linkage (e.g., phosphodiester or phosphorothioate ester) or other linkage.

III.A.2.b. Ethylene Glycols (HEG, TEG, PEG)

[0298] In some aspects, the linker combination comprises a non-cleavable linker, wherein the non-cleavable linker comprises a polyethylene glycol (PEG) characterized by a formula $R^3-(O-CH_2-CH_2)_n-$ or $R^3-(O-CH_2-CH_2)_n-O-$ with R^3 being hydrogen, methyl or ethyl and n having a value from 2 to 200. In some aspects, the linker comprises a spacer, wherein the spacer is PEG.

[0299] In some aspects, the PEG linker is an oligo-ethylene glycol, e.g., diethylene glycol, triethylene glycol, tetra ethylene glycol (TEG), pentaethylene glycol, or a hexaethylene glycol (HEG) linker.

[0300] In some aspects, n has a value of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42,

43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, or 200.

[0301] In some aspects, n is between 2 and 10, between 10 and 20, between 20 and 30, between 30 and 40, between 40 and 50, between 50 and 60, between 60 and 70, between 70 and 80, between 80 and 90, between 90 and 100, between 100 and 110, between 110 and 120, between 120 and 130, between 130 and 140, between 140 and 150, between 150 and 160, between 160 and 170, between 170 and 180, between 180 and 190, or between 190 and 200.

[0302] In some specific aspects, n has a value from 3 to 200, from 3 to 20, from 10 to 30, or from 9 to 45.

[0303] In some aspects, the PEG is a branched PEG. Branched PEGs have three to ten PEG chains emanating from a central core group.

[0304] In certain embodiments, the PEG moiety is a monodisperse polyethylene glycol. In the context of the present disclosure, a monodisperse polyethylene glycol (mdPEG) is a PEG that has a single, defined chain length and molecular weight. mdPEGs are typically generated by separation from the polymerization mixture by chromatography. In certain formulae, a monodisperse PEG moiety is assigned the abbreviation mdPEG.

[0305] In some aspects, the PEG is a Star PEG. Star PEGs have 10 to 100 PEG chains emanating from a central core group.

[0306] In some aspects, the PEG is a Comb PEGs. Comb PEGs have multiple PEG chains normally grafted onto a polymer backbone.

[0307] In certain aspects, the PEG has a molar mass between 100 g/mol and 3000 g/mol, particularly between 100 g/mol and 2500 g/mol, more particularly of approx. 100 g/mol to 2000 g/mol. In certain aspects, the PEG has a molar mass between 200 g/mol and 3000 g/mol, particularly between 300 g/mol and 2500 g/mol, more particularly of approx. 400 g/mol to 2000 g/mol.

[0308] In some aspects, the PEG is PEG₁₀₀, PEG₂₀₀, PEG₃₀₀, PEG₄₀₀, PEG₅₀₀, PEG₆₀₀, PEG₇₀₀, PEG₈₀₀, PEG₉₀₀, PEG₁₀₀₀, PEG₁₁₀₀, PEG₁₂₀₀, PEG₁₃₀₀, PEG₁₄₀₀, PEG₁₅₀₀, PEG₁₆₀₀, PEG₁₇₀₀,

PEG₁₈₀₀, PEG₁₉₀₀, PEG₂₀₀₀, PEG₂₁₀₀, PEG₂₂₀₀, PEG₂₃₀₀, PEG₂₄₀₀, PEG₂₅₀₀, PEG₁₆₀₀, PEG₁₇₀₀, PEG₁₈₀₀, PEG₁₉₀₀, PEG₂₀₀₀, PEG₂₁₀₀, PEG₂₂₀₀, PEG₂₃₀₀, PEG₂₄₀₀, PEG₂₅₀₀, PEG₂₆₀₀, PEG₂₇₀₀, PEG₂₈₀₀, PEG₂₉₀₀, or PEG₃₀₀₀. In one particular aspect, the PEG is PEG₄₀₀. In another particular aspect, the PEG is PEG₂₀₀₀.

[0309] In some aspects, a linker combination of the present disclosure can comprise several PEG linkers, e.g., a cleavable linker flanked by PEG, HEG, or TEG linkers.

[0310] In some aspects, the linker combination comprises (HEG)_n and/or (TEG)_n, wherein n is an integer between 1 and 50, and each unit is connected, e.g., via a phosphate ester linker, a phosphorothioate ester linkage, or a combination thereof.

III.A.2.c. Glycerol and Polyglycerols (PG)

[0311] In some aspects, the linker combination comprises a non-cleavable linker comprising a glycerol unit or a polyglycerol (PG) described by the formula ((R₃—O—(CH₂—CHOH—CH₂O)_n—) with R₃ being hydrogen, methyl or ethyl, and n having a value from 3 to 200. In some aspects, n has a value from 3 to 20. In some aspects, n has a value from 10 to 30.

[0312] In some aspects, the PG linker is a diglycerol, triglycerol, tetraglycerol (TG), pentaglycerol, or a hexaglycerol (HG) linker.

[0313] In some aspects, n has a value of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, or 200.

[0314] In some aspects, n is between 2 and 10, between 10 and 20, between 20 and 30, between 30 and 40, between 40 and 50, between 50 and 60, between 60 and 70, between 70 and 80, between 80 and 90, between 90 and 100, between 100 and 110, between 110 and 120, between 120 and 130, between 130 and 140, between 140 and 150, between 150 and 160, between 160 and 170, between 170 and 180, between 180 and 190, or between 190 and 200.

[0315] In some alternatives of these embodiments, n has a value from 9 to 45. In some aspects, the heterologous moiety is a branched polyglycerol described by the formula $(R^3-O-(CH_2-CHOR^5-CH_2-O)_n-)$ with R^5 being hydrogen or a linear glycerol chain described by the formula $(R^3-O-(CH_2-CHOH-CH_2-O)_n-)$ and R^3 being hydrogen, methyl or ethyl. In some aspects, the heterologous moiety is a hyperbranched polyglycerol described by the formula $(R^3-O-(CH_2-CHOR^5-CH_2-O)_n-)$ with R^5 being hydrogen or a glycerol chain described by the formula $(R^3-O-(CH_2-CHOR^6-CH_2-O)_n-)$, with R^6 being hydrogen or a glycerol chain described by the formula $(R^3-O-(CH_2-CHOR^7-CH_2-O)_n-)$, with R^7 being hydrogen or a linear glycerol chain described by the formula $(R^3-O-(CH_2-CHOH-CH_2-O)_n-)$ and R^3 being hydrogen, methyl or ethyl. Hyperbranched glycerol and methods for its synthesis are described in Oudshorn et al. (2006) *Biomaterials* 27:5471-5479; Wilms et al. (2010) *Acc. Chem. Res.* 43, 129-41, and references cited therein.

[0316] In certain aspects, the PG has a molar mass between 100 g/mol and 3000 g/mol, particularly between 100 g/mol and 2500 g/mol, more particularly of approx. 100 g/mol to 2000 g/mol. In certain aspects, the PG has a molar mass between 200 g/mol and 3000 g/mol, particularly between 300 g/mol and 2500 g/mol, more particularly of approx. 400 g/mol to 2000 g/mol.

[0317] In some aspects, the PG is PG₁₀₀, PG₂₀₀, PG₃₀₀, PG₄₀₀, PG₅₀₀, PG₆₀₀, PG₇₀₀, PG₈₀₀, PG₉₀₀, PG₁₀₀₀, PG₁₁₀₀, PG₁₂₀₀, PG₁₃₀₀, PG₁₄₀₀, PG₁₅₀₀, PG₁₆₀₀, PG₁₇₀₀, PG₁₈₀₀, PG₁₉₀₀, PG₂₀₀₀, PG₂₁₀₀, PG₂₂₀₀, PG₂₃₀₀, PG₂₄₀₀, PG₂₅₀₀, PG₁₆₀₀, PG₁₇₀₀, PG₁₈₀₀, PG₁₉₀₀, PG₂₀₀₀, PG₂₁₀₀, PG₂₂₀₀, PG₂₃₀₀, PG₂₄₀₀, PG₂₅₀₀, PG₂₆₀₀, PG₂₇₀₀, PG₂₈₀₀, PG₂₉₀₀, or PG₃₀₀₀. In one particular aspect, the PG is PG₄₀₀. In another particular aspect, the PG is PG₂₀₀₀.

[0318] In some aspects, the linker combination comprises (glycerol) $_n$, and/or (HG) $_n$ and/or (TG) $_n$, wherein n is an integer between 1 and 50, and each unit is connected, e.g., via a phosphate ester linker, a phosphorothioate ester linkage, or a combination thereof.

III.A.2.d. Aliphatic (Alkyl) linkers

[0319] In some aspects, the linker combination comprises at least one aliphatic (alkyl) linker, e.g., propyl, butyl, hexyl, or C₂-C₁₂ alkyl, such as C₂-C₁₀ alkyl or C₂-C₆ alkyl.

III.A.3. Cleavable linkers

[0320] In some aspects, different components of an ASO disclosed herein can be linker by a cleavable linker. The term cleavable linker refers to a linker comprising at least one linkage or chemical bond that can be broken or cleaved. As used herein, the term cleave refers to the breaking

of one or more chemical bonds in a relatively large molecule in a manner that produces two or more relatively smaller molecules. Cleavage may be mediated, e.g., by a nuclease, peptidase, protease, phosphatase, oxidase, or reductase, for example, or by specific physicochemical conditions, e.g., redox environment, pH, presence of reactive oxygen species, or specific wavelengths of light.

[0321] In some aspects, the term "cleavable," as used herein, refers, e.g., to rapidly degradable linkers, such as, e.g., phosphodiester and disulfides, while the term "non-cleavable" refers, e.g., to more stable linkages, such as, e.g., nuclease-resistant phosphorothioates.

[0322] In some aspects, the cleavable linker is a dinucleotide or trinucleotide linker, a disulfide, an imine, a thioketal, a val-cit dipeptide, or any combination thereof.

[0323] In some aspects, the cleavable linker comprises valine-alanine-p-aminobenzylcarbamate or valine-citrulline-p-aminobenzylcarbamate.

III.A.4. Specific examples and topologies

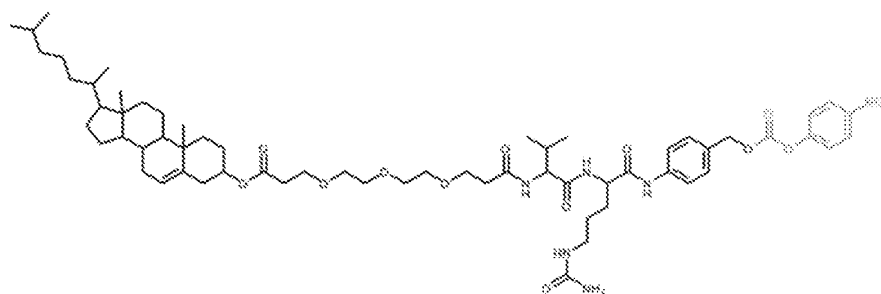
[0324] In specific aspects of the present disclosure, the linker combination consists of a linker of formula



wherein m, n, and o are 0 or 1, and at least one of m, n, or o is not zero. Exemplary linker combinations according to such formula are C6-TEG-HEG, C6-HEG, C6-TEG, C6, TEG-HEG, TEG, C8-TEG-HEG, C8-HEG, C8-TEG, and C8.

[0325] In some aspects, the linker combination comprises a non-cleavable linker (e.g., TEG or HEG) in combination with one or more cleavable linkers, e.g., an enzymatic cleavable linker and a self immolative linker.

[0326] In a specific aspect, the linker combination the linker combination comprises the linker combination TEG (non-cleavable linker)-Val-Cit(cleavable linker)-pAB(self-immolative linker), as shown below



[0327] Specific combinations of anchoring moieties and linker combinations are illustrated in the tables below.

Table 2.

Anchoring moiety	Linker combination		
	1 st Linker	2 nd Linker	3 rd Linker
Cholesterol	C6	TEG	HEG
Cholesterol	C6	HEG	No
Cholesterol	C6	TEG	No
Cholesterol	C6	No	No
Cholesterol	TEG	HEG	No
Cholesterol	TEG	No	No
Tocopherol	C8	TEG	HEG
Tocopherol	C8	HEG	No
Tocopherol	C8	TEG	No
Tocopherol	C8	No	No
Tocopherol	TEG	HEG	No
Tocopherol	HEG	No	No
Tocopherol	TEG	No	No
Tocopherol	No	No	No
Palmitate	C6	TEG	HEG
Palmitate	C6	HEG	No

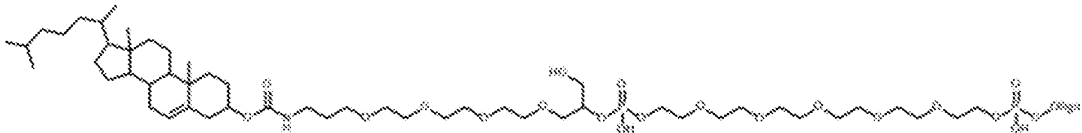
Palmitate	C6	TEG	No
Palmitate	C6	No	No
Cholesterol	TEG	Glycerol	HEG

Table 3.

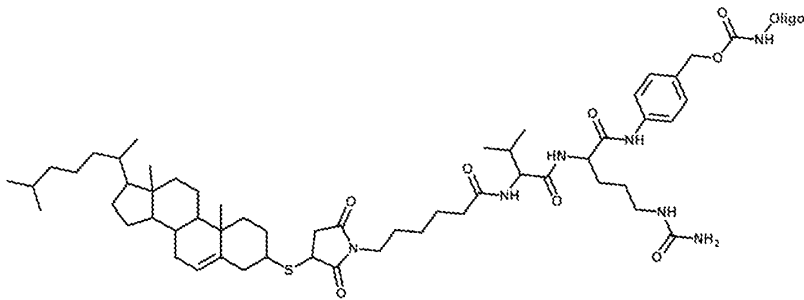
Linker Combination		
Linker 1	Cleavable Linker 2	Linker 3
C6	Disulfide Imine Thioketal Tri/Dinucleotide Val-Cit	C6
None		None
TEG		TEG
HEG		HEG
TEG-HEG		TEG-HEG

[0328] Specific oligonucleotides such as ASOs of the present disclosure are exemplified below

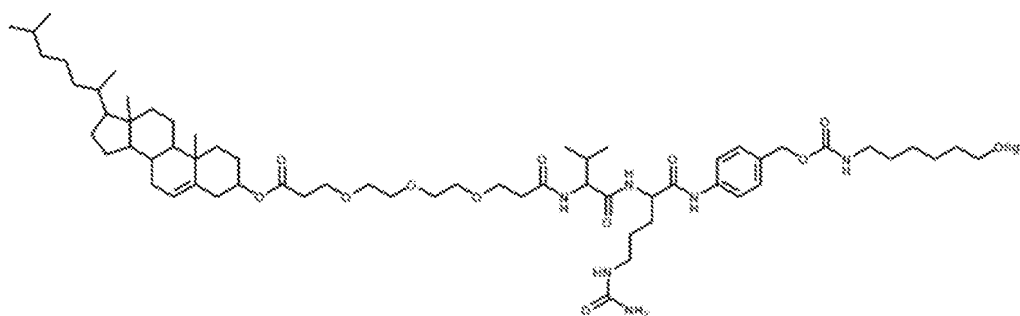
[Cholesterol]-[TEG]-[HEG]-[ASO]



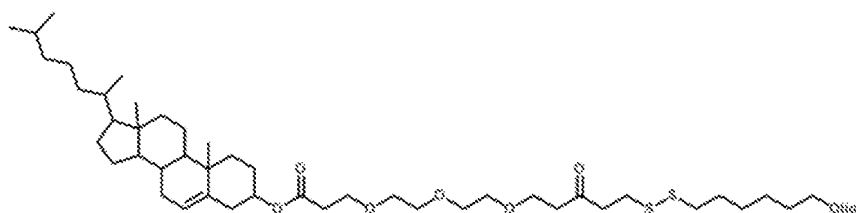
[Cholesterol]-[SMal]-[Val-Cit]-[pAB]-[ASO]



[Cholesterol]-[TEG]-[Val-Cit]-[C6]-[ASO]



[Cholesterol]-[TEG]-[SS]-[C6]-[ASO]



wherein [Cholesterol] is a cholesterol anchoring moiety, [TEG] is a TEG non-cleavable linker, [HEG] is a HEG non-cleavable linker, [SS] is a disulfide redox cleavable linker, [C6] is an alkyl non-cleavable linker, [SMal] is S-maleimide, [Val-Cit] is a valine-citrulline cleavable linker, [pAB] is a pAB self-immolative linker. In some aspects, an ASO of the present disclosure has a structure according to the exemplary structures provided above, in which one or more components has been replaced by a component in the same class as those depicted in the example. For example, the [cholesterol] anchoring moiety can be substituted by another anchoring moiety disclosed herein, a [TEG] can be substituted by another polymeric non-cleavable linker disclosed herein (e.g., HEG, PEG, PG), [Val-Cit] can be replaced by another peptidase cleavable linker, or [pAB] can be substituted by another self-immolative linker.

III.B. Scaffold Moieties

[0329] One or more scaffold moieties can be expressed in the EVs. In some aspects, one or more scaffold moieties are used to anchor an ASO to the EV of the present disclosure. In other aspects, one or more scaffold moieties are used to anchor a protein or a molecule to the EVs in addition to the ASOs. Therefore, an EV of the present disclosure comprises an anchoring moiety linking an ASO and a scaffold moiety linking a protein or a molecule, e.g., a targeting moiety. In some aspects, the ASO is linked to the scaffold moiety. In some aspects, the EV comprises more than one scaffold moiety. In some aspects, a first ASO is linked to a first scaffold moiety and a

second ASO is linked to a second scaffold moiety. In some aspects, the first scaffold moiety and the second scaffold moiety are the same type of scaffold moiety, *e.g.*, the first and second scaffold moieties are both a Scaffold X protein. In some aspects, the first scaffold moiety and the second scaffold moiety are different types of scaffold moiety, *e.g.*, the first scaffold moiety is a Scaffold Y protein and the second scaffold moiety is a Scaffold X protein. In some aspects, the first scaffold moiety is a Scaffold Y, disclosed herein. In some aspects, the first scaffold moiety is a Scaffold X, disclosed herein. In some aspects, the second scaffold moiety is a Scaffold Y, disclosed herein. In some aspects, the second scaffold moiety is a Scaffold X, disclosed herein.

[0330] In some aspects, the EV comprises one or more scaffold moieties, which are capable of anchoring an ASO to the EV, *e.g.*, exosome, (*e.g.*, either on the luminal surface or on the exterior surface). In certain aspects, the scaffold moiety is a polypeptide ("scaffold protein"). In certain aspects, the scaffold protein comprises an exosome protein or a fragment thereof. In other aspects, scaffold moieties are non-polypeptide moieties. In some aspects, scaffold proteins include various membrane proteins, such as transmembrane proteins, integral proteins and peripheral proteins, enriched on the exosome membranes. They can include various CD proteins, transporters, integrins, lectins, and cadherins. In certain aspects, a scaffold moiety (*e.g.*, scaffold protein) comprises Scaffold X. In other aspects, a scaffold moiety (*e.g.*, exosome protein) comprises Scaffold Y. In further aspects, a scaffold moiety (*e.g.*, exosome protein) comprises both a Scaffold X and a Scaffold Y.

[0331] In some aspects, EVs, *e.g.*, exosomes, of the present disclosure comprise a membrane modified in its composition. For example, their membrane compositions can be modified by changing the protein, lipid, or glycan content of the membrane.

[0332] In some aspects, the surface-engineered EVs, *e.g.*, exosomes, are generated by chemical and/or physical methods, such as PEG-induced fusion and/or ultrasonic fusion. In other aspects, the surface-engineered EVs, *e.g.*, exosomes, are generated by genetic engineering. EVs, *e.g.*, exosomes, produced from a genetically-modified producer cell or a progeny of the genetically-modified cell can contain modified membrane compositions. In some aspects, surface-engineered EVs, *e.g.*, exosomes, have scaffold moiety (*e.g.*, exosome protein, *e.g.*, Scaffold X) at a higher or lower density (*e.g.*, higher number) or include a variant or a fragment of the scaffold moiety.

[0333] For example, surface (*e.g.*, Scaffold X)-engineered EVs, can be produced from a cell (*e.g.*, HEK293 cells) transformed with an exogenous sequence encoding a scaffold moiety (*e.g.*, exosome proteins, *e.g.*, Scaffold X) or a variant or a fragment thereof. EVs including scaffold moiety expressed from the exogenous sequence can include modified membrane compositions.

[0334] Various modifications or fragments of the scaffold moiety can be used for the aspects of the present disclosure. For example, scaffold moiety modified to have enhanced affinity to a binding agent can be used for generating surface-engineered EV that can be purified using the binding agent. Scaffold moieties modified to be more effectively targeted to EVs and/or membranes can be used. Scaffold moieties modified to comprise a minimal fragment required for specific and effective targeting to exosome membranes can be also used.

[0335] Scaffold moieties can be engineered to be expressed as a fusion molecule, *e.g.*, fusion molecule of Scaffold X to an ASO. For example, the fusion molecule can comprise a scaffold moiety disclosed herein (*e.g.*, Scaffold X, *e.g.*, PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, SLC3A2, ATP transporter, or a fragment or a variant thereof) linked to an ASO.

[0336] In some aspects, the Scaffold X comprises Prostaglandin F2 receptor negative regulator (the PTGFRN polypeptide). The PTGFRN protein can be also referred to as CD9 partner 1 (CD9P-1), Glu-Trp-Ile EWI motif-containing protein F (EWI-F), Prostaglandin F2-alpha receptor regulatory protein, Prostaglandin F2-alpha receptor-associated protein, or CD315. The full length amino acid sequence of the human PTGFRN protein (Uniprot Accession No. Q9P2B2) is shown at Table 2 as SEQ ID NO: 301. The PTGFRN polypeptide contains a signal peptide (amino acids 1 to 25 of SEQ ID NO: 301), the extracellular domain (amino acids 26 to 832 of SEQ ID NO: 301), a transmembrane domain (amino acids 833 to 853 of SEQ ID NO: 301), and a cytoplasmic domain (amino acids 854 to 879 of SEQ ID NO: 301). The mature PTGFRN polypeptide consists of SEQ ID NO: 301 without the signal peptide, *i.e.*, amino acids 26 to 879 of SEQ ID NO: 301.

Table 3. Exemplary Scaffold X Protein Sequences

Protein	Sequence
The PTGFRN Protein (SEQ ID NO: 301)	MGRLASRPLLLALLSLALCRGVVRVPTATLVRVVGTELVIPCNVSDYDGPSEQNFDWSFS SLGSSFVELASTWEVGFPALYQERLQRGEILLRRTANDAVELHIKNVQPSDQGHYKCSTP STDATVQGNIEDTVQVKVLADSLHVGPSARPPPSLSLREGEPPFELRCTAASASPLHHTLAL LWEVHRGPARRSVLALTHEGRFHPGLGYEQRYHSGDVRLDTVGSDAYRLSVSRALSADQGS YRCIVSEWIAEQGNWQEIQEKAVEVATVVIQPSVLRAAVPKNVSAEGKELDLTCNITTDR ADDVRPEVTWSFSRMPDSTLPGSRVLARLDRDSLHSSPHVALSHVDARSYHLLVRDVSKE NSGYYYCHVSLWAPGHNRSWHKVAEAVSSPAGVGVTWLEPDYQVYLNASKVPGFADDPTEL ACRVVDTKSGEANVRFTVSWYYRMNRRSDNVVTSELLAVMDGDWTLKYGERSKQRAQDGF IFSKEHTDTFNRIQRTTEEDRGNYCVVSAWTKQRNNSWVSKDVFSKPVNIFWALEDSV LVVKARQPKPFFAAGNTFEMTCKVSSKNIKSPRYSVLIMAEKPVGDLSSPNETKYIISLDQ DSVVKLENWTDASRVGCVLEKVQEDEFYRMYQTQVSDAGLYRCMVTAWSPVRGSLWREA ATLSLNPIEIDFQTSGPIFNASVHSDTPSVIRGDLIKLFCIITVEGAALDPDDMAFDVSWF AVHSFGLDKAPVLLSSLDKGIIVTTSRRDWKSDLSLERSVLEFLLQVHGSEDDQDFGNYYC SVTPWVKSPGTSWQKEAEIHSKPVFITVKMDVLNAFKYPLLIGVGLSTVIGLLSCLIGYCS SHWCKKEVQETRERRRRLMSMEMD

The PTGFRN protein Fragment (SEQ ID NO: 302)	GPIFNASVHSDTPSVIRGDLIKLFCIITVEGAALDPDDMAFDVSWFAVHSFGLDKAPVLLS SLDRKGIVTTSRRDWKSDLSLERVSVLEFLLQVHGSEDQDFGNYYCSVTPWVKSPGTSWQK EAEIHSKPVFITVKMDVLNAFKYPLLIGVGLSTVIGLLSCLIGYCSSHWCCKKEVQETRRE RRRLMSMEM 687-878 of SEQ ID NO: 301
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III.C. Targeting Moiety

[0337] In some aspects, the EV, *e.g.*, exosome, comprises a targeting moiety, *e.g.*, an exogenous targeting moiety. In some aspects, the exogenous targeting moiety comprises a peptide, an antibody or an antigen-binding fragment thereof, a chemical compound, or any combination thereof. In some aspects, the targeting moiety comprises a microprotein, a designed ankyrin repeat protein (darpin), an anticalin, an adnectin, an aptamer, a peptide mimetic molecule, a natural ligand for a receptor, a camelid nanobody, or any combination thereof. In some aspects, the exogenous targeting moiety comprises a full-length antibody, a single domain antibody, a heavy chain only antibody (VHH), a single chain antibody, a shark heavy chain only antibody (VNAR), an scFv, a Fv, a Fab, a Fab', a F(ab')₂, or any combination thereof. In some aspects, the antibody is a single chain antibody.

[0338] In some aspects, a tropism moiety of the present disclosure targets a transferrin receptor (TfR). Transferrin receptors, *e.g.*, TfR1 or TfR2, are carrier proteins for transferrin. Transferrin receptors import iron by internalizing the transferrin-iron complex through receptor-mediated endocytosis.

[0339] TfR1 (see, *e.g.*, UniProt P02786 TFR1_Human) or transferrin receptor 1 (also known as cluster of differentiation 71 or CD71) is expressed on the endothelial cells of the blood-brain barrier (BBB). In some aspects, a tropism moiety of the present disclosure can comprise a ligand that can target TfR, *e.g.*, target TfR1, such as transferrin, or an antibody or other binding molecule capable of specifically binding to TfR. In some aspects, the antibody targeting a transferrin receptor is a low affinity anti-transferring receptor antibody (see, *e.g.*, US20190202936A1 which is herein incorporated by reference in its entirety).

[0340] In some aspects, the tropism moiety comprises all or a portion (*e.g.*, a binding portion) of a ligand for a transferrin receptor, for example a human transferrin available in GenBank as Accession numbers NM001063, XM002793, XM039847, NM002343 or NM013900, among others, or a variant, fragment, or derivative thereof.

[0341] In some aspects, the tropism moiety comprises a transferrin-receptor-targeting moiety, i.e., a targeting moiety directed to a transferrin receptor. Suitable transferrin-receptor-targeting moieties include a transferrin or transferrin variant, such as, but not limited to, a serum transferrin, lacto transferrin (lactoferrin) ovotransferrin, or melanotransferrin. Transferrins are a family of nonheme iron-binding proteins found in vertebrates, including serum transferrins, lacto transferrins (lactoferrins), ovotransferrins, and melanotransferrins. Serum transferrin is a glycoprotein with a molecular weight of about 80 kDa, comprising a single polypeptide chain with two N-linked polysaccharide chains that are branched and terminate in multiple antennae, each with terminal sialic acid residues. There are two main domains, the N domain of about 330 amino acids, and the C domain of about 340 amino acids, each of which is divided into two subdomains, N1 and N2, and C1 and C2. Receptor binding of transferrin occurs through the C domain, regardless of glycosylation.

[0342] In some aspects, the tropism moiety is a serum transferrin or transferrin variant such as, but not limited to a hexasialo transferrin, a pentasialo transferrin, a tetrasialo transferrin, a trisialo transferrin, a disialo transferrin, a monosialo transferrin, or an asialo transferrin, or a carbohydrate-deficient transferrin (CDT) such as an asialo, monosialo or disialo transferrin, or a carbohydrate-free transferrin (CFT) such as an asialo transferrin. In some aspects, the tropism moiety is a transferrin variant having the N-terminal domain of transferrin, the C-terminal domain of transferrin, the glycosylation of native transferrin, reduced glycosylation as compared to native (wild-type) transferrin, no glycosylation, at least two N terminal lobes of transferrin, at least two C terminal lobes of transferrin, at least one mutation in the N domain, at least one mutation in the C domain, a mutation wherein the mutant has a weaker binding avidity for transferrin receptor than native transferrin, and/or a mutation wherein the mutant has a stronger binding avidity for transferrin receptor than native transferrin, or any combination of the foregoing.

[0343] In some aspects, the tropism moiety targeting a transferrin receptor comprises an anti-transferrin receptor variable new antigen receptor (vNAR), e.g., a binding domain with a general motif structure (FW1-CDR1-FW2-3-CDR3-FW4). See, e.g., U.S. 2017-0348416, which is herein incorporated by reference in its entirety. vNARs are key component of the adaptive immune system of sharks. At only 11 kDa, these single-domain structures are the smallest IgG-like proteins in the animal kingdom and provide an excellent platform for molecular engineering and biologics drug discovery. vNAR attributes include high affinity for target, ease of expression, stability, solubility, multi-specificity, and increased potential for solid tissue penetration. See Ubah et al. *Biochem. Soc. Trans.* (2018) 46(6):1559-1565.

[0344] In some aspects, the tropism moiety comprises a vNAR domain capable of specifically binding to TfR1, wherein the vNAR domain comprises or consists essentially of a vNAR scaffold with any one CDR1 peptide in Table 1 of U.S. 2017-0348416 in combination with any one CDR3 peptide in Table 1 of U.S. 2017-0348416.

[0345] In some aspects, the targeting moiety is linked to the EV, *e.g.*, the exosome, by a scaffold protein. In some aspects, the scaffold protein is any scaffold protein disclosed herein. In some aspects, the scaffold protein is a Scaffold X. In some aspects, the scaffold protein is a Scaffold Y.

III.D. Linkers

[0346] As described *supra*, extracellular vesicles (EVs) of the present disclosure (*e.g.*, exosomes and nanovesicles) can comprises one or more linkers that link a molecule of interest (*e.g.*, an ASO) to the EVs (*e.g.*, to the exterior surface or on the luminal surface). In some aspects, an ASO is linked to the EVs directly or via a scaffold moiety (*e.g.*, Scaffold X or Scaffold Y). In certain aspects, the ASO is linked to the scaffold moiety by a linker. In certain aspects, the ASO is linked to the second scaffold moiety by a linker.

[0347] In certain aspects, an ASO is linked to the exterior surface of an exosome via Scaffold X. In further aspects, an ASO is linked to the luminal surface of an exosome via Scaffold X or Scaffold Y. The linker can be any chemical moiety known in the art.

[0348] As used herein, the term "linker" refers to a peptide or polypeptide sequence (*e.g.*, a synthetic peptide or polypeptide sequence) or to a non-polypeptide, *e.g.*, an alkyl chain. In some aspects, two or more linkers can be linked in tandem. When multiple linkers are present, each of the linkers can be the same or different. Generally, linkers provide flexibility or prevent/ameliorate steric hindrances. Linkers are not typically cleaved; however, in certain aspects, such cleavage can be desirable. Accordingly, in some aspects, a linker can comprise one or more protease-cleavable sites, which can be located within the sequence of the linker or flanking the linker at either end of the linker sequence.

[0349] In some aspects, the linker is a peptide linker. In some aspects, the peptide linker can comprise at least about two, at least about three, at least about four, at least about five, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, at least about 90, at least about 95, or at least about 100 amino acids.

[0350] In some aspects, the peptide linker is synthetic, *i.e.*, non-naturally occurring. In one aspect, a peptide linker includes peptides (or polypeptides) (*e.g.*, natural or non-naturally occurring peptides) which comprise an amino acid sequence that links or genetically fuses a first linear sequence of amino acids to a second linear sequence of amino acids to which it is not naturally linked or genetically fused in nature. For example, in one aspect the peptide linker can comprise non-naturally occurring polypeptides which are modified forms of naturally occurring polypeptides (*e.g.*, comprising a mutation such as an addition, substitution or deletion).

[0351] Linkers can be susceptible to cleavage ("cleavable linker") thereby facilitating release of the biologically active molecule (*e.g.*, an ASO).

[0352] In some aspects, the linker is a "reduction-sensitive linker." In some aspects, the reduction-sensitive linker contains a disulfide bond. In some aspects, the linker is an "acid labile linker." In some aspects, the acid labile linker contains hydrazone. Suitable acid labile linkers also include, for example, a cis-aconitic linker, a hydrazide linker, a thiocarbamoyl linker, or any combination thereof.

[0353] In some aspects, the linker comprises a non-cleavable linker.

[0354] In some aspects, the linker comprises acrylic phosphoramidite (*e.g.*, ACRYDITE™), adenylation, azide (NHS Ester), digoxigenin (NHS Ester), cholesterol-TEG, I-LINKER™, an amino modifier (*e.g.*, amino modifier C6, amino modifier C12, amino modifier C6 dT, or Uni-Link™ amino modifier), alkyne, 5' Hexynyl, 5-Octadiynyl dU, biotinylation (*e.g.*, biotin, biotin (Azide), biotin dT, biotin-TEG, dual biotin, PC biotin, or desthiobiotin), thiol modification (thiol modifier C3 S-S, dithiol or thiol modifier C6 S-S), or any combination thereof.

[0355] In some aspects, the linker comprises a terpene such as nerolidol, farnesol, limonene, linalool, geraniol, carvone, fenchone, or menthol; a lipid such as palmitic acid or myristic acid; cholesterol; oleyl; retinyl; cholesteryl residues; cholic acid; adamantane acetic acid; 1-pyrene butyric acid; dihydrotestosterone; 1,3-Bis-O(hexadecyl)glycerol; geranyloxyhexyl group; hexadecylglycerol; borneol; 1,3-propanediol; heptadecyl group; O3-(oleoyl)lithocholic acid; O3-(oleoyl)cholenic acid; dimethoxytrityl; phenoxazine, a maleimide moiety, a glucorinidase type, a CL2A-SN38 type, folic acid; a carbohydrate; vitamin A; vitamin E; vitamin K, or any combination thereof.

III.E. Modified EVs Comprising Tropism Moieties

[0356] In some aspects, an EV, *e.g.*, exosome, disclosed herein can be surface engineered to adjust its properties, *e.g.*, biodistribution, *e.g.*, via incorporation of immuno-affinity ligands or cognate receptor ligands. For example, EV, *e.g.*, exosomes, disclosed herein can be surface

engineered to direct them to a specific cellular type, e.g., Schwann cells, sensory neurons, motor neurons, meningeal macrophages, or a tumor cell, or can be surface engineered to enhance their migration to a specific compartment, e.g., to the CNS (in order to improve intrathecal compartment retention) or to a tumor microenvironment.

[0357] In some aspects, an EV, e.g., exosome, comprises (i) an ASO disclosed herein and (ii) a bio-distribution modifying agent or targeting moiety. In some aspects, the bio-distribution modifying agent or targeting moiety comprises a single-domain antigen-binding moiety, e.g., a VHH and/or a vNAR. As used here, the terms "bio-distribution modifying agent" and "targeting moiety" are used interchangeably and refer to an agent that can modify the distribution of extracellular vesicles (e.g., exosomes, nanovesicles) *in vivo* or *in vitro* (e.g., in a mixed culture of cells of different varieties). In some aspects, the targeting moiety alters the tropism of the EV (e.g., exosome), i.e., the target moiety is a "tropism moiety". As used herein, the term "tropism moiety" refers to a targeting moiety that when expressed on an EV (e.g., exosome) alters and/or enhances the natural movement of the EV. For example, in some aspects, a tropism moiety can promote the EV (e.g., exosome) to be taken up by a particular cell, tissue, or organ.

[0358] EVs, e.g., exosomes, exhibit preferential uptake in discrete cell types and tissues, and their tropism can be directed by adding proteins to their surface that interact with receptors on the surface of target cells. The tropism moiety can comprise a biological molecule, such as a protein, a peptide, a lipid, or a carbohydrate, or a synthetic molecule. For example, in some aspects the tropism moiety can comprise an affinity ligand, e.g., an antibody (such as an anti-CD19 nanobody, an anti-CD22 nanobody, an anti-CLEC9A nanobody, or an anti-CD3 nanobody), a VHH domain, a phage display peptide, a fibronectin domain, a camelid nanobody, and/or a vNAR. In some aspects, the tropism moiety can comprise, e.g., a synthetic polymer (e.g., PEG), a natural ligand/molecule (e.g., CD40L, albumin, CD47, CD24, CD55, CD59), and/or a recombinant protein (e.g., XTEN).

[0359] In some aspects, a tropism moiety can increase uptake of the EV, e.g., an exosome, by a cell.

[0360] In some aspects, when tropism to the central nervous system is desired, an EV, e.g., exosome, of the present disclosure can comprise a tissue or cell-specific target ligand, which increases EV, e.g., exosome, tropism to a specific central nervous system tissue or cell. In some aspects, the cell is a glial cell. In some aspects, the glial cell is an oligodendrocyte, an astrocyte, an ependymal cell, a microglia cell, a Schwann cell, a satellite glial cell, an olfactory ensheathing cell, or a combination thereof. In some aspects, the cell is a neural stem cell. In some aspects, the cell-

specific target ligand, which increases EV, e.g., exosome, tropism to a Schwann cells binds to a Schwann cell surface marker such as Myelin Basic Protein (MBP), Myelin Protein Zero (P0), P75NTR, NCAM, PMP22, or any combination thereof. In some aspects, the cell-specific tropism moiety comprises an antibody or an antigen-binding portion thereof, an aptamer, or an agonist or antagonist of a receptor expressed on the surface of the Schwann cell.

[0361] In principle, the EVs, e.g., exosomes of the present disclosure comprising at least one tropism moiety that can direct the EV, e.g., exosome, to a specific target cell or tissue (e.g., a cell in the CNS or a Schwann cell in peripheral nerves) can be administered using any suitable administration method known in the art (e.g., intravenous injection or infusion) since the presence of the tropism moiety (alone or in combination with the presence of an antiphagocytic signal such as CD47 and the use of a specific administration route) will induce a tropism of the EVs, e.g., exosomes, towards the desired target cell or tissue.

[0362] Pharmacokinetics, biodistribution, and in particular tropism and retention in the desired tissue or anatomical location can also be accomplished by selecting the appropriate administration route (e.g., intrathecal administration or intraocular administration to improve tropism to the central nervous system).

[0363] In some aspects, the EV, e.g., exosome, comprises at least two different tropism moieties. In some aspects, the EV, e.g., exosome, comprises three different tropism moieties. In some aspects, the EV, e.g., exosome, comprises four different tropism moieties. In some aspects, the EV, e.g., exosome, comprises five or more different tropism moieties. In some aspects, one or more of the tropism moieties increases uptake of the EV, e.g., exosome, by a cell. In some aspects, each tropism moiety is attached to a scaffold moiety, e.g., a Scaffold X protein or a fragment thereof. In some aspects, multiple tropism moieties can be attached to the same scaffold moiety, e.g., a Scaffold X protein or a fragment thereof. In some aspects, several tropism moieties can be attached in tandem to a scaffold moiety, e.g., a Scaffold X protein or a fragment thereof. In some aspects, a tropism moiety disclosed herein or a combination thereof is attached to a scaffold moiety, e.g., a Scaffold X protein or a fragment thereof, via a linker or spacer. In some aspects, a linker or spacer or a combination thereof is interposed between two tropism moieties disclosed herein.

[0364] Non-limiting examples of tropism moieties capable of directing EVs, e.g., exosomes, of the present disclosure to different nervous system cell types are disclosed below.

III.E.1. Tropism moieties targeting Schwann cells

[0365] In some aspects, a tropism moiety can target a Schwann cell. In some aspects, the tropism moiety that directs an EV, e.g., exosome, disclosed herein to a Schwann cell targets, e.g.,

a transferrin receptor (TfR), apolipoprotein D (ApoD), Galectin 1 (LGALS1), Myelin proteolipid protein (PLP), Glypican 1, or Syndecan 3. In some aspects, the tropism moiety directing an EV, e.g., exosome, of the present disclosure to a Schwann cell is a transferrin, or a fragment, variant or derivative thereof.

[0366] In some aspects, a tropism moiety of the present disclosure targets a transferrin receptor (TfR). Transferrin receptors, e.g., TfR1 or TfR2, are carrier proteins for transferrin. Transferrin receptors import iron by internalizing the transferrin-iron complex through receptor-mediated endocytosis.

[0367] TfR1 (see, e.g., UniProt P02786 TFR1_Human) or transferrin receptor 1 (also known as cluster of differentiation 71 or CD71) is expressed on the endothelial cells of the blood-brain barrier (BBB). TfR1 is known to be expressed in a variety of cells such as red blood cells, monocytes, hepatocytes, intestinal cells, and erythroid cells, and is upregulated in rapidly dividing cells such as tumor cells (non small cell lung cancer, colon cancer, and leukemia) as well as in tissue affected by disorders such as acute respiratory distress syndrome (ARDS). TfR2 is primarily expressed in liver and erythroid cells, is found to a lesser extent in lung, spleen and muscle, and has a 45% identity and 66% similarity with TfR1. TfR1 is a transmembrane receptor that forms a homodimer of 760 residues with disulfide bonds and a molecular weight of 90 kDa. Affinity for transferrin varies between the two receptor types, with the affinity for TfR1 being at least 25-30 fold higher than that of TfR2.

[0368] Binding to TfR1 allows the transit of large molecules, e.g., antibodies, into the brain. Some TfR1-targeting antibodies have been shown to cross the blood-brain barrier, without interfering with the uptake of iron. Amongst those are the mouse anti rat-TfR antibody OX26 and the rat anti mouse-TfR antibody 8D3. The affinity of the antibody-TfR interaction is important to determine the success of transcytotic transport over endothelial cells of the BBB. Monovalent TfR interaction favors BBB transport due to altered intracellular sorting pathways. Avidity effects of bivalent interactions redirecting transport to the lysosome. Also, reducing TfR binding affinity directly promote dissociation from the TfR which increase brain parenchymal exposure of the TfR binding antibody. See, e.g., U.S. Patent No. 8,821,943, which is herein incorporated by reference in its entirety. Accordingly, in some aspects, a tropism moiety of the present disclosure can comprise a ligand that can target TfR, e.g., target TfR1, such as transferrin, or an antibody or other binding molecule capable of specifically binding to TfR. In some aspects, the antibody targeting a transferrin receptor is a low affinity anti-transferring receptor antibody (see, e.g., US20190202936A1 which is herein incorporated by reference in its entirety).

[0369] In some aspects, the tropism moiety comprises all or a portion (e.g., a binding portion) of a ligand for a transferrin receptor, for example a human transferrin available in GenBank as Accession numbers NM001063, XM002793, XM039847, NM002343 or NM013900, among others, or a variant, fragment, or derivative thereof.

[0370] In some aspects, the tropism moiety comprises a transferrin-receptor-targeting moiety, i.e., a targeting moiety directed to a transferrin receptor. Suitable transferrin-receptor-targeting moieties include a transferrin or transferrin variant, such as, but not limited to, a serum transferrin, lacto transferrin (lactoferrin) ovotransferrin, or melanotransferrin. Transferrins are a family of nonheme iron-binding proteins found in vertebrates, including serum transferrins, lacto transferrins (lactoferrins), ovotransferrins, and melanotransferrins. Serum transferrin is a glycoprotein with a molecular weight of about 80 kDa, comprising a single polypeptide chain with two N-linked polysaccharide chains that are branched and terminate in multiple antennae, each with terminal sialic acid residues. There are two main domains, the N domain of about 330 amino acids, and the C domain of about 340 amino acids, each of which is divided into two subdomains, N1 and N2, and C1 and C2. Receptor binding of transferrin occurs through the C domain, regardless of glycosylation.

[0371] In some aspects, the tropism moiety is a serum transferrin or transferrin variant such as, but not limited to a hexasialo transferrin, a pentasialo transferrin, a tetrasialo transferrin, a trisialo transferrin, a disialo transferrin, a monosialo transferrin, or an asialo transferrin, or a carbohydrate-deficient transferrin (CDT) such as an asialo, monosialo or disialo transferrin, or a carbohydrate-free transferrin (CFT) such as an asialo transferrin. In some aspects, the tropism moiety is a transferrin variant having the N-terminal domain of transferrin, the C-terminal domain of transferrin, the glycosylation of native transferrin, reduced glycosylation as compared to native (wild-type) transferrin, no glycosylation, at least two N terminal lobes of transferrin, at least two C terminal lobes of transferrin, at least one mutation in the N domain, at least one mutation in the C domain, a mutation wherein the mutant has a weaker binding avidity for transferrin receptor than native transferrin, and/or a mutation wherein the mutant has a stronger binding avidity for transferrin receptor than native transferrin, or any combination of the foregoing.

[0372] In some aspects, the tropism moiety targeting a transferrin receptor comprises an anti-transferrin receptor variable new antigen receptor (vNAR), e.g., a binding domain with a general motif structure (FW1-CDR1-FW2-3-CDR3-FW4). See, e.g., U.S. 2017-0348416, which is herein incorporated by reference in its entirety. vNARs are key component of the adaptive immune system of sharks. At only 11 kDa, these single-domain structures are the smallest IgG-like proteins

in the animal kingdom and provide an excellent platform for molecular engineering and biologics drug discovery. vNAR attributes include high affinity for target, ease of expression, stability, solubility, multi-specificity, and increased potential for solid tissue penetration. See Ubah et al. *Biochem. Soc. Trans.* (2018) 46(6):1559-1565.

[0373] In some aspects, the tropism moiety comprises a vNAR domain capable of specifically binding to TfR1, wherein the vNAR domain comprises or consists essentially of a vNAR scaffold with any one CDR1 peptide in Table 1 of U.S. 2017-0348416 in combination with any one CDR3 peptide in Table 1 of U.S. 2017-0348416.

[0374] In some aspects, a tropism moiety of the present disclosure targets ApoD. Unlike other lipoproteins, which are mainly produced in the liver, apolipoprotein D is mainly produced in the brain, cerebellum, and peripheral nerves. ApoD is 169 amino acids long, including a secretion peptide signal of 20 amino acids. It contains two glycosylation sites (asparagines 45 and 78) and the molecular weight of the mature protein varies from 20 to 32 kDa. ApoD binds steroid hormones such as progesterone and pregnenolone with a relatively strong affinity, and to estrogen with a weaker affinity. Arachidonic acid (AA) is an ApoD ligand with a much better affinity than that of progesterone or pregnenolone. Other ApoD ligands include E-3-methyl-2-hexenoic acid, retinoic acid, sphingomyelin and sphingolipids. Accordingly, in some aspects, a tropism moiety of the present disclosure comprises a ligand that can target ApoD, e.g., an antibody or other binding molecule capable of specifically binding to ApoD.

[0375] In some aspects, a tropism moiety of the present disclosure targets Galectin 1. The galectin-1 protein is 135 amino acids in length. Accordingly, in some aspects, a tropism moiety of the present disclosure comprises a ligand that can target Galectin 1, e.g., an antibody or other binding molecule capable of specifically binding to Galectin 1.

[0376] In some aspects, a tropism moiety of the present disclosure targets PLP. PLP is the major myelin protein from the CNS. It plays an important role in the formation or maintenance of the multilamellar structure of myelin. The myelin sheath is a multi-layered membrane, unique to the nervous system that functions as an insulator to greatly increase the efficiency of axonal impulse conduction. PLP is a highly conserved hydrophobic protein of 276 to 280 amino acids which contains four transmembrane segments, two disulfide bonds and which covalently binds lipids (at least six palmitate groups in mammals). Accordingly, in some aspects, a tropism moiety of the present disclosure comprises a ligand that can target PLP, e.g., an antibody or other binding molecule capable of specifically binding to PLP.

[0377] In some aspects, a tropism moiety of the present disclosure targets Glypican 1. Accordingly, in some aspects, a tropism moiety of the present disclosure comprises a ligand that can target Glypican 1, e.g., an antibody or other binding molecule capable of specifically binding to Glypican 1. In some aspects, a tropism moiety of the present disclosure targets Syndecan 3. Accordingly, in some aspects, a tropism moiety of the present disclosure comprises a ligand that can target Syndecan 3, e.g., an antibody or other binding molecule capable of specifically binding to Syndecan 3.

III.E.2. Tropism moieties targeting sensory neurons

[0378] In some aspects, a tropism moiety disclosed herein can direct an EV, e.g., exosome, disclosed herein to a sensory neuron. In some aspects, the tropism moiety that directs an EV, e.g., exosome, disclosed herein to a sensory neuron targets a Trk receptor, e.g., TrkA, TrkB, TrkC, or a combination thereof.

[0379] Trk (tropomyosin receptor kinase) receptors are a family of tyrosine kinases that regulates synaptic strength and plasticity in the mammalian nervous system. The common ligands of Trk receptors are neurotrophins, a family of growth factors critical to the functioning of the nervous system. The binding of these molecules is highly specific. Each type of neurotrophin has different binding affinity toward its corresponding Trk receptor. Accordingly, in some aspects, the tropism moiety directing an EV, e.g., exosome, disclosed herein to a sensory neuron, comprises a neurotrophin.

[0380] Neurotrophins bind to Trk receptors as homodimers. Accordingly, in some aspects, the tropism moiety comprises at least two neurotrophins disclosed herein, e.g., in tandem. In some aspects, the tropism moiety comprises at least two neurotrophins disclosed herein, e.g., in tandem, that are attached to a scaffold protein, for example, Protein X, via a linker. In some aspects, the linker connecting the scaffold protein, e.g., Protein X, to the neurotrophin (e.g., a neurotrophin homodimer) has a length of at least 10 amino acids. In some aspects, the linker connecting the scaffold protein, e.g., Protein X, to the neurotrophin (e.g., a neurotrophin homodimer) has a length of at least about 25 amino acids, about 30 amino acids, about 35 amino acids, about 40 amino acids, about 45 amino acids, or about 50 amino acids.

[0381] In some aspects, the neurotrophin is a neurotrophin precursor, i.e., a proneurotrophin, which is later cleaved to produce a mature protein.

[0382] Nerve growth factor (NGF) is the first identified and probably the best characterized member of the neurotrophin family. It has prominent effects on developing sensory and sympathetic neurons of the peripheral nervous system. Brain-derived neurotrophic factor (BDNF)

has neurotrophic activities similar to NGF, and is expressed mainly in the CNS and has been detected in the heart, lung, skeletal muscle and sciatic nerve in the periphery (Leibrock, J. et al., Nature, 341:149-152 (1989)). Neurotrophin-3 (NT-3) is the third member of the NGF family and is expressed predominantly in a subset of pyramidal and granular neurons of the hippocampus, and has been detected in the cerebellum, cerebral cortex and peripheral tissues such as liver and skeletal muscles (Ernfors, P. et al., Neuron 1: 983-996 (1990)). Neurotrophin-4 (also called NT-4) is the most variable member of the neurotrophin family. Neurotrophin-6 (NT-6) was found in teleost fish and binds to p75 receptor.

[0383] In some aspects, the neurotrophin targeting TrkB comprises, e.g., NT-4 or BDNF, or a fragment, variant, or derivative thereof. In some aspects, the neurotrophin targeting TrkA comprises, e.g., NGF or a fragment, variant, or derivative thereof. In some aspects, the neurotrophin targeting TrkC comprises, e.g., NT-3 or a fragment, variant, or derivative thereof.

[0384] In some aspects, the tropism moiety comprises brain derived neurotrophic factor (BDNF). In some aspects, the BDNF is a variant of native BDNF, such as a two amino acid carboxyl-truncated variant. In some aspects, the tropism moiety comprises the full-length 119 amino acid sequence of BDNF (HSDPARRGELSVCDSEWVTAADKKTAVDMSGGTVTVLEKVPVSKGQLKQYFYETK CNPMGYTKEGCRGIDKRHWNSQCRTTQSYVRALTMDSKKRIGWRFIRIDTSCVCTLTIK RGR; SEQ ID NO: 161). In some aspects, a one amino-acid carboxy-truncated variant of BDNF is utilized (amino acids 1-118 of SEQ ID NO: 161).

[0385] In some aspects, the tropism moiety comprises a carboxy-truncated variant of the native BDNF, e.g., a variant in which 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 amino acids are absent from the carboxy-terminus of the BDNF. BDNF variants include the complete 119 amino acid BDNF, the 117 or 118 amino acid variant with a truncated carboxyl terminus, variants with a truncated amino terminus, or variants with up to about 20%, about 30, or about 40% change in amino acid composition, as long as the protein variant still binds to the TrkB receptor with high affinity.

[0386] In some aspects, the tropism moiety comprises a two amino-acid carboxy-truncated variant of BDNF (amino acids 1-117 of SEQ ID NO: 161). In some aspects, the tropism moiety comprises a three amino-acid carboxy-truncated variant of BDNF (amino acids 1-116 of SEQ ID NO: 161). In some aspects, the tropism moiety comprises a four amino-acid carboxy-truncated variant of BDNF (amino acids 1-115 of SEQ ID NO: 161). In some aspects, the tropism moiety comprises a five amino-acid carboxy-truncated variant of BDNF (amino acids 1-114 of SEQ ID

NO: 161). In some aspects, the tropism moiety comprises a BDNF that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or about 100% identical with the sequence of SEQ ID NO: 161, or a truncated version thereof, e.g., the 117 or 118 amino acid variant with a one- or two-amino acid truncated carboxyl terminus, or variants with a truncated amino terminus. See, e.g., U.S. Pat. No. 8,053,569B2, which is herein incorporated by reference in its entirety.

[0387] In some aspects, the tropism moiety comprises nerve growth factor (NGF). In some aspects, the NGF is a variant of native NGF, such as a truncated variant. In some aspects, the tropism moiety comprises the 26-kDa beta subunit of protein, the only component of the 7S NGF complex that is biologically active. In some aspects, the tropism moiety comprises the full-length 120 amino acid sequence of beta NGF (SSSHPIFHRGEFSVCDSSVWVGDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCRDPNPVDSGCRGIDSKHWNSYCTTTHTFVKALTMGKQAAWRFIRIDTACVCVLSRKAVRRA; SEQ ID NO: 162). In some aspects, the tropism moiety comprises a carboxy-truncated variant of the native NGF, e.g., a variant in which 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 amino acids are absent from the carboxy-terminus of NGF. NGF variants include the complete 120 amino acid NGF, the shorter amino acid variants with a truncated carboxyl terminus, variants with a truncated amino terminus, or variants with up to about 20%, about 30%, or about 40% change in amino acid composition, as long as the tropism moiety still binds to the TrkB receptor with high affinity. In some aspects, the tropism moiety comprises an NGF that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or about 100% identical with the sequence of SEQ ID NO: 162, or a truncated version thereof.

[0388] In some aspects, the tropism moiety comprises neurotrophin-3 (NT-3). In some aspects, the NT-3 is a variant of native NT-3, such as a truncated variant. In some aspects, the tropism moiety comprises the full-length 119 amino acid sequence of NT-3 (YAEHKSHRGEYSVCDSESLWVTDKSSAIDIRGHQVTVLGEIKTGNSPVKQYFYETRCKE ARPVKNGCRGIDDKHWNSQCKTSQTYVRALTSENNKLVGWRWIRIDTSCVCALSRKIGRT; SEQ ID NO: 163). In some aspects, the tropism moiety comprises a carboxy-truncated variant of the native NT-3, e.g., a variant in which 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 amino acids are absent from the carboxy-terminus of NT-3. NT-3 variants include the complete 119 amino acid NT-3, the shorter amino acid variants with a truncated carboxyl terminus, variants with a truncated amino terminus, or variants with up to about 20%, about 30%, or about 40% change in amino acid

composition, as long as the tropism moiety still binds to the TrkC receptor with high affinity. In some aspects, the tropism moiety comprises an NT-3 that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or about 100% identical with the sequence of SEQ ID NO: 163, or a truncated version thereof.

[0389] In some aspects, the tropism moiety comprises neurotrophin-4 (NT-4). In some aspects, the NT-4 is a variant of native NT-4, such as a truncated variant. In some aspects, the tropism moiety comprises the full-length 130 amino acid sequence of NT-4 (GVSETAPASRRGELAVCDAVSGWVTDRRTAVDLRGREVEVLGEVPAAGGSPLRQYFFE TRCKADNAEEGGPGAGGGGCRGVDRRHVSECKAKQSYVRALTADAQGRVGWRWIR IDTACVCTLLSRTGRA; SEQ ID NO: 164). In some aspects, the tropism moiety comprises a carboxy-truncated variant of the native NT-4, e.g., a variant in which 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 amino acids are absent from the carboxy-terminus of NT-4. NT-4 variants include the complete 130 amino acid NT-4, the shorter amino acid variants with a truncated carboxyl terminus, variants with a truncated amino terminus, or variants with up to about 20%, about 30%, or about 40% change in amino acid composition, as long as the tropism moiety still binds to the TrkB receptor with high affinity. In some aspects, the tropism moiety comprises an NT-4 that is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or about 100% identical with the sequence of SEQ ID NO: 164, or a truncated version thereof.

[0390] Structure/function relationship studies of NGF and NGF-related recombinant molecules demonstrated that mutations in NGF region 25-36, along with other β -hairpin loop and non-loop regions, significantly influenced NGF/NGF-receptor interactions (Ibanez et al., EMBO J., 10, 2105-2110, (1991)). Small peptides derived from this region have been demonstrated to mimic NGF in binding to Mock receptor and affecting biological responses (LeSauter et al. J. Biol. Chem. 270, 6564-6569, 1995). Dimers of cyclized peptides corresponding to β -loop regions of NGF were found to act as partial NGF agonists in that they had both survival-promoting and NGF-inhibiting activity while monomer and linear peptides were inactive (Longo et al., J. Neurosci. Res., 48, 1-17, 1997). Accordingly, in some aspects, a tropism moiety of the present disclosure comprises such peptides.

[0391] Cyclic peptides have also been designed and synthesized to mimic the β -loop regions of NGF, BDNF, NT3 and NT-4/5. Certain monomers, dimers or polymers of these cyclic peptides can have a three-dimensional structure, which binds to neurotrophin receptors under

physiological conditions. All of these structural analogs of neurotrophins that bind to nerve cell surface receptors and are internalized can serve as the binding agent B of the compound according to the present disclosure to deliver the conjugated therapeutic moiety TM to the nervous system. Accordingly, in some aspects, a tropism moiety of the present disclosure comprises such cyclic peptides or combinations thereof.

[0392] In some aspects, antibodies against nerve cell surface receptors that are capable of binding to the receptors and being internalized can also serve as tropism moieties binding to a Trk receptor. For example, monoclonal antibody (MAb) 5C3 is specific for the NGF docking site of the human p140 TrkA receptor, with no cross-reactivity with human TrkB receptor. MAb 5C3 and its Fab mimic the effects of NGF in vitro, and image human Trk-A positive tumors in vivo (Kramer et al., Eur. J. Cancer, 33, 2090-2091, (1997)). Molecular cloning, recombination, mutagenesis and modeling studies of MAb 5C3 variable region indicated that three or less of its complementarity determining regions (CDRs) are relevant for binding to TrkA. Assays with recombinant CDRs and CDR-like synthetic polypeptides demonstrated that they had agonistic bioactivities similar to intact MAb 5C3. Monoclonal antibody MC192 against p75 receptor has also been demonstrated to have neurotrophic effects. Therefore, these antibodies and their functionally equivalent fragments can also serve as tropism moieties of the present disclosure.

[0393] In some aspects, peptidomimetics that are synthesized by incorporating unnatural amino acids or other organic molecules can also serve tropism moieties of the present disclosure.

[0394] Other neurotrophins are known in the art. Accordingly, in some aspects, the target moiety comprises a neurotrophin selected from the group consisting of fibroblast growth factor (FGF)-2 and other FGFs, erythropoietin (EPO), hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor (TGF)- α , TGF- β , vascular endothelial growth factor (VEGF), interleukin-1 receptor antagonist (IL-1ra), ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), neurturin, platelet-derived growth factor (PDGF), heregulin, neuregulin, artemin, persephin, interleukins, granulocyte-colony stimulating factor (CSF), granulocyte-macrophage-CSF, netrins, cardiotrophin-1, hedgehogs, leukemia inhibitory factor (LIF), midkine, pleiotrophin, bone morphogenetic proteins (BMPs), netrins, saposins, semaphorins, and stem cell factor (SCF).

[0395] In some aspects, the tropism moiety directing an EV, e.g. exosome, disclosed herein to a sensory neuron, comprises a varicella zoster virus (VZV) peptide.

III.E.3. Tropism moieties targeting motor neurons

[0396] In some aspects, a tropism moiety disclosed herein can direct an EV, e.g., exosome, disclosed herein to a motor neuron. In some aspects, the tropism moiety that directs an EV, e.g., exosome, disclosed herein to a motor comprises a Rabies Virus Glycoprotein (RVG) peptide, a Targeted Axonal Import (TAXI) peptide, a P75R peptide, or a Tet-C peptide.

[0397] In some aspects, the tropism moiety comprises a Rabies Virus Glycoprotein (RVG) peptide. See, e.g., U.S. Pat. App. Publ. 2014-00294727, which is herein incorporated by reference in its entirety. In some aspects, the RVG peptide comprises amino acid residues 173-202 of the RVG (YTIWMPENPRPGTPCDIFTNSRGKCRASNG; SEQ ID NO: 601) or a variant, fragment, or derivative thereof. In some aspects, the tropism moiety is a fragment of SEQ ID NO: 601. Such a fragment of SEQ ID NO: 601 can have, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids deleted from the N-terminal and/or the C-terminal of SEQ ID NO: 601. A functional fragment derived from SEQ ID NO: 601 can be identified by sequentially deleting N- and/or C-terminal amino acids from SEQ ID NO: 601 and assessing the function of the resulting peptide fragment, such as function of the peptide fragment to bind acetylcholine receptor and/or ability to transmit through the blood brain barrier. In some aspects, the tropism moiety comprises a fragment of SEQ ID NO: 601 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16 or 15 amino acids in length. In some aspects, the tropism moiety comprises a fragment of SEQ ID NO: 601 less than 15 peptides in length.

[0398] A “variant” of a RVG peptide, for example SEQ ID NO: 601, is meant to refer to a molecule substantially similar in structure and function, i.e., where the function is the ability to pass or transit through the BBB, to either the entire molecule, or to a fragment thereof. A variant of an RVG peptide can contain a mutation or modification that differs from a reference amino acid in SEQ ID NO: 601. In some aspects, a variant of SEQ ID NO: 601 is a fragment of SEQ ID NO: 601 as disclosed herein. In some aspects, an RVG variant can be a different isoform of SEQ ID NO: 601 or can comprise different isomer amino acids. Variants can be naturally-occurring, synthetic, recombinant, or chemically modified polynucleotides or polypeptides isolated or generated using methods well known in the art. RVG variants can include conservative or non-conservative amino acid changes. See, e.g., U.S. Pat. No. 9,757,470, which is herein incorporated by reference in its entirety.

[0399] In some aspects, the tropism moiety comprises a Targeted Axonal Import (TAXI) peptide. In some aspects, the TAXI peptide is cyclized TAXI peptide of sequence SACQSQSQMRCGGG (SEQ ID NO: 602). See, e.g., Sellers et al. (2016) Proc. Natl. Acad. Sci.

USA 113:2514-2519, and U.S. Pat. No. 9,056,892, which are herein incorporated by reference in their entireties. TAxI transport peptides as described herein may be of any length. Typically, the transport peptide will be between 6 and 50 amino acids in length, more typically between 10 and 20 amino acids in length. In some aspects, the TAxI transport peptide comprises the amino acid sequence QSQSQMR (SEQ ID NO: 603), ASGAQAR (SEQ ID NO: 604), PF, or TSTAPHLRLRLTSR (SEQ ID NO: 605). Optionally, the TAxI transport peptide further includes a flanking sequence to facilitate incorporation into a delivery construct or carrier, e.g., a linker. In one aspect, the peptide is flanked with cysteines. In some aspects, the TAxI transport peptide further comprises additional sequence selected to facilitate delivery into nuclei. For example, a peptide that facilitates nuclear delivery is a nuclear localizing signal (NLS). Typically, this signal consists of a few short sequences of positively charged lysines or arginines, such as PPKRKKV (SEQ ID NO: 606). In one aspect, the NLS has the amino acid sequence PPKRKKV (SEQ ID NO: 607).

[0400] In some aspects, a tropism moiety of the present disclosure comprises a peptide BBB shuttle having a sequence selected from SEQ ID NOs: 608-627 and any combination thereof. See, e.g., Oller-Salvia et al. (2016) Chem. Soc. Rev. 45, 4690-4707, and Jafari et al. (2019) Expert Opinion on Drug Delivery 16:583-605 which are herein incorporated by reference in their entireties.

SEQ ID NO	Peptide	Sequence
608	Angiopep-2	TFFYGGSRGKRNNFKTEEY-OH
609	ApoB (3371-3409)	SSVIDALQYKLEGTTTLTRK-RGLKLATALSLSNKFVEGS
610	ApoE (159-167) ₂	(LRKLKRLL) ₂
611	Peptide-22	Ac-C(&)MPRLRGC(&)-NH ₂
612	THR	THRPPMWSPVWP-NH ₂
613	THR retro-enantio	pwvpswmpprht-NH ₂
614	CRT	C(&)RTIGPSVC(&)
615	Leptin30	YQQILTSMPSRNVIQISND-LENLRDLLHVL
616	RVG29	YTIWMPENPRPGTPCDIFT-NSRGKRASNG-OH
617	^D CDX	GreirtGraerwsekf-OH
618	Apamin	C(& ₁)NC(& ₂)KAPETALC(& ₁)-AR-RC(& ₂)QQH-NH ₂
619	MiniAp-4	[Dap](&)KAPETALD(&)
620	GSH	γ-L-glutamyl-CG-OH
621	G23	HLNILSTLWKYRC
622	g7	GFtGFLS(O-β-Glc)-NH ₂
623	TGN	TGNYKALHPHNG
624	TAT (47-57)	YGRKKRRQRRR-NH ₂
625	SynB1	RGGRLSYRRRFSTSTGR
626	Diketopiperazines	&(N-MePhe)-(N-MePhe)Diketo-piperazines
627	PhPro	(Phenylproline) ₄ -NH ₂

Nomenclature for cyclic peptides (&) is adapted to the 3-letter amino acid code from the one described by Spengler *et al. Pept. Res.*, 2005, 65, 550-555

[Dap] stands for diaminopropionic acid.

III. F. Anti-phagocytic Signal

[0401] Clearance of administered EVs, *e.g.*, exosomes, by the body's immune system can reduce the efficacy of an administered EV, *e.g.*, exosome, therapy. In some aspects, the surface of the EV, *e.g.*, exosome, is modified to limit or block uptake of the EV, *e.g.*, exosome, by cells of the immune system, *e.g.*, macrophages. In some aspects, the surface of the EV, *e.g.*, exosome, is modified to express one or more surface antigen that inhibits uptake of the EV, *e.g.*, exosome, by a macrophage. In some aspects, the surface antigen is associated with the exterior surface of the EV, (*e.g.*, exosome).

[0402] Surface antigens useful in the present disclosure include, but are not limited to, antigens that label a cell as a "self" cell. In some aspects, the surface antigen comprises an anti-phagocytic signal. In some aspects, the anti-phagocytic signal is selected from CD47, CD24, a fragment thereof, and any combination thereof. In certain aspects, the anti-phagocytic signal comprises CD24, *e.g.*, human CD24. In some aspects, the anti-phagocytic signal comprises a fragment of CD24, *e.g.*, human CD24. In certain aspects, the EV, *e.g.*, exosome, is modified to express CD47 or a fragment thereof on the exterior surface of the EV, *e.g.*, exosome.

[0403] CD47, also referred to as leukocyte surface antigen CD47 and integrin associated protein (IAP), as used herein, is a transmembrane protein that is found on many cells in the body. CD47 is often referred to as the "don't eat me" signal, as it signals to immune cells, in particular myeloid cells, that a particular cell expressing CD47 is not a foreign cell. CD47 is the receptor for SIRPA, binding to which prevents maturation of immature dendritic cells and inhibits cytokine production by mature dendritic cells. Interaction of CD47 with SIRPG mediates cell-cell adhesion, enhances superantigen-dependent T-cell-mediated proliferation and costimulates T-cell activation. CD47 is also known to have a role in both cell adhesion by acting as an adhesion receptor for THBS1 on platelets, and in the modulation of integrins. CD47 also plays an important role in memory formation and synaptic plasticity in the hippocampus (by similarity). In addition, CD47 can play a role in membrane transport and/or integrin dependent signal transduction, prevent premature elimination of red blood cells, and be involved in membrane permeability changes induced following virus infection.

[0404] In some aspects, an EV, e.g., exosome, disclosed herein is modified to express a human CD47 on the surface of the EV, e.g., exosome. The canonical amino acid sequence for human CD47 and various known isoforms (UniProtKB - Q08722) are provided herein as SEQ ID NOs: 629-632. In some aspects, the EV, e.g., exosome, is modified to express a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 629 or a fragment thereof. In some aspects, the EV, e.g., exosome, is modified to express a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 630 or a fragment thereof. In some aspects, the EV, e.g., exosome, is modified to express a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 631 or a fragment thereof. In some aspects, the EV, e.g., exosome, is modified to express a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 632 or a fragment thereof.

[0405] In some aspects, the EV, e.g., exosome, is modified to express full length CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, is modified to express a fragment of CD47 on the surface of the EV, e.g., exosome, wherein the fragment comprises the extracellular domain of CD47, e.g., human CD47. Any fragment of CD47 that retains an ability to block and/or inhibit phagocytosis by a macrophage can be used in the EVs, e.g., exosomes, disclosed herein. In some aspects, the fragment comprises amino acids 19 to about 141 of the canonical human CD47 sequence (e.g., amino acids 19-141 of SEQ ID NO 629). In some aspects, the fragment comprises amino acids 19 to about 135 of the canonical human CD47 sequence (e.g., amino acids 19-135 of SEQ ID NO 629). In some aspects, the fragment comprises amino acids 19 to about 130 of the canonical human CD47 sequence (e.g., amino acids 19-130 of SEQ ID NO 629). In some aspects, the fragment comprises amino acids 19 to about 125 of the canonical human CD47 sequence (e.g., amino acids 19-125 of SEQ ID NO 629).

[0406] In some aspects, the EV, e.g., exosome, is modified to express a polypeptide having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to amino acids 19 to about 141 of the canonical human CD47 sequence (e.g., amino acids 19-141 of SEQ ID NO 629). In some aspects, the EV, e.g., exosome, is modified to express a polypeptide having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to amino acids 19 to about 135 of the canonical human CD47 sequence (e.g., amino acids 19-135 of SEQ ID NO 629). In some aspects, the EV, e.g., exosome, is modified to express a polypeptide having at least about 70%, at least about 75%,

at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to amino acids 19 to about 130 of the canonical human CD47 sequence (e.g., amino acids 19-130 of SEQ ID NO 629). In some aspects, the EV, e.g., exosome, is modified to express a polypeptide having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to amino acids 19 to about 125 of the canonical human CD47 sequence (e.g., amino acids 19-125 of SEQ ID NO 629).

[0407] In some aspects, the CD47 or the fragment thereof is modified to increase the affinity of CD47 and its ligand SIRP α . In some aspects, the fragment of CD47 comprises a Velcro-CD47 (see, e.g., Ho et al., JBC 290:12650-63 (2015), which is incorporated by reference herein in its entirety). In some aspects, the Velcro-CD47 comprises a C15S substitution relative to the wild-type human CD47 sequence (SEQ ID NO: 629).

[0408] In some aspects, the EV, e.g., exosome, comprises a CD47 or a fragment thereof expressed on the surface of the EV, e.g., exosome, at a level that is higher than an unmodified EV, e.g., exosome. In some aspects, the CD47 or the fragment thereof is fused with a scaffold protein. Any scaffold protein disclosed herein can be used to express the CD47 or the fragment thereof on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, is modified to express a fragment of CD47 fused to the N-terminus of a Scaffold X protein. In some aspects, the EV, e.g., exosome, is modified to express a fragment of CD47 fused to the N-terminus of PTGFRN.

[0409] In some aspects, the EV, e.g., exosome, comprises at least about 20 molecules, at least about 30 molecules, at least about 40, at least about 50, at least about 75, at least about 100, at least about 125, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at least about 450, at least about 500, at least about 750, or at least about 1000 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, comprises at least about 20 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, comprises at least about 30 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, comprises at least about 40 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, comprises at least about 50 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, comprises at least about 100 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, comprises at least about 200 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV,

e.g., exosome, comprises at least about 300 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, comprises at least about 400 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, comprises at least about 500 molecules of CD47 on the surface of the EV, e.g., exosome. In some aspects, the EV, e.g., exosome, comprises at least about 1000 molecules of CD47 on the surface of the EV, e.g., exosome.

[0410] In some aspects, expression CD47 or a fragment thereof on the surface of the EV, e.g., exosome, results in decreased uptake of the EV, e.g., exosome, by myeloid cells as compared to an EV, e.g., exosome, not expressing CD47 or a fragment thereof. In some aspects, uptake by myeloid cells of the EV, e.g., exosome, expressing CD47 or a fragment thereof is decreased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95%, relative to uptake by myeloid cells of EVs, e.g., exosomes, that do not express CD47 or a fragment thereof.

[0411] In some aspects, expression CD47 or a fragment thereof on the surface of the EV, e.g., exosome, results in decreased localization of the EV, e.g., exosome, to the liver, as compared to an EV, e.g., exosome, not expressing CD47 or a fragment thereof. In some aspects, localization to the liver of EVs, e.g., exosomes, expressing CD47 or a fragment thereof is decreased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95%, relative to the localization to the liver of EVs, e.g., exosomes, not expressing CD47 or a fragment thereof.

[0412] In some aspects, the in vivo half-life of an EV, e.g., exosome, expressing CD47 or a fragment thereof is increased relative to the in vivo half-life of an EV, e.g., exosome, that does not express CD47 or a fragment thereof. In some aspects, the in vivo half-life of an EV, e.g., exosome, expressing CD47 or a fragment thereof is increased by at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least about 10-fold, relative to the in vivo half-life of an EV, e.g., exosome, that does not express CD47 or a fragment thereof.

[0413] In some aspects, an EV, *e.g.*, exosome, expressing CD47 or a fragment thereof has an increased retention in circulation, *e.g.*, plasma, relative to the retention of an EV, *e.g.*, exosome, that does not express CD47 or a fragment thereof in circulation, *e.g.*, plasma. In some aspects, retention in circulation, *e.g.*, plasma, of an EV, *e.g.*, exosome, expressing CD47 or a fragment thereof is increased by at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, or at least about 10-fold, relative to the retention in circulation, *e.g.*, plasma, of an EV, *e.g.*, exosome, that does not express CD47 or a fragment thereof.

[0414] In some aspects, an EV, *e.g.*, exosome, expressing CD47 or a fragment thereof has an altered biodistribution when compared with an exosome that does not express CD47 or a fragment. In some aspects, the altered biodistribution leads to increased uptake into endothelial cells, T cells, or increased accumulation in various tissues, including, but not limited to skeletal muscle, cardiac muscle, diaphragm, kidney, bone marrow, central nervous system, lungs, cerebral spinal fluid (CSF), or any combination thereof.

IV. Pharmaceutical Compositions

[0415] Some aspects of the present disclosure are directed to methods of treating a peripheral neuropathy, *e.g.*, CIPN, in a subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising an EV comprising an exogenous NLRP3 antagonist, as disclosed herein. As such, provided herein are pharmaceutical compositions comprising an EV, *e.g.*, exosome, of the present disclosure having the desired degree of purity, and a pharmaceutically acceptable carrier or excipient, in a form suitable for administration to a subject. Pharmaceutically acceptable excipients or carriers can be determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions comprising a plurality of extracellular vesicles. (*See, e.g.*, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. 21st ed. (2005)). The pharmaceutical compositions are generally formulated sterile and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[0416] In some aspects, a pharmaceutical composition comprises one or more therapeutic agents and an exosome described herein. In certain aspects, the EVs, *e.g.*, exosomes, are co-administered with one or more additional therapeutic agents in a pharmaceutically acceptable

carrier. In some aspects, the ASO and the one or more additional therapeutic agents for the present disclosure can be administered in the same EV. In other aspects, the ASO and the one or more additional therapeutic agents for the present disclosure are administered in different EVs. For example, the present disclosure includes a pharmaceutical composition comprising an EV comprising an ASO and an EV comprising an additional therapeutic agent. In some aspects, the pharmaceutical composition comprising the EV, *e.g.*, exosome, is administered prior to administration of the additional therapeutic agent(s). In other aspects, the pharmaceutical composition comprising the EV, *e.g.*, exosome, is administered after the administration of the additional therapeutic agent(s). In further aspects, the pharmaceutical composition comprising the EV, *e.g.*, exosome, is administered concurrently with the additional therapeutic agent(s).

[0417] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients (*e.g.*, animals or humans) at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0418] Examples of carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. The use of such media and compounds for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or compound is incompatible with the extracellular vesicles described herein, use thereof in the compositions is contemplated. Supplementary therapeutic agents can also be incorporated into the compositions. Typically, a pharmaceutical composition is formulated to be compatible with its intended route of administration. The EVs, *e.g.*, exosomes, can be administered by parenteral, topical, intravenous, oral, subcutaneous, intra-arterial, intradermal, transdermal, rectal, intracranial, intraperitoneal, intranasal, intratumoral, intramuscular route or as inhalants. In certain aspects, the pharmaceutical composition comprising exosomes is administered

intravenously, *e.g.* by injection. The EVs, *e.g.*, exosomes, can optionally be administered in combination with other therapeutic agents that are at least partly effective in treating the disease, disorder or condition for which the EVs, *e.g.*, exosomes, are intended.

[0419] Solutions or suspensions can include the following components: a sterile diluent such as water, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0420] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (if water soluble) or dispersions and sterile powders. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The composition is generally sterile and fluid to the extent that easy syringeability exists. The carrier can be a solvent or dispersion medium containing, *e.g.*, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, *e.g.*, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal compounds, *e.g.*, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. If desired, isotonic compounds, *e.g.*, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride can be added to the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition a compound which delays absorption, *e.g.*, aluminum monostearate and gelatin.

[0421] Sterile injectable solutions can be prepared by incorporating the EVs, *e.g.*, exosomes, in an effective amount and in an appropriate solvent with one or more ingredients enumerated herein or known in the art, as desired. Generally, dispersions are prepared by incorporating the EVs, *e.g.*, exosomes, into a sterile vehicle that contains a basic dispersion medium and any desired other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The EVs, *e.g.*, exosomes, can be administered in the form of a depot

injection or implant preparation which can be formulated in such a manner to permit a sustained or pulsatile release of the EV, *e.g.*, exosome.

[0422] Systemic administration of compositions comprising exosomes can also be by transmucosal means. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, *e.g.*, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of, *e.g.*, nasal sprays.

[0423] In certain aspects the pharmaceutical composition comprising EVs, *e.g.*, exosomes is administered intravenously into a subject that would benefit from the pharmaceutical composition. In certain other aspects, the composition is administered to the lymphatic system, *e.g.*, by intralymphatic injection or by intranodal injection (*see e.g.*, Senti *et al.*, PNAS 105(46): 17908 (2008)), or by intramuscular injection, by subcutaneous administration, by intratumoral injection, by direct injection into the thymus, or into the liver.

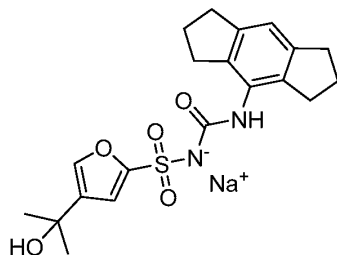
[0424] In certain aspects, the pharmaceutical composition comprising exosomes is administered as a liquid suspension. In certain aspects, the pharmaceutical composition is administered as a formulation that is capable of forming a depot following administration. In certain preferred aspects, the depot slowly releases the EVs, *e.g.*, exosomes, into circulation, or remains in depot form.

[0425] Typically, pharmaceutically-acceptable compositions are highly purified to be free of contaminants, are biocompatible and not toxic, and are suited to administration to a subject. If water is a constituent of the carrier, the water is highly purified and processed to be free of contaminants, *e.g.*, endotoxins.

[0426] The pharmaceutically-acceptable carrier can be lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginates, gelatin, calcium silicate, micro-crystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and/or mineral oil, but is not limited thereto. The pharmaceutical composition can further include a lubricant, a wetting agent, a sweetener, a flavor enhancer, an emulsifying agent, a suspension agent, and/or a preservative.

[0427] In some aspects, the pharmaceutical compositions described herein comprise a pharmaceutically acceptable salt. In some aspects, the pharmaceutically acceptable salt comprises a sodium salt, a potassium salt, an ammonium salt, or any combination thereof.

[0428] The pharmaceutical compositions described herein comprise the EVs, *e.g.*, exosomes, described herein and optionally an additional pharmaceutically active or therapeutic agent. The additional therapeutic agent can be a biological agent, a small molecule agent, or a nucleic acid agent. In some aspects, the additional therapeutic agent is an additional NLRP3 antagonist. In some aspects, the NLRP3 antagonist is any NLRP3 antagonist disclosed herein. In some aspects, the additional NLRP3 antagonist is an anti-NLRP3 antibody. In some aspects, the additional NLRP3 antagonist is a small molecule. In some aspects, the additional NLRP3 antagonist is a small molecule disclosed herein. In some aspects, the additional NLRP3 antagonist is selected from MCC950, Tanilast, Oridonin, CY-09, Bay 11-7082, Parthenolide, 3,4-methylenedioxy- β -nitrostyrene (MNB), β -hydroxybutyrate (BHB), dimethyl sulfoxide (DMSO), type I interferon, and any combination thereof. In some aspects, the additional NLRP3 antagonist comprises the following formula:



[0429]

[0430] In some aspects, the additional NLRP3 antagonist comprises MCC950.

[0431] In some aspects, the additional NLRP3 antagonist comprises an ASO. In some aspects, the additional NLRP3 antagonist comprises any ASO described herein.

[0432] Dosage forms are provided that comprise a pharmaceutical composition comprising the EVs, *e.g.*, exosomes, described herein. In some aspects, the dosage form is formulated as a liquid suspension for intravenous injection. In some aspects, the dosage form is formulated as a liquid suspension for intratumoral injection.

[0433] In certain aspects, the preparation of exosomes is subjected to radiation, *e.g.*, X rays, gamma rays, beta particles, alpha particles, neutrons, protons, elemental nuclei, UV rays in order to damage residual replication-competent nucleic acids.

[0434] In certain aspects, the preparation of exosomes is subjected to gamma irradiation using an irradiation dose of more than 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more than 100 kGy.

[0435] In certain aspects, the preparation of exosomes is subjected to X-ray irradiation using an irradiation dose of more than 0.1, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90,

100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, or greater than 10000 mSv.

V. Kits

[0436] Also provided herein are kits comprising one or more exosomes described herein and instructions to administer the one or more exosomes to a subject according to a method disclosed herein (*e.g.*, to a subject having a peripheral neuropathy, *e.g.*, CIPN). In some aspects, provided herein is a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions described herein, such as one or more exosomes provided herein and instructions for use according to any method disclosed herein (*e.g.*, to a subject having a peripheral neuropathy, *e.g.*, CIPN). In some aspects, the kits contain a pharmaceutical composition described herein and any prophylactic or therapeutic agent, such as those described herein. In some aspects, the kit is for use in the treatment of a disease or condition associated with a peripheral neuropathy. In some aspects, the kit is for use in the treatment of CIPN. In some aspects, the kit is a diagnostic kit.

[0437] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. *See*, for example, Sambrook *et al.*, ed. (1989) Molecular Cloning A Laboratory Manual (2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook *et al.*, ed. (1992) Molecular Cloning: A Laboratory Manual, (Cold Springs Harbor Laboratory, NY); D. N. Glover ed., (1985) DNA Cloning, Volumes I and II; Gait, ed. (1984) Oligonucleotide Synthesis; Mullis *et al.* U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) Nucleic Acid Hybridization; Hames and Higgins, eds. (1984) Transcription And Translation; Freshney (1987) Culture Of Animal Cells (Alan R. Liss, Inc.); Immobilized Cells And Enzymes (IRL Press) (1986); Perbal (1984) A Practical Guide To Molecular Cloning; the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) Gene Transfer Vectors For Mammalian Cells, (Cold Spring Harbor Laboratory); Wu *et al.*, eds., Methods In Enzymology, Vols. 154 and 155; Mayer and Walker, eds. (1987) Immunochemical Methods In Cell And Molecular Biology (Academic Press, London); Weir and Blackwell, eds., (1986) Handbook Of Experimental Immunology, Volumes I-IV; Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986);); Crooke, Antisense drug Technology:

Principles, Strategies and Applications, 2nd Ed. CRC Press (2007) and in Ausubel *et al.* (1989) Current Protocols in Molecular Biology (John Wiley and Sons, Baltimore, Md.).

[0438] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties.

[0439] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1: In vivo analysis of exoASONLRP3 in CIPN mouse model

[0440] A mouse model for chemotherapy induced peripheral neuropathy (CIPN) was used to test the efficacy of exoASONLRP3 *in vivo*. Exosomes comprising exoASONLRP3 were administered by intrathecal injection (FIG. 2). CIPN was simulated by administering cisplatin to mice on days 0-5 and 10-15, as indicated in FIG. 3A. Mice were administered a negative control (sham; no cisplatin); cisplatin and PBS; cisplatin and MCC950 (days 15-20); cisplatin and control exosomes (no ASO; day 15); cisplatin and ASONLRP3 (free ASO; day 15); cisplatin and exoASONLRP3 (day 5); cisplatin and exoASONLRP3 (day 15); or cisplatin and egabapentin (days 8 and 21); as indicated (FIGs. 3A-3B). A Von Frey test was used to measure pain in treated mice. Mice treated with cisplatin followed by an NLRP3 inhibitor showed decreased pain, with mice administered exoASONLRP3 displaying pain levels that were comparable to mice that were not administered cisplatin.

[0441] These results indicate that inhibition of NLRP3 through administration of exoASONLRP3 is capable of reducing pain associated with CIPN.

INCORPORATION BY REFERENCE

[0442] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

EQUIVALENTS

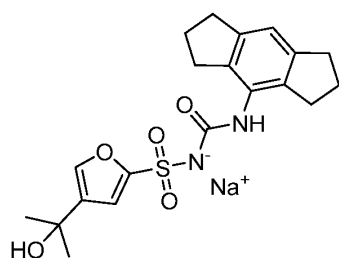
[0443] While various specific aspects have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s). Many variations will become apparent to those skilled in the art upon review of this specification.

What is Claimed:

1. A method of treating peripheral neuropathy in a subject in need thereof, comprising administering to the subject an extracellular vesicle comprising an exogenous NLRP3 antagonist.
2. A method of reducing, ameliorating, or treating one or more symptoms of a peripheral neuropathy in a subject in need thereof, comprising administering to the subject an extracellular vesicle comprising an exogenous NLRP3 antagonist.
3. The method of claim 1 or 2, wherein the exogenous NLRP3 antagonist is a chemical compound, an siRNA, an shRNA, an antisense oligonucleotide, a protein, or any combination thereof.
4. The method of any one of claims 1 to 3, wherein the extracellular vesicle targets a cell selected from the group consisting of a macrophage, a myeloid-derived suppressor cell (MDSC), a monocyte, a basophil, a neutrophil, an eosinophil, and any combination thereof.
5. The method of any one of claims 1 to 4, wherein the extracellular vesicle comprising the ASO or the ASO induces M2 macrophage polarization in the subject.
6. The method of any one of claims 1 to 5, wherein the extracellular vesicle comprising the ASO or the ASO reduces myeloid inflammation in a nerve, meningeal myeloid inflammation, nerve sheath inflammation, or any combination thereof.
7. The method of any one of claims 1 to 6, wherein the extracellular vesicle comprising the ASO reduces myeloid inflammation in a sheath.
8. The method of any one of claims 1 to 7, wherein the extracellular vesicle comprising the ASO reduces macrophage influx in one or more of a root, nerve, and/or muscle.
9. The method of any one of claims 1 to 8, wherein the extracellular vesicle comprising the ASO reduces macrophage phagocytosis in one or more of a root, nerve, and/or muscle.
10. The method of any one of claims 1 to 9, wherein the exogenous NLRP3 antagonist is a small molecule.
11. The method of claim 10, wherein the small molecule is selected from the group consisting of MCC950, Tanilast, Oridonin, CY-09, Bay 11-7082, Parthenolide, 3,4-methylenedioxy- β -

nitrostyrene (MNB), β -hydroxybutyrate (BHB), dimethyl sulfoxide (DMSO), type I interferon, and any combination thereof.

12. The method of claim 10 or 11, wherein the exogenous NLRP3 antagonist comprises the formula (I):



(I).

13. The method of any one of claims 10 to 10, wherein the exogenous NLRP3 antagonist comprises MCC950.

14. The method of any one of claims 1 to 13, wherein the exogenous NLRP3 antagonist comprises an antisense oligonucleotide (ASO).

15. The method of claim 14, wherein the ASO comprises a contiguous nucleotide sequence of 10 to 30 nucleotides in length that is complementary to a nucleic acid sequence within a *NLRP3* transcript.

16. The method of claim 15, wherein the contiguous nucleotide sequence is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% complementary to the nucleic acid sequence within the *NLRP3* transcript.

17. The method of any one of claims 14 to 16, wherein the ASO is capable of reducing NLRP3 protein expression in a human cell (*e.g.*, an immune cell), wherein the human cell expresses the NLRP3 protein.

18. The method of claim 17, wherein the NLRP3 protein expression is reduced by at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to NLRP3 protein expression in a human cell that is not exposed to the ASO.

19. The method of any one of claims 14 to 18, wherein the ASO is capable of reducing a level of *NLRP3* mRNA in a human cell (e.g., an immune cell), wherein the human cell expresses the *NLRP3* mRNA.
20. The method of claim 19, wherein the level of *NLRP3* mRNA is reduced by at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to the level of the *NLRP3* mRNA in a human cell that is not exposed to the ASO.
21. The method of any one of claims 14 to 20, wherein the ASO is a gapmer, a mixmer, or a totalmer.
22. The method of any one of claims 14 to 21, wherein the ASO comprises one or more nucleoside analogs.
23. The method of claim 22, wherein one or more of the nucleoside analogs comprises a 2'-O-alkyl-RNA; 2'-O-methyl RNA (2'-OMe); 2'-alkoxy-RNA; 2'-O-methoxyethyl-RNA (2'-MOE); 2'-amino-DNA; 2'-fluoro-RNA; 2'-fluoro-DNA; arabino nucleic acid (ANA); 2'-fluoro-ANA; or bicyclic nucleoside analog.
24. The method of claim 22 or 23, wherein one or more of the nucleoside analogs is a sugar modified nucleoside.
25. The method of claim 24, wherein the sugar modified nucleoside is an affinity enhancing 2' sugar modified nucleoside.
26. The method of any one of claims 22 to 25, wherein one or more of the nucleoside analogs comprises a nucleoside comprising a bicyclic sugar.
27. The method of any one of claims 22 to 25, wherein one or more of the nucleoside analogs comprises an LNA.
28. The method of any one of claims 22 to 27, wherein one or more of the nucleotide analogs is selected from the group consisting of constrained ethyl nucleoside (cEt), 2',4'-constrained 2'-O-methoxyethyl (cMOE), α -L-LNA, β -D-LNA, 2'-O,4'-C-ethylene-bridged nucleic acids (ENA), amino-LNA, oxy-LNA, thio-LNA, and any combination thereof.

29. The method of any one of claims 14 to 28, wherein the ASO comprises one or more 5'-methyl-cytosine nucleobases.

30. The method of any one of claims 15 to 29, wherein the contiguous nucleotide sequence is complementary to a nucleic acid sequence within (i) a 5' untranslated region (UTR); (ii) a coding region; or (iii) a 3' UTR of the *NLRP3* transcript.

31. The method of any one of claims 15 to 30, wherein the contiguous nucleotide sequence is complementary to a nucleic acid sequence comprising (i) nucleotides 1 – 534 of SEQ ID NO: 3; (ii) nucleotides 448 – 2193 of SEQ ID NO: 3; (iii) nucleotides 2125 – 3036 of SEQ ID NO: 3; (iv) nucleotides 2987 – 3990 of SEQ ID NO: 3; (v) 3996 – 4456 of SEQ ID NO: 3; (vi) nucleotides 106 – 334 of SEQ ID NO: 3; (vii) nucleotides 648 – 2113 of SEQ ID NO: 3; (viii) nucleotides 2225 – 2956 of SEQ ID NO: 3; (ix) nucleotides 2987 – 3810 of SEQ ID NO: 3; (x) 3996 – 4376 of SEQ ID NO: 3; (xi) nucleotides 156 – 284 of SEQ ID NO: 3; (xii) nucleotides 698 – 2063 of SEQ ID NO: 3; (xiii) nucleotides 2275 – 2906 of SEQ ID NO: 3; (xiv) nucleotides 3037 – 3760 of SEQ ID NO: 3; (xv) 4046 – 4326 of SEQ ID NO: 3; (xvi) nucleotides 196 – 244 of SEQ ID NO: 3; (xvii) nucleotides 738 – 2003 of SEQ ID NO: 3; (xviii) nucleotides 2315 – 2866 of SEQ ID NO: 3; (xix) nucleotides 3077 – 3720 of SEQ ID NO: 3; or (xx) 4086 – 4286 of SEQ ID NO: 3.

32. The method of any one of claims 15 to 31, wherein the contiguous nucleotide sequence is complementary to a nucleic acid sequence within (i) nucleotides 206 – 234 of SEQ ID NO: 3; (ii) nucleotides 748-2013 of SEQ ID NO: 3; (iii) nucleotides 2325 – 2856 of SEQ ID NO: 3; (iv) nucleotides 3087 – 3710 of SEQ ID NO: 3; or (v) 4096 – 4276 of SEQ ID NO: 3.

33. The method of any one of claims 15 to 32, wherein the contiguous nucleotide sequence comprises a nucleotide sequence complementary to a sequence selected from the sequences in FIGs. 1A and 1B.

34. The method of any one of claims 15 to 33, wherein the continuous nucleotide sequence is fully complementary to a nucleotide sequence within the *NLRP3* transcript.

35. The method of any one of claims 14 to 34, wherein the ASO comprises a nucleotide sequence selected from SEQ ID NOs: 101-200, with one or two mismatches.

36. The method of any one of claims 14 to 35, wherein the ASO comprises a nucleotide sequence selected from SEQ ID NOs: 101-200.

37. The method of any one of claims 14 to 36, wherein the ASO is from 14 to 20 nucleotides in length.
38. The method of any one of claims 15 to 37, wherein the contiguous nucleotide sequence comprises one or more modified internucleoside linkages.
39. The method of claim 38, wherein the one or more modified internucleoside linkages is a phosphorothioate linkage.
40. The method of claim 38 or 39, wherein at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% of internucleoside linkages are modified.
41. The method of claim 40, wherein each of the internucleoside linkages in the ASO is a phosphorothioate linkage.
42. The method of any one of claims 1 to 41, wherein the extracellular vesicle comprises an anchoring moiety.
43. The method of claim 42, wherein the NLRP3 antagonist is linked to the anchoring moiety.
44. The method of any one of claims 1 to 43, wherein the extracellular vesicle comprises an exogenous targeting moiety.
45. The method of claim 44, wherein the exogenous targeting moiety comprises a peptide, an antibody or an antigen-binding fragment thereof, a chemical compound, an RNA aptamer, or any combination thereof.
46. The method of claim 44 or 45, wherein the exogenous targeting moiety comprises a peptide.
47. The method of any one of claims 44 to 46, wherein the exogenous targeting moiety comprises a microprotein, a designed ankyrin repeat protein (darpin), an anticalin, an adnectin, an aptamer, a peptide mimetic molecule, a natural ligand for a receptor, a camelid nanobody, or any combination thereof.
48. The method of any one of claims 44 to 47, wherein the exogenous targeting moiety comprises a full-length antibody, a single domain antibody, a heavy chain only antibody (VHH), a

single chain antibody, a shark heavy chain only antibody (VNAR), an scFv, a Fv, a Fab, a Fab', a F(ab')₂, or any combination thereof.

49. The method of claim 48, wherein the antibody is a single chain antibody.

50. The method of any one of claims 44 to 49, wherein the EV comprises a scaffold moiety linking the exogenous targeting moiety to the EV.

51. The method of any one of claims 42 to 50, wherein the anchoring moiety and/or the scaffold moiety is a Scaffold X or a Scaffold Y.

52. The method of any one of claims 42 to 51, wherein the exogenous NLRP3 antagonist is linked to the anchoring moiety and/or the scaffold moiety on the exterior surface of the EV.

53. The method of any one of claims 42 to 52, wherein the exogenous NLRP3 antagonist is linked to the anchoring moiety and/or the scaffold moiety on the luminal surface of the EV.

54. The method of any one of claims 42 to 53, wherein the anchoring moiety comprises sterol, GM1, a lipid, a vitamin, a small molecule, a peptide, or a combination thereof.

55. The method of any one of claims 42 to 53, wherein the anchoring moiety comprises cholesterol.

56. The method of any one of claims 42 to 53, wherein the anchoring moiety comprises a phospholipid, a lysophospholipid, a fatty acid, a vitamin (e.g., vitamin D and/or vitamin E), or any combination thereof.

57. The method of any one of claims 42 to 56, wherein the exogenous NLRP3 antagonist is linked to the anchoring moiety and/or the scaffold moiety by a linker.

58. The method of any one of claims 1 to 57, wherein the exogenous NLRP3 antagonist is linked to the EV by a linker.

59. The method of claim 57 or 58, wherein the linker is a polypeptide.

60. The method of claim 57 or 58, wherein the linker is a non-polypeptide moiety.

61. The method of claim 57 or 58, wherein the linker comprise ethylene glycol.

62. The method of claim 61, wherein the linker comprises HEG, TEG, PEG, or any combination thereof.
63. The method of claim 57 or 58, wherein the linker comprises acrylic phosphoramidite (e.g., ACRYDITE™), adenylation, azide (NHS Ester), digoxigenin (NHS Ester), cholesterol-TEG, I-LINKER™, an amino modifier (e.g., amino modifier C6, amino modifier C12, amino modifier C6 dT, or Uni-Link™ amino modifier), alkyne, 5' Hexynyl, 5-Octadiynyl dU, biotinylation (e.g., biotin, biotin (Azide), biotin dT, biotin-TEG, dual biotin, PC biotin, or desthiobiotin), thiol modification (thiol modifier C3 S-S, dithiol or thiol modifier C6 S-S), or any combination thereof.
64. The method of any one of claims 57 to 63, wherein the linker is a cleavable linker.
65. The method of claim 64, wherein the linker comprises valine-alanine-p-aminobenzylcarbamate or valine-citrulline-p-aminobenzylcarbamate.
66. The method of any one of claims 57 to 65, wherein the linker comprises (i) a maleimide moiety and (ii) valine-alanine-p-aminobenzylcarbamate or valine-citrulline-p-aminobenzylcarbamate.
67. The method of any one of claims 1 to 66, wherein the EV is an exosome.
68. The method of any one of claims 1 to 67, wherein the peripheral neuropathy is associated with diabetes, a trauma, an autoimmune disorder, a kidney disorder, a liver disorder, hypothyroidism, a vascular disorder, an abnormal vitamin level, alcohol use, or any combination thereof.
69. The method of any one of claims 1 to 68, wherein the peripheral neuropathy comprises chemotherapy-induced peripheral neuropathy (CIPN).
70. The method of any one of claims 1 to 69, wherein the subject was previously administered a chemotherapy.
71. The method of claim 70, wherein the chemotherapy comprises a platinum derivative, a vinca alkaloid, bortezomib, a taxane, or any combination thereof.
72. The method of claim 70 or 71, wherein the chemotherapy comprises cisplatin, carboplatin, oxaliplatin, docetaxel, vincristine, paclitaxel, gemcitabine, or any combination thereof.

73. The method of any one of claims 1 to 72, wherein the extracellular vesicle reduces the severity or occurrence of one or more symptom in the subject selected from tingling, pain, burning, numbness, sensitivity to hot, sensitivity to cold, difficulty with fine motor skills, and any combination thereof.