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(54) Title: SUBCUTANEOUS DELIVERY OF MESSENGER RNA

(57) **Abrégé/Abstract:**

The present invention provides, among other things, methods of formulating nucleic acid-containing nanoparticles with an mRNA encoding an enzyme to afford efficient delivery of payload to a cell or tissue of interest via subcutaneous administration. The resulting payload can be efficiently delivered to the liver and other organs or tissues of a treated subject.

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(54) Title: SUBCUTANEOUS DELIVERY OF MESSENGER RNA

(57) Abstract: The present invention provides, among other things, methods of formulating nucleic acid-containing nanoparticles with an mRNA encoding an enzyme to afford efficient delivery of payload to a cell or tissue of interest via subcutaneous administration. The resulting payload can be efficiently delivered to the liver and other organs or tissues of a treated subject.

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SUBCUTANEOUS DELIVERY OF MESSENGER RNA

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/671,820, filed May 15, 2018, which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The present specification makes reference to a Sequence Listing (submitted electronically as a .txt file named “MRT-1252WO_ST25” on May 13, 2019). The .txt file was generated May 13, 2019 and is 26,169 bytes in size. The entire contents of the Sequence Listing are herein incorporated by reference.

BACKGROUND

[0003] Messenger RNA therapy (MRT) is becoming an increasingly important approach for the treatment of a variety of diseases. MRT involves administration of messenger RNA (mRNA) to a patient in need of the therapy for production of the protein encoded by the mRNA within the patient's body. Lipid nanoparticles are commonly being used to deliver mRNA for efficient *in vivo* delivery of mRNA and it is now possible to deliver specific mRNA-loaded lipid nanoparticles systemically via intravenous delivery. However, for increase in patient comfort and compliance, improvements in subcutaneous methods of delivery of therapeutic mRNA are greatly needed.

SUMMARY OF INVENTION

[0004] The present invention provides, among other things, improved methods and compositions for the effective *in vivo* delivery of mRNA via subcutaneous administration. In particular, an mRNA encoding a protein of therapeutic interest is injected subcutaneously with an mRNA encoding an enzyme that is capable of degrading extracellular matrices such as a hyaluronidase, for efficient exposure of the therapeutic mRNA to the circulation. As described herein, a first mRNA encoding a protein of therapeutic interest when administered with a second mRNA encoding hyaluronidase, results in unexpectedly efficient delivery of the first therapeutic mRNA, accompanied with its efficient protein expression *in vivo*, particularly in the liver. The mRNAs are encapsulated in lipid nanoparticles (LNPs). In some embodiments the therapeutic mRNA is encapsulated in lipid nanoparticles (LNPs). In some embodiments both the therapeutic mRNA and the hyaluronidase mRNA are encapsulated in lipid nanoparticles (LNPs). Although hyaluronidase had been used to enhance subcutaneous delivery of small molecule and protein drugs, it was uncertain prior to the inventors' recent investigations if hyaluronidase could also be effective in facilitating subcutaneous delivery of mRNA, in particular, mRNA encapsulated in lipid nanoparticles (LNPs), in view of the significant size differences and the complexity of the LNP-mRNA formulations. Many mRNA-loaded LNPs have sizes close to or around about 100 nM, which is at least five times as large as a typical protein (typical proteins including antibodies have an average size below 20 nm). It was further uncertain whether delivery of mRNA-LNPs in presence of an mRNA encoding hyaluronidase could be effective in augmenting subcutaneous uptake and delivery of mRNA-LNPs. In view of efficient mRNA delivery and high protein expression in the liver following subcutaneous delivery using hyaluronidase enzyme, which was recently reported for the first time in the Applicant's application PCT/US17/61176, filed on 11-10-2017, hereby fully incorporated by reference), the present invention is particularly useful in treating metabolic diseases such as ornithine transcarbamylase (OTC) deficiency. Using an mRNA encoding a hyaluronidase in the same or a separate formulation to deliver a therapeutic mRNA, a robust and sustained delivery and distribution of the therapeutic mRNA can be achieved with surprising ease and cost-effectiveness. Without wishing to be bound by a theory, it is likely that the mRNA encoding hyaluronidase is readily distributed and translated at the site of administration and in turn helps in uptake and efficient distribution of the therapeutic mRNA as a result of the function of the translated hyaluronidase *in situ*. The hyaluronidase based administration as provided in the present application increases the efficiency of subcutaneous delivery of mRNA, which is more patient friendly compared to other administration routes such

as intravenous (IV) or intramuscular (IM), can reduce healthcare costs and increase patient compliance and throughput at the hospital.

[0005] In one aspect, the present invention provides a method for subcutaneous delivery of a messenger RNA (mRNA) to a subject in need thereof, the method comprising: administering subcutaneously to the subject a composition comprising: an mRNA encoding a protein or polypeptide, and an mRNA encoding a hyaluronidase.

[0006] In some embodiments, the mRNA encoding a protein or polypeptide is a therapeutic mRNA. In some embodiments, the protein or polypeptide encoded by the mRNA, i.e. the therapeutic mRNA as described herein, encodes a protein or polypeptide selected from a group consisting of: erythropoietin (EPO), Phenylalanine hydroxylase (PAH), argininosuccinate synthase 1 (ASS1), α 1-anti-trypsin (A1AT), Factor IX (FIX), Factor VIII (FVIII), carboxypeptidase N, alpha galactosidase (GLA), ornithine carbamoyltransferase (OTC), human growth hormone (hOtt), *SLC3A1* encoded protein, *SLC3A9* encoded protein, *COL4A5* encoded protein, *FXN* encoded protein, *GNS* encoded protein, *HGSNAT* encoded protein, *NAGLU* encoded protein, *SGSH* encoded protein, *MUT* encoded protein methyl malonyl CoA mutase and *ATP7B* encoded protein ATPase 2.

[0007] In some embodiments, the mRNA encoding a protein or a polypeptide, which is a therapeutic mRNA, has a length of or greater than about 0.5 kb, 1 kb, 1.5 kb, 2 kb, 2.5 kb, 3 kb, 3.5 kb, 4 kb, 4.5 kb, 5 kb, 6kb, 7 kb, 8 kb, 9 kb, 10 kb, 11 kb, 12 kb, 13 kb, 14 kb, or 15 kb.

[0008] In some embodiments, the mRNA encoding hyaluronidase is a helper mRNA, which encodes a mammalian hyaluronidase selected from a bovine hyaluronidase, a porcine hyaluronidase, an equine hyaluronidase, an ovine hyaluronidase and a human hyaluronidase.

[0009] In some embodiments, the mRNA encoding the hyaluronidase comprises a polynucleotide sequence having at least 80% identity to SEQ ID NO: 9, 10 or 12.

[0010] In some embodiments the mRNA encoding the protein or polypeptide and the mRNA encoding a hyaluronidase enzyme are individually capped and tailed.

[0011] In some embodiments the mRNA encoding the protein or polypeptide and the mRNA encoding a hyaluronidase enzyme are encapsulated in a lipid nanoparticles (LNPs).

[0012] In some embodiments, the lipid nanoparticles comprise a cationic lipid, which is selected from a group consisting of cKK-E12 (3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine-2,5-dione), OF-02, Target 23, Target 24, ICE, HGT5000, HGT5001, HGT4003, DOTAP (1,2-dioleoyl-3-trimethylammonium propane), DODAP (1,2-dioleoyl-3-dimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DlinDMA, DODAC, DDAB, DMRIE, DOSPA, DOGS,

DODMA, DMDMA, DODAC, DLenDMA, DMRIE, CLinDMA, CpLinDMA, DMOBA, DOcarbDAP, DLinDAP, DLincarbDAP, DLinCDAP, KLin-K-DMA, DLin-K-XTC2-DMA, DLin-KC2-DMA, dialkylamino-based, imidazole-based, and guanidinium-based cationic lipids.

[0013] In some embodiments, the lipid nanoparticle comprises one or more non-cationic lipids. In some embodiments, the one or more non-cationic lipids are selected from the group consisting of DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), DOPC (1,2-dioleoyl-sn-glycero-3-phosphatidylcholine) DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)) and combinations thereof.

[0014] In some embodiments, the liposome comprises a PEGylated lipid. In some embodiments, the PEGylated lipid constitutes at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, or at least 10% of the total lipids in the liposome. In some embodiments, the PEGylated lipid constitutes at least 5% of the total lipids in the liposome. In some embodiments, the PEGylated lipid constitutes about 5% of the total lipids in the liposome. In some embodiments, the PEGylated lipid constitutes 10% or less, 9% or less, 8% or less, 7% or less, 6% or less, 5% or less, 4% or less, or 3% or less of the total lipids in the liposome. In some embodiments, the PEGylated lipid constitutes 5% or less of the total lipids in the liposome.

[0015] In some embodiments, the mRNA comprises unmodified nucleotides. In some embodiments, the mRNA comprises one or more modified nucleotides. In some embodiments, the one or more modified nucleotides comprise pseudouridine, N-1-methyl-pseudouridine, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 4'-thiouridine, 4'-thiocytidine, and/or 2-thiocytidine.

[0016] In some embodiments the mRNA encoding the protein or polypeptide and the mRNA encoding a hyaluronidase enzyme are encapsulated in the lipid nanoparticle (LNP). In some embodiments, the mRNA encoding the protein or polypeptide and the mRNA encoding a hyaluronidase enzyme are encapsulated in the separate LNPs. In some embodiments, the mRNA encoding the protein or polypeptide and the mRNA encoding a hyaluronidase enzyme are encapsulated in separate LNPs having non-identical compositions.

[0017] In some embodiments, the therapeutic mRNA and the hyaluronidase-encoding mRNA are administered simultaneously. In some embodiments, the therapeutic mRNA and the hyaluronidase-encoding mRNA are administered sequentially. In some embodiments the hyaluronidase-encoding mRNA is administered 0.1 hours, 0.2 hours, 0.3 hours, 0.4 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours or 6 hours prior to administering the therapeutic mRNA composition. In some embodiments, the hyaluronidase-encoding mRNA is administered 1 hour, 2 hours, 3 hours, 4 hours, 5 hours or 6 hours prior to administering the therapeutic mRNA composition.

[0018] In some embodiments, the protein encoded by the therapeutic mRNA is expressed in the liver. In some embodiments, the protein encoded by the therapeutic mRNA is expressed in the kidney. In some embodiments, the protein encoded by the therapeutic mRNA is expressed in the lung. In some embodiments, the protein encoded by the therapeutic mRNA is detectable in the serum. In some embodiments, the expression of the protein encoded by the therapeutic mRNA is detectable at least 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, or 1 week after single administration.

[0019] In some embodiments, the therapeutic mRNA is administered at a dose of at least 0.5 mg/Kg of body weight. In some embodiments, the therapeutic mRNA is administered at a dose of about 1 mg/Kg, about 2 mg/Kg, about 3 mg/Kg, about 4 mg/Kg, about 5 mg/Kg, about 6 mg/Kg, about 7 mg/Kg, about 8 mg/Kg, about 9 mg/Kg, about 10 mg/Kg, about 11 mg/Kg, about 12 mg/Kg, about 13 mg/Kg, about 14 mg/Kg, about 15 mg/Kg, about 16 mg/Kg, about 17 mg/Kg, about 18 mg/Kg, about 19 mg/Kg, about 20 mg/Kg, about 25 mg/Kg, about 30 mg/Kg or about 50 mg/Kg of body weight.

[0020] In some embodiments, about 0.1 - 100 mg of mRNA encoding the hyaluronidase is administered. In some embodiments, about 0.5 - 90 mg of mRNA encoding the hyaluronidase is administered. In some embodiments, about 1- 80 mg of mRNA encoding the hyaluronidase is administered. In some embodiments, about 2- 70 mg of mRNA encoding the hyaluronidase is administered. In some embodiments, about 3- 60 mg of mRNA encoding the hyaluronidase is administered. In some embodiments, about 4- 50 mg of mRNA encoding the hyaluronidase is administered. In some embodiments, about 5- 50 mg of mRNA encoding the hyaluronidase is administered.

[0021] In some embodiments, the mRNA encoding the hyaluronidase is administered at a dose amount equivalent for translating to produce an expected amount of at least about 1U hyaluronidase enzyme per mg of the therapeutic RNA to be delivered. In some embodiments, hyaluronidase mRNA is administered at a dose equivalent of at least 2U per mg of the

therapeutic RNA, at least 5U per mg of the therapeutic RNA, at least 10U per mg of the therapeutic RNA, at least 20U per mg of the therapeutic mRNA, at least 30U per mg of the therapeutic mRNA, at least 40U per mg of the therapeutic mRNA, at least 50U per mg of the therapeutic mRNA, at least 100U per mg of the therapeutic mRNA, at least 200U per mg of the therapeutic mRNA, at least 300U per mg of the therapeutic mRNA, at least 400U per mg of the therapeutic mRNA, at least 500U per mg of the therapeutic mRNA, at least 1000U per mg of the therapeutic RNA, at least 2000U per mg of the therapeutic RNA, at least 3000U per mg of the therapeutic RNA, at least 4000U per mg of the therapeutic RNA, or at least 5000U per mg of the therapeutic RNA delivered. In one aspect, the present invention provides a method for treating a disease, disorder or condition in a subject, comprising delivering subcutaneously to the subject a therapeutic mRNA encoding a protein or a polypeptide, and a helper mRNA encoding a hyaluronidase, wherein the therapeutic mRNA-encoded protein or polypeptide is deficient in the subject. The disease, disorder or condition herein is selected from ornithine transcarbamylase (OTC) deficiency, Phenylalanine hydroxylase (PAH) deficiency (phenylketonuria, PKU), argininosuccinate synthase 1 (ASS1) deficiency, erythropoietin (EPO) deficiency, Fabry disease; hemophilic diseases (such as, e.g., hemophilia B (FIX), hemophilia A (FVIII); SMN1-related spinal muscular atrophy (SMA); amyotrophic lateral sclerosis (ALS); GALT-related galactosemia; COL4A5-related disorders including Alport syndrome; galactocerebrosidase deficiencies; X-linked adrenoleukodystrophy; Friedreich's ataxia; Pelizaeus-Merzbacher disease; TSC1 and TSC2-related tuberous sclerosis; Sanfilippo B syndrome (MPS IIIB); the FMR1-related disorders which include Fragile X syndrome, Fragile X-Associated Tremor/Ataxia Syndrome and Fragile X Premature Ovarian Failure Syndrome; Prader-Willi syndrome; hereditary hemorrhagic telangiectasia (AT); Niemann-Pick disease Type C1; the neuronal ceroid lipofuscinoses-related diseases including Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), Juvenile Batten disease, Santavuori-Haltia disease, Jansky-Bielschowsky disease, and PTT-1 and TPP1 deficiencies; EIF2B1, EIF2B2, EIF2B3, EIF2B4 and EIF2B5-related childhood ataxia with central nervous system hypomyelination/vanishing white matter; CACNA1A and CACNB4-related Episodic Ataxia Type 2; the MECP2-related disorders including Classic Rett Syndrome, MECP2-related Severe Neonatal Encephalopathy and PPM-X Syndrome; CDKL5-related Atypical Rett Syndrome; Kennedy's disease (SBMA); Notch-3 related cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL); SCN1A and SCN1B-related seizure disorders; the Polymerase G-related disorders which include Alpers-Huttenlocher syndrome, POLG-related sensory ataxic neuropathy, dysarthria, and ophthalmoparesis, and autosomal dominant and recessive progressive external

ophthalmoplegia with mitochondrial DNA deletions; X-Linked adrenal hypoplasia; X-linked agammaglobulinemia; and Wilson's disease.

[0022] In one embodiment, the present disclosure provides a method of treating ornithine transcarbamylase (OTC deficiency) by mRNA therapy. The method comprises administering to a subject in need of treatment a composition for subcutaneous delivery comprising messenger RNA encoding OTC protein and an mRNA encoding a hyaluronidase enzyme.

[0023] In some embodiments, the OTC mRNA is encapsulated within a nanoparticle. In some embodiments, the nanoparticle is a lipid-based or polymer-based nanoparticle. In some embodiments, the lipid-based nanoparticle is a liposome.

[0024] In some embodiments, the subcutaneous injection results in expression of the OTC protein in the liver of the subject.

[0025] In some embodiments, the subcutaneous injection delivers mRNA to hepatocytes. In some embodiments, the subcutaneous injection results in OTC expression in hepatocytes.

[0026] In some embodiments, the subcutaneous injection results in expression of the OTC protein in the serum of the subject.

[0027] In some embodiments, the expression of the protein encoded by the mRNA is detectable at least 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 4 weeks, or 1 month post-administration.

[0028] In some embodiments, OTC expression after mRNA administration can be detected by a functional assay.

[0029] In some embodiments, the administering of the composition results in an increased OTC protein expression or activity level in serum of the subject as compared to a control level. In some embodiments, the control level is a baseline serum OTC protein expression or activity level in the subject prior to the treatment. In some embodiments, the control level is a reference level indicative of the average serum OTC protein expression or activity level in OTC patients without treatment.

[0030] In some embodiments, the administering of the composition results in a reduced urinary orotic acid level in the subject as compared to a control orotic acid level. In some embodiments, the control orotic acid level is a baseline urinary orotic acid level in the subject prior to the treatment. In some embodiments, the control orotic acid level is a reference level indicative of the average urinary orotic acid level in OTC patients without treatment.

[0031] In some embodiments, wherein the administering of the composition results in an increased citrulline level in serum of the subject as compared to a control citrulline level. In some embodiments, the control citrulline level is a baseline serum citrulline level in the subject

prior to the treatment. In some embodiments, the control citrulline level is a reference level indicative of the average serum citrulline level in OTC patients without treatment.

[0032] In some embodiments, the mRNA encoding the OTC protein and the mRNA encoding the hyaluronidase enzyme are injected simultaneously.

[0033] In some embodiments, the mRNA encoding the OTC protein and the mRNA encoding the hyaluronidase enzyme are injected in one composition.

[0034] In some embodiments, the mRNA encoding the OTC protein and the mRNA encoding the hyaluronidase enzyme are injected in separate compositions.

[0035] In some embodiments, the mRNA encoding the OTC protein and the mRNA encoding the hyaluronidase enzyme are injected sequentially.

[0036] In some embodiments, the mRNA encoding the OTC protein and the mRNA encoding the hyaluronidase enzyme are injected in a volume of less than 20 ml, less than 15 ml, less than 10 ml, less than 5ml, less than 4 ml, less than 3 ml, less than 2 ml, or less than 1 ml.

[0037] In some embodiments, the subcutaneous injection is performed once a week or less frequently. In some embodiments, the subcutaneous injection is performed twice a month or less frequently. In some embodiments, the subcutaneous injection is performed once a month or less frequently.

[0038] In another aspect, the present invention provides for a composition for treating ornithine transcarbamylase (OTC deficiency), comprising an mRNA encoding an ornithine transcarbamylase (OTC) protein, and an mRNA encoding a hyaluronidase enzyme.

[0039] In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 20 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 18 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 16 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 14 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 12 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 10 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 9 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 8 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 7 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 6 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 5 mg/mL or less. In some embodiments, the mRNA encoding

hyaluronidase enzyme is administered at a dose 4 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 3 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 2 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose 1 mg/mL or less. In some embodiments, the mRNA encoding hyaluronidase enzyme is administered at a dose ranging between 1- 20 mg/mL.

[0040] In some embodiments, the mRNA is encapsulated within a nanoparticle.

[0041] In some embodiments, the nanoparticle is a lipid-based or polymer-based nanoparticle.

[0042] In some embodiments, the composition is a liquid form.

[0043] In another embodiment the composition is a lyophilized powder.

[0044] In one aspect, the invention provides a container containing a composition described above. The container is a vial or a syringe. The syringe may be prefilled for single subcutaneous administration. The vial may contain lyophilized powder or liquid form of the composition.

[0045] In this application, the use of “or” means “and/or” unless stated otherwise. As used in this disclosure, the term “comprise” and variations of the term, such as “comprising” and “comprises,” are not intended to exclude other additives, components, integers or steps. As used in this application, the terms “about” and “approximately” are used as equivalents. Both terms are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0046] Other features, objects, and advantages of the present invention are apparent in the detailed description, drawings and claims that follow. It should be understood, however, that the detailed description, the drawings, and the claims, while indicating embodiments of the present invention, are given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art.

DEFINITIONS

[0047] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0048] *Animal*: As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans, at any stage of development. In

some embodiments, “animal” refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0049] *Approximately or about:* As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0050] *Delivery:* As used herein, the term “delivery” encompasses both local and systemic delivery. For example, delivery of mRNA encompasses situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and retained within the target tissue (also referred to as “local distribution” or “local delivery”), and situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and secreted into patient’s circulation system (*e.g.*, serum) and systematically distributed and taken up by other tissues (also referred to as “systemic distribution” or “systemic delivery”).

[0051] *Encapsulation:* As used herein, the term “encapsulation,” or grammatical equivalent, refers to the process of confining an individual mRNA molecule within a nanoparticle.

[0052] *Expression:* As used herein, “expression” of a nucleic acid sequence refers to translation of an mRNA into a polypeptide, assemble multiple polypeptides into an intact protein (*e.g.*, enzyme) and/or post-translational modification of a polypeptide or fully assembled protein (*e.g.*, enzyme). In this application, the terms “expression” and “production,” and grammatical equivalent, are used inter-changeably.

[0053] *Half-life:* As used herein, the term “half-life” is the time required for a quantity such as nucleic acid or protein concentration or activity to fall to half of its value as measured at the beginning of a time period.

[0054] *Hyaluronidase:* As used herein, the term “hyaluronidase” refers to the family of enzymes that are capable of degrading hyaluronic acid (hyaluronan).

[0055] *Improve, increase, or reduce:* As used herein, the terms “improve,” “increase” or “reduce,” or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control subject (or multiple control subject) in the absence of the treatment described herein. A “control subject” is a subject afflicted with the same form of disease as the subject being treated, who is about the same age as the subject being treated.

[0056] *In Vitro:* As used herein, the term “*in vitro*” refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, *etc.*, rather than within a multi-cellular organism.

[0057] *In Vivo:* As used herein, the term “*in vivo*” refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).

[0058] *Local distribution or delivery:* As used herein, the terms “local distribution,” “local delivery,” or grammatical equivalent, refer to tissue specific delivery or distribution. Typically, local distribution or delivery requires a protein (*e.g.*, enzyme) encoded by mRNAs be translated and expressed intracellularly or with limited secretion that avoids entering the patient’s circulation system.

[0059] *Messenger RNA (mRNA):* As used herein, the term “messenger RNA (mRNA)” refers to a polynucleotide that encodes at least one polypeptide. mRNA as used herein encompasses both modified and unmodified RNA. mRNA may contain one or more coding and non-coding regions. mRNA can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, *etc.* Where appropriate, *e.g.*, in the case of chemically synthesized molecules, mRNA can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, *etc.* An mRNA sequence is presented in the 5’ to 3’ direction unless otherwise indicated. In some embodiments, an mRNA is or comprises natural nucleosides (*e.g.*, adenosine, guanosine, cytidine, uridine); nucleoside analogs (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (*e.g.*, methylated bases); intercalated

bases; modified sugars (*e.g.*, 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (*e.g.*, phosphorothioates and 5'-*N*-phosphoramidite linkages).

[0060] *Patient:* As used herein, the term “patient” or “subject” refers to any organism to which a provided composition may be administered, *e.g.*, for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. A human includes pre- and post-natal forms.

[0061] *Pharmaceutically acceptable:* The term “pharmaceutically acceptable” as used herein, refers to substances that, within the scope of sound medical judgment, are suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0062] *Subcutaneous administration:* As used herein, the term “subcutaneous administration” or “subcutaneous injection” refers to a bolus injection into the subcutis which is the tissue layer between the skin and the muscle.

[0063] *Subject:* As used herein, the term “subject” refers to a human or any non-human animal (*e.g.*, mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term “subject” is used herein interchangeably with “individual” or “patient.” A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0064] *Substantially:* As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0065] *Systemic distribution or delivery:* As used herein, the terms “systemic distribution,” “systemic delivery,” or grammatical equivalent, refer to a delivery or distribution mechanism or approach that affect the entire body or an entire organism. Typically, systemic distribution or delivery is accomplished via body's circulation system, *e.g.*, blood stream. Compared to the definition of “local distribution or delivery.”

[0066] *Target tissues:* As used herein, the term “target tissues” refers to any tissue that is affected by a disease to be treated. In some embodiments, target tissues include those tissues that display disease-associated pathology, symptom, or feature.

[0067] *Therapeutic mRNA:* As used herein, the term therapeutic mRNA is used to designate the mRNA that is intended for mRNA therapy. A therapeutic mRNA may designate an mRNA which encodes a protein or polypeptide which is deficient in a subject in need for therapy. It is interchangeably used with the term ‘first mRNA’ throughout the specification, without any presumption as to the temporal sequence of delivery with respect to, for example, a second mRNA.

[0068] *Therapeutically effective amount:* As used herein, the term “therapeutically effective amount” of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.

[0069] *Treating:* As used herein, the term “treat,” “treatment,” or “treating” refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

DETAILED DESCRIPTION

[0070] The present invention provides, among other things, improved methods and compositions of mRNA delivery for messenger RNA therapy via subcutaneous route by administering the mRNA of interest (the first mRNA) with a second mRNA encoding a hyaluronidase enzyme. The second mRNA helps or augments the cellular uptake and distribution of the mRNA. The mRNA payload was efficiently delivered to the livers (and other organs or tissues) of treated animals. Such a hyaluronidase based method has major benefits to creating new delivery profiles of otherwise intolerable drugs.

[0071] Among other things, the present invention provides methods and compositions for the treatment of ornithine transcarbamylase (OTC) deficiency by administering via subcutaneous

injection to a subject in need of treatment an mRNA encoding an ornithine transcarbamylase (OTC) protein and a second mRNA encoding a hyaluronidase enzyme. The invention may also be used to treat various other diseases, disorders and conditions in particular metabolic diseases, disorders and conditions.

[0072] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of “or” means “and/or” unless stated otherwise.

Hyaluronidase enzymes

[0073] Various hyaluronidase enzymes may be used to practice the present invention. For example, there are three groups of hyaluronidases based on their mechanisms of action. Two of the groups are endo- β -N-acetyl-hexosaminidases. One group includes the vertebrate enzymes that utilize substrate hydrolysis. The vertebrate hyaluronidases (EC 3.2.1.35) are endo- β -N-acetyl-hexosaminidases employing substrate hydrolysis for catalysis. The vertebrate hyaluronans also have transglycosidase activities, with the ability to cross-link chains of HA and the potential ability to cross-link chains of HA with ChS or Ch. The vertebrate hyaluronidases degrade HA through a non-processive endolytic process, generating mostly tetrasaccharides. Mammalian hyaluronidases are members of the group of carbohydrate-active enzymes (CAZy), termed glycosidase family 56, defined as endo- β -acetyl-hexosaminidases that utilize hydrolysis in catalysis of HA at the β 1,4 glycosidic linkages.

[0074] The second group, which is predominantly bacterial, includes the eliminases that function by β -elimination of the glycosidic linkage with introduction of an unsaturated bond. Bacterial hyaluronidases are also endo- β -acetyl-hexosaminidases, but utilize the lyase mechanism. They belong to a different CAZy family, to polysaccharide lyase family 8. In general, these polysaccharide lyases (EC 4.2.2.*) cleave by β -elimination, resulting in a double bond at the new non-reducing end. The hyaluronate lyases (EC 4.2.2.1; bacterial Hyal) consists of only one subgroup within family 8 that also include: chondroitin ABC lyases (EC 4.2.2.4), chondroitin AC lyases (EC 4.2.2.5), and xanthan lyases (EC 4.2.2.12). All of these bacterial enzymes, hyaluronidases, chondroitinases, and xanthanases, share significant sequence, structural, and mechanistic homology.

[0075] The third group is the endo- β -glucuronidases. These are found in leeches, which are annelids, and in certain crustaceans.

[0076] In addition, there are six known genes coding for hyaluronidase-like sequences in human genome, Hyal-1, Hyal-2, Hyal-3, Hyal-4, and PH-20/Spam1 and a pseudogene Phyal1

(not translated), all of which have high degree of homology. Mice also have six genes coding for hyaluronidases which have high degree of homology with human genes (Stern et al., Chem. Rev. 2006, 106(3): 818-839). In some embodiments, hyaluronidase may also be obtained from cows or pigs as a sterile preparation which is free of any other animal substance.

[0077] Bovine PH-20 is a commonly used hyaluronidase, and is available commercially in a reasonably pure form (Sigma catalog no. H3631, Type VI-S, from bovine testes, with an activity of 3,000 to 15,000 national formulary units (NFU) units/mg).

[0078] Hyaluronidase for injection can be obtained commercially in powder form or in solution. For example, an FDA approved bovine testicular hyaluronidase enzyme is available as a colorless odorless solution.

[0079] In some embodiments, an International Unit for hyaluronidase may be defined as the activity of 0.1 mg of the International Standard Preparation, and is equal to one turbidity reducing unit (TRU) (Humphrey JH et al., "International Standard for Hyaluronidase," Bull World Health Organ. 1957; 16(2): 291-294) based on the following reaction:

Hyaluronidase

Hyaluronic acid —————> **Di and monosaccharides + smaller hyaluronic acid fragments**

Accordingly, one unit of Hyaluronidase activity will cause a change in A_{600} of 0.330 per minute at pH 5.3 at 37 °C in a 2.0 ml reaction mixture (45 minute assay). % Transmittance is determined at 600 nm, Light path = 1 cm.

[0080] In some embodiments, an artificially synthesized bovine hyaluronidase PH-20 mRNA may be used for the present purpose.

[0081] In some embodiments, the bovine hyaluronidase mRNA used herein has a greater than 80% sequence identity to SEQ ID NO: 9 (GenBank ID No.: BC110183.1). In some embodiments, the bovine hyaluronidase mRNA used herein has greater than 90% sequence identity to SEQ ID NO: 9. In some embodiments, the mRNA has a sequence identity of greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, or greater than 98% sequence identity to SEQ ID NO: 9. In some embodiments, the bovine hyaluronidase mRNA used herein has 100% identity to SEQ ID NO: 9. In some embodiments the bovine hyaluronidase mRNA encodes for a PH-20 hyaluronidase which is about 90% identical to SEQ ID NO: 10 (GenBank ID No.: BC110183.1, cds sequence). In some embodiments, the mRNA encoded PH-20 hyaluronidase has a sequence identity of greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, or greater than 98% sequence identity to the sequence of SEQ ID NO: 10. In some embodiments, the bovine hyaluronidase has 100% identity to SEQ ID NO: 10. In some embodiments, the bovine hyaluronidase mRNA encodes a

protein which has an amino acid sequence having at least about 90% sequence identity with that of SEQ ID NO: 11. (GenBank ID No.: AAI10184.1). In some embodiments, the mRNA encodes a protein having amino acid sequence identity of greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, or greater than 98% sequence identity to SEQ ID NO: 11.

An exemplary bovine hyaluronidase mRNA sequence is given below:

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GGTTTATCTCTGTTCTTGGTGAGGAGACAGACAGAATTGACTGCTGTGCTCATCCGC
GAGGGTAAATGTG CTCAGCTCTT TATGGAGTAGTGGAGACGGGCAGAGATGACAA
GATGAAGCAACTTGCAAAACATTCCTAAATACGAAGGAAGAAGAATATTTAAATGA
AATCATCATTATTCATTTTTATCCATCAAAG TGGCTTCATTCTGTGTTTCATATCTTGC
ATCAAATATTAGGTACACCAAAGCGTGTAGGAG AAAAAAGTGCCTTTCACAGTCAT
CGCTCTTTGTGATGAGAATGCTGAGGC GCCACCATAT CTCCTTTCGGAGCTTTGCTG
GGTCTAGCGGAACACCCCAGGCAGTGTTACCTTCCTTCTGCTTCCGTGTTGTTTGGC
TCTGGACTTCAGAGCACCCCCTCTTATTTCAAACACTTCTTTCCTCTGGGCCTGGAAT
GCCCCAGTTGAACGTTGTGTTAACAGAAGATTTCAACTACCTCCAGATCTGAGACTC
TTCTCTGTAAAAGGAAGCCCCCAGAAAAGTGCTACCGGACAATTTATTACATTATTT
TATGCTGATAGACTTGGCTACTATCCTCATATAGATGAAA AAACAGGCAAAACCGT
ATTCGGAGGAATTCCCCAGTTGGGAAACTTAAAAAGTCATATGGAGAAAGCAAAAA
ATGACATTGCCTATTACATAACCAAATGACAGCGTGGGCTTGGCGGTCATTGACTGGG
AAAACCTGGAGGCCTACCTGGGCAAGAACTGGAAACCTAAAGATGTTTACAGGGAT
GAGTCAGTTGAGTTGGTTCTGCAAAAAAATCCGCAACTCAGTTTCCCAGAGGCTTCC
AAGATTGCAAAAGTGGAATTTTGAGACAGCAGGAAAGAGTTTCATGCAAGAGACTTT
AAAACCTGGGAAAATTACTTCGGCCAAATCACTTATGGGGTTATTATCTTTTTCTGA
TTGTTACAATCATAATCATAACCAACCTACTTACAATGGAAATTGCCCTGATGTGAA
AAAAGGAGAAATGATGATCTCGAGTGGTTGTGGAAGGAAAGCACTGCCCTTTTCCC
TTCTGTTTATTTGAATATCAGGTTAAAATCTACTCAAAATGCTGCCTTGTATGTTTCGT
AATCGTGTCCAGGAAGCCATTCGGTTGTCTAAAATAGCGAGTGTGAAAGTCCACTT
CCGGTTTTTGTATATGCCCGTCCAGTTTTTACTGATGGGTCTTCAACATATCTTTCTC
AGGGTGACCTTGTGAATTCGGTTGGTGAGATCGTTTCTCTAGGTGCCTCTGGGATTA
TAATGTGGGGCAGTCTCAATCTAAGCTTATCTATGCAATCTTGCATGAACCTAGGCA
CT
TACTTGAACACTACACTGAATCCTTACATAATCAACGTCACCCTAGCCGCCAAAATG
TGCAG CCAAGTGCTTTGCCACAATGAAGGAGTGTGTACAAGGAAACACTGGAATTC
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AAGCGACTATCTTCACCTGAACCCAATGAATTTTGCTATTCAAACCTGGGGAAGGTGG
 AAAATACACAGTACCTGGGACAGTCACACTTGAAGACTTGCAAAAGTTTCTGATAC
 ATTTTATTGCAGTTGTTATGCCAACATCCACTGTAAGAAGAGAGTTGATATAAAAAA
 TGTTTCATAGTGTTAACGTGTGTATGGCAGAAGACATTTGTATAGACAGCCCTGTGAA
 GTTACAACCCAGTGATCATTCCCTCCAGCCAGGAGGCATCTACTACCACCTTCAGCAG
 TATCTCACCCCTCCACTACAACCTGCCACAGTATCTCCATGTACTCCTGAGAAACACTC
 CCCTGAGTGCCTCAAAGTCAGGTGTTTCGGAAGTCATCCCCAACGTCACCCAAAAGG
 CGTGTCAAAGTGTTAAATTGAAGAACATTTCCCTATCAGTCACCTATTCAAAATATTA
 AAAATCAAACAACCTATTAATAATTAAATTCAGTAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGAAAAAA
 AAAAAAA (SEQ ID NO: 9)

Another exemplary bovine hyaluronidase mRNA sequence is given below:

ATGAGAATGCTGAGGCGCCACCATATCTCCTTTCGGAGCTTTGCTGGGTCTAGCGG
 AACACCCCAGGCAGTGTTACCTTCCTTCTGCTTCCGTGTTGTTTGGCTCTGGACTTC
 AGAGCACCCCCTCTTATTTCAAACACTTCTTTCCTCTGGGCCTGGAATGCCCCAGTTG
 AACGTTGTGTTAACAGAAGATTTCAACTACCTCCAGATCTGAGACTCTTCTCTGTAA
 AAGGAAGCCCCCAGAAAAGTGCTACCGGACAATTTATTACATTATTTTATGCTGATA
 GACTTGGCTACTATCCTCATATAGATGAAAAACAGGCAAAACCGTATTCGGAGGA
 ATTCCCCAGTTGGGAACTTAAAAAGTCATATGGAGAAAGCAAAAAATGACATTGC
 CTATTACATACCAAATGACAGCGTGGGCTTGGCGGTCATTGACTGGGAAAACCTGGA
 GGCCTACCTGGGCAAGAACTGGAAACCTAAAGATGTTTACAGGGATGAGTCAGTT
 GAGTTGGTTCTGCAAAAAAATCCGCAACTCAGTTTCCCAGAGGCTTCCAAGATTGCA
 AAAGTGGATTTTGAGACAGCAGGAAAGAGTTTCATGCAAGAGACTTTAAACCTGGG
 AAAATTACTTCGGCCAAATCACTTATGGGGTTATTATCTTTTTCCTGATTGTT
 ACAATCATAATCATAACCAACCTACTTACAATGGAAATTGCCCTGATGTAGAAAAA
 AGGAGAAATGATGATCTCGAGTGGTTGTGGAAGGAAAGCACTGCCCTTTTCCCTTCT
 GTTTATTTGAATATCAGGTAAAATCTACTCAAATGCTGCCTTGTATGTTTCGTAATC
 GTGTCCAGGAAGCCATTTCGGTTGTCTAAAATAGCGAGTGTCGAAAGTCCACTTCCGG
 TTTTGTATATGCCCCGTCCAGTTTTTACTGATGGGTCTTCAACATATCTTCTCAGGG
 TGACCTTGTGAATTCGGTTGGTGAGATCGTTTCTCTAGGTGCCTCTGGGATTATAATG
 TGGGGCAGTCTCAATCTAAGCTTATCTATGCAATCTTGCAATGAACCTAGGCACTTAC
 TTGAACACTACACTGAATCCTTACATAATCAACGTCACCCTAGCCGCCAAAATGTGC

AGCCAAGTGCTTTGCCACAATGAAGGAGTGTGTACAAGGAAACACTGGAATTCAAG
 CGACTATCTTCACCTGAACCCAATGAATTTTGCTATTCAAACCTGGGGAAGGTGGAAA
 ATACACAGTACCTGGGACAGTCACACTTGAAGACTTGCAAAAGTTTTCTGATACATT
 TTATTGCAGTTGTTATGCCAACATCCACTGTAAGAAGAGAGTTGATATAAAAAATGT
 TCATAGTGTTAACGTGTGTATGGCAGAAGACATTTGTATAGACAGCCCTGTGAAGTT
 ACAACCCAGTGATCATTCTCCAGCCAGGAGGCATCTACTACCACCTTCAGCAGTAT
 CTCACCCTCCACTACAACCTGCCACAGTATCTCCATGTACTCCTGAGAAACACTCCCC
 TGAGTGCCTCAAAGTCAGGTGTTTCGGAAGTCATCCCCAACGTCACCCAAAAGGCGT
 GTCAAAGTGTTAAATTGAAGAACATTTCTATCAGTCACCTATTCAAATATTAAAA
 ATCAAACAACCTATTA (SEQ ID NO: 10)

An exemplary translated protein sequence is:

MRMLRRHHISFRSFAGSSGTPQAVFTFLLLPCCLALDFRAPPLISNTSFLWAWNAPVERC
 VNRRFQLPPDLRLFSVKGSPQKSATGQFITLFYADRLGYYPHIDEKTGKTVFGGIPQLGN
 LKSHMEKAKNDIAYYIPN DSVGLAVIDWENWRPTWARNWKPKDVYRDESVLVLQK
 NPQLSFPEASKIAKVDFETAGKSFMQETLKLGLLRPNHLWGYLFPDCYNHNHNQPT
 YNGNCPDVEKRRNDLEWLWKESTALFPSVYLNIRLKSTQNAALYVRNRVQEAIRLSKI
 ASVESPLPVFVYARPVFTDGSSTYLSQGDVNSVGEIVSLGASGIIMWGSNLNLSMQSC
 MNLGTYLNTTLNPHYIINVTLAAKMCSQVLCHNEGVCTRKHWNSSDYLHLNPMNFAIQT
 GEGGKYTVPGTVTLEDLQKFSDTFYCSCYANIHCKKRVDIKNVHSVNVCMAEDICIDSP
 VKLQPSDHSSSQEASTTTFSSISPSTTTATVSPCTPEKHSPECLKVRCSEVIPNVTQKACQS
 VKLKNISYQSPIQNIKNQTTY (SEQ ID NO: 11).

[0082] In some embodiments, an artificially synthesized human hyaluronidase mRNA is administered for subcutaneous delivery of a therapeutic mRNA. The human hyaluronidase mRNA administered for subcutaneous delivery of a therapeutic mRNA has greater than 80% sequence identity to SEQ ID NO: 12 (GenBank ID No: AF040710). In some embodiments, the human hyaluronidase mRNA used herein has greater than 90% sequence identity to SEQ ID NO: 12. In some embodiments, the mRNA has a sequence identity of greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, or greater than 98% sequence identity to SEQ ID NO: 12. In some embodiments, the human hyaluronidase mRNA used herein has 100% identity to SEQ ID NO: 12. In some embodiments, the human hyaluronidase mRNA encodes a protein which has an amino acid sequence having at least about 90% sequence identity with that of SEQ ID NO: 13. (GenBank ID No: AAC70915.1). In some embodiments, the

mRNA encodes a protein having amino acid sequence identity of greater than 91%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, or greater than 98% sequence identity to SEQ ID NO: 13.

An exemplary human hyaluronidase mRNA sequence is given below:

ATGACCACGCAACTGGGCCCAGCCCTGGTGCTGGGGGTGGCCCTGTGCCTGGGTTGT
GGCCAGCCCCTACCACAGGTCCCTGAACGCCCCTTCTCTGTGCTGTGGAATGTACCC
TCAGCACACTGTGAGGCCCGCTTTGGTGTGCACCTGCCACTCAATGCTCTGGGCATC
ATAGCCAACCGTGGCCAGCATTTTCACGGTCAGAACATGACCATTTTCTACAAGAAC
CAACTCGGCCTCTATCCCTACTTTGGACCCAGGGGCACAGCTCACAATGGGGGCATC
CCCCAGGCTTTGCCCCTTGACCGCCACCTGGCACTGGCTGCCTACCAGATCCACCAC
AGCCTGAGACCTGGCTTTGCTGGCCCAGCAGTGCTGGATTGGGAGGAGTGGTGTCC
ACTCTGGGCTGGGAACTGGGGCCGCCGCCGAGCTTATCAGGCAGCCTCTTGGGCTTG
GGCACAGCAGGTATTCCCTGACCTGGACCCTCAGGAGCAGCTCTACAAGGCCTATA
CTGGCTTTGAGCAGGCGGCCCGTGCACTGATGGAGGATACGCTGCGGGTGGCCCAG
GCACTACGGCCCCATGGACTCTGGGGCTTCTATCACTACCCAGCCTGTGGCAATGGC
TGGCATAGTATGGCTTCCA ACTATAACCGGCCGCTGCCATGCAGCCACCCTTGCCCGC
AACACTCAACTGCATTGGCTCTGGGCCGCCTCCAGTGCCCTCTTCCCCAGCATCTAC
CTCCCACCCAGGCTGCCACCTGCCACCAACCAGGCCTTTGTCCGACATCGCCTGGAG
GAGGCCTTCCGTGTGGCCCTTGTTGGGCACCGACATCCCCTGCCTGTCCTGGCCTAT
GTCCGCCTCACACACCGGAGATCTGGGAGGTTCTGTCCCAGGATGACCTTGTGCAG
TCCATTGGTGTGAGTGCAGCACTAGGGGCAGCCGGCGTGGTGCTCTGGGGGGACCT
GAGCCTCTCCAGCTCTGAGGAGGAGTGCTGGCATCTCCATGACTACCTGGTGGACAC
CTTGGGGCCCCTATGTGATCAATGTGACCAGGGCAGCGATGGCCTGCAGTCACCAGC
GGTGCCATGGCCACGGGCGCTGTGCCCCGGCGAGATCCAGGACAGATGGAAGCCTTT
CTACACCTGTGGCCAGACGGCAGCCTTGGAGATTGGAAGTCCTTCAGCTGCCACTGT
TACTGGGGCTGGGCTGGCCCCACCTGCCAGGAGCCCAGCCTGGGCCTAAAGAAGCA
GTATAAAGCCAGGGCCCCCTGCCACTGCCTCTTCTTTTCCCTGCTGCCACTTTTCCAGT
CCTGGA ACTACTCTGTCCC ACTCTTGCTCTATT CAGTTTACAGTCAACCCTCCCAAGC
ACACACCCCGCTTCCCTTGGAATCCCTGA (SEQ ID NO: 12)

An exemplary human hyaluronidase protein sequence is given below:

MTTQLGPALVLGVALCLGCGQLPQVPERPFSVLWNVPSAHCEARFGVHLPLNALGIIA
NRGQHFHGGQNMIFYKNQLGLYPYFGPRGTAHNGGIPQALPLDRHLALAAAYQIHSLRP

GFAGPAVLDWEEWCPLWAGNWGRRRAYQAASWAWAQQVFPDLDPQEQLYKAYTGF
 EQAARALMEDTLRVAQALRPHGLWGFYHYPACGNGWHSMASNYTGRCHAATLARNT
 QLHWLWAASSALFPSIYLPRLPPAHHQAFVRHRLEEAFRVALVGHRHPLPVLAYVRLT
 HRRSGRFLSQDDLVSIGVSAALGAAGVVLWGDLSLSSSEEECWHLHDYLVDTLGPYVI
 NVTRAAMACSHQRCHGHGRCARRDPGQMEAFHLWPDGSLGDWKSFSCHCYWGWA
 GPTCQEPSLGLKKQYKARAPATASSFPCCHFSSPGTTLSHSCSIQFTVNPPKHTPRFPWNP
 (SEQ ID NO: 13)

[0083] In some embodiments, an mRNA encoding the full length or a fragment of the hyaluronidase is used.

[0084] An exemplary recombinant hyaluronidase dose of hyaluronidase is about 1 Unit to 50,000 Units. Accordingly, the hyaluronidase mRNA is administered at a dose equivalent so as to translate to a protein of the amount of less than 40,000U, less than 30,000U, less than 20,000U, less than 10,000U, less than 9000U, less than 8000U, less than 7000U, less than 6000U, less than 5000U less than 4000U, less than 3000U, less than 2000U, less than 1000U, less than 900U, less than 800U, less than 700U, less than 600U, or less than 500U. In some embodiments, the hyaluronidase mRNA is administered at a dose equivalent so as to translate to a protein of the amount of at least 1U, at least 5U, at least 10U, at least 20U, at least 30U, at least 40U, at least 50U, at least 60U, at least 70U, at least 80U, at least 100U, or at least 150U. In some other embodiments, the hyaluronidase mRNA is administered at a dose equivalent so as to translate to a protein of the amount of at least 160U, at least 180U, at least 200U, at least 220U, at least 240U, at least 260U, at least 280U, at least 300U, at least 320U, at least 340U, at least 360U, at least 380U, or at least 400U. In one or more embodiments, a porcine (pig) hyaluronidase is used at a dose ranging between 1-50,000 Units. The hyaluronidase mRNA is administered at a dose equivalent so as to translate to a protein of the amount of less than 40,000U, less than 30,000U, less than 20,000U, less than 10,000U, less than 9000U, less than 8000U, less than 7000U, less than 6000U, less than 5000U less than 4000U, less than 3000U, less than 2000U, less than 1000U, less than 900U, less than 800U, less than 700U, less than 600U, or less than 500U. The method of any one of the preceding claims, wherein the hyaluronidase mRNA is administered at a dose equivalent so as to translate to a protein of the of at least 1U, at least 5U, at least 10U, at least 20U, at least 30U, at least 40U, at least 50U, at least 60U, at least 70U, at least 80U, at least 100U, or at least 150U. In some other embodiments, the hyaluronidase mRNA is administered at a dose equivalent so as to translate to a protein of the amount of at least 160U, at least 180U, at least 200U, at least 220U, at least 240U, at least 260U,

at least 280U, at least 300U, at least 320U, at least 340U, at least 360U, at least 380U, or at least 400U.

[0085] In one or more embodiments, hyaluronidase mRNA is administered simultaneously with the therapeutic mRNA. In some embodiments, hyaluronidase may be administered prior to the administration of the mRNA. In some embodiments, the mRNA and the hyaluronidase enzyme are part of the same formulation. In some embodiments, the RNA and the hyaluronidase enzyme are injected as separate formulations.

[0086] In some embodiments, the mRNA encoding hyaluronidase may be administered in an aqueous solution. In some embodiments, the mRNA encoding hyaluronidase in saline solution. In some embodiments the hyaluronidase enzyme is part of the mRNA formulation and is present in the same solution, the solution comprising mRNA-encapsulated lipid nanoparticles. In some embodiments a lyophilized preparation comprising the mRNA-encapsulated lipid and the hyaluronidase enzyme is formulated for therapeutic use.

Messenger RNA (mRNA)

[0087] The present invention may be used to deliver any mRNA. As used herein, mRNA is the type of RNA that carries information from DNA to the ribosome for translation of the encoded protein. mRNAs may be synthesized according to any of a variety of known methods. For example, mRNAs according to the present invention may be synthesized via *in vitro* transcription (IVT). Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (e.g., T3, T7 or SP6 RNA polymerase), DNaseI, pyrophosphatase, and/or RNase inhibitor. The exact conditions will vary according to the specific application.

[0088] In some embodiments, *in vitro* synthesized mRNA may be purified before formulation and encapsulation to remove undesirable impurities including various enzymes and other reagents used during mRNA synthesis.

[0089] The present invention may be used to deliver mRNAs of a variety of lengths. In some embodiments, the present invention may be used to deliver *in vitro* synthesized mRNA of or greater than about 1 kb, 1.5 kb, 2 kb, 2.5 kb, 3 kb, 3.5 kb, 4 kb, 4.5 kb, 5 kb, 6 kb, 7 kb, 8 kb, 9 kb, 10 kb, 11 kb, 12 kb, 13 kb, 14 kb, 15 kb, or 20 kb in length. In some embodiments, the present invention may be used to deliver *in vitro* synthesized mRNA ranging from about 1-20 kb, about 1-15 kb, about 1-10 kb, about 5-20 kb, about 5-15 kb, about 5-12 kb, about 5-10 kb, about 8-20 kb, or about 8-15 kb in length.

[0090] The present invention may be used to deliver mRNA that is unmodified or mRNA containing one or more modifications that typically enhance stability. In some embodiments, modifications are selected from modified nucleotides, modified sugar phosphate backbones, and 5' and/or 3' untranslated region (UTR).

[0091] In some embodiments, modifications of mRNA may include modifications of the nucleotides of the RNA. A modified mRNA according to the invention can include, for example, backbone modifications, sugar modifications or base modifications. In some embodiments, mRNAs may be synthesized from naturally occurring nucleotides and/or nucleotide analogues (modified nucleotides) including, but not limited to, purines (adenine (A), guanine (G)) or pyrimidines (thymine (T), cytosine (C), uracil (U)), and as modified nucleotides analogues or derivatives of purines and pyrimidines, such as e.g. 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N-6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydrouracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queosine, .beta.-D-mannosyl-queosine, wybutoxosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine. The preparation of such analogues is known to a person skilled in the art e.g. from the U.S. Pat. No. 4,373,071, U.S. Pat. No. 4,401,796, U.S. Pat. No. 4,415,732, U.S. Pat. No. 4,458,066, U.S. Pat. No. 4,500,707, U.S. Pat. No. 4,668,777, U.S. Pat. No. 4,973,679, U.S. Pat. No. 5,047,524, U.S. Pat. No. 5,132,418, U.S. Pat. No. 5,153,319, U.S. Pat. Nos. 5,262,530 and 5,700,642, the disclosure of which is included here in its full scope by reference.

[0092] In some embodiments, mRNAs may contain RNA backbone modifications. Typically, a backbone modification is a modification in which the phosphates of the backbone of the nucleotides contained in the RNA are modified chemically. Exemplary backbone modifications typically include, but are not limited to, modifications from the group consisting of methylphosphonates, methylphosphoramidates, phosphoramidates, phosphorothioates (e.g. cytidine 5'-O-(1-thiophosphate)), boranophosphates, positively charged guanidinium groups etc.,

which means by replacing the phosphodiester linkage by other anionic, cationic or neutral groups.

[0093] In some embodiments, mRNAs may contain sugar modifications. A typical sugar modification is a chemical modification of the sugar of the nucleotides it contains including, but not limited to, sugar modifications chosen from the group consisting of 2'-deoxy-2'-fluoro-oligoribonucleotide (2'-fluoro-2'-deoxycytidine 5'-triphosphate, 2'-fluoro-2'-deoxyuridine 5'-triphosphate), 2'-deoxy-2'-deamine-oligoribonucleotide (2'-amino-2'-deoxycytidine 5'-triphosphate, 2'-amino-2'-deoxyuridine 5'-triphosphate), 2'-O-alkyloligoribonucleotide, 2'-deoxy-2'-C-alkyloligoribonucleotide (2'-O-methylcytidine 5'-triphosphate, 2'-methyluridine 5'-triphosphate), 2'-C-alkyloligoribonucleotide, and isomers thereof (2'-aracytidine 5'-triphosphate, 2'-arauridine 5'-triphosphate), or azidotriphosphates (2'-azido-2'-deoxycytidine 5'-triphosphate, 2'-azido-2'-deoxyuridine 5'-triphosphate).

[0094] In some embodiments, mRNAs may contain modifications of the bases of the nucleotides (base modifications). A modified nucleotide which contains a base modification is also called a base-modified nucleotide. Examples of such base-modified nucleotides include, but are not limited to, 2-amino-6-chloropurine riboside 5'-triphosphate, 2-aminoadenosine 5'-triphosphate, 2-thiocytidine 5'-triphosphate, 2-thiouridine 5'-triphosphate, 4-thiouridine 5'-triphosphate, 5-aminoallylcytidine 5'-triphosphate, 5-aminoallyluridine 5'-triphosphate, 5-bromocytidine 5'-triphosphate, 5-bromouridine 5'-triphosphate, 5-iodocytidine 5'-triphosphate, 5-iodouridine 5'-triphosphate, 5-methylcytidine 5'-triphosphate, 5-methyluridine 5'-triphosphate, 6-azacytidine 5'-triphosphate, 6-azauridine 5'-triphosphate, 6-chloropurine riboside 5'-triphosphate, 7-deazaadenosine 5'-triphosphate, 7-deazaguanosine 5'-triphosphate, 8-azaadenosine 5'-triphosphate, 8-azidoadenosine 5'-triphosphate, benzimidazole riboside 5'-triphosphate, N1-methyladenosine 5'-triphosphate, N1-methylguanosine 5'-triphosphate, N6-methyladenosine 5'-triphosphate, O6-methylguanosine 5'-triphosphate, pseudouridine 5'-triphosphate, puromycin 5'-triphosphate or xanthosine 5'-triphosphate.

[0095] Typically, mRNA synthesis includes the addition of a “cap” on the 5' end, and a “tail” on the 3' end. The presence of the cap is important in providing resistance to nucleases found in most eukaryotic cells. The presence of a “tail” serves to protect the mRNA from exonuclease degradation.

[0096] Thus, in some embodiments, mRNAs include a 5' cap structure. A 5' cap is typically added as follows: first, an RNA terminal phosphatase removes one of the terminal phosphate groups from the 5' nucleotide, leaving two terminal phosphates; guanosine triphosphate (GTP) is then added to the terminal phosphates via a guanylyl transferase,

producing a 5'-5' inverted triphosphate linkage; and the 7-nitrogen of guanine is then methylated by a methyltransferase. 2'-O-methylation may also occur at the first base and/or second base following the 7-methyl guanosine triphosphate residues. Examples of cap structures include, but are not limited to, m⁷GpppNp-RNA, m⁷GpppNmp-RNA and m⁷GpppNmpNmp-RNA (where m indicates 2'-O-methyl residues).

[0097] In some embodiments, mRNAs include a 3' poly(A) tail structure. A poly-A tail on the 3' terminus of mRNA typically includes about 10 to 300 adenosine nucleotides (e.g., about 10 to 200 adenosine nucleotides, about 10 to 150 adenosine nucleotides, about 10 to 100 adenosine nucleotides, about 20 to 70 adenosine nucleotides, or about 20 to 60 adenosine nucleotides). In some embodiments, mRNAs include a 3' poly(C) tail structure. A suitable poly-C tail on the 3' terminus of mRNA typically include about 10 to 200 cytosine nucleotides (e.g., about 10 to 150 cytosine nucleotides, about 10 to 100 cytosine nucleotides, about 20 to 70 cytosine nucleotides, about 20 to 60 cytosine nucleotides, or about 10 to 40 cytosine nucleotides). The poly-C tail may be added to the poly-A tail or may substitute the poly-A tail.

[0098] In some embodiments, mRNAs include a 5' and/or 3' untranslated region. In some embodiments, a 5' untranslated region includes one or more elements that affect an mRNA's stability or translation, for example, an iron responsive element. In some embodiments, a 5' untranslated region may be between about 50 and 500 nucleotides in length.

[0099] In some embodiments, a 3' untranslated region includes one or more of a polyadenylation signal, a binding site for proteins that affect an mRNA's stability of location in a cell, or one or more binding sites for miRNAs. In some embodiments, a 3' untranslated region may be between 50 and 500 nucleotides in length or longer.

Cap structure

[0100] In some embodiments, mRNAs include a 5' cap structure. A 5' cap is typically added as follows: first, an RNA terminal phosphatase removes one of the terminal phosphate groups from the 5' nucleotide, leaving two terminal phosphates; guanosine triphosphate (GTP) is then added to the terminal phosphates via a guanylyl transferase, producing a 5'-5' inverted triphosphate linkage; and the 7-nitrogen of guanine is then methylated by a methyltransferase. Examples of cap structures include, but are not limited to, m⁷G(5')ppp (5'(A,G(5')ppp(5')A and G(5')ppp(5')G.

[0101] Naturally occurring cap structures comprise a 7-methyl guanosine that is linked via a triphosphate bridge to the 5'-end of the first transcribed nucleotide, resulting in a dinucleotide cap of m⁷G(5')ppp(5')N, where N is any nucleoside. *In vivo*, the cap is added

enzymatically. The cap is added in the nucleus and is catalyzed by the enzyme guanylyl transferase. The addition of the cap to the 5' terminal end of RNA occurs immediately after initiation of transcription. The terminal nucleoside is typically a guanosine, and is in the reverse orientation to all the other nucleotides, i.e., G(5')ppp(5')GpNpNp.

[0102] A common cap for mRNA produced by *in vitro* transcription is m⁷G(5')ppp(5')G, which has been used as the dinucleotide cap in transcription with T7 or SP6 RNA polymerase *in vitro* to obtain RNAs having a cap structure in their 5'-termini. The prevailing method for the *in vitro* synthesis of capped mRNA employs a pre-formed dinucleotide of the form m⁷G(5')ppp(5')G (“m⁷GpppG”) as an initiator of transcription.

[0103] To date, a usual form of a synthetic dinucleotide cap used in *in vitro* translation experiments is the Anti-Reverse Cap Analog (“ARCA”) or modified ARCA, which is generally a modified cap analog in which the 2' or 3' OH group is replaced with -OCH₃.

[0104] Additional cap analogs include, but are not limited to, a chemical structures selected from the group consisting of m⁷GpppG, m⁷GpppA, m⁷GpppC; unmethylated cap analogs (e.g., GpppG); dimethylated cap analog (e.g., m^{2,7}GpppG), trimethylated cap analog (e.g., m^{2,2,7}GpppG), dimethylated symmetrical cap analogs (e.g., m⁷Gpppm⁷G), or anti reverse cap analogs (e.g., ARCA; m^{7,2'Ome}GpppG, m^{7,2'd}GpppG, m^{7,3'Ome}GpppG, m^{7,3'd}GpppG and their tetraphosphate derivatives) (see, e.g., Jemielity, J. et al., “Novel ‘anti-reverse’ cap analogs with superior translational properties”, RNA, 9: 1108-1122 (2003)).

[0105] In some embodiments, a suitable cap is a 7-methyl guanylate (“m⁷G”) linked via a triphosphate bridge to the 5'-end of the first transcribed nucleotide, resulting in m⁷G(5')ppp(5')N, where N is any nucleoside. A preferred embodiment of a m⁷G cap utilized in embodiments of the invention is m⁷G(5')ppp(5')G.

[0106] In some embodiments, the cap is a Cap0 structure. Cap0 structures lack a 2'-O-methyl residue of the ribose attached to bases 1 and 2. In some embodiments, the cap is a Cap1 structure. Cap1 structures have a 2'-O-methyl residue at base 2. In some embodiments, the cap is a Cap2 structure. Cap2 structures have a 2'-O-methyl residue attached to both bases 2 and 3.

[0107] A variety of m⁷G cap analogs are known in the art, many of which are commercially available. These include the m⁷GpppG described above, as well as the ARCA 3'-OCH₃ and 2'-OCH₃ cap analogs (Jemielity, J. et al., RNA, 9: 1108-1122 (2003)). Additional cap analogs for use in embodiments of the invention include N7-benzylated dinucleoside tetraphosphate analogs (described in Grudzien, E. et al., RNA, 10: 1479-1487 (2004)), phosphorothioate cap analogs (described in Grudzien-Nogalska, E., et al., RNA, 13: 1745-1755

(2007)), and cap analogs (including biotinylated cap analogs) described in U.S. Patent Nos. 8,093,367 and 8,304,529, incorporated by reference herein.

Tail structure

[0108] Typically, the presence of a “tail” serves to protect the mRNA from exonuclease degradation. The poly A tail is thought to stabilize natural messengers and synthetic sense RNA. Therefore, in certain embodiments a long poly A tail can be added to an mRNA molecule thus rendering the RNA more stable. Poly A tails can be added using a variety of art-recognized techniques. For example, long poly A tails can be added to synthetic or *in vitro* transcribed RNA using poly A polymerase (Yokoe, *et al.* Nature Biotechnology. 1996; 14: 1252-1256). A transcription vector can also encode long poly A tails. In addition, poly A tails can be added by transcription directly from PCR products. Poly A may also be ligated to the 3' end of a sense RNA with RNA ligase (see, e.g., Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1991 edition)).

[0109] In some embodiments, mRNAs include a 3' tail structure. Typically, a tail structure includes a poly(A) and/or poly(C) tail. A poly-A or poly-C tail on the 3' terminus of mRNA typically includes at least 50 adenosine or cytosine nucleotides, at least 150 adenosine or cytosine nucleotides, at least 200 adenosine or cytosine nucleotides, at least 250 adenosine or cytosine nucleotides, at least 300 adenosine or cytosine nucleotides, at least 350 adenosine or cytosine nucleotides, at least 400 adenosine or cytosine nucleotides, at least 450 adenosine or cytosine nucleotides, at least 500 adenosine or cytosine nucleotides, at least 550 adenosine or cytosine nucleotides, at least 600 adenosine or cytosine nucleotides, at least 650 adenosine or cytosine nucleotides, at least 700 adenosine or cytosine nucleotides, at least 750 adenosine or cytosine nucleotides, at least 800 adenosine or cytosine nucleotides, at least 850 adenosine or cytosine nucleotides, at least 900 adenosine or cytosine nucleotides, at least 950 adenosine or cytosine nucleotides, or at least 1 kb adenosine or cytosine nucleotides, respectively. In some embodiments, a poly-A or poly-C tail may be about 10 to 800 adenosine or cytosine nucleotides (e.g., about 10 to 200 adenosine or cytosine nucleotides, about 10 to 300 adenosine or cytosine nucleotides, about 10 to 400 adenosine or cytosine nucleotides, about 10 to 500 adenosine or cytosine nucleotides, about 10 to 550 adenosine or cytosine nucleotides, about 10 to 600 adenosine or cytosine nucleotides, about 50 to 600 adenosine or cytosine nucleotides, about 100 to 600 adenosine or cytosine nucleotides, about 150 to 600 adenosine or cytosine nucleotides, about 200 to 600 adenosine or cytosine nucleotides, about 250 to 600 adenosine or cytosine nucleotides, about 300 to 600 adenosine or cytosine nucleotides, about 350 to 600 adenosine or

cytosine nucleotides, about 400 to 600 adenosine or cytosine nucleotides, about 450 to 600 adenosine or cytosine nucleotides, about 500 to 600 adenosine or cytosine nucleotides, about 10 to 150 adenosine or cytosine nucleotides, about 10 to 100 adenosine or cytosine nucleotides, about 20 to 70 adenosine or cytosine nucleotides, or about 20 to 60 adenosine or cytosine nucleotides) respectively. In some embodiments, a tail structure includes is a combination of poly(A) and poly(C) tails with various lengths described herein. In some embodiments, a tail structure includes at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% adenosine nucleotides. In some embodiments, a tail structure includes at least 50%, 55%, 65%, 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% cytosine nucleotides.

[0110] In some embodiments, the length of the poly A or poly C tail is adjusted to control the stability of a modified sense mRNA molecule of the invention and, thus, the transcription of protein. For example, since the length of the poly A tail can influence the half-life of a sense mRNA molecule, the length of the poly A tail can be adjusted to modify the level of resistance of the mRNA to nucleases and thereby control the time course of polynucleotide expression and/or polypeptide production in a target cell.

5' and 3' Untranslated Region

[0111] In some embodiments, mRNAs include a 5' and/or 3' untranslated region. In some embodiments, a 5' untranslated region includes one or more elements that affect an mRNA's stability or translation, for example, an iron responsive element. In some embodiments, a 5' untranslated region may be between about 50 and 500 nucleotides in length.

[0112] In some embodiments, a 3' untranslated region includes one or more of a polyadenylation signal, a binding site for proteins that affect an mRNA's stability of location in a cell, or one or more binding sites for miRNAs. In some embodiments, a 3' untranslated region may be between 50 and 500 nucleotides in length or longer.

[0113] Exemplary 3' and/or 5' UTR sequences can be derived from mRNA molecules which are stable (e.g., globin, actin, GAPDH, tubulin, histone, or citric acid cycle enzymes) to increase the stability of the sense mRNA molecule. For example, a 5' UTR sequence may include a partial sequence of a CMV immediate-early 1 (IE1) gene, or a fragment thereof to improve the nuclease resistance and/or improve the half-life of the polynucleotide. Also contemplated is the inclusion of a sequence encoding human growth hormone (hGH), or a fragment thereof to the 3' end or untranslated region of the polynucleotide (e.g., mRNA) to further stabilize the polynucleotide. Generally, these modifications improve the stability and/or

pharmacokinetic properties (e.g., half-life) of the polynucleotide relative to their unmodified counterparts, and include, for example modifications made to improve such polynucleotides' resistance to *in vivo* nuclease digestion.

[0114] While mRNA provided from *in vitro* transcription reactions may be desirable in some embodiments, other sources of mRNA are contemplated as within the scope of the invention including mRNA produced from bacteria, fungi, plants, and/or animals.

[0115] The present invention may be used to deliver mRNAs encoding a variety of proteins. Non-limiting examples of mRNAs suitable for the present invention include mRNAs encoding target proteins such as argininosuccinate synthetase (ASS1), firefly luciferase (FFL), phenylalanine hydroxylase (PAH), and Ornithine transcarbamylase (OTC).

Exemplary mRNA sequences

[0116] In some embodiments, the present invention provides methods and compositions for delivering mRNA encoding a target protein to a subject for the treatment of the target protein deficiency. Exemplary mRNA sequences are shown below.

Construct design:

X – mRNA coding sequence – Y

5' and 3' UTR Sequences

X (5' UTR Sequence) =

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAAGACACC
GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUCCTCCG
UGCCAAGAGUGACUCACCGUCCUUGACACG (**SEQ ID NO: 1**)

Y (3' UTR Sequence) =

CGGGUGGCAUCCCUGUGACCCUCCCCAGUGCCUCUCCUGGCCCUUGGAAGUUGCC
ACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCAUCAAGCU (**SEQ ID NO: 2**)

OR

GGGUGGCAUCCCUGUGACCCUCCCCAGUGCCUCUCCUGGCCCUUGGAAGUUGCCA
CUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCAUCAAGCU (**SEQ ID NO: 3**)

An exemplary full-length codon-optimized human ornithine transcarbamylase (OTC) messenger RNA sequence is shown below:

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAAGACACC
 GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUCCCCG
 UGCCAAGAGUGACUCACCGUCCUUGACACGAUGCUGUUCAACCUUCGGAUCUUGC
 UGAACAACGCUGCGUUCGGAUUGGUCACAACUUCAUGGUCCGGAACUUCAGAUG
 CGGCCAGCCGCUCCAGAACAAGGUGCAGCUCAAGGGGAGGGACCUCACCCUG
 AAAACUUCACCGGAGAAGAGAUCAAGUACAUGCUGUGGCUGUCAGCCGACCUC
 AAUUCGGAUCAAGCAGAAGGGCGAAUACCUUCCUUUGCUGCAGGGAAAGUCCCU
 GGGGAUGAUCUUCGAGAAGCGCAGCACUCGCACUAGACUGUCAAACUGAAACCGGC
 UUCGCGCUGCUGGGAGGACACCCUGCUUCCUGACCACCCAAGAUAUCCAUCUGG
 GUGUGAACGAAUCCCUACCGACACAGCGCGGGUGCUGUCGUCCAUGGCAGACGC
 GGUCCUCGCCCCGCGUGUACAAGCAGUCUGAUCUGGACACUCUGGCCAAGGAAGCC
 UCCAUUCCUAUCAUUAUUGGAUUGUCCGACCUCUACCAUCCCAUCCAGAUUCUGG
 CCGAUUAUCUGACUCUGCAAGAACAUAUACAGCUCCCUGAAGGGGCUUACCCUUUC
 GUGGAUCGGCGACGGCAACAACAUUCUGCACAGCAUUAUGAUGAGCGCUGCCAAG
 UUUGGAAUGCACCUCCAAGCAGCGACCCCGAAGGGAUACGAGCCAGACGCCUCCG
 UGACGAAGCUGGCUGAGCAGUACGCCAAGGAGAACGGCACUAAGCUGCUGCUCAC
 CAACGACCCUCUCGAAGCCGCCACGGUGGCAACGUGCUGAUCACCGAUACCUGG
 AUCUCCAUGGGACAGGAGGAGGAAAAGAAGAAGCGCCUGCAAGCAUUUCAGGGG
 UACCAGGUGACUAUGAAAACCGCCAAGGUCGCCGCCUCGGACUGGACCUUCUUGC
 ACUGUCUGCCCAGAAAGCCCGAAGAGGUGGACGACGAGGUGUUCUACAGCCCGCG
 GUCGCUGGUCUUUCCGGAGGCCGAAAACAGGAAGUGGACUAUCAUGGCCGUGAU
 GGUGUCCCUUGCUGACCGAUUACUCCCCGCAGCUGCAGAAACCAAAGUUCUGA
 CGGGUGGCAUCCCUUGUGACCCCUCCCAGUGCCUCUCCUGGCCCUUGGAAGUUGCC
 ACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCAUCAAGCU (SEQ
 ID NO: 4).

An exemplary full length codon-optimized human ornithine transcarbamylase (OTC) messenger RNA sequence is shown below:

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAAGACACC
 GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUCCCCG
 UGCCAAGAGUGACUCACCGUCCUUGACACGAUGCUGUUCAACCUUCGGAUCUUGC

UGAACAACGCUGCGUUCCGGAAUGGUCACAACUUCAUGGUCCGGAACUUCAGAUG
 CGGCCAGCCGCUCCAGAACAAAGGUGCAGCUCAAGGGGAGGGACCUCUACCCUG
 AAAAACUUCACCGGAGAAGAGAUCAAGUACAUGCUGUGGCUGUCAGCCGACCUCA
 AAUUCCGGAUCAAGCAGAAGGGCGAAUACCUUCCUUUGCUGCAGGGAAAGUCCCU
 GGGGAUGAUCUUCGAGAAGCGCAGCACUCGCACUAGACUGUCAACUGAAACCGGC
 UUCGCGCUGCUGGGAGGACACCCUGCUUCCUGACCACCCAAGAUAUCCAUCUGG
 GUGUGAACGAAUCCCUACCGACACAGCGCGGGUGCUGUCGUCCAUGGCAGACGC
 GGUCCUCGCCCCGCGUGUACAAGCAGUCUGAUCUGGACACUCUGGCCAAGGAAGCC
 UCCAUUCCUAUCAUUAUGGAUUGUCCGACCUCUACCAUCCCAUCCAGAUUCUGG
 CCGAUUAUCUGACUCUGCAAGAACAUAACAGCUCCCUGAAGGGGCUUACCCUUUC
 GUGGAUCGGCGACGGCAACAACAUUCUGCACAGCAUUAUGAUGAGCGCUGCCAAG
 UUUGGAAUGCACCUCCAAGCAGCGACCCCGAAGGGAUACGAGCCAGACGCCUCCG
 UGACGAAGCUGGCUGAGCAGUACGCCAAGGAGAACGGCACUAAGCUGCUGCUCAC
 CAACGACCCUCUCGAAGCCGCCACGGUGGCAACGUGCUGAUCACCGAUACCUGG
 AUCUCCAUGGGACAGGAGGAGGAAAAGAAGAAGCGCCUGCAAGCAUUUCAGGGG
 UACCAGGUGACUAUGAAAACCGCCAAGGUCGCCGCCUCGGACUGGACCUUCUUGC
 ACUGUCUGCCCAGAAAGCCCGAAGAGGUGGACGACGAGGUGUUCUACAGCCCGCG
 GUCGCUGGUCUUUCCGGAGGCCGAAAACAGGAAGUGGACUAUCAUGGCCGUGAU
 GGUGUCCCUUGCUGACCGAUUACUCCCCGCAGCUGCAGAAACCAAAGUUCUGA
 GGGUGGCAUCCCUUGUGACCCCUCCCCAGUGCCUCUCCUGGCCCUUGGAAGUUGCCA
 CUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCAUCAAAGCU (SEQ
 ID NO: 5).

Another exemplary full length codon-optimized human ornithine transcarbamylase (OTC)
 messenger RNA sequence is shown below:

GGACAGAUCGCCUGGAGACGCCAUCCACGCUGUUUUGACCUCCAUAGAAGACACC
 GGGACCGAUCCAGCCUCCGCGGCCGGGAACGGUGCAUUGGAACGCGGAUUCCTCG
 UGCCAAGAGUGACUCACCGUCCUUGACACGAUGCUGUUUAACCGAGAGAAUUCUGC
 UGAACAACGCCGCGUUCAGGAACGGCCACAAUUUCAUGGUCCGCAACUUUAGAUG
 CGGACAGCCUCUCCAAAACAAGGUCCAGCUCAAGGGGCGGGACUUGCUGACCCUU
 AAGAACUUUACCGGCGAAGAGAUCAAGUACAUGCUGUGGUUGUCAGCGGACCUG
 AAGUCCGCAUCAAGCAGAAAGGGGAGUAUCUGCCGCGUCUCCAAGGAAAGUCGC
 UCGGCAUGAUCUUCGAGAAGCGCUCGACCAGAACCCGGCUGUCCACUGAAACUGG

UUUCGCCCCUUCUGGGUGGACACCCUUGUUUCCUGACAACCCAGGACAUCCAUCUG
 GGCGUGAACGAAAGCCUCACUGACACCGCCAGGGUGCUGAGCUCCAUGGCCGACG
 CUGUCCUUGCCCCGGGUGUACAAGCAGUCCGAUCUGGACACUCUGGCCAAGGAAGC
 GUCCAUCCCCGAUCAUUAACGGACUGUCCGACCUGUACCACCCGAUCCAGAUUCUG
 GCCGACUACCUGACCUUGCAAGAGCACUACAGCUCACUGAAGGGGCUUGACCCUGA
 GCUGGAUCGGCGACGGAAACAACAUUCUGCAUUCGAUCAUGAUGUCCGCGGCCAA
 GUUCGGAAUGCAUCUGCAGGCCGCAACUCCCAAGGGAUACGAACCUGAUGCGUCC
 GUGACUAAGCUGGCCGAGCAGUACGCAAAGGAAAACGGCACCAAGCUGCUGCUGA
 CCAACGACCCGCUCGAAGCUGCCCACGGAGGGGAACGUGCUCAUUACCGACACUUG
 GAUCUCCAUGGGGCAGGAAGAAGAGAAGAAGAAGCGGCUCCAGGCAUUCCAGGG
 UUACCAGGUCACCAUGAAAACGGCCAAAGUGGCCGCUUCGGAUUGGACUUUCCUC
 CACUGCCUUCCTCCGCAAACCUGAGGAAGUGGAUGAUGAAGUGUUCUACUCCCCAC
 GCUCCCUCGUGUUCCTCCGAGGCCGAGAAUCGGAAGUGGACCAUUAUGGCCGUGAU
 GGUGUCACUGCUGACCGACUACAGCCCCAACUGCAAAAAGCCGAAGUUCUGA
 CGGGUGGCAUCCCUGUGACCCCUCCTCCAGUGCCUCUCCUGGCCCUUGGAAGUUGCC
 ACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCAUCAAGCU (SEQ
 ID NO: 6)

Exemplary codon-optimized Human ASS1 (CO-hASS1) Coding Sequence

AUGAGCAGCAAGGGCAGCGUGGUGCUGGCCUACAGCGGCGGCCUGGACACCAGCU
 GCAUCCUGGUGUGGCUGAAGGAGCAGGGCUACGACGUGAUCGCCUACCUGGCCAA
 CAUCGGCCAGAAGGAGGACUUCGAGGAGGCCCGCAAGAAGGCCCUGAAGCUGGGC
 GCCAAGAAGGUGUUCAUCGAGGACGUGAGCCGCGAGUUCGUGGAGGAGUUCAUC
 UGGCCCGCCAUCCAGAGCAGCGCCUGUACGAGGACCGCUACCUGCUGGGCACCA
 GCCUGGCCCGCCCCUGCAUCGCCCCGCAAGCAGGUGGAGAUCGCCAGCGCGAGGG
 CGCCAAGUACGUGAGCCACGGCGCCACCGGCAAGGGCAACGACCAGGUGCGCUUC
 GAGCUGAGCUGCUACAGCCUGGCCCCCAGAUCAAGGUGAUCGCCCCCUGGCGCA
 UGCCCCGAGUUCUACAACCGCUUCAAGGGCCGCAACGACCUGAUGGAGUACGCCAA
 GCAGCACGGCAUCCCCAUCCCCGUGACCCCCAAGAACCCUGGAGCAUGGACGAG
 AACCUGAUGCACAUCAGCUACGAGGCCGGCAUCCUGGAGAACCCCAAGAACCAGG
 CCCCCCCCCGGCCUGUACACCAAGACCCAGGACCCCGCCAAGGCCCCCAACACCCCC
 GACAUCCUGGAGAUCGAGUUCAAGAAGGGCGUGCCCGUGAAGGUGACCAACGUG
 AAGGACGGCACCAACCACAGACCAGCCUGGAGCUGUUCAUGUACCUGAACGAGG

UGGCCGGCAAGCACGGCGUGGGCCGCAUCGACAUCGUGGAGAACCGCUUCAUCGG
 CAUGAAGAGCCGCGGCAUCUACGAGACCCCCGCCGGCACCAUCCUGUACCACGCC
 CACCUGGACAUCGAGGCCUUCACCAUGGACCGCGAGGUGCGCAAGAUCAAGCAGG
 GCCUGGGCCUGAAGUUCGCCGAGCUGGUGUACACCGGCUUCUGGCACAGCCCCGA
 GUGCGAGUUCGUGCGCCACUGCAUCGCCAAGAGCCAGGAGCGCGUGGAGGGCAAG
 GUGCAGGUGAGCGUGCUGAAGGGCCAGGUGUACAUCCUGGGCCGCGAGAGCCCCC
 UGAGCCUGUACAACGAGGAGCUGGUGAGCAUGAACGUGCAGGGCGACUACGAGC
 CCACCGACGCCACCGGCUUCAUCAACAUAACAGCCUGCGCCUGAAGGAGUACCA
 CCGCCUGCAGAGCAAGGUGACCGCCAAGUGA (SEQ ID NO: 7)

Exemplary codon-optimized Human PAH (CO-hPAH) Coding Sequence

AUGAGCACCGCCGUGCUGGAGAACCCCGGCCUGGGCCGCAAGCUGAGCGACUUCG
 GCCAGGAGACCAGCUACAUCGAGGACAACUGCAACCAGAACGGCGCCAUCAGCCU
 GAUCUUCAGCCUGAAGGAGGAGGUGGGCGCCUGGCCAAGGUGCUGCGCCUGUUC
 GAGGAGAACGACGUGAACCUGACCCACAUCGAGAGCCGCCCCAGCCGCCUGAAGA
 AGGACGAGUACGAGUUCUUCACCCACCUGGACAAGCGCAGCCUGCCCGCCUGAC
 CAACAUCAUCAAGAUCUGCGCCACGACAUCGGCGCCACCGUGCACGAGCUGAGC
 CGCGACAAGAAGAAGGACACCGUGCCUGGUUCCCCCGCACCAUCCAGGAGCUGG
 ACCGCUUCGCCAACCAGAUCCUGAGCUACGGCGCCGAGCUGGACGCCGACCACCC
 CGGCUUCAAGGACCCCGUGUACCGCGCCCGCCGCAAGCAGUUCGCCGACAUCGCC
 UACAACUACCGCCACGGCCAGCCCAUCCCCCGCGUGGAGUACAUGGAGGAGGAGA
 AGAAGACCUGGGGCACCGUGUUAAGACCCUGAAGAGCCUGUACAAGACCCACGC
 CUGCUACGAGUACAACCACAUCUCCCCCUGCUGGAGAAGUACUGCGGCUUCCAC
 GAGGACAACAUCCCCCAGCUGGAGGACGUGAGCCAGUUCCUGCAGACCUGCACCG
 GCUUCCGCCUGCGCCCCGUGGCCGGCCUGCUGAGCAGCCGCGACUUCCUGGGCGG
 CCUGGCCUUCGCGUGUUCCACUGCACCCAGUACAUCCGCCACGGCAGCAAGCCC
 AUGUACACCCCCGAGCCCGACAUCUGCCACGAGCUGCUGGGCCACGUGCCCCUGU
 UCAGCGACCGCAGCUUCGCCCAGUUCAGCCAGGAGAUCCGGCCUGGCCAGCCUGGG
 CGCCCCCGACGAGUACAUCGAGAAGCUGGGCCACCAUCUACUGGUUACCGUGGAG
 UUCGGCCUGUGCAAGCAGGGCGACAGCAUCAAGGCCUACGGCGCCGGCCUGCUGA
 GCAGCUUCGGCGAGCUGCAGUACUGCCUGAGCGAGAAGCCCAAGCUGCUGCCCCU
 GGAGCUGGAGAAGACCGCCAUCCAGAACUACACCGUGACCGAGUUCAGCCCCUG
 UACUACGUGGCCGAGAGCUUCAACGACGCCAAGGAGAAGGUGCGCAACUUCGCCG

CCACCAUCCCCCGCCCCUUCAGCGUGCGCUACGACCCCUACACCCAGCGCAUCGAG
 GUGCUGGACAACACCCAGCAGCUGAAGAUCUGGCCGACAGCAUCAACAGCGAGA
 UCGGCAUCCUGUGCAGCGCCCUGCAGAAGAUCAAGUAA (SEQ ID NO: 8)

[0117] In some embodiments, a suitable mRNA sequence may encode a homolog or an analog of target protein. For example, a homolog or an analog of target protein may be a modified target protein containing one or more amino acid substitutions, deletions, and/or insertions as compared to a wild-type or naturally-occurring target protein while retaining substantial target protein activity. In some embodiments, an mRNA suitable for the present invention encodes an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to the above exemplary sequences. In some embodiments, an mRNA suitable for the present invention encodes a protein substantially identical to target protein. In some embodiments, an mRNA suitable for the present invention encodes an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the above exemplary sequences. In some embodiments, an mRNA suitable for the present invention encodes a fragment or a portion of target protein. In some embodiments, an mRNA suitable for the present invention encodes a fragment or a portion of target protein, wherein the fragment or portion of the protein still maintains target activity similar to that of the wild-type protein. In some embodiments, an mRNA suitable for the present invention has a nucleotide sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the above exemplary sequences.

[0118] In some embodiments, a suitable mRNA encodes a fusion protein comprising a full length, fragment or portion of a target protein fused to another protein (e.g., an N or C terminal fusion). In some embodiments, the protein fused to the mRNA encoding a full length, fragment or portion of a target protein encodes a signal or a cellular targeting sequence.

Lipid Nanoparticles

[0119] According to the present invention, mRNA may be encapsulated or complexed in nanoparticles. In some embodiments, nanoparticles are also referred to as “delivery vehicle,” “transfer vehicle”, or grammatical equivalents.

[0120] According to various embodiments, suitable nanoparticles include, but are not limited to polymer based carriers, such as polyethylenimine (PEI), lipid nanoparticles and

liposomes, nanoliposomes, ceramide-containing nanoliposomes, proteoliposomes, both natural and synthetically-derived exosomes, natural, synthetic and semi-synthetic lamellar bodies, nanoparticulates, calcium phosphor-silicate nanoparticulates, calcium phosphate nanoparticulates, silicon dioxide nanoparticulates, nanocrystalline particulates, semiconductor nanoparticulates, poly(D-arginine), sol-gels, nanodendrimers, starch-based delivery systems, micelles, emulsions, niosomes, multi-domain-block polymers (vinyl polymers, polypropyl acrylic acid polymers, dynamic polyconjugates), dry powder formulations, plasmids, viruses, calcium phosphate nucleotides, aptamers, peptides and other vectorial tags.

[0121] In some embodiments, the mRNA is encapsulated within one or more liposomes. As used herein, the term “liposome” refers to any lamellar, multilamellar, or solid nanoparticle vesicle. Typically, a liposome as used herein can be formed by mixing one or more lipids or by mixing one or more lipids and polymer(s). Thus, the term “liposome” as used herein encompasses both lipid and polymer based nanoparticles. In some embodiments, a liposome suitable for the present invention contains cationic, non-cationic lipid(s), cholesterol-based lipid(s) and/or PEG-modified lipid(s).

PEGylated Lipids

[0122] In some embodiments, a suitable lipid solution includes one or more PEGylated lipids. For example, the use of polyethylene glycol (PEG)-modified phospholipids and derivatized lipids such as derivatized ceramides (PEG-CER), including N-Octanoyl-Sphingosine-1-[Succinyl(Methoxy Polyethylene Glycol)-2000] (C8 PEG-2000 ceramide) is also contemplated by the present invention. Contemplated PEG-modified lipids include, but are not limited to, a polyethylene glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C₆-C₂₀ length. In some embodiments, a PEG-modified or PEGylated lipid is PEGylated cholesterol or PEG-2K. In some embodiments, particularly useful exchangeable lipids are PEG-ceramides having shorter acyl chains (e.g., C₁₄ or C₁₈).

[0123] PEG-modified phospholipid and derivatized lipids may constitute at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, or at least 10% of the total lipids in the liposome.

Cationic Lipids

[0124] As used herein, the phrase “cationic lipids” refers to any of a number of lipid species that have a net positive charge at a selected pH, such as physiological pH. Several cationic lipids have been described in the literature, many of which are commercially available.

Particularly suitable cationic lipids for use in the compositions and methods of the invention include those described in international patent publications WO 2010/053572 (and particularly, C12-200 described at paragraph [00225]) and WO 2012/170930, both of which are incorporated herein by reference. In certain embodiments, cationic lipids suitable for the compositions and methods of the invention include an ionizable cationic lipid described in U.S. provisional patent application 61/617,468, filed March 29, 2012 (incorporated herein by reference), such as, e.g., (15Z, 18Z)-N,N-dimethyl-6-(9Z, 12Z)-octadeca-9, 12-dien-1-yl)tetracos-15,18-dien-1-amine (HGT5000), (15Z, 18Z)-N,N-dimethyl-6-((9Z, 12Z)-octadeca-9, 12-dien-1-yl)tetracos-4,15,18-trien-1-amine (HGT5001), and (15Z,18Z)-N,N-dimethyl-6-((9Z, 12Z)-octadeca-9, 12-dien-1-yl)tetracos-5, 15, 18-trien-1-amine (HGT5002).

[0125] In some embodiments, cationic lipids suitable for the compositions and methods of the invention include cationic lipids such as 3,6-bis(4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)piperazine-2,5-dione (OF-02).

[0126] In some embodiments, cationic lipids suitable for the compositions and methods of the invention include a cationic lipid described in WO 2015/184256 A2 entitled "Biodegradable lipids for delivery of nucleic acids" which is incorporated by reference herein such as 3-(4-(bis(2-hydroxydodecyl)amino)butyl)-6-(4-((2-hydroxydodecyl)(2-hydroxyundecyl)amino)butyl)-1,4-dioxane-2,5-dione (Target 23), 3-(5-(bis(2-hydroxydodecyl)amino)pentan-2-yl)-6-(5-((2-hydroxydodecyl)(2-hydroxyundecyl)amino)pentan-2-yl)-1,4-dioxane-2,5-dione (Target 24).

[0127] In some embodiments, cationic lipids suitable for the compositions and methods of the invention include a cationic lipid described in WO 2013/063468 and in U.S. provisional application entitled "Lipid Formulations for Delivery of Messenger RNA", both of which are incorporated by reference herein.

[0128] In some embodiments, one or more cationic lipids suitable for the present invention may be N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride or "DOTMA". (Feigner et al. (Proc. Nat'l Acad. Sci. 84, 7413 (1987); U.S. Pat. No. 4,897,355). Other suitable cationic lipids include, for example, 5-carboxyspermylglycinedioctadecylamide or "DOGS," 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium or "DOSPA" (Behr et al. Proc. Nat'l Acad. Sci. 86, 6982 (1989); U.S. Pat. No. 5,171,678; U.S. Pat. No. 5,334,761), 1,2-Dioleoyl-3-Dimethylammonium-Propane or "DODAP", 1,2-Dioleoyl-3-Trimethylammonium-Propane or "DOTAP".

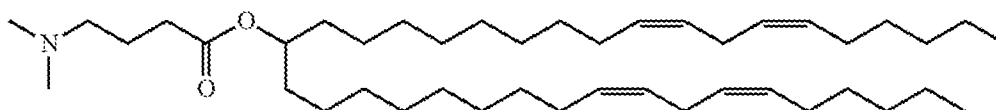
[0129] Additional exemplary cationic lipids also include 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane or "DSDMA", 1,2-dioleoyloxy-N,N-dimethyl-3-aminopropane or "DODMA", 1

,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane or "DLinDMA", 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane or "DLenDMA", N-dioleoyl-N,N-dimethylammonium chloride or "DODAC", N,N-distearyl-N,N-dimethylammonium bromide or "DDAB", N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide or "DMRIE", 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-octadecadienoxy)propane or "CLinDMA", 2-[5'-(cholest-5-en-3-beta-oxy)-3'-oxapentoxo]-3-dimethyl-1-(cis,cis-9',12'-octadecadienoxy)propane or "CpLinDMA", N,N-dimethyl-3,4-dioleoyloxybenzylamine or "DMOBA", 1,2-N,N'-dioleoylcarbonyl-3-dimethylaminopropane or "DOcarbDAP", 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine or "DLinDAP", 1,2-N,N'-Dilinoleoylcarbonyl-3-dimethylaminopropane or "DLincarbDAP", 1,2-Dilinoleoylcarbonyl-3-dimethylaminopropane or "DLinCDAP", 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane or "DLin-DMA", 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane or "DLin-K-XTC2-DMA", and 2-(2,2-di((9Z,12Z)-octadeca-9,12-dien-1-yl)-1,3-dioxolan-4-yl)-N,N-dimethylethanamine (DLin-KC2-DMA)) (see, WO 2010/042877; Semple et al., *Nature Biotech.* 28: 172-176 (2010)), or mixtures thereof. (Heyes, J., et al., *J Controlled Release* 107: 276-287 (2005); Morrissey, DV., et al., *Nat. Biotechnol.* 23(8): 1003-1007 (2005); PCT Publication WO2005/121348A1). In some embodiments, one or more of the cationic lipids comprise at least one of an imidazole, dialkylamino, or guanidinium moiety.

[0130] In some embodiments, one or more cationic lipids may be chosen from XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane), MC3 (((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate), ALNY-100 ((3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine)), NC98-5 (4,7,13-tris(3-oxo-3-(undecylamino)propyl)-N1,N16-diundecyl-4,7,10,13-tetraazahexadecane-1,16-diamide),

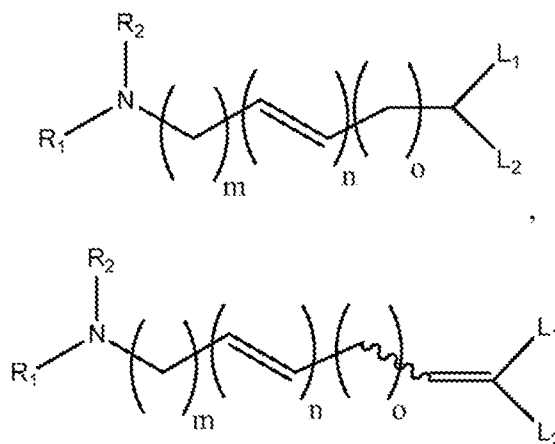
[0131] The term "cationic lipids" refers to any of a number of lipid and lipidoid species that have a net positive charge at a selected pH, such as at physiological pH.

[0132] Suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2010/14474, which is incorporated herein by reference. In certain embodiments, the compositions and methods of the present invention include a cationic lipid, (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino) butanoate, having a compound structure of:

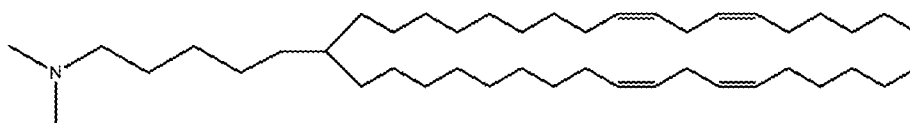


and pharmaceutically acceptable salts thereof.

[0133] Other suitable cationic lipids for use in the compositions and methods of the present invention include ionizable cationic lipids as described in International Patent Publication WO 2013/149140, which is incorporated herein by reference. In some embodiments, the compositions and methods of the present invention include a cationic lipid of one of the following formulas:

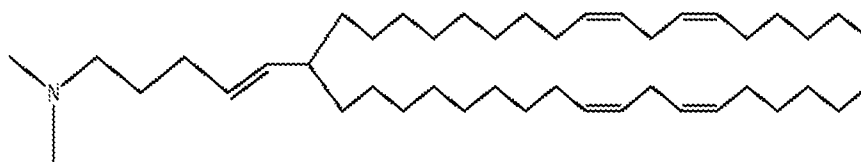


or a pharmaceutically acceptable salt thereof, wherein R_1 and R_2 are each independently selected from the group consisting of hydrogen, an optionally substituted, variably saturated or unsaturated C_1 - C_{20} alkyl and an optionally substituted, variably saturated or unsaturated C_6 - C_{20} acyl; wherein L_1 and L_2 are each independently selected from the group consisting of hydrogen, an optionally substituted C_1 - C_{30} alkyl, an optionally substituted variably unsaturated C_1 - C_{30} alkenyl, and an optionally substituted C_1 - C_{30} alkynyl; wherein m and o are each independently selected from the group consisting of zero and any positive integer (e.g., where m is three); and wherein n is zero or any positive integer (e.g., where n is one). In certain embodiments, the compositions and methods of the present invention include the cationic lipid (15Z, 18Z)-N,N-dimethyl-6-(9Z,12Z)-octadeca-9,12-dien-1-yl) tetracos-15,18-dien-1-amine (“HGT5000”), having a compound structure of:



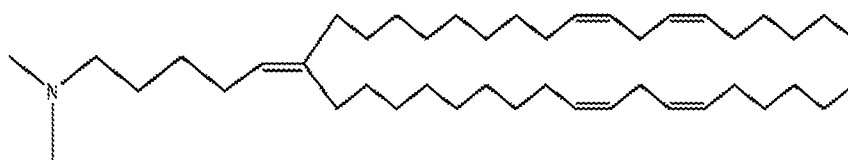
(HGT-5000)

and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include the cationic lipid (15Z, 18Z)-N,N-dimethyl-6-((9Z,12Z)-octadeca-9,12-dien-1-yl) tetracos-4,15,18-trien-1-amine (“HGT5001”), having a compound structure of:



(HGT-5001)

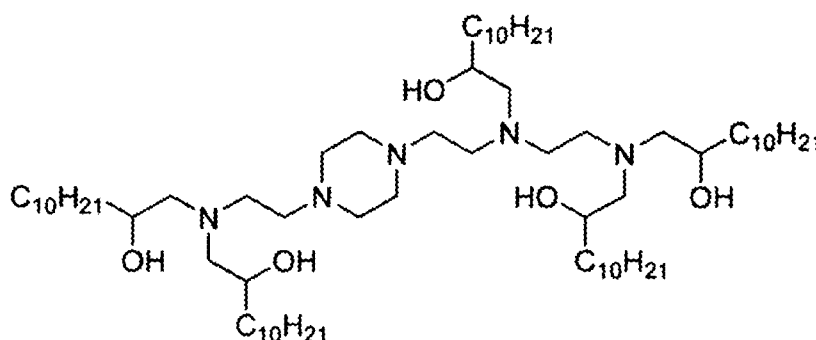
and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include the cationic lipid and (15Z,18Z)-N,N-dimethyl-6-((9Z,12Z)-octadeca-9,12-dien-1-yl) tetracos-5,15,18-trien-1-amine (“HGT5002”), having a compound structure of:



(HGT-5002)

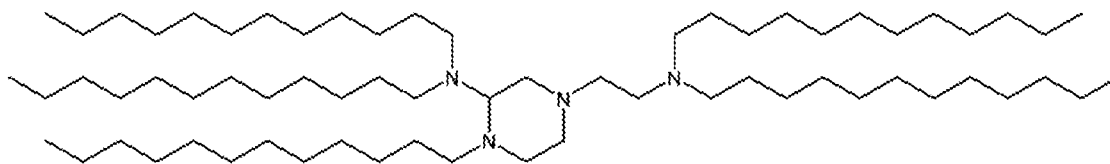
and pharmaceutically acceptable salts thereof.

[0134] Other suitable cationic lipids for use in the compositions and methods of the invention include cationic lipids described as aminoalcohol lipidoids in International Patent Publication WO 2010/053572, which is incorporated herein by reference. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



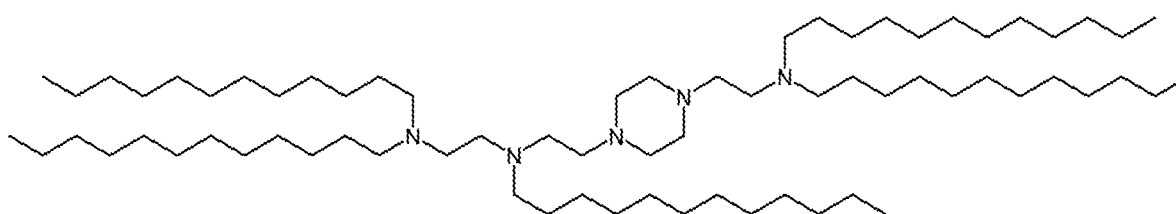
and pharmaceutically acceptable salts thereof.

[0135] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2016/118725, which is incorporated herein by reference. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



and pharmaceutically acceptable salts thereof.

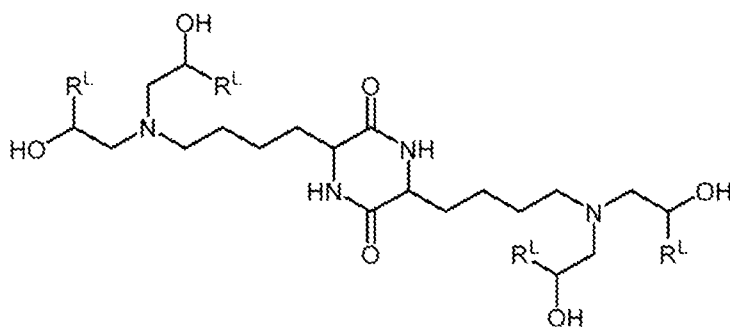
[0136] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2016/118724, which is incorporated herein by reference. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



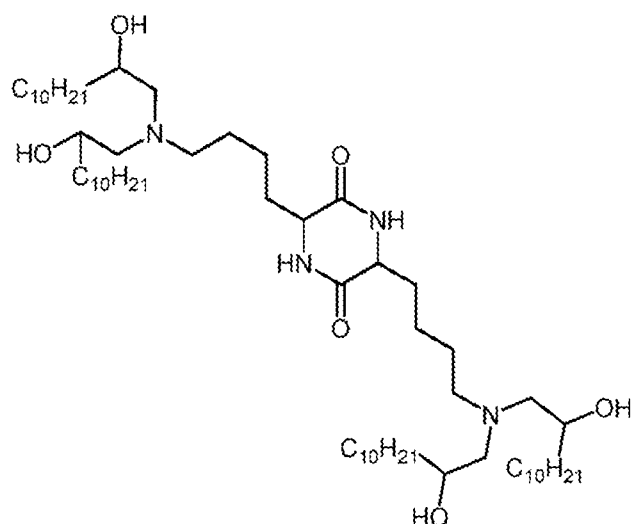
and pharmaceutically acceptable salts thereof.

[0137] Other suitable cationic lipids for use in the compositions and methods of the invention include a cationic lipid having the formula of 14, 25-ditridecyl 15,18,21,24-tetraaza-octatriacontane, and pharmaceutically acceptable salts thereof.

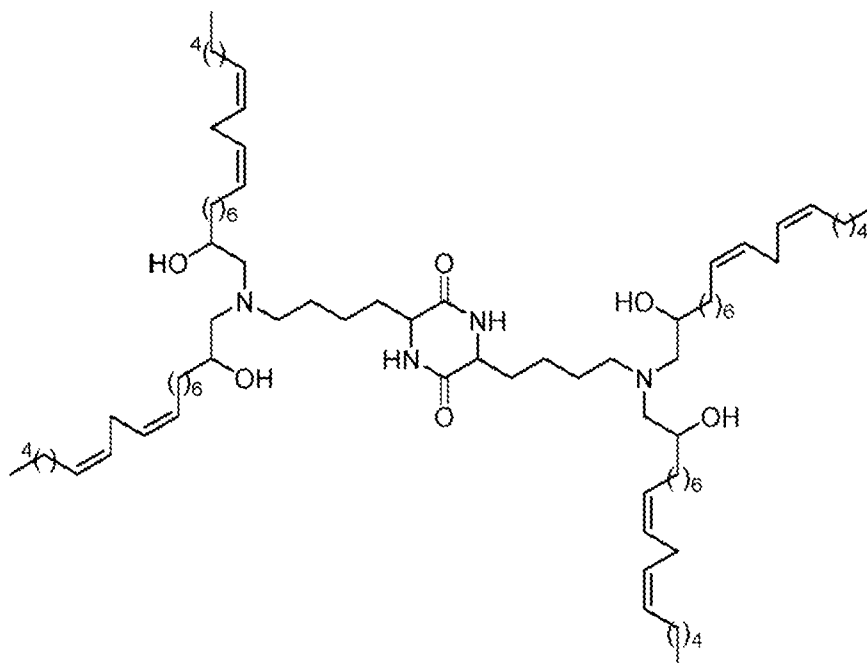
[0138] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publications WO 2013/063468 and WO 2016/205691, each of which are incorporated herein by reference. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:



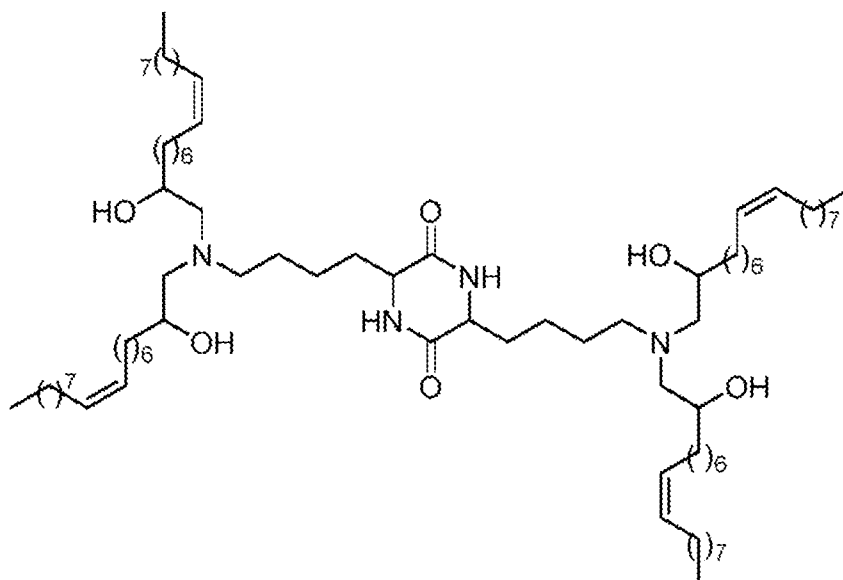
or pharmaceutically acceptable salts thereof, wherein each instance of R^L is independently optionally substituted C_6 - C_{40} alkenyl. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



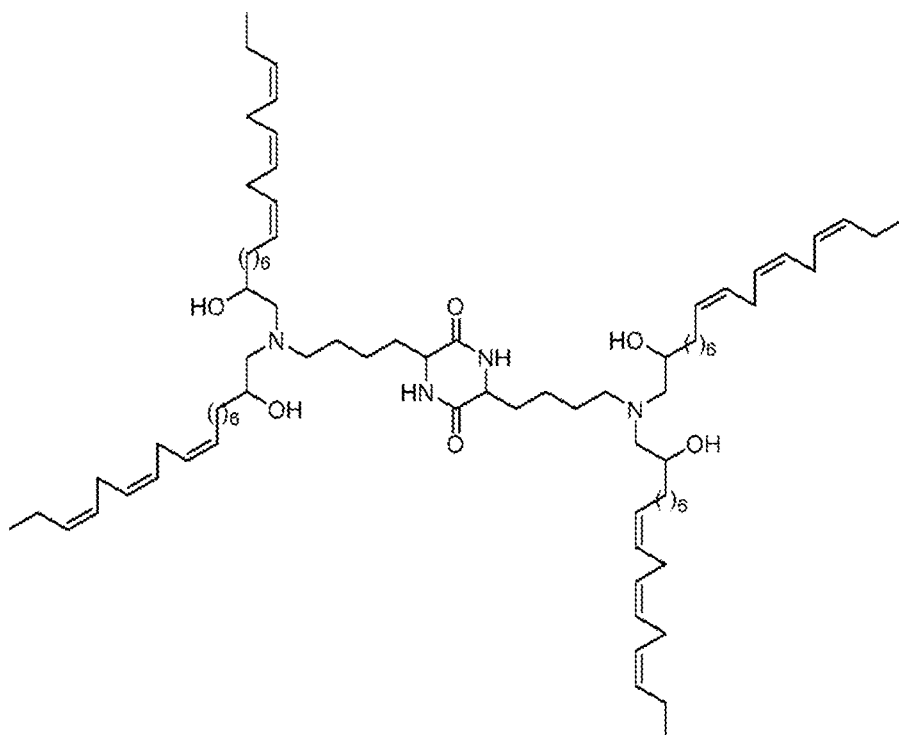
and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:

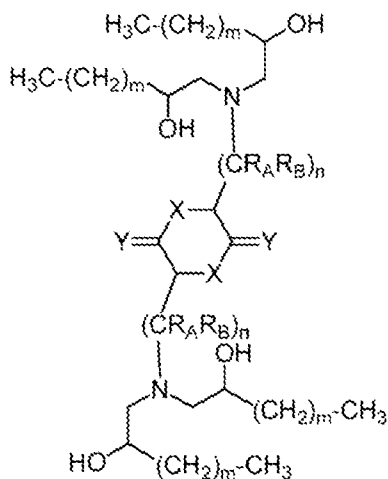


and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:

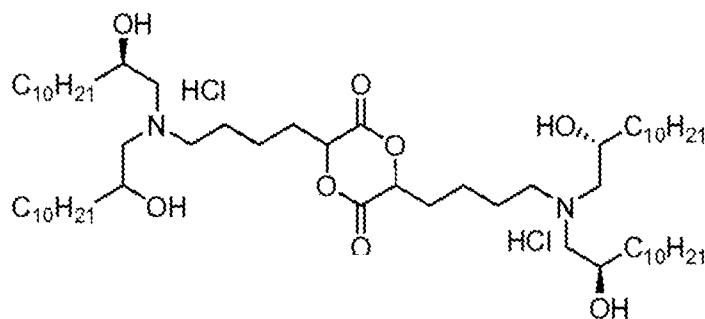


and pharmaceutically acceptable salts thereof.

[0139] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2015/184256, which is incorporated herein by reference. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:



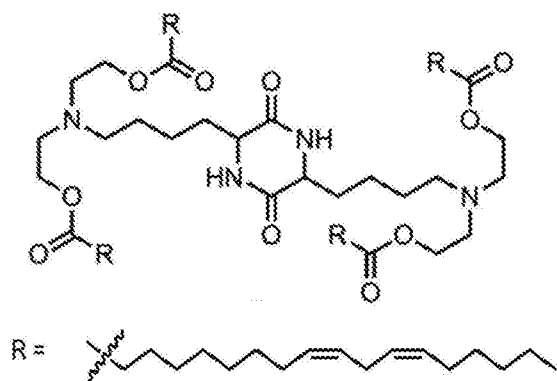
or a pharmaceutically acceptable salt thereof, wherein each X independently is O or S; each Y independently is O or S; each m independently is 0 to 20; each n independently is 1 to 6; each R_A is independently hydrogen, optionally substituted C1-50 alkyl, optionally substituted C2-50 alkenyl, optionally substituted C2-50 alkynyl, optionally substituted C3-10 carbocyclyl, optionally substituted 3-14 membered heterocyclyl, optionally substituted C6-14 aryl, optionally substituted 5-14 membered heteroaryl or halogen; and each R_B is independently hydrogen, optionally substituted C1-50 alkyl, optionally substituted C2-50 alkenyl, optionally substituted C2-50 alkynyl, optionally substituted C3-10 carbocyclyl, optionally substituted 3-14 membered heterocyclyl, optionally substituted C6-14 aryl, optionally substituted 5-14 membered heteroaryl or halogen. In certain embodiments, the compositions and methods of the present invention include a cationic lipid, “Target 23”, having a compound structure of:



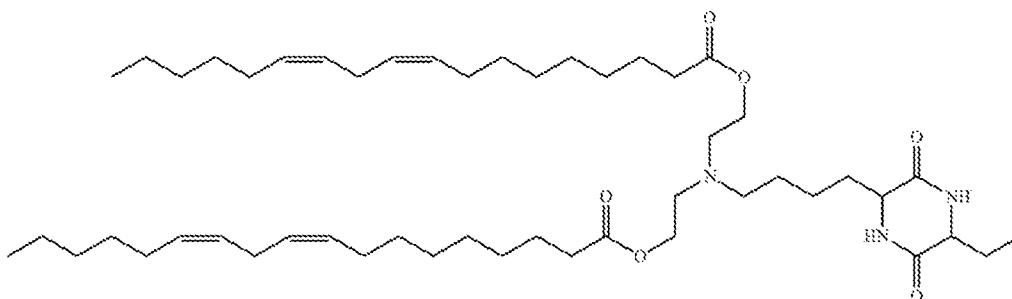
(Target 23)

[0140] and pharmaceutically acceptable salts thereof.

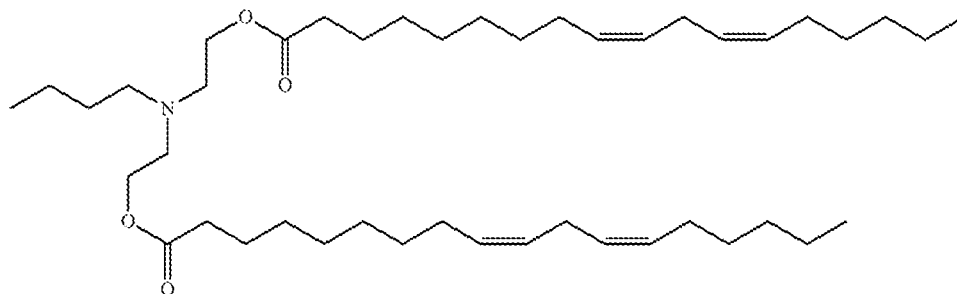
[0141] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2016/004202, which is incorporated herein by reference. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



or a pharmaceutically acceptable salt thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

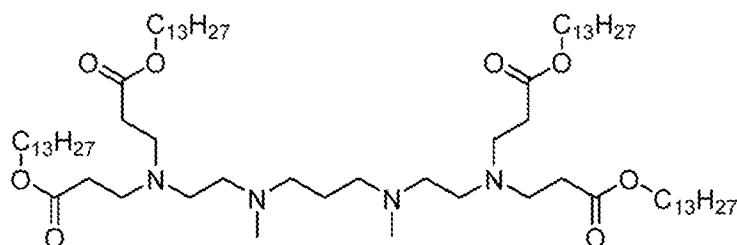


or a pharmaceutically acceptable salt thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



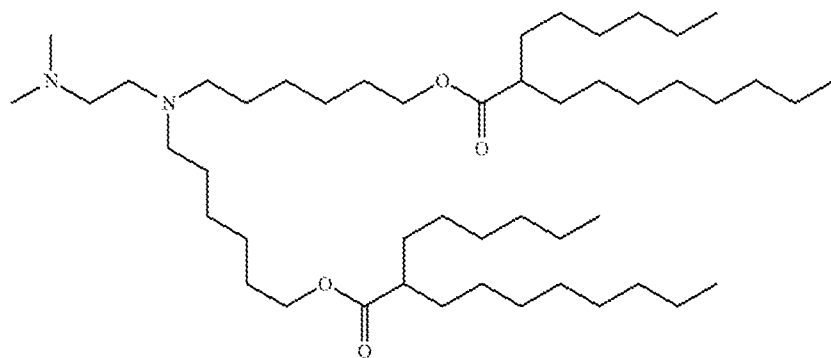
or a pharmaceutically acceptable salt thereof.

[0142] Other suitable cationic lipids for use in the compositions and methods of the present invention include the cationic lipids as described in J. McClellan, M. C. King, Cell 2010, 141, 210-217 and in Whitehead et al., Nature Communications (2014) 5:4277, which is incorporated herein by reference. In certain embodiments, the cationic lipids of the compositions and methods of the present invention include a cationic lipid having a compound structure of:

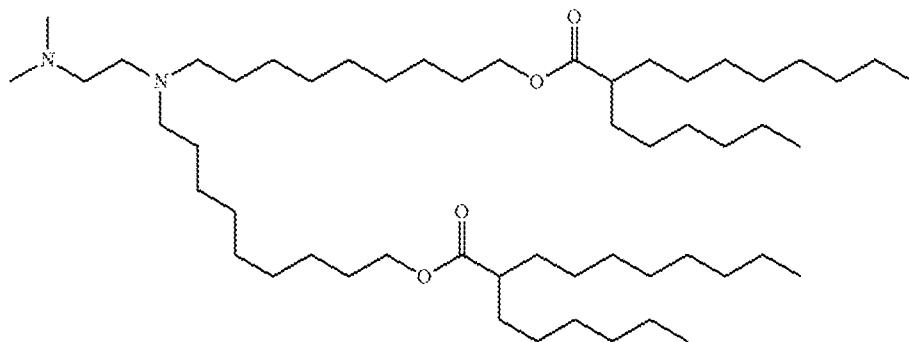


and pharmaceutically acceptable salts thereof.

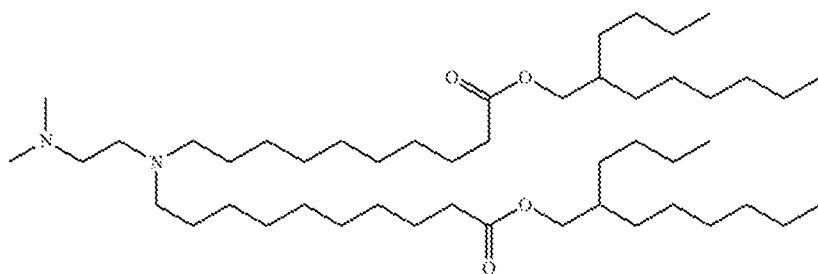
[0143] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2015/199952, which is incorporated herein by reference. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



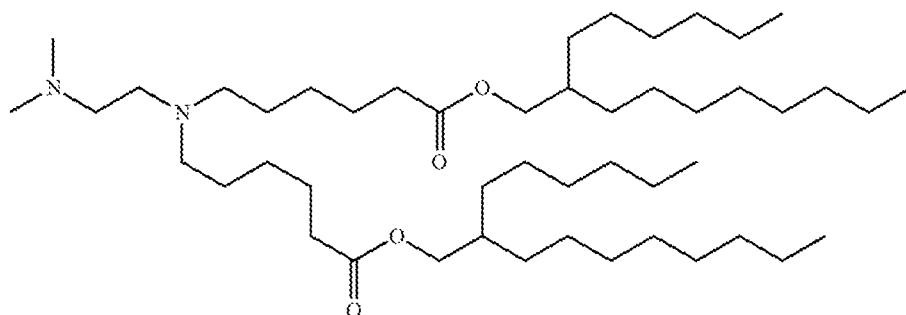
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



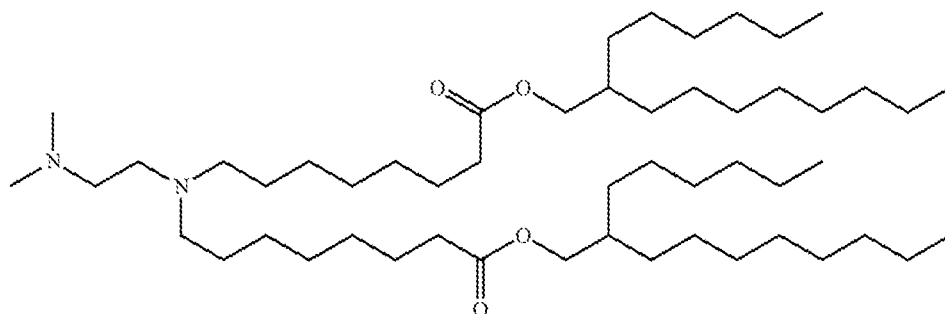
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



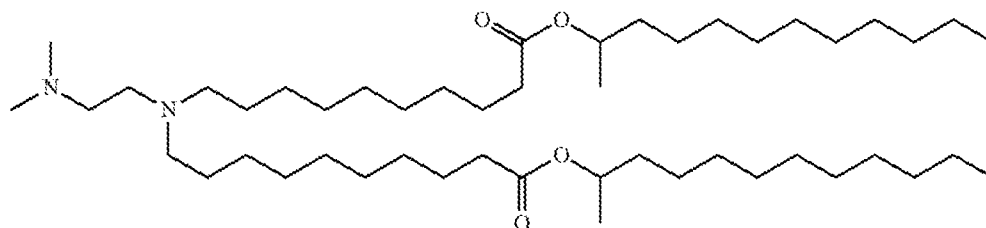
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



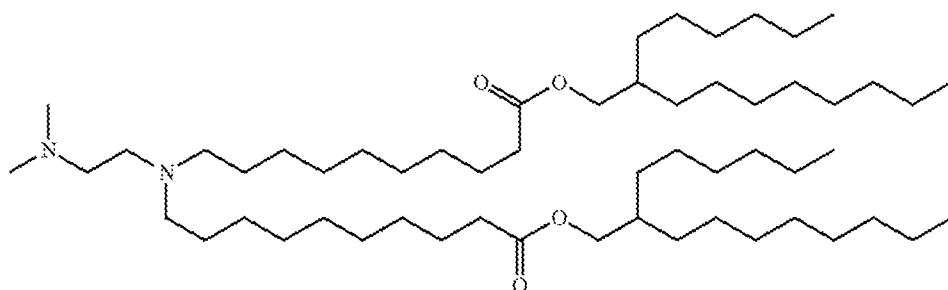
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



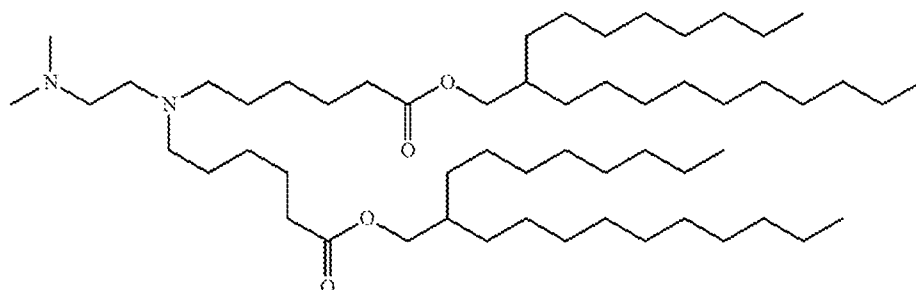
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



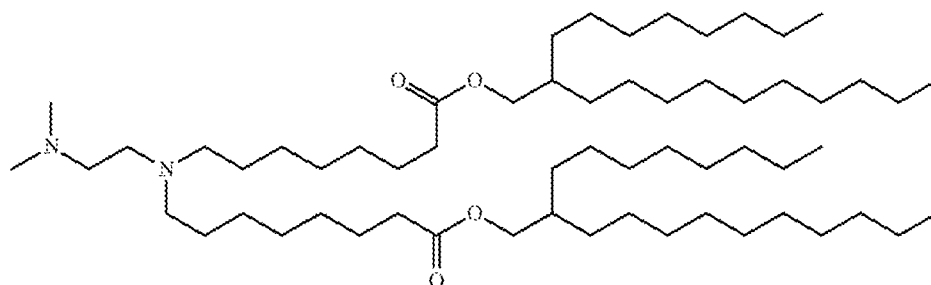
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



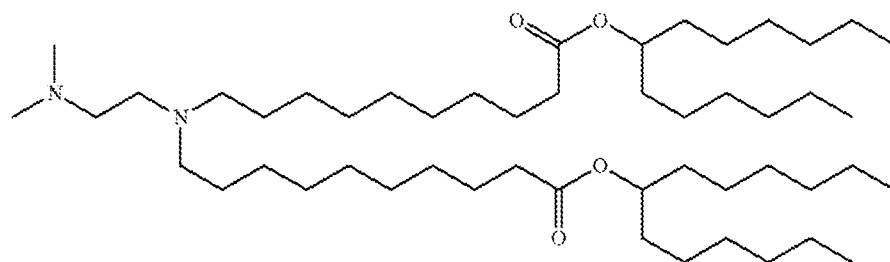
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



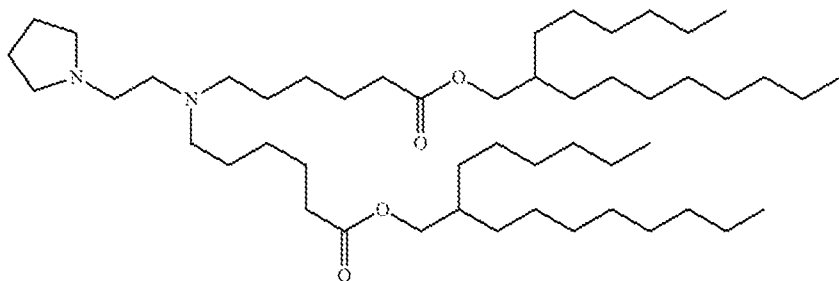
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



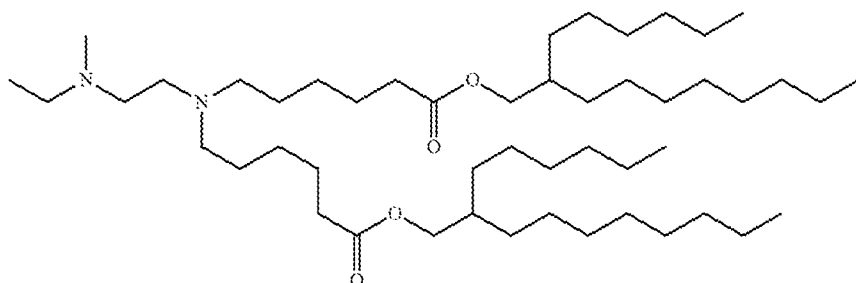
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



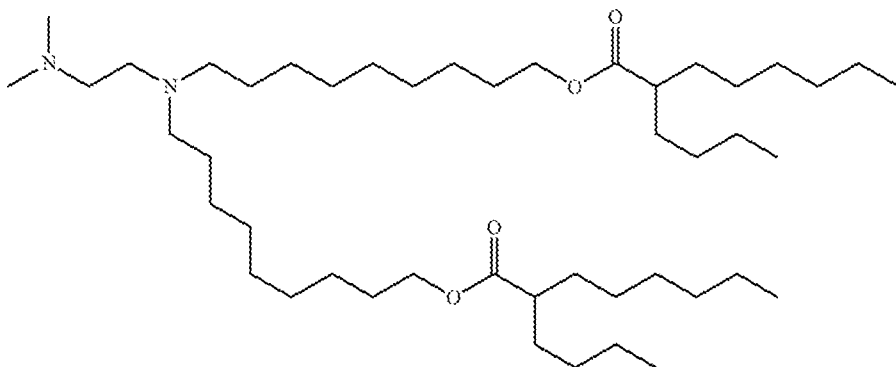
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

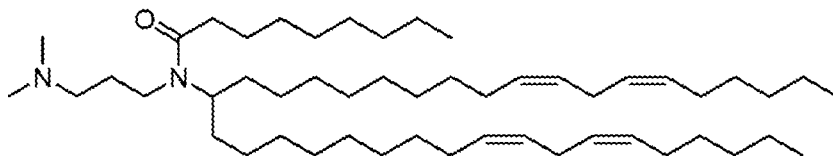


and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

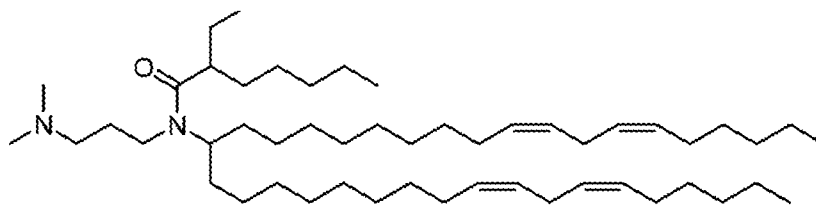


and pharmaceutically acceptable salts thereof.

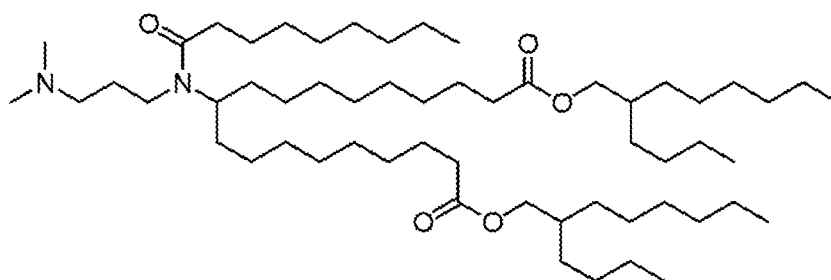
[0144] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/004143, which is incorporated herein by reference. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



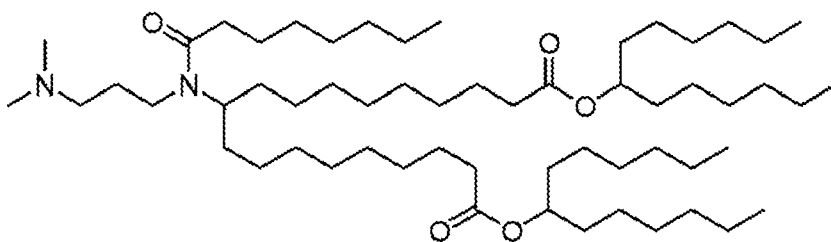
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



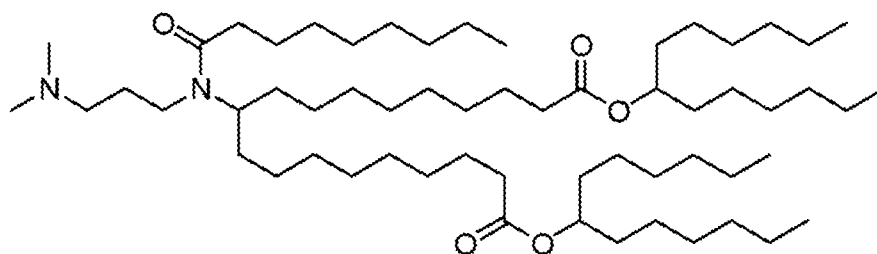
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



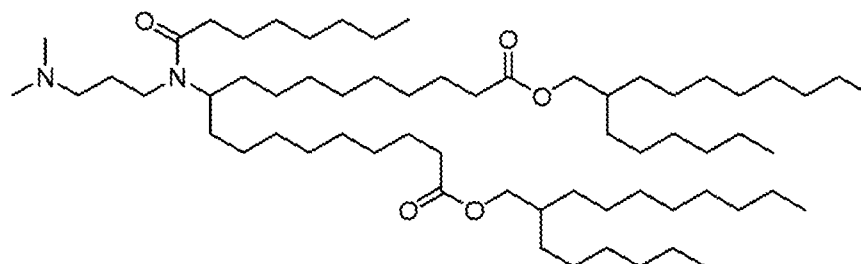
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



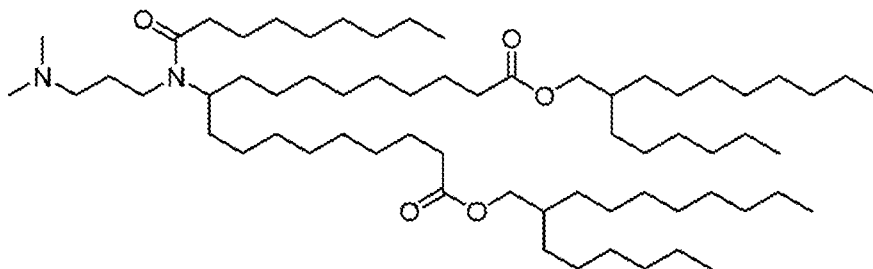
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



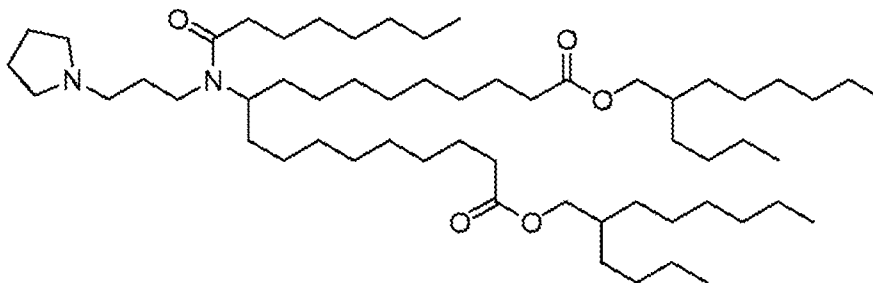
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



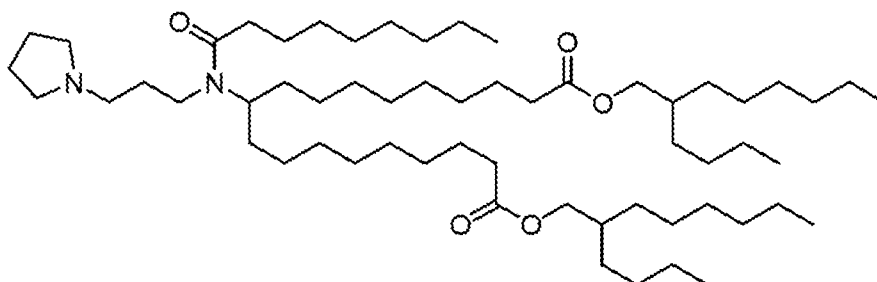
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



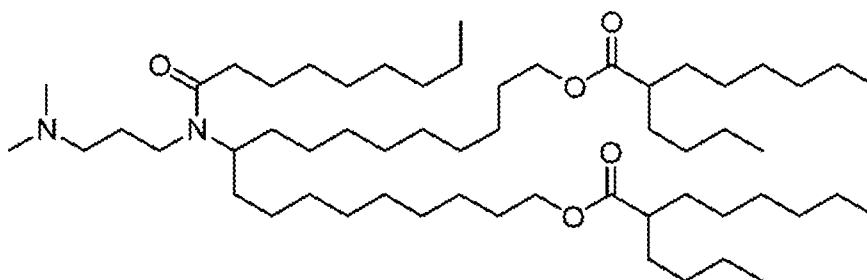
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



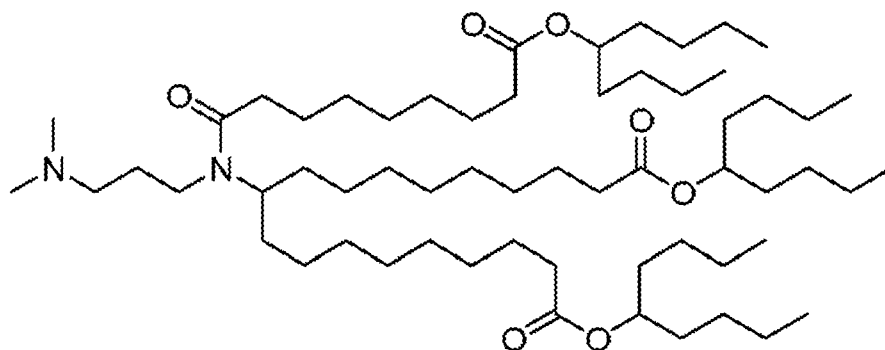
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



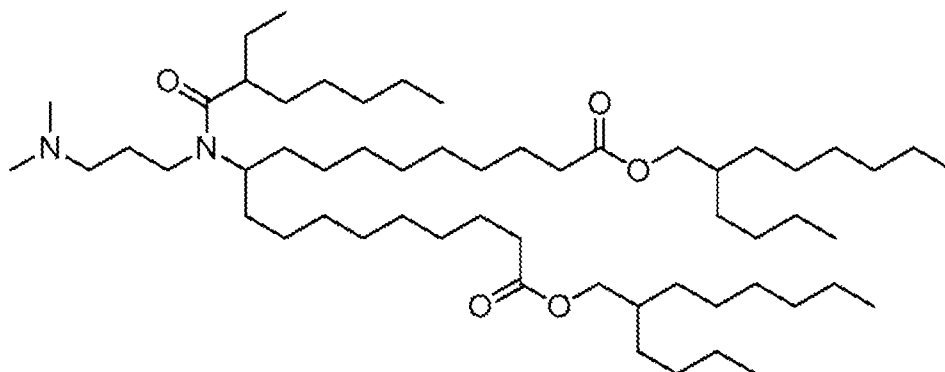
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



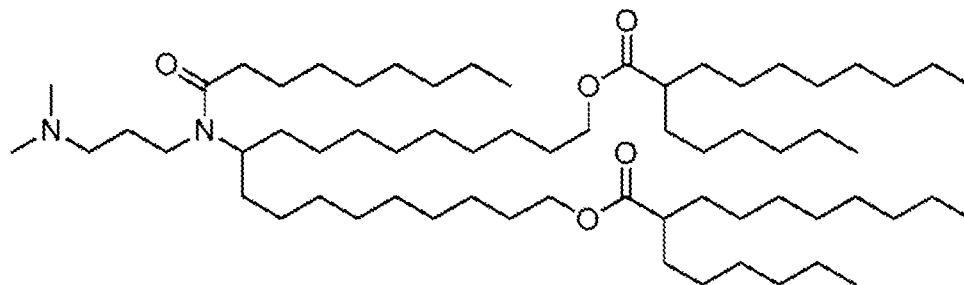
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



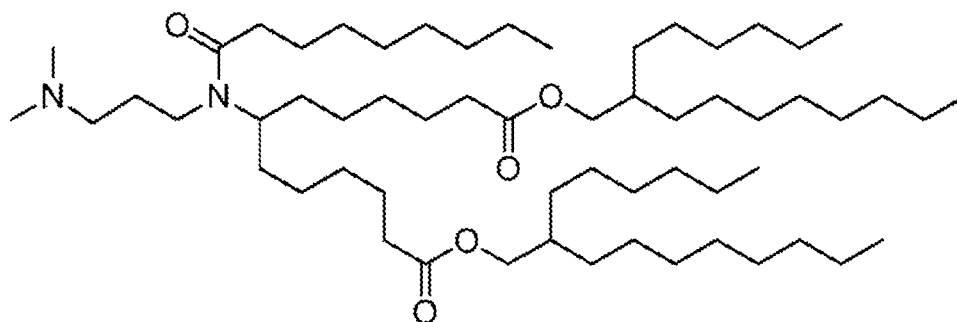
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



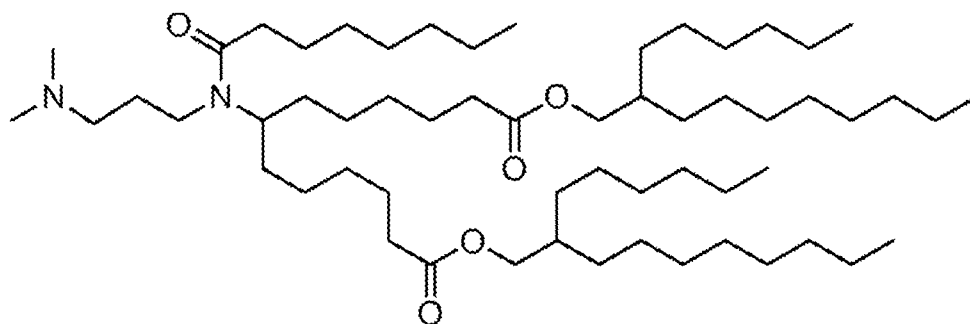
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



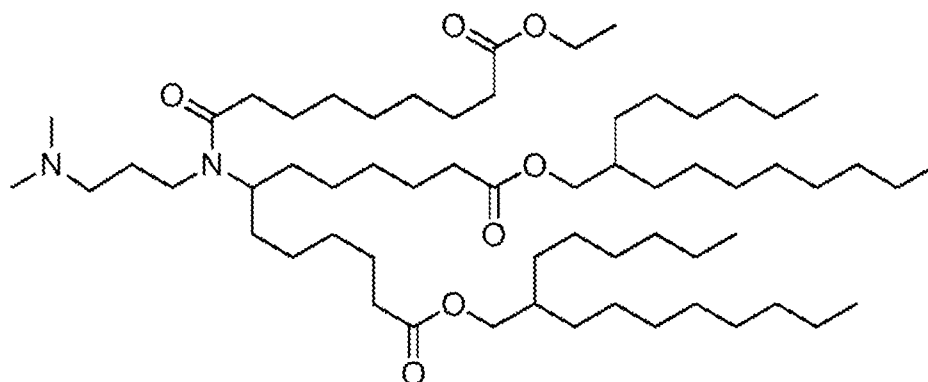
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



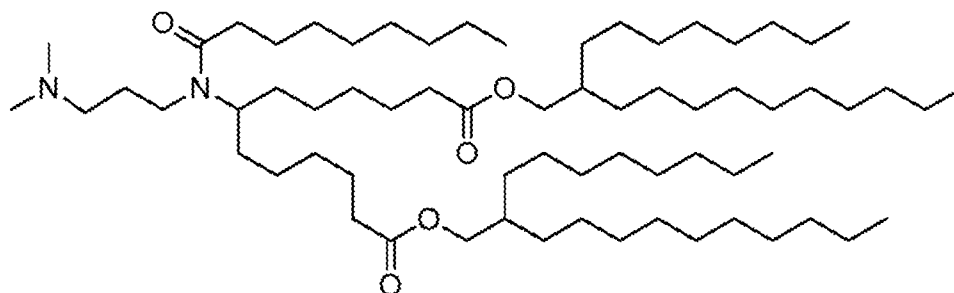
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

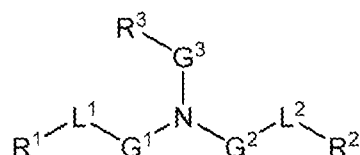


and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



and pharmaceutically acceptable salts thereof.

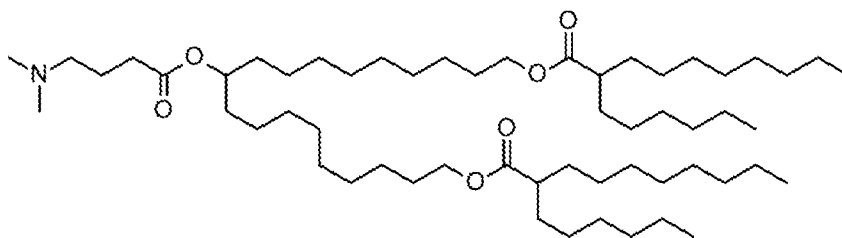
[0145] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/075531, which is incorporated herein by reference. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:



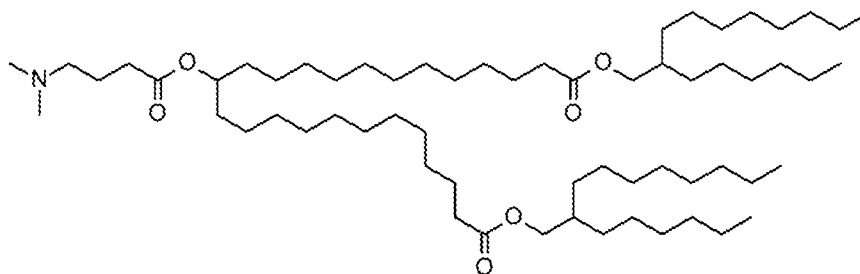
or a pharmaceutically acceptable salt thereof, wherein one of L^1 or L^2 is $-O(C=O)-$, $-(C=O)O-$, $-C(=O)-$, $-O-$, $-S(O)_x$, $-S-S-$, $-C(=O)S-$, $-SC(=O)-$, $-NR^aC(=O)-$, $-C(=O)NR^a$, $NR^aC(=O)NR^a$, $-OC(=O)NR^a$, or $-NR^aC(=O)O-$; and the other of L^1 or L^2 is $-O(C=O)-$, $-(C=O)O-$, $-C(=O)-$, $-O-$,

-S(O)_x, -S-S-, -C(=O)S-, SC(=O)-, -NR^aC(=O)-, -C(=O)NR^a-, ,NR^aC(=O)NR^a-, -OC(=O)NR^a- or -NR^aC(=O)O- or a direct bond; G¹ and G² are each independently unsubstituted C₁-C₁₂ alkylene or C₁-C₁₂ alkenylene; G³ is C₁-C₂₄ alkylene, C₁-C₂₄ alkenylene, C₃-C₈ cycloalkylene, C₃-C₈ cycloalkenylene; R^a is H or C₁-C₁₂ alkyl; R¹ and R² are each independently C₆-C₂₄ alkyl or C₆-C₂₄ alkenyl; R³ is H, OR⁵, CN, -C(=O)OR⁴, -OC(=O)R⁴ or -NR⁵C(=O)R⁴; R⁴ is C₁-C₁₂ alkyl; R⁵ is H or C₁-C₆ alkyl; and x is 0, 1 or 2.

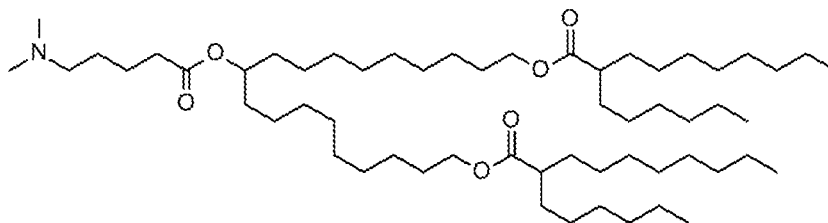
[0146] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/117528, which is incorporated herein by reference. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



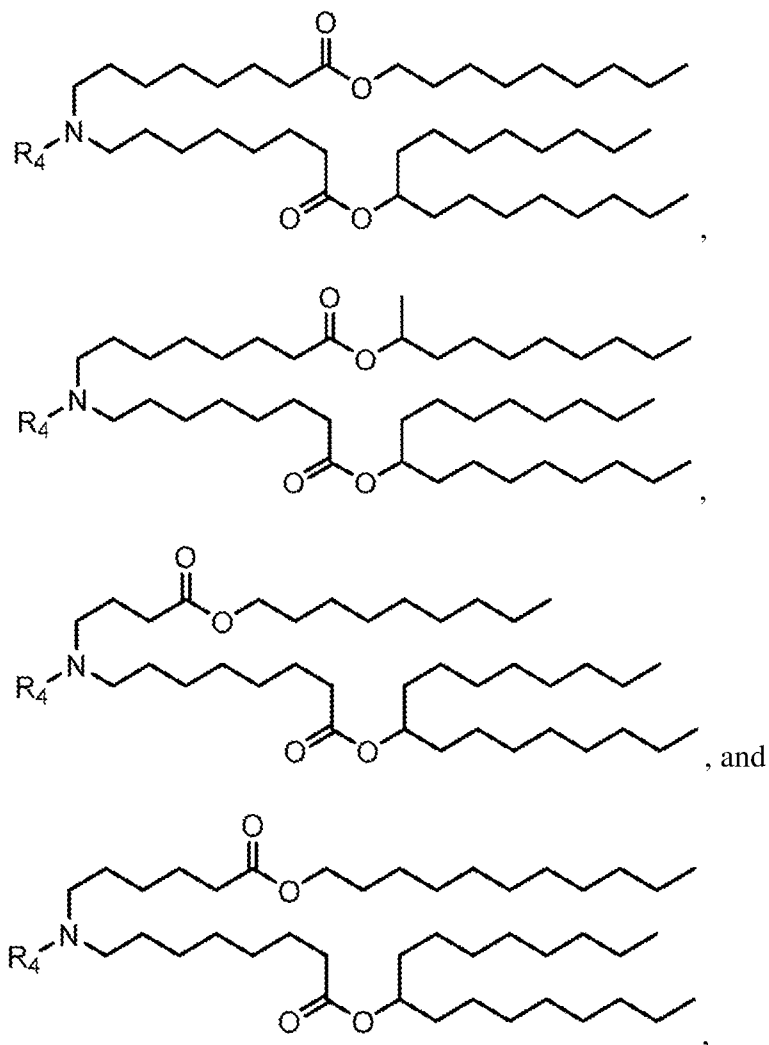
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



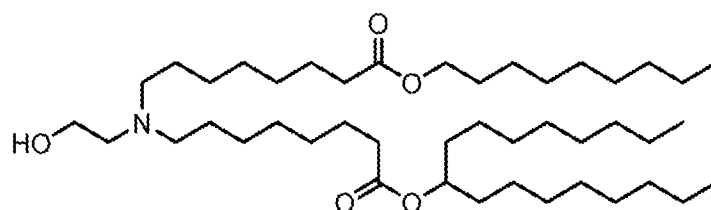
and pharmaceutically acceptable salts thereof.

[0147] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/049245, which is incorporated herein by reference. In some embodiments, the cationic lipids of the

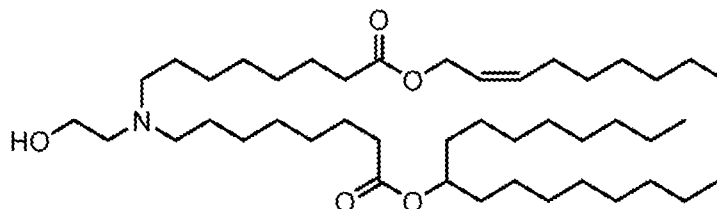
compositions and methods of the present invention include a compound of one of the following formulas:



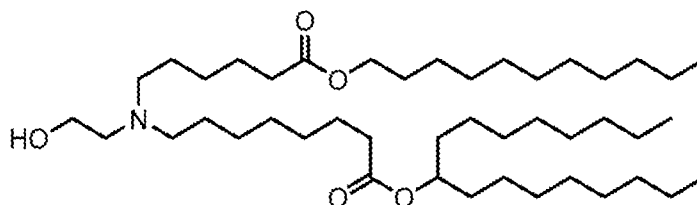
and pharmaceutically acceptable salts thereof. For any one of these four formulas, R_4 is independently selected from $-(CH_2)_nQ$ and $-(CH_2)_nCHQR$; Q is selected from the group consisting of $-OR$, $-OH$, $-O(CH_2)_nN(R)_2$, $-OC(O)R$, $-CX_3$, $-CN$, $-N(R)C(O)R$, $-N(H)C(O)R$, $-N(R)S(O)_2R$, $-N(H)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(H)C(O)N(R)_2$, $-N(H)C(O)N(H)(R)$, $-N(R)C(S)N(R)_2$, $-N(H)C(S)N(R)_2$, $-N(H)C(S)N(H)(R)$, and a heterocycle; and n is 1, 2, or 3. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



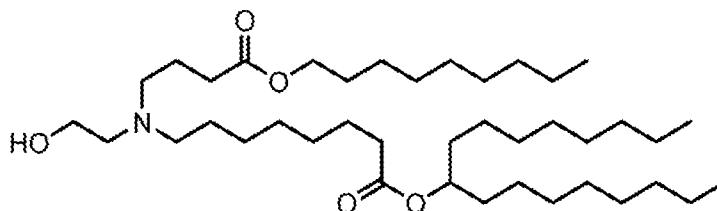
and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:

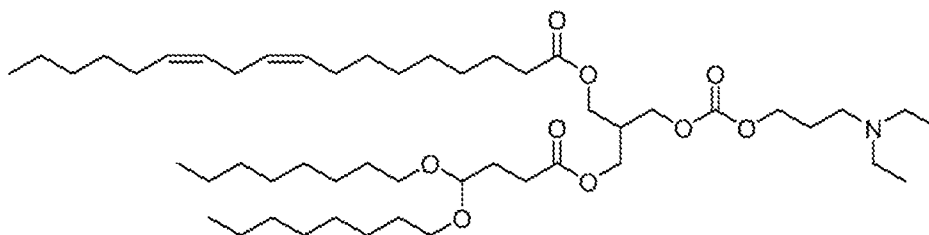


and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:

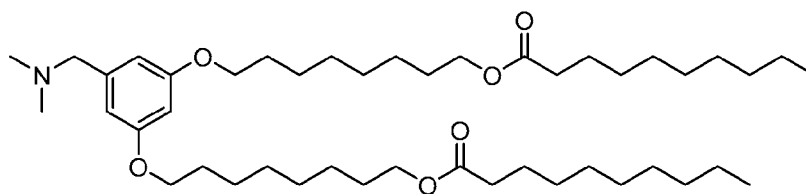


and pharmaceutically acceptable salts thereof.

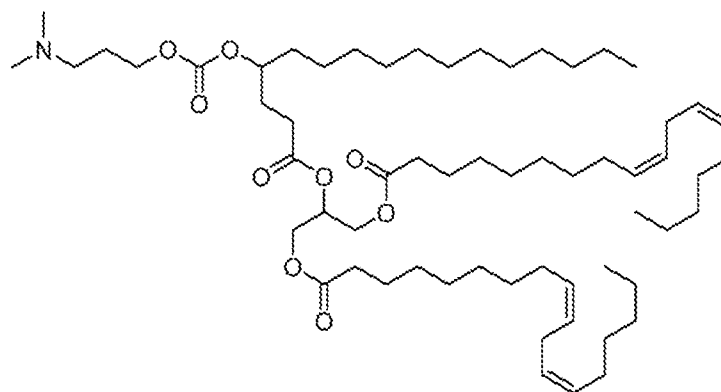
[0148] Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/173054 and WO 2015/095340, each of which is incorporated herein by reference. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



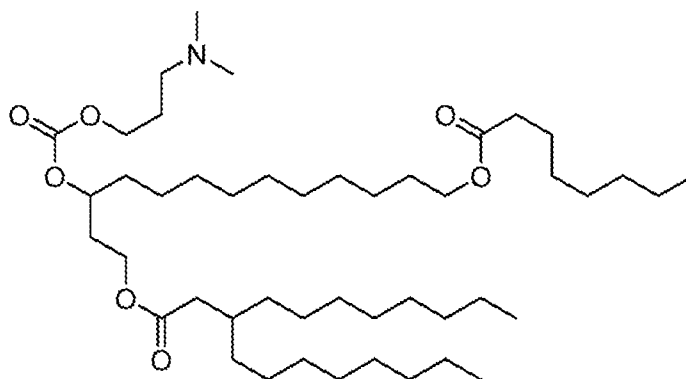
and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:

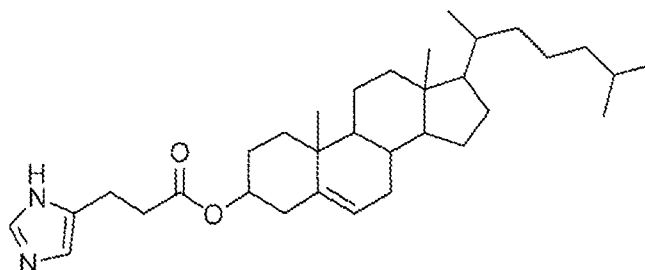


and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



and pharmaceutically acceptable salts thereof.

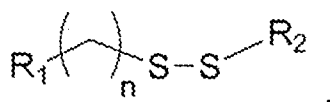
[0149] Other suitable cationic lipids for use in the compositions and methods of the invention include cholesterol-based cationic lipids. In certain embodiments, the compositions and methods of the present invention include imidazole cholesterol ester or “ICE”, having a compound structure of:



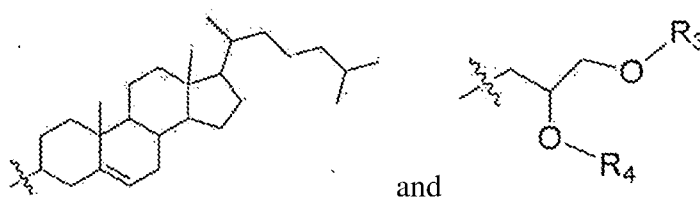
(ICE)

and pharmaceutically acceptable salts thereof.

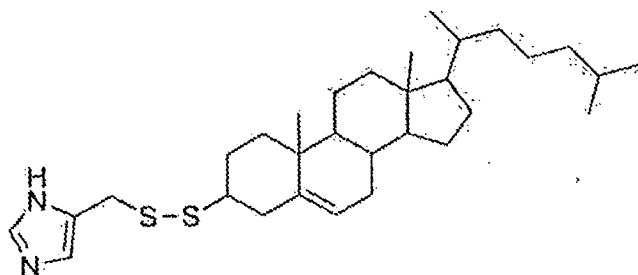
[0150] Other suitable cationic lipids for use in the compositions and methods of the present invention include cleavable cationic lipids as described in International Patent Publication WO 2012/170889, which is incorporated herein by reference. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:



wherein R_1 is selected from the group consisting of imidazole, guanidinium, amino, imine, enamine, an optionally-substituted alkyl amino (e.g., an alkyl amino such as dimethylamino) and pyridyl; wherein R_2 is selected from the group consisting of one of the following two formulas:

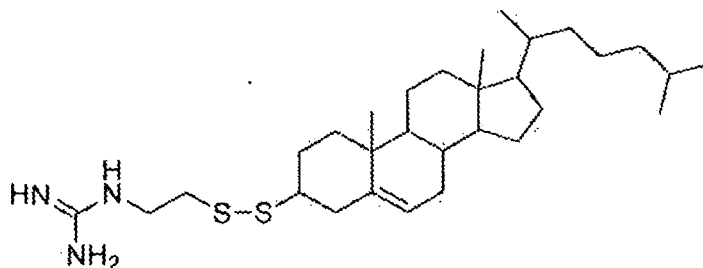


and wherein R_3 and R_4 are each independently selected from the group consisting of an optionally substituted, variably saturated or unsaturated C_6 - C_{20} alkyl and an optionally substituted, variably saturated or unsaturated C_6 - C_{20} acyl; and wherein n is zero or any positive integer (e.g., one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty or more). In certain embodiments, the compositions and methods of the present invention include a cationic lipid, “HGT4001”, having a compound structure of:



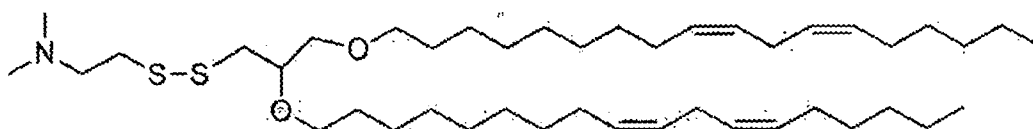
(HGT4001)

and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid, "HGT4002", having a compound structure of:



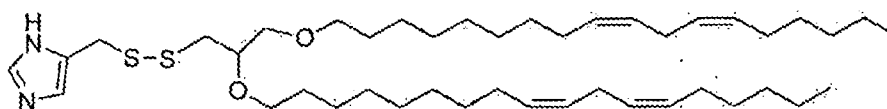
(HGT4002)

and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid, "HGT4003", having a compound structure of:



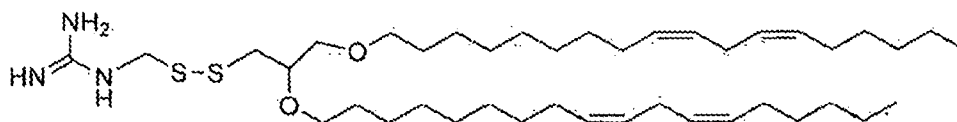
(HGT4003)

and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid, "HGT4004", having a compound structure of:



(HGT4004)

and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid "HGT4005", having a compound structure of:



(HGT4005)

and pharmaceutically acceptable salts thereof.

[0151] In some embodiments, the compositions and methods of the present invention include the cationic lipid, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (“DOTMA”). (Feigner et al. (Proc. Nat'l Acad. Sci. 84, 7413 (1987); U.S. Pat. No. 4,897,355, which is incorporated herein by reference). Other cationic lipids suitable for the compositions and methods of the present invention include, for example, 5-carboxyspermylglycinedioctadecylamide (“DOGS”); 2,3-dioleoyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium (“DOSPA”) (Behr et al. Proc. Nat'l Acad. Sci. 86, 6982 (1989), U.S. Pat. No. 5,171,678; U.S. Pat. No. 5,334,761); 1,2-Dioleoyl-3-Dimethylammonium-Propane (“DODAP”); 1,2-Dioleoyl-3-Trimethylammonium-Propane (“DOTAP”).

[0152] Additional exemplary cationic lipids suitable for the compositions and methods of the present invention also include: 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane (“DSDMA”); 1,2-dioleoyloxy-N,N-dimethyl-3-aminopropane (“DODMA”); 1,2-dilinoleoyloxy-N,N-dimethyl-3-aminopropane (“DLinDMA”); 1,2-dilinenyloxy-N,N-dimethyl-3-aminopropane (“DLinDMA”); N-dioleoyl-N,N-dimethylammonium chloride (“DODAC”); N,N-distearyl-N,N-dimethylammonium bromide (“DDAB”); N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (“DMRIE”); 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(cis,cis-9,12-octadecadienoxy)propane (“CLinDMA”); 2-[5'-(cholest-5-en-3-beta-oxy)-3'-oxapentoxo]-3-dimethyl-1-(cis,cis-9',1'-2'-octadecadienoxy)propane (“CpLinDMA”); N,N-dimethyl-3,4-dioleoyloxybenzylamine (“DMOBA”); 1,2-N,N'-dioleoylcarbonyl-3-dimethylaminopropane (“DOcarbDAP”); 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine (“DLinDAP”); 1,2-N,N'-Dilinoleoylcarbonyl-3-dimethylaminopropane (“DLincarbDAP”); 1,2-Dilinoleoylcarbonyl-3-dimethylaminopropane (“DLinCDAP”); 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (“DLin-K-DMA”); 2-((8-[(3P)-cholest-5-en-3-yloxy]octyl)oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propane-1-amine (“Octyl-CLinDMA”); (2R)-2-((8-[(3beta)-cholest-5-en-3-yloxy]octyl)oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (“Octyl-CLinDMA (2R)”); (2S)-2-((8-[(3P)-cholest-5-en-3-yloxy]octyl)oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (“Octyl-CLinDMA (2S)”); 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (“DLin-K-XTC2-DMA”); and 2-(2,2-di((9Z,12Z)-octadeca-9,12-dien-1-yl)-1,3-dioxolan-4-yl)-N,N-dimethylethanamine (“DLin-KC2-DMA”) (see, WO 2010/042877, which is incorporated herein by reference; Semple et al., Nature Biotech. 28: 172-176 (2010)). (Heyes, J., et al., J Controlled Release 107: 276-287 (2005); Morrissey, DV., et al., Nat. Biotechnol. 23(8): 1003-1007 (2005); International Patent Publication WO 2005/121348). In some embodiments,

one or more of the cationic lipids comprise at least one of an imidazole, dialkylamino, or guanidinium moiety.

[0153] In some embodiments, one or more cationic lipids suitable for the compositions and methods of the present invention include 2,2-Dilinoley1-4-dimethylaminoethyl-[1,3]-dioxolane ("XTC"); (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d] [1,3]dioxol-5-amine ("ALNY-100") and/or 4,7,13-tris(3-oxo-3-(undecylamino)propyl)-N1,N16-diundecyl-4,7,10,13-tetraazahexadecane-1,16-diamide ("NC98-5").

[0154] In some embodiments, the compositions of the present invention include one or more cationic lipids that constitute at least about 5%, 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70%, measured by weight, of the total lipid content in the composition, e.g., a lipid nanoparticle. In some embodiments, the compositions of the present invention include one or more cationic lipids that constitute at least about 5%, 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70%, measured as a mol %, of the total lipid content in the composition, e.g., a lipid nanoparticle. In some embodiments, the compositions of the present invention include one or more cationic lipids that constitute about 30-70 % (e.g., about 30-65%, about 30-60%, about 30-55%, about 30-50%, about 30-45%, about 30-40%, about 35-50%, about 35-45%, or about 35-40%), measured by weight, of the total lipid content in the composition, e.g., a lipid nanoparticle. In some embodiments, the compositions of the present invention include one or more cationic lipids that constitute about 30-70 % (e.g., about 30-65%, about 30-60%, about 30-55%, about 30-50%, about 30-45%, about 30-40%, about 35-50%, about 35-45%, or about 35-40%), measured as mol %, of the total lipid content in the composition, e.g., a lipid nanoparticle.

Non-cationic/Helper Lipids

[0155] As used herein, the phrase "non-cationic lipid" refers to any neutral, zwitterionic or anionic lipid. As used herein, the phrase "anionic lipid" refers to any of a number of lipid species that carry a net negative charge at a selected pH, such as physiological pH. Non-cationic lipids include, but are not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-

ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), or a mixture thereof.

[0156] In some embodiments, non-cationic lipids may constitute at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or 70% of the total lipids in a suitable lipid solution by weight or by molar. In some embodiments, non-cationic lipid(s) constitute(s) about 30-50 % (e.g., about 30-45%, about 30-40%, about 35-50%, about 35-45%, or about 35-40%) of the total lipids in a suitable lipid solution by weight or by molar.

Cholesterol-based Lipids

[0157] In some embodiments, a suitable lipid solution includes one or more cholesterol-based lipids. For example, suitable cholesterol-based cationic lipids include, for example, DC-Choi (N,N-dimethyl-N-ethylcarboxamidcholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao, et al. Biochem. Biophys. Res. Comm. 179, 280 (1991); Wolf et al. BioTechniques 23, 139 (1997); U.S. Pat. No. 5,744,335), or ICE. In some embodiments, cholesterol-based lipid(s) constitute(s) at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, or 70% of the total lipids in a suitable lipid solution by weight or by molar. In some embodiments, cholesterol-based lipid(s) constitute(s) about 30-50 % (e.g., about 30-45%, about 30-40%, about 35-50%, about 35-45%, or about 35-40%) of the total lipids in a suitable lipid solution by weight or by molar.

[0158] Exemplary combinations of cationic lipids, non-cationic lipids, cholesterol-based lipids, and PEG-modified lipids are described in the Examples section. For example, a suitable lipid solution may contain cKK-E12, DOPE, cholesterol, and DMG-PEG2K; C12-200, DOPE, cholesterol, and DMG-PEG2K; HGT5000, DOPE, cholesterol, and DMG-PEG2K; HGT5001, DOPE, cholesterol, and DMG-PEG2K; cKK-E12, DPPC, cholesterol, and DMG-PEG2K; C12-200, DPPC, cholesterol, and DMG-PEG2K; HGT5000, DPPC, cholesterol, and DMG-PEG2K; or HGT5001, DPPC, cholesterol, and DMG-PEG2K. The selection of cationic lipids, non-cationic lipids and/or PEG-modified lipids which comprise the lipid mixture as well as the relative molar ratio of such lipids to each other, is based upon the characteristics of the selected lipid(s) and the nature of the and the characteristics of the mRNA to be encapsulated. Additional considerations include, for example, the saturation of the alkyl chain, as well as the size, charge, pH, pKa, fusogenicity and toxicity of the selected lipid(s). Thus the molar ratios may be adjusted accordingly.

mRNA-loaded Nanoparticles

[0159] Any desired lipids may be mixed at any ratios suitable for encapsulating mRNAs. In some embodiments, a suitable lipid solution contains a mixture of desired lipids including cationic lipids, non-cationic lipids, cholesterol and/or PEGylated lipids.

[0160] In some embodiments, a process for encapsulating mRNA in lipid nanoparticles comprises mixing an mRNA solution and a lipid solution, wherein the mRNA solution and/or the lipid solution are heated to a pre-determined temperature greater than ambient temperature prior to mixing to form lipid nanoparticles that encapsulate mRNA (see U.S. Patent Application Serial No. 14/790,562 entitled “Encapsulation of messenger RNA”, filed July 2, 2015 and its provisional U.S. patent application Serial No. 62/020,163, filed July 2, 2014, the disclosure of which are hereby incorporated in their entirety).

[0161] In some embodiments, a process for encapsulating mRNA in lipid nanoparticles comprises combining pre-formed lipid nanoparticles with mRNA (see U.S. Provisional Application Serial No. 62/420,413, filed November 10, 2016 and U.S. Provisional Application Serial No. 62/580,155, filed November 1, 2017, the disclosures of which are hereby incorporated by reference). In some embodiments, combining pre-formed lipid nanoparticles with mRNA results in lipid nanoparticles that show improved efficacy of intracellular delivery of the mRNA. In some embodiments, combining pre-formed lipid nanoparticles with mRNA results in very high encapsulation efficiencies of mRNA encapsulated in lipid nanoparticles (i.e., in the range of 90-95%). In some embodiments, combining pre-formed lipid nanoparticles with mRNA is achieved with pump systems which maintain the lipid/mRNA (N/P) ratio constant throughout the process and which also afford facile scale-up.

[0162] Suitable liposomes in accordance with the present invention may be made in various sizes. In some embodiments, provided liposomes may be made smaller than previously known mRNA encapsulating liposomes. In some embodiments, decreased size of liposomes is associated with more efficient delivery of mRNA. Selection of an appropriate liposome size may take into consideration the site of the target cell or tissue and to some extent the application for which the liposome is being made.

[0163] In some embodiments, an appropriate size of liposome is selected to facilitate systemic distribution of antibody encoded by the mRNA. In some embodiments, it may be desirable to limit transfection of the mRNA to certain cells or tissues. For example, to target hepatocytes a liposome may be sized such that its dimensions are smaller than the fenestrations of the endothelial layer lining hepatic sinusoids in the liver; in such cases the liposome could readily penetrate such endothelial fenestrations to reach the target hepatocytes.

[0164] Alternatively or additionally, a liposome may be sized such that the dimensions of the liposome are of a sufficient diameter to limit or expressly avoid distribution into certain cells or tissues. For example, a liposome may be sized such that its dimensions are larger than the fenestrations of the endothelial layer lining hepatic sinusoids to thereby limit distribution of the liposomes to hepatocytes.

[0165] In some embodiments, the size of a liposome is determined by the length of the largest diameter of the liposome particle. In some embodiments, a suitable liposome has a size no greater than about 250 nm (e.g., no greater than about 225 nm, 200 nm, 175 nm, 150 nm, 125 nm, 100 nm, 75 nm, or 50 nm). In some embodiments, a suitable liposome has a size ranging from about 10 - 250 nm (e.g., ranging from about 10 – 225 nm, 10 – 200 nm, 10 – 175 nm, 10 – 150 nm, 10 – 125 nm, 10 – 100 nm, 10 – 75 nm, or 10 – 50 nm). In some embodiments, a suitable liposome has a size ranging from about 100 - 250 nm (e.g., ranging from about 100 – 225 nm, 100 – 200 nm, 100 – 175 nm, 100 – 150 nm). In some embodiments, a suitable liposome has a size ranging from about 10 - 100 nm (e.g., ranging from about 10 – 90 nm, 10 – 80 nm, 10 – 70 nm, 10 – 60 nm, or 10 – 50 nm). In a particular embodiment, a suitable liposome has a size less than about 100 nm.

[0166] A variety of alternative methods known in the art are available for sizing of a population of liposomes. One such sizing method is described in U.S. Pat. No. 4,737,323, incorporated herein by reference. Sonicating a liposome suspension either by bath or probe sonication produces a progressive size reduction down to small ULV less than about 0.05 microns in diameter. Homogenization is another method that relies on shearing energy to fragment large liposomes into smaller ones. In a typical homogenization procedure, MLV are recirculated through a standard emulsion homogenizer until selected liposome sizes, typically between about 0.1 and 0.5 microns, are observed. The size of the liposomes may be determined by quasi-electric light scattering (QELS) as described in Bloomfield, *Ann. Rev. Biophys. Bioeng.*, 10:421-150 (1981), incorporated herein by reference. Average liposome diameter may be reduced by sonication of formed liposomes. Intermittent sonication cycles may be alternated with QELS assessment to guide efficient liposome synthesis.

Pharmaceutical Compositions

[0167] To facilitate expression of mRNA *in vivo*, delivery vehicles such as lipid nanoparticles, including liposomes, can be formulated in combination with one or more additional nucleic acids, carriers, targeting ligands or stabilizing reagents, or in pharmacological compositions where it is mixed with suitable excipients. In some embodiments, the lipid

nanoparticles encapsulating mRNA are simultaneously administered with hyaluronidase. Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition.

[0168] Provided liposomally-encapsulated or associated mRNAs, and compositions containing the same, may be administered and dosed in accordance with current medical practice, taking into account the clinical condition of the subject, the site and method of administration, the scheduling of administration, the subject's age, sex, body weight and other factors relevant to clinicians of ordinary skill in the art. The "effective amount" for the purposes herein may be determined by such relevant considerations as are known to those of ordinary skill in experimental clinical research, pharmacological, clinical and medical arts. In some embodiments, the amount administered is effective to achieve at least some stabilization, improvement or elimination of symptoms and other indicators as are selected as appropriate measures of disease progress, regression or improvement by those of skill in the art. For example, a suitable amount and dosing regimen is one that causes at least transient protein (e.g., enzyme) production.

[0169] Although the current invention focuses on subcutaneous delivery, which is a bolus injection into the subcutis (the tissue layer between the skin and the muscle), other suitable routes of administration include, for example, oral, rectal, vaginal, transmucosal, pulmonary including intratracheal or inhaled, or intestinal administration; parenteral delivery, including intradermal, transdermal (topical), intramuscular, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, or intranasal. In particular embodiments, the intramuscular administration is to a muscle selected from the group consisting of skeletal muscle, smooth muscle and cardiac muscle. In some embodiments, the administration results in delivery of the mRNA to a muscle cell. In some embodiments the administration results in delivery of the mRNA to a hepatocyte (i.e., liver cell). In a particular embodiment, the intramuscular administration results in delivery of the mRNA to a muscle cell.

[0170] Alternatively or additionally, liposomally encapsulated mRNAs and compositions of the invention may be administered in a local rather than systemic manner.

[0171] Provided methods of the present invention contemplate single as well as multiple administrations of a therapeutically effective amount of the therapeutic agents (e.g., mRNA encoding a therapeutic protein) described herein. Therapeutic agents can be administered at regular intervals, depending on the nature, severity and extent of the subject's condition (e.g., OTC deficiency). In some embodiments, a therapeutically effective amount of the therapeutic agent (e.g., mRNA encoding a therapeutic protein) of the present invention may be administered

subcutaneously periodically at regular intervals (e.g., once every year, once every six months, once every five months, once every three months, bimonthly (once every two months), monthly (once every month), biweekly (once every two weeks), twice a month, once every 30 days, once every 28 days, once every 14 days, once every 10 days, once every 7 days, weekly, twice a week, daily or continuously.

[0172] In some embodiments, provided liposomes and/or compositions are formulated such that they are suitable for extended-release of the mRNA contained therein. Such extended-release compositions may be conveniently administered to a subject at extended dosing intervals. For example, in some embodiments, the compositions of the present invention are administered to a subject twice a day, daily or every other day. In a preferred embodiment, the compositions of the present invention are administered to a subject twice a week, once a week, once every 7 days, once every 10 days, once every 14 days, once every 28 days, once every 30 days, once every two weeks, once every three weeks, or more preferably once every four weeks, once a month, twice a month, once every six weeks, once every eight weeks, once every other month, once every three months, once every four months, once every six months, once every eight months, once every nine months or annually. Also contemplated are compositions and liposomes which are formulated for depot administration (e.g., intramuscularly, subcutaneously, intravitreally) to either deliver or release mRNA over extended periods of time. Preferably, the extended-release means employed are combined with modifications made to the mRNA to enhance stability.

[0173] As used herein, the term “therapeutically effective amount” is largely based on the total amount of the therapeutic agent contained in the pharmaceutical compositions of the present invention. Generally, a therapeutically effective amount is sufficient to achieve a meaningful benefit to the subject (e.g., treating, modulating, curing, preventing and/or ameliorating OTC deficiency). For example, a therapeutically effective amount may be an amount sufficient to achieve a desired therapeutic and/or prophylactic effect. Generally, the amount of a therapeutic agent (e.g., mRNA encoding a therapeutic protein) administered to a subject in need thereof will depend upon the characteristics of the subject. Such characteristics include the condition, disease severity, general health, age, sex and body weight of the subject. One of ordinary skill in the art will be readily able to determine appropriate dosages depending on these and other related factors. In addition, both objective and subjective assays may optionally be employed to identify optimal dosage ranges.

[0174] A therapeutically effective amount is commonly administered in a dosing regimen that may comprise multiple unit doses. For any particular therapeutic protein, a therapeutically

effective amount (and/or an appropriate unit dose within an effective dosing regimen) may vary, for example, depending on route of administration, on combination with other pharmaceutical agents. Also, the specific therapeutically effective amount (and/or unit dose) for any particular patient may depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific pharmaceutical agent employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and/or rate of excretion or metabolism of the specific protein employed; the duration of the treatment; and like factors as is well known in the medical arts.

[0175] In some embodiments, the therapeutically effective dose ranges from about 0.005 mg/kg to 500 mg/kg body weight, e.g., from about 0.005 mg/kg to 400 mg/kg body weight, from about 0.005 mg/kg to 300 mg/kg body weight, from about 0.005 mg/kg to 200 mg/kg body weight, from about 0.005 mg/kg to 100 mg/kg body weight, from about 0.005 mg/kg to 90 mg/kg body weight, from about 0.005 mg/kg to 80 mg/kg body weight, from about 0.005 mg/kg to 70 mg/kg body weight, from about 0.005 mg/kg to 60 mg/kg body weight, from about 0.005 mg/kg to 50 mg/kg body weight, from about 0.005 mg/kg to 40 mg/kg body weight, from about 0.005 mg/kg to 30 mg/kg body weight, from about 0.005 mg/kg to 25 mg/kg body weight, from about 0.005 mg/kg to 20 mg/kg body weight, from about 0.005 mg/kg to 15 mg/kg body weight, from about 0.005 mg/kg to 10 mg/kg body weight.

[0176] In some embodiments, the therapeutically effective dose is greater than about 0.1 mg/kg body weight, greater than about 0.5 mg/kg body weight, greater than about 1.0 mg/kg body weight, greater than about 3 mg/kg body weight, greater than about 5 mg/kg body weight, greater than about 10 mg/kg body weight, greater than about 15 mg/kg body weight, greater than about 20 mg/kg body weight, greater than about 30 mg/kg body weight, greater than about 40 mg/kg body weight, greater than about 50 mg/kg body weight, greater than about 60 mg/kg body weight, greater than about 70 mg/kg body weight, greater than about 80 mg/kg body weight, greater than about 90 mg/kg body weight, greater than about 100 mg/kg body weight, greater than about 150 mg/kg body weight, greater than about 200 mg/kg body weight, greater than about 250 mg/kg body weight, greater than about 300 mg/kg body weight, greater than about 350 mg/kg body weight, greater than about 400 mg/kg body weight, greater than about 450 mg/kg body weight, greater than about 500 mg/kg body weight. In a particular embodiment, the therapeutically effective dose is 1.0 mg/kg body weight. In some embodiments, the therapeutically effective dose of 1.0 mg/kg body weight is administered intramuscularly or intravenously.

[0177] Also contemplated herein are lyophilized pharmaceutical compositions comprising one or more of the liposomes disclosed herein and related methods for the use of such compositions as disclosed for example, in International Patent Application PCT/US12/41663, filed June 8, 2012, the teachings of which are incorporated herein by reference in their entirety. For example, lyophilized pharmaceutical compositions according to the invention may be reconstituted prior to administration or can be reconstituted *in vivo*. For example, a lyophilized pharmaceutical composition can be formulated in an appropriate dosage form (e.g., an intradermal dosage form such as a disk, rod or membrane) and administered such that the dosage form is rehydrated over time *in vivo* by the individual's bodily fluids.

[0178] Provided liposomes and compositions may be administered to any desired tissue. In some embodiments, the provided liposomes and compositions comprising mRNA are delivered subcutaneously and the mRNA is expressed in a cell or tissue type other than the subcutis. In some embodiments, the mRNA encoding a target protein delivered by provided liposomes or compositions is expressed in the tissue in which the liposomes and/or compositions were administered. In some embodiments, the mRNA delivered is expressed in a tissue different from the tissue in which the liposomes and/or compositions were administered. Exemplary tissues in which delivered mRNA may be delivered and/or expressed include, but are not limited to, the liver, kidney, heart, spleen, serum, brain, skeletal muscle, lymph nodes, skin, and/or cerebrospinal fluid.

[0179] In some embodiments, administering a provided composition results in increased expression of the mRNA administered, or increased activity level of the mRNA-encoded protein in a biological sample from a subject as compared to a baseline expression or activity level before treatment or administration. In some embodiments, administering a provided composition results in increased expression or activity level of the therapeutic protein encoded by the mRNA of a provided composition in a biological sample from a subject as compared to a baseline expression or activity level before treatment. Typically, the baseline level is measured immediately before treatment. Biological samples include, for example, whole blood, serum, plasma, urine and tissue samples (e.g., muscle, liver, skin fibroblasts). In some embodiments, administering a provided composition results in increased therapeutic protein (protein encoded by administered mRNA) expression or activity level by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% as compared to the baseline level immediately before treatment. In some embodiments, administering a provided composition results in increased mRNA expression or activity level in a biological sample from a subject as compared to subjects who were not treated. In some embodiments, administering a provided composition results in

increased expression or activity level of the therapeutic protein encoded by the mRNA of a provided composition in a biological sample from a subject as compared to subjects who were not treated.

[0180] According to various embodiments, the timing of expression of delivered mRNAs can be tuned to suit a particular medical need. In some embodiments, the expression of the protein encoded by delivered mRNA is detectable 1, 2, 3, 6, 12, 24, 48, 72, 96 hours, 1 week, 2 weeks, or 1 month after administration of provided liposomes and/or compositions.

[0181] In some embodiments, a therapeutically effective dose of the provided composition, when administered regularly, results in increased citrulline production in a subject as compared to baseline citrulline production before treatment. Typically, the citrulline level before or after the treatment may be measured in a biological sample obtained from the subject such as blood, plasma or serum, urine, or solid tissue extracts. In some embodiments, treatment according to the present invention results in an increase of the citrulline level in a biological sample (e.g., plasma, serum, or urine) by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1-fold, 1.5-fold, 2-fold, 2.5-fold, or 3-fold as compared to the base line citrulline level.

[0182] According to the present invention, a therapeutically effective dose of the provided composition, when administered regularly, results in at least one symptom or feature of a protein deficiency being reduced in intensity, severity, or frequency or having delayed onset.

Therapeutic Application

[0183] The present invention may be used to treat various diseases, disorders and conditions. Of particular interest, monogenic disorders and disorders where administering an mRNA encoding a protein reduces one or more disease related symptoms, or ameliorates the disease symptoms, are candidates for therapeutic application using the present invention. Exemplary therapeutic messenger RNAs for subcutaneous administration as delineated in the present application disclosure can be selected from any of the corresponding exemplary genes listed in Tables 1, 2, 3, 4, 5 or 6 having the related functions, or implicated in the disease or conditions as described.

TABLE 1

DISEASE/DISORDERS	GENE(S)
Neoplasia	PTEN; ATM; ATR; EGFR; ERBB2; ERBB3; ERBB4; Notch1; Notch2; Notch3; Notch4; AKT; AKT2; AKT3; HIF; H1Fla; HIF3a; Met; HRG; Bcl2; PPARalpha; PPAR gamma; WT1 (Wilms Tumor); FGF Receptor Family members (5 members: 1,

	2, 3, 4, 5); CDKN2a; APC; RB (retinoblastoma); MEN1; VHL; BRCA1; BRCA2; AR (Androgen Receptor); TSG101; IGF; IGF Receptor; Igfl (4 variants); Igf2 (3 variants); Igfl Receptor; Igf2 Receptor; Bax; Bcl2; caspases family (9 members: 1, 2, 3, 4, 6, 7, 8, 9, 12); Kras; Apc
Age-related Macular Degeneration	Aber; Ccl2; Cc2; cp (ceruloplasmin); Timp3; cathepsinD; Vldlr; Ccr2
Schizophrenia Disorders	Neuregulin1 (Nrg1); Erb4 (receptor for Neuregulin); Complexin1 (Cplx1); Tph1 Tryptophan hydroxylase; Tph2 Tryptophan hydroxylase 2; Neurexin 1; GSK3; GSK3a; GSK3b; 5-HTT (Slc6a4); COMT; DRD (Drdla); SLC6A3; DAOA; DTNBP1; Dao (Dao1)
Trinucleotide Repeat Disorders	HTT (Huntington's Dx); SBMA/SMAXI/AR (Kennedy's Dx); FXN/X25 (Friedrich's Ataxia); ATX3 (Machado-Joseph's Dx); ATXN1 and ATXN2 (spinocerebellar ataxias); DMPK (myotonic dystrophy); Atrophin-1 and Atn1(DRPLA Dx); CBP (Creb-BP-global instability); VLDLR (Alzheimer's); Atxn7; Atxn10
Fragile X Syndrome	FMR2; FXR1; FXR2; mGLUR5
Secretase Related Disorders	APH-1 (alpha and beta); Presenilin (Psen1); nicastrin (Ncstn); PEN-2
Others	Nos1; Parp1; Nat1; Nat2
Prion-related Disorders	Prp
ALS	SOD1; ALS2; STEX; FUS; TARD BP; VEGF (VEGF-a; VEGF-b; VEGF-c)
Drug Addiction	Prkce (alcohol); Drd2; Drd4; ABAT (alcohol); GRIA2; Grm5; Grin1; Htr1b; Grin2a; Drd3; Pdyn; Gria1 (alcohol)
Autism	Mecp2; BZRAP1; MDGA2; Sema5A; Neurexin 1; Fragile X (FMR2 (AFF2); FXR1; FXR2; Mglur5)
Alzheimer's Disease	E1; CHIP; UCH; UBB; Tau; LRP; PICALM; Clusterin; PS1; SORL1; CR1; Vldlr; Uba1; Uba3; CHIP28 (Aqp1, Aquaporin 1); Uchl1; Uchl3; APP
Inflammation	IL-10; IL-1 (IL-1a; IL-1b); IL-13; IL-17 (IL-17a (CTLA8); IL-17b; IL-17c; IL-17d; IL-171); 11-23; Cx3crl; ptpn22; TNFa; NOD2/CARD15 for IBD; IL-6; IL-12 (IL-12a; IL-12b); CTLA4; Cx3cell
Parkinson's Disease	x-Synuclein; DJ-1; LRRK2; Parkin; PINK1

TABLE 2

CELLULAR FUNCTION	GENES
Blood and coagulation diseases and disorders	Anemia (CRAN1, CDA1, RPS19, DBA, PKLR, PK1, NT5C3, UMPH1, PSN1, RHAG, RH50A, NRAMP2, SPTB, ALAS2, ANH1, ASB, ABCB7, ABC7, ASAT); Bare lymphocyte syndrome (TAPBP, TPSN, TAP2, ABCB3, PSF2, RING11, MHC2TA, C2TA, RFX5, RFXAP, RFX5), Bleeding disorders (TBXA2R, P2RX1, P2X1); Factor Hand factor H-like 1 (HF1, CFH, HUS); Factor V and Factor VIII (MCFD2); Factor VII deficiency (F7); Factor X deficiency (F10); Factor XI deficiency (F11); Factor XII deficiency (F12, HAF); Factor XIIIa deficiency (F13A1, F13A); Factor XIIIb deficiency (F13B); Fanconi anemia (FANCA, FACA, FA1, FA, FAA, FAAP95, FAAP90, FLJ34064, FANCB, FANCC, FACC, BRCA2, FANCD1, FANCD2, FANCD,

	FACD, FAD, FANCE, FACE, FANCF, XRCC9, FANCG, BR1PI, BACH1, FANCF, PHF9, FANCL, FANCM, KIAA1596); Hemophagocytic lymphohistiocytosis disorders (PRF1, HPLH2, UNC13D, MUNC13-4, HPLH3, HLH3, FHL3); Hemophilia A (F8, FSC, HEMA); Hemophilia B (F9, HEMB), Hemorrhagic disorders (PI, ATT, F5); Leukocyte deficiencies and disorders (ITGB2, CD18, LCAMB, LAD, EIF2B1, EIF2BA, EIF2B2, EIF2B3, EIF2B5, LVWM, CACH, CLE, EIF2B4); Sickle cell anemia (HBB); Thalassemia (HBA2, HBB, HBD, LCRB, HBA1).
Cell dysregulation and oncology diseases and disorders	B-cell non-Hodgkin lymphoma (BCL7A, BCL7); Leukemia (TALI, TCL5, SCL, TAL2, FLT3, NBS1, NBS, ZNFN1A1, 1KI, LYF1, HOXD4, HOX4B, BCR, CML, PHL, ALL, ARNT, KRAS2, RASK2, GMPS, AFIO, ARHGEF12, LARG, KIAA0382, CALM, CLTH, CEBPA, CEBP, CHIC2, BTL, FLT3, KIT, PBT, LPP, NPML, NUP214, D9S46E, CAN, CAIN, RUNX1, CBFA2, AML1, WHSC1L1, NSD3, FLT3, AF1Q, NPM1, NUMA1, ZNF145, PLZF, PML, MYL, STAT5B, AF1Q, CALM, CLTH, ARL11, ARLTS1, P2RX7, P2X7, BCR, CML, PHL, ALL, GRAF, NF1, VRNF, WSS, NFNS, PTPN11, PTP2C, SHP2, NS1, BCL2, CCND1, PRAD1, BCL1, TCRA, GATA1, GF1, ERYF1, NFE1, ABLI, NQO1, DIA4, NMOR1, NUP214, D9S46E, CAN, CAIN).
Inflammation and immune related diseases and disorders	AIDS (KIR3DL1, NKAT3, NKB1, AMB11, K1R3DS1, IFNG, CXCL12, SD F1); Autoimmune lymphoproliferative syndrome (TNFRSF6, APT1, FAS, CD95, ALPS1A); Combined immunodeficiency, (IL2RG, SCIDX1, SCIDX, IMD4); HN-1 (CCL5, SCYA5, D17S136E, TCP228), HIV susceptibility or infection (IL10, CSIF, CMKBR2, CCR2, CMKBR5, CCCR5 (CCR5)); Immunodeficiencies (CD3E, CD3G, AICDA, AID, HIGM2, TNFRSF5, CD40, UNG, DGU, HIGM4, TNFSF5, CD40LG, HIGM1, IGM, FOXP3, IPEX, AIID, XPID, PIDX, TNFRSF14B, TACI; Inflammation (IL-10, IL-1 (IL-1a, IL-1b), IL-13, IL-17 (IL-17a (CTLA8), IL-17b, IL-17c, IL-17d, IL-171), 11-23, Cx3cr1, ptpn22, TNFa, NOD2/CARD15 for IBD, IL-6, IL-12 (IL-12a, IL-12b), CTLA4, Cx3cl1); Severe combined immunodeficiencies (SCIDs)(JAK3, JAKL, DCLREIC, ARTEMIS, SCIDA, RAG1, RAG2, ADA, PTPRC, CD45, LCA, IL7R, CD3D, T3D, IL2RG, SCIDX1, SCIDX, IMD4).
Metabolic, liver, kidney and protein diseases and disorders	Amyloid neuropathy (TTR, PALB); Amyloidosis (APOA1, APP, AAA, CVAP, AD1, GSN, FGA, LYZ, TTR, PALB); Cirrhosis (KRT18, KRT8, CIRH1A, NAIC, TEX292, KIAA1988); Cystic fibrosis (CFTR, ABCC7, CF, MRP7); Glycogen storage diseases (SLC2A2, GLUT2, G6PC, G6PT, G6PT1, GAA, LAMP2, LAMPB, AGL, GDE, GBE1, GYS2, PYGL, PFKM); Hepatic adenoma, 142330 (TCF1, HNF1A, MODY3), Hepatic failure, early onset, and neurologic disorder (SCOD1, SCO1), Hepatic lipase deficiency (LIPC), Hepatoblastoma, cancer and carcinomas (CTNNB1, PDGFRL, PDGRL, PRLTS, AX1N1, AXIN, CTNNB1, TP53, P53, LFS1, IGF2R, MPRI, MET, CASP8, MCH5; Medullary cystic kidney disease (UMOD, HNFJ, FJHN, MCKD2, ADMCKD2); Phenylketonuria (PAH, PKU1, QDPR, DHPR, PTS); Polycystic kidney and hepatic disease

	(FCYT, PKHD1, ARPKD, PKD1, PKD2, PKD4, PKDTS, PRKCSH, G19P1, PCLD, SEC63).
Muscular/skeletal diseases and disorders	Becker muscular dystrophy (DMD, BMD, MYF6), Duchenne Muscular Dystrophy (DMD, BMD); Emery-Dreifuss muscular dystrophy (LMNA, LMN1, EMD2, FPLD, CMD1A, HGPS, LGMD1B, LMNA, LMN1, EMD2, FPLD, CMD1A); Facioscapulohumeral muscular dystrophy (FSHMD1A, FSHD1A); Muscular dystrophy (FKRP, MDC1C, LGMD2I, LAMA2, LAMM, LARGE, KIAA0609, MDC1D, FCMD, TTID, MYOT, CAPN3, CANP3, DYSF, LGMD2B, SGCG, LGMD2C, DMDA1, SCG3, SGCA, ADL, DAG2, LGMD2D, DMDA2, SGCB, LGMD2E, SGCD, SGD, LGMD2F, CMD1L, TCAP, LGMD2G, CMD1N, TRIM32, HT2A, LGMD2H, FKRP, MDC1C, LCMD21, TTN, CMD1G, TMD, LGMD2J, POMT1, CAV3, LGMD1C, SEPN1, SELN, RSMD1, PLEC1, PLTN, EBS1); Osteopetrosis (LRP5, BMND1, LRP7, LR3, OPPG, VBCH2, CLCN7, CLC7, OPTA2, OSTM1, GL, TCIRG1, TIRC7, OC116, OPTB1); Muscular atrophy (VAPB, VAPC, ALS8, SMN1, SMA1, SMA2, SMA3, SMA4, BSCL2, SPG17, GARS, SMAD1, CMT2D, HEXB, IGHMBP2, SMUBP2, CATF1, SMARD1).
Neurological and neuronal diseases and disorders	ALS (SOD1, ALS2, STEX, FUS, TARDBP, VEGF (VEGF-a, VEGF-b, VEGF-c); Alzheimer disease (APP, AAA, CVAP, AD1, APOE, AD2, PSEN2, AD4, STM2, APBB2, FE65L1, NOS3, PLAUI, URK, ACE, DCPI, ACEI, MPO, PAC1PI, PAXIPIL, PTIP, A2M, BLMH, BMH, PSEN1, AD3); Autism (Mecp2, BZRAP1, MDGA2, Sema5A, Neurexin 1, GLO1, MECP2, RTT, PPMX, MRX16, MRX79, NLGN3, NLGN4, KIAA1260, AUTSX2); Fragile X Syndrome (FMR2, FXR1, FXR2, mGLUR5), Huntington's disease and disease like disorders (HD, IT15, PRNP, PRIP, JPH3, JP3, HDL2, TBP, SCA17); Parkinson disease (NR4A2, NURR1, NOT, TINUR, SNCAIP, TBP, SCA17, SNCA, NACP, PARK1, PARK4, DJ1, PARK7, LRRK2, PARK8, PINK1, PARK6, UCHL1, PARK5, SNCA, NACP, PARK1, PARK4, PRKN, PARK2, PDJ, DBH, NDUFV2); Rett syndrome (MECP2, RTT, PPMX, MRX16, MRX79, CDKL5, STK9, MECP2, RTT, PPMX, MRX16, MRX79, x-Synuclein, DJ-1); Schizophrenia (Neuregulin1 (Nrg1), Erb4 (receptor for Neuregulin), Complexin1 (Cplx1), Tph1 Tryptophan hydroxylase, Tph2, Tryptophan hydroxylase 2, Neurexin 1, GSK3, GSK3a, GSK3b, 5-HTT (Slc6a4), CONT, DRD (Drd1a), SLC6Aß, DAOA, DTNBP1, Dao (Dao1)); Secretase Related Disorders (APH-1 (alpha and beta), Presenilin (Psen1), nicastrin, (Ncstn), PEN-2, Nos1, Parp1, Nat1, Nat2); Trinucleotide Repeat Disorders (HTT (Huntington's Dx), SBMA/SMAX1/AR (Kennedy's Dx), FXN/X25 (Friedrich's Ataxia), ATX3 (Machado-Joseph's Dx), ATXN1 and ATXN2 (spinocerebellar ataxias), DMPK (myotonic dystrophy), Atrophin-1 and Atn1 (DRPLA Dx), CBP (Creb-BP-global instability), VLDLR (Alzheimer's), Atxn7, Atxn10)
Ocular diseases and disorders	Age-related macular degeneration (Aber, Ccl2, Cc2, cp (ceruloplasmin), Timp3, cathepsinD, Vldlr, Ccr2); Cataract (CRYAA, CRYA1, CRYBB2, CRYB2, PITX3, BFSP2,

	CP49, CP47, CRYAA, CRYA1, PAX6, AN2 MGDA, CRYBA1, CRYB1, CRYGC, CRYG3, CCL, LIM2, MP19, CRYGD, CRYG4, BFSP2, CP49, CP47, HSF4, CTM, HSF4, CTM, MIP, AQPO, CRYAB, CRYA2, CTPP2, CRYBB1, CRYGD, CRYG4, CRYBB2, CRYB2, CRYGC, CRYG3, CCL, CRYAA, CRYA1, GJA8, CX50, CAE1, GJA3, CX46, CZP3, CAE3, CCM1, CAM, KRIT1); Corneal clouding and dystrophy (APOA1, TGFB1, CSD2, CDGG1, CSD, BIGH3, CDG2, TACSTD2, TROP2, M1SI, VSX1, RINX, PPCD, PPD, KTCN, COL8A2, FECD, PPCD2, PIP5K3, CFD); Cornea plana congenital (KERA, CNA2); Glaucoma (MYOC, TIGR, GLCIA, JOAG, GPOA, OPTN, GLC1E, FIP2, HYPL, NRP, CYP1B1, GLC3A, OPA1, NTG, NPG, CYP1B1, GLC3A); Leber congenital amaurosis (CRB1, RP12, CRX, CORD2, CRD, RPGRIP1, LCA6, CORD9, RPE65, RP20, AIPL1, LCA4, GUCY2D, GUC2D, LCA1, CORD6, RDH12, LCA3); Macular dystrophy (ELOVL4, ADMD, STGD2, STGD3, RDS, RP7, PRPH2, PRPH, AVMD, AOFMD, VMD2).
Epilepsy	NHLRC1, EPM2A, EPM2B
Duchenne muscular dystrophy	DMD, BMD
AIDS	KIR3DL1, NKAT3, NKB1, AMB11, KIR3DS1, IFNG, CDDCL12, SDF1
Alpha 1-Antitrypsin Deficiency	SERPINA1 [serpin peptidase inhibitor, cladeA (alpha-1 antiproteinase, antitrypsin), member 1]; SERPINA2 [serpin peptidase inhibitor, cladeA (alpha-1 antiproteinase, antitrypsin), member 2]; SERPINA3 [serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3]; SERPINA5 [serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5]; SERPINA6 [serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6]; SERPINA7 [serpin peptidase inhibitor, Glade A (alpha-1 antiproteinase, antitrypsin), member 7]; SERPINA6 (serpin peptidase inhibitor, cladeA (alpha-1 antiproteinase, antitrypsin), member 6)

TABLE 3

CELLULAR FUNCTION	GENES
PI3K/AKT Signaling	PRKCE; ITGAM; ITGA5; IRAK1; PRKAA2; EIF2AK2; PTEN; EIF4E; PRKCZ; GRK6; MAPK1; TSC1; PLK1; AKT2; IKBKB; PIK3CA; CDK8; CDKN1B; NFKB2; BCL2; PIK3CB; PPP2R1A; MAPK8; BCL2L1; MAPK3; TSC2; ITGA1; KRAS; EIF4EBP1; RELA; PRKCD; NOS3; PRKAA1; MAPK9; CDK2; PPP2CA; PIM1; ITGB7; YWHAZ; ILK; TP53; RAF1; IKBKG; RELB; DYRK1A; CDKN1A; ITGB1; MAP2K2; JAK1; AKT1; JAK2; PIK3R1; CHUK; PDPK1; PPP2R5C; CTNNB1; MAP2K1; NFKB1; PAK3; ITGB3; CCND1; GSK3A; FRAP1; SFN; ITGA2; TTK; CSNK1A1; BRAF; GSK3B; AKT3; FOXO1; SGK; HSP90AA1; RPS6KB1

ERK/MAPK Signaling	PRKCE; ITGAM; ITGA5; HSPB1; IRAK1; PRKAA2; EIF2AK2; RAC1; RAP1A; TLN1; EIF4E; ELK1; GRK6; MAPK1; RAC2; PLK1; AKT2; PIK3CA; CDK8; CREB1; PRKC1; PTK2; FOS; RPS6KA4; PIK3CB; PPP2R1A; PIK3C3; MAPK8; MAPK3; ITGA1; ETSI; KRAS; MYCN; EIF4EBP1; PPARG; PRKCD; PRKAA1; MAPK9; SRC; CDK2; PPP2CA; PIM1; PIK3C2A; ITGB7; YWHAZ; PPP1CC; KSR1; PXN; RAF1; FYN; DYRK1A; ITGB1; MAP2K2; PAK4; PIK3R1; STAT3; PPP2R5C; MAP2K1; PAK3; ITGB3; ESR1; ITGA2; MYC; TTK; CSNK1A1; CRKL; BRAF; ATF4; PRKCA; SRF; STAT1; SGK
Glucocorticoid Receptor Signaling	RAC1; TAF4B; EP300; SMAD2; TRAF6; PCAF; ELK1; MAPK1; SMAD3; AKT2; IKBKB; NCOR2; UBE21; PIK3CA; CREB1; FOS; HSPA5; NFKB2; BCL2; MAP3K14; STAT5B; PIK3CB; PIK3C3; MAPK8; BCL2L1; MAPK3; TSC22D3; MAPK10; NRIP1; KRAS; MAPK13; RELA; STAT5A; MAPK9; NOS2A; PBX1; NR3C1; PIK3C2A; CDKN1C; TRAF2; SERPINE1; NCOA3; MAPK14; TNF; RAF1; IKBKG; MAP3K7; CREBBP; CDKN1A; MAP2K2; JAK1; IL8; NCOA2; AKT1; JAK2; PIK3R1; CHUK; STAT3; MAP2K1; NFKB1; TGFB1; ESR1; SMAD4; CEBPB; WN; AR; AKT3; CCL2; MMP1; STAT1; IL6; HSP90AA1
Axonal Guidance Signaling	PRKCE; ITGAM; ROCK1; ITGA5; CXCR4; ADAM12; IGF1; RAC1; RAP1A; EIF4E; PRKCZ; NRP1; NTRK2; ARHGEF7; SMO; ROCK2; MAPK1; PGF; RAC2; PTPN11; GNAS; AKT2; PIK3CA; ERBB2; PRKCI; PTK2; CFL1; GNAQ; PIK3CB; CXCL12; PIK3C3; WNT11; PRKD1; GNB2L1; ABL1; MAPK3; ITGA1; KRAS; RHOA; PRKCD; PIK3C2A; ITGB7; GLI2; PXN; VASP; RAF1; FYN; ITGB1; MAP2K2; PAK4; ADAM17; AKT1; PIK3R1; GLI1; WNT5A; ADAM10; MAP2K1; PAK3; ITGB3; CDC42; VEGFA; ITGA2; EPHA8; CRKL; RND1; GSK3B; AKT3; PRKCA
Ephrin Receptor Signaling	PRKCE; ITGAM; ROCK1; ITGA5; CXCR4; IRAK1; PRKAA2; EIF2AK2; RAC1; RAP1A; GRK6; ROCK2; MAPK1; PGF; RAC2; PTPN11; GNAS; PLK1; AKT2; DOK1; CDK8; CREB1; PTK2; CFL1; GNAQ; MAP3K14; CXCL12; MAPK8; GNB2L1; ABL1; MAPK3; ITGA1; KRAS; RHOA; PRKCD; PRKAA1; MAPK9; SRC; CDK2; PIM1; ITGB7; PXN; RAF1; FYN; DYRK1A; ITGB1; MAP2K2; PAK4; AKT1; JAK2; STAT3; ADAM10; MAP2K1; PAK3; ITGB3; CDC42; VEGFA; ITGA2; EPHA8; TTK; CSNK1A1; CRKL; BRAF; PTPN13; ATF4; AKT3; SGK
Actin Cytoskeleton Signaling	ACTN4; PRKCE; ITGAM; ROCK1; ITGA5; IRAK1; PRKAA2; EIF2AK2; RAC1; INS; ARHGEF7; GRK6; ROCK2; MAPK1; RAC2; PLK1; AKT2; PIK3CA; CDK8; PTK2; CPL1; PIK3CB; MYH9; DIAPH1; PIK3C3; MAPK8; F2R; MAPK3; SLC9A1; ITGA1; KRAS; RHOA; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A; ITGB7; PPP1CC; PXN; VIL2; RAF1; GSN; DYRK1A; ITGB1; MAP2K2; PAK4; PIP5K1A; PIK3R1; MAP2K1; PAK3; ITGB3; CDC42; APC; ITGA2; TTK; CSNK1A1; CRKL; BRAF; VAV3; SGK
Huntington's Disease Signaling	PRKCE; IGF1; EP300; RCOR1; PRKCZ; HDAC4; TGM2; MAPK1; CAPNS1; AKT2; EGFR; NCOR2; SPI1; CAPN2; PIK3CA; HDAC5; CREB1; PRKCI; HSPA5; REST; GNAQ;

	PIK3CB; PIK3C3; MAPK8; IGF1R; PRKD1; GNB2L1; BCL2L1; CAPN1; MAPK3; CASP8; HDAC2; HDAC7A; PRKCD; HDAC11; MAPK9; HDAC9; PIK3C2A; HDAC3; TP53; CASP9; CREBBP; AKT1; PIK3R1; PDPK1; CASP1; APAF1; FRAP1; CASP2; JUN; BAX; ATF4; AKT3; PRKCA; CLTC; SGK; HDAC6; CASP3
Apoptosis Signaling	PRKCE; ROCK1; BID; IRAK1; PRKAA2; EIF2AK2; BAK1; BIRC4; GRK6; MAPK1; CAPNS1; PLK1; AKT2; IKBKB; CAPN2; CDK8; FAS; NFKB2; BCL2; MAP3K14; MAPK8; BCL2L1; CAPN1; MAPK3; CASP8; KRAS; RELA; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; TP53; TNF; RAF1; IKBKG; RELB; CASP9; DYRK1A; MAP2K2; CHUK; APAF1; MAP2K1; NFKB1; PAK3; LMNA; CASP2; BIRC2; TTK; CSNK1A1; BRAF; BAX; PRKCA; SGK; CASP3; BIRC3; PARP1
B Cell Receptor Signaling	RAC1; PTEN; LYN; ELK1; MAPK1; RAC2; PTPN11; AKT2; IKBKB; PIK3CA; CREB1; SYK; NFKB2; CAMK2A; MAP3K14; PIK3CB; PIK3C3; MAPK8; BCL2L1; ABL1; MAPK3; ETS1; KRAS; MAPK13; RELA; PTPN6; MAPK9; EGR1; PIK3C2A; BTK; MAPK14; RAF1; IKBKG; RELB; MAP3K7; MAP2K2; AKT1; PIK3R1; CHUK; MAP2K1; NFKB1; CDC42; GSK3A; FRAP1; BCL6; BCL10; JUN; GSK3B; ATF4; AKT3; VAV3; RPS6KB1
Leukocyte Extravasation Signaling	ACTN4; CD44; PRKCE; ITGAM; ROCK1; CXCR4; CYBA; RAC1; RAP1A; PRKCZ; ROCK2; RAC2; PTPN11; MMP14; PIK3CA; PRKCI; PTK2; PIK3CB; CXCL12; PIK3C3; MAPK8; PRKD1; ABL1; MAPK10; CYBB; MAPK13; RHOA; PRKCD; MAPK9; SRC; PIK3C2A; BTK; MAPK14; NOX1; PXN; VIL2; VASP; ITGB1; MAP2K2; CTNND1; PIK3R1; CTNNB1; CLDN1; CDC42; F11R; ITK; CRKL; VAV3; CTTN; PRKCA; MMP1; MMP9
Integrin Signaling	ACTN4; ITGAM; ROCK1; ITGA5; RAC1; PTEN; RAP1A; TLN1; ARHGEF7; MAPK1; RAC2; CAPNS1; AKT2; CAPN2; PIK3CA; PTK2; PIK3CB; PIK3C3; MAPK8; CAV1; CAPN1; ABL1; MAPK3; ITGA1; KRAS; RHOA; SRC; PIK3C2A; ITGB7; PPP1CC; ILK; PXN; VASP; RAF1; FYN; ITGB1; MAP2K2; PAK4; AKT1; PIK3R1; TNK2; MAP2K1; PAK3; ITGB3; CDC42; RND3; ITGA2; CRKL; BRAF; GSK3B; AKT3
Acute Phase Response Signaling	IRAK1; SOD2; MYD88; TRAF6; ELK1; MAPK1; PTPN11; AKT2; IKBKB; PIK3CA; FOS; NFKB2; MAP3K14; PIK3CB; MAPK8; RIPK1; MAPK3; IL6ST; KRAS; MAPK13; IL6R; RELA; SOCS1; MAPK9; FTL; NR3C1; TRAF2; SERPINE1; MAPK14; TNF; RAF1; PDK1; IKBKG; RELB; MAP3K7; MAP2K2; AKT1; JAK2; PIK3R1; CHUK; STAT3; MAP2K1; NFKB1; FRAP1; CEBPB; JUN; AKT3; IL1R1; IL6
PTEN Signaling	ITGAM; ITGA5; RAC1; PTEN; PRKCZ; BCL2L11; MAPK1; RAC2; AKT2; EGFR; IKBKB; CBL; PIK3CA; CDKN1B; PTK2; NFKB2; BCL2; PIK3CB; BCL2L1; MAPK3; ITGA1; KRAS; ITGB7; ILK; PDGFRB; INSR; RAF1; IKBKG; CASP9; CDKN1A; ITGB1; MAP2K2; AKT1; PIK3R1; CHUK; PDGFRA; PDPK1; MAP2K1; NFKB1; ITGB3; CDC42; CCND1; GSK3A; ITGA2; GSK3B; AKT3; FOXO1; CASP3; RPS6KB1

p53 Signaling	PTEN; EP300; BBC3; PCAF; FASN; BRCA1; GADD45A; BIRC5; AKT2; PIK3CA; CHEK1; TP53INP1; BCL2; PIK3CB; PIK3C3; MAPK8; THBS1; ATR; BCL2L1; E2F1; PMAIP1; CHEK2; TNFRSF10B; TP73; RB1; HDAC9; CDK2; PIK3C2A; MAPK14; TP53; LRDD; CDKN1A; HIPK2; AKT1; PIK3R1; RRM2B; APAF1; CTNNB1; SIRT1; CCND1; PRKDC; ATM; SFN; CDKN2A; JUN; SNAI2; GSK3B; BAX; AKT3
Aryl Hydrocarbon Receptor Signaling	HSPR1; EP300; FASN; TGM2; RXRA; MAPK1; NQO1; NCOR2; SP1; ARNT; CDKN1B; FOS; CHEK1; SMARCA4; NEKB2; MAPK8; ALDH1A1; ATR; E2F1; MAPK3; NRIP1; CHEK2; RELA; TP73; GSTP1; RB1; SRC; CDK2; AHR; NFE2L2; NCOA3; TP53; TNF; CDKN1A; NCOA2; APAF1; NFKB1; CCND1; ATM; ESR1; CDKN2A; MYC; JUN; ESR2; BAX; IL6; CYP1B1; HSP90AA1
Xenobiotic Metabolism Signaling	PRKCE; EP300; PRKCZ; RXRA; MAPK1; NQO1; NCOR2; PIK3CA; ARNT; PRKCI; NFKB2; CAMK2A; PIK3CB; PPP2R1A; PIK3C3; MAPK8; PRKD1; ALDH1A1; MAPK3; NRIP1; KRAS; MAPK13; PRKCD; GSTP1; MAPK9; NOS2A; ABCB1; AHR; PPP2CA; FTL; NFE2L2; PIK3C2A; PPARGC1A; MAPK14; TNF; RAF1; CREBBP; MAP2K2; PIK3R1; PPP2R5C; MAP2K1; NFKB1; KEAP1; PRKCA; EIF2AK3; IL6; CYP1B1; HSP90AA1
SAPK/JNK Signaling	PRKCE; IRAK1; PRKAA2; EIF2AK2; RAC1; ELK1; GRK6; MAPK1; GADD45A; RAC2; PLK1; AKT2; PIK3CA; FADD; CDK8; PIK3CB; PIK3C3; MAPK8; RIPK1; GNB2L1; IRS1; MAPK3; MAPK10; DAXX; KRAS; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A; TRAF2; TP53; LCK; MAP3K7; DYRK1A; MAP2K2; PIK3R1; MAP2K1; PAK3; CDC42; JUN; TTK; CSNK1A1; CRKL; BRAF; SGK
PPAr/RXR Signaling	PRKAA2; EP300; INS; SMAD2; TRAF6; PPARA; FASN; RXRA; MAPK1; SMAD3; GNAS; IKBKB; NCOR2; ABCA1; GNAQ; NFKB2; MAP3K14; STAT5B; MAPK8; IRS1; MAPK3; KRAS; RELA; PRKAA1; PPARGC1A; NCOA3; MAPK14; INSR; RAF1; IKBKG; RELB; MAP3K7; CREBBP; MAP2K2; JAK2; CHUK; MAP2K1; NFKB1; TGFBR1; SMAD4; JUN; IL1R1; PRKCA; IL6; HSP90AA1; ADIPOQ
NF-KB Signaling	IRAK1; EIF2AK2; EP300; INS; MYD88; PRKCZ; TRAF6; TBK1; AKT2; EGFR; IKBKB; PIK3CA; BTRC; NFKB2; MAP3K14; PIK3CB; PIK3C3; MAPK8; RIPK1; HDAC2; KRAS; RELA; PIK3C2A; TRAF2; TLR4; PDGFRB; TNF; INSR; LCK; IKBKG; RELB; MAP3K7; CREBBP; AKT1; PIK3R1; CHUK; PDGFRA; NFKB1; TLR2; BCL10; GSK3B; AKT3; TNFAIP3; IL1R1
Neuregulin Signaling	ERBB4; PRKCE; ITGAM; ITGA5; PTEN; PRKCZ; ELK1; MAPK1; PTPN11; AKT2; EGFR; ERBB2; PRKCI; CDKN1B; STAT5B; PRKD1; MAPK3; ITGA1; KRAS; PRKCD; STAT5A; SRC; ITGB7; RAF1; ITGB1; MAP2K2; ADAM17; AKT1; PIK3R1; PDPK1; MAP2K1; ITGB3; EREG; FRAP1; PSEN1; ITGA2; MYC; NRG1; CRKL; AKT3; PRKCA; HSP90AA1; RPS6KB1
Wnt & Beta catenin Signaling	CD44; EP300; LRP6; DVL3; CSNK1E; GJA1; SMO; AKT2; PIN1; CDH1; BTRC; GNAQ; MARK2; PPP2R1A; WNT11; SRC; DKK1; PPP2CA; SOX6; SFRP2; ILK; LEF1; SOX9;

	TP53; MAP3K7; CREBBP; TCF7L2; AKT1; PPP2R5C; WNT5A; LRP5; CTNNB1; TGFBR1; CCND1; GSK3A; DVL1; APC; CDKN2A; MYC; CSNK1A1; GSK3B; AKT3; SOX2
Insulin Receptor Signaling	PTEN; INS; EIF4E; PTPN1; PRKCZ; MAPK1; TSC1; PTPN11; AKT2; CBL; PIK3CA; PRKCI; PIK3CB; PIK3C3; MAPKS; IRS1; MAPK3; TSC2; KRAS; EIF4EBP1; SLC2A4; PIK3C2A; PPP1CC; INSR; RAF1; FYN; MAP2K2; JAK1; AKT1; JAK2; PIK3R1; PDPK1; MAP2K1; GSK3A; FRAP1; CRKL; GSK3B; AKT3; FOXO1; SGK; RPS6KB1
IL-6 Signaling	HSPB1; TRAF6; MAPKAPK2; ELK1; MAPK1; PTPN11; IKBKB; FOS; NFKB2; MAP3K14; MAPKS; MAPK3; MAPK10; IL6ST; KRAS; MAPK13; IL6R; RELA; SOCS1; MAPK9; ABCB1; TRAF2; MAPK14; TNF; RAF1; IKBKG; RELB; MAP3K7; MAP2K2; IL8; JAK2; CHUK; STAT3; MAP2K1; NFKB1; CEBPB; JUN; IL1R1; SRF; IL6
Hepatic Cholestasis	PRKCE; IRAK1; INS; MYDSS; PRKCZ; TRAF6; PPARA; RXRA; IKBKB; PRKCI; NFKB2; MAP3K14; MAPKS; PRKD1; MAPK10; RELA; PRKCD; MAPK9; ABCB1; TRAF2; TLR4; TNF; INSR; IKBKG; RELB; MAP3K7; IL8; CHUK; NR1H2; TJP2; NFKB1; ESR1; REBF1; FGFR4; JUN; IL1R1; PRKCA; IL6
IGF-1 Signaling	IGF1; PRKCZ; ELK1; MAPK1; PTPN11; NEDD4; AKT2; PIK3CA; PRKCI; PTK2; FOS; PIK3CB; PIK3C3; MAPKS; IGF1R; IRS1; MAPK3; IGFBP7; KRAS; PIK3C2A; YWHAZ; PXN; RAF1; CASP9; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; IGFBP2; SFN; JUN; CYR61; AKT3; FOXO1; SRF; CTGF; RPS6KB1
NRF2-mediated Oxidative Stress Response	PRKCE; EP300; SOD2; PRKCZ; MAPK1; SQSTM1; NQO1; PIK3CA; PRKCI; FOS; PIK3CB; PIK3C3; MAPK8; PRKD1; MAPK3; KRAS; PRKCD; GSTP1; MAPK9; FTL; NFE2L2; PIK3C2A; MAPK14; RAF1; MAP3K7; CREBBP; MAP2K2; AKT1; PIK3R1; MAP2K1; PPIB; JUN; KEAP1; GSK3B; ATF4; PRKCA; EIF2AK3; HSP90AA1
Hepatic Fibrosis/Hepatic Stellate Cell Activation	EDN1; IGF1; KDR; FLT1; SMAD2; FGFR1; MET; PGF; SMAD3; EGFR; FAS; CSF1; NFKB2; BCL2; MYH9; IGF1R; IL6R; RELA; TLR4; PDGFRB; TNF; RELB; IL8; PDGFRA; NFKB1; TGFBR1; SMAD4; VEGFA; BAX; IL1R1; CCL2; HGF; MMP1; STAT1; IL6; CTGF; MMP9
PPAR Signaling	EP300; INS; TRAF6; PPARA; RXRA; MAPK1; IKBKB; NCOR2; FOS; NFKB2; MAP3K14; STAT5B; MAPK3; NRIP1; KRAS; PPARG; RELA; STAT5A; TRAF2; PPARGC1A; PDGFRB; TNF; INSR; RAF1; IKBKG; RELB; MAP3K7; CREBBP; MAP2K2; CHUK; PDGFRA; MAP2K1; NFKB1; JUN; IL1R1; HSP90AA1
Fc Epsilon R1 Signaling	PRKCE; RAC1; PRKCZ; LYN; MAPK1; RAC2; PTPN11; AKT2; PIK3CA; SYK; PRKCI; PIK3CB; PIK3C3; MAPK8; PRKD1; MAPK3; MAPK10; KRAS; MAPK13; PRKCD; MAPK9; PIK3C2A; BTK; MAPK14; TNF; RAF1; FYN; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; AKT3; VAV3; PRKCA
G-Protein Coupled Receptor Signaling	PRKCE; RAP1A; RGS16; MAPK1; GNAS; AKT2; IKBKB; PIK3CA; CREB1; GNAQ; NFKB2; CAMK2A; PIK3CB; PIK3C3; MAPK3; KRAS; RELA; SRC; PIK3C2A; RAF1; IKBKG; RELB; FYN; MAP2K2; AKT1; PIK3R1; CHUK;

	PDPK1; STAT3; MAP2K1; NFKB1; BRAF; ATF4; AKT3; PRKCA
Inositol Phosphate Metabolism	PRKCE; IRAK1; PRKAA2; EIF2AK2; PTEN; GRK6; MAPK1; PLK1; AKT2; PIK3CA; CDK8; PIK3CB; PIK3C3; MAPK8; MAPK3; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A; DYRK1A; MAP2K2; PIP5K1A; PIK3R1; MAP2K1; PAK3; ATM; TTK; CSNK1A1; BRAF; SGK
PDGF Signaling	EIF2AK2; ELK1; ABL2; MAPK1; PIK3CA; FOS; PIK3CB; PIK3C3; MAPK8; CAV1; ABL1; MAPK3; KRAS; SRC; PIK3C2A; PDGFRB; RAF1; MAP2K2; JAK1; JAK2; PIK3R1; PDGFRA; STAT3; SPHK1; MAP2K1; MYC; JUN; CRKL; PRKCA; SRF; STAT1; SPHK2
VEGF Signaling	ACTN4; ROCK1; KDR; FLT1; ROCK2; MAPK1; PGF; AKT2; PIK3CA; ARNT; PTK2; BCL2; PIK3CB; PIK3C3; BCL2L1; MAPK3; KRAS; HIF1A; NOS3; PIK3C2A; PXN; RAF1; MAP2K2; ELAVL1; AKT1; PIK3R1; MAP2K1; SFN; VEGFA; AKT3; FOXO1; PRKCA
Natural Killer Cell Signaling	PRKCE; RAC1; PRKCZ; MAPK1; RAC2; PTPN11; KIR2DL3; AKT2; PIK3CA; SYK; PRKCI; PIK3CB; PIK3C3; PRKD1; MAPK3; KRAS; PRKCD; PTPN6; PIK3C2A; LCK; RAF1; FYN; MAP2K2; PAK4; AKT1; PIK3R1; MAP2K1; PAK3; AKT3; VAV3; PRKCA
Cell Cycle: G1/S Checkpoint Regulation	HDAC4; SMAD3; SUV39H1; HDAC5; CDKN1B; BTRC; ATR; ABL1; E2F1; HDAC2; HDAC7A; RB1; HDAC11; HDAC9; CDK2; E2F2; HDAC3; TP53; CDKN1A; CCND1; E2F4; ATM; RBL2; SMAD4; CDKN2A; MYC; NRG1; GSK3B; RBL1; HDAC6
T Cell Receptor Signaling	RAC1; ELK1; MAPK1; IKBKB; CBL; PIK3CA; FOS; NFKB2; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; RELA; PIK3C2A; BTK; LCK; RAF1; IKBKG; RELB; FYN; MAP2K2; PIK3R1; CHUK; MAP2K1; NFKB1; ITK; BCL10; JUN; VAV3
Death Receptor Signaling	CRADD; HSPB1; BID; BIRC4; TBK1; IKBKB; FADD; FAS; NFKB2; BCL2; MAP3K14; MAPK8; RIPK1; CASP8; DAXX; TNFRSF10B; RELA; TRAF2; TNF; IKBKG; RELB; CASP9; CHUK; APAF1; NFKB1; CASP2; BIRC2; CASP3; BIRC3
FGF Signaling	RAC1; FGFR1; MET; MAPKAPK2; MAPK1; PTPN11; AKT2; PIK3CA; CREB1; PIK3CB; PIK3C3; MAPK8; MAPK3; MAPK13; PTPN6; PIK3C2A; MAPK14; RAF1; AKT1; PIK3R1; STAT3; MAP2K1; FGFR4; CRKL; ATF4; AKT3; PRKCA; HGF
GN-CSF Signaling	LYN; ELK1; MAPK1; PTPN11; AKT2; PIK3CA; CAMK2A; STAT5B; PIK3CB; PIK3C3; GNB2L1; BCL2L1; MAPK3; ETS1; KRAS; RUNX1; PIM1; PIK3C2A; RAF1; MAP2K2; AKT1; JAK2; PIK3R1; STAT3; MAP2K1; CCND1; AKT3; STAT1
Amyotrophic Lateral Sclerosis Signaling	BID; IGF1; RAC1; BIRC4; PGF; CAPNS1; CAPN2; PIK3CA; BCL2; PIK3CB; PIK3C3; BCL2L1; CAPN1; PIK3C2A; TP53; CASP9; PIK3R1; RAB5A; CASP1; APAF1; VEGFA; BIRC2; BAX; AKT3; CASP3; BIRC3
JAK/Stat Signaling	PTPN1; MAPK1; PTPN11; AKT2; PIK3CA; STAT5B; PIK3CB; PIK3C3; MAPK3; KRAS; SOCS1; STAT5A; PTPN6; PIK3C2A; RAF1; CDKN1A; MAP2K2; JAK1;

	AKT1; JAK2; PIK3R1; STAT3; MAP2K1; FRAP1; AKT3; STAT1
Nicotinate and Nicotinamide Metabolism	PRKCE; IRAK1; PRKAA2; EIF2AK2; GRK6; MAPK1; LK1; AKT2; T2; CDK8; MAPK8; MAPK3; PRKCD; PRKAA1; PBEF1; MAPK9; CDK2; PIMI; DYRK1A; MAP2K2; MAP2K1; PAK3; NT5E; TTK; CSNK1A1; BRAF; SGK
Chemokine Signaling	CXCR4; ROCK2; MAPK1; PTK2; FOS; CFL1; GNAQ; CAMK2A; CXCL12; MAPK8; MAPK3; KRAS; MAPK13; RHOA; CCR3; SRC; PPP1CC; MAPK14; NOX1; RAF1; MAP2K2; MAP2K1; JUN; CCL2; PRKCA
IL-2 Signaling	ELK1; MAPK1; PTPN11; AKT2; PIK3CA; SYK; FOS; STAT5B; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; SOCS1; STAT5A; PIK3C2A; LCK; RAF1; MAP2K2; JAK1; AKT1; PIK3R1; MAP2K1; JUN; AKT3
Synaptic Long Term Depression	PRKCE; IGF1; PRKCZ; PRDX6; LYN; MAPK1; GNAS; PRKCI; GNAQ; PPP2R1A; IGF1R; PRKD1; MAPK3; KRAS; GRN; PRKCD; NOS3; NOS2A; PPP2CA; YWHAZ; RAF1; MAP2K2; PPP2R5C; MAP2K1; PRKCA
Estrogen Receptor Signaling	TAF4B; EP300; CARM1; PCAF; MAPK1; NCOR2; SMARCA4; MAPK3; NRIP1; KRAS; SRC; NR3C1; HDAC3; PPARGG1A; RBM9; NCOA3; RAF1; CREBBP; MAP2K2; NCOA2; MAP2K1; PRKDC; ESR1; ESR2
Protein Ubiquitination Pathway	TRAF6; SMURF1; BIRC4; BRCA1; UCHL1; NEDD4; CBL; UBE2I; BTRC; HSPA5; USP7; USP10; FBXW7; USP9X; STUB1; USP22; B2M; BIRC2; PARK2; USP8; USP1; VHL; HSP90AA1; BIRC3
IL-10 Signaling	TRAF6; CCR1; ELK1; IKBKB; SP1; FOS; NFKB2; MAP3K14; MAPK8; MAPK13; RELA; MAPK14; TNF; IKBKG; RELB; MAP3K7; JAK1; CHUK; STAT3; NFKB1; JUN; IL1R1; IL6
VDR/RXR Activation	PRKCE; EP300; PRKCZ; RXRA; GADD45A; HES1; NCOR2; SP1; PRKCI; CDKN1B; PRKD1; PRKCD; RUNX2; KLF4; YY1; NCOA3; CDKN1A; NCOA2; SPP1; LRP5; CEBPB; FOXO1; PRKCA
TGF-beta Signaling	EP300; SMAD2; SMURF1; MAPK1; SMAD3; SMAD1; FOS; MAPK8; MAPK3; KRAS; MAPK9; RUNX2; SERPINE1; RAF1; MAP3K7; CREBBP; MAP2K2; MAP2K1; TGFB1; SMAD4; JUN; SMAD5
Toll-like Receptor Signaling	IRAK1; EIF2AK2; MYD88; TRAF6; PPARA; ELK1; IKBKB; FOS; NFKB2; MAP3K14; MAPK8; MAPK13; RELA; TLR4; MAPK14; IKBKG; RELB; MAP3K7; CHUK; NFKB1; TLR2; JUN
P38 MAPK Signaling	HSPB1; IRAK1; TRAF6; MAPKAPK2; ELK1; FADD; FAS; CREB1; DDIT3; RPS6KA4; DAXX; MAPK13; TRAF2; MAPK14; TNF; MAP3K7; TGFB1; MYC; ATF4; IL1R1; SRF; STAT1
Neurotrophin/TRK Signaling	NTRK2; MAPK1; PTPN11; PIK3CA; CREB1; FOS; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; PIK3C2A; RAF1; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; CDC42; JUN; ATF4
FXR/RXR Activation	INS; PPARA; FASN; RXRA; AKT2; SDC1; MAPK8; APOB; MAPK10; PPARG; MTPP; MAPK9; PPARGC1A; TNF; CREBBP; AKT1; SREBF1; FGFR4; AKT3; FOXO1

Synaptic Long Term Potentiation	PRKCE; RAP1A; EP300; PRKCZ; MAPK1; CREB1; PRKCI; GNAQ; CAMK2A; PRKD1; MAPK3; KRAS; PRKCD; PPP1CC; RAF1; CREBBP; MAP2K2; MAP2K1; ATF4; PRKCA
Calcium Signaling	RAP1A; EP300; HDAC4; MAPK1; HDAC5; CREB1; CAMK2A; MYH9; MAPK3; HDAC2; HDAC7A; HDAC11; HDAC9; HDAC3; CREBBP; CALR; CAMKK2; ATF4; HDAC6
EGF Signaling	ELK1; MAPK1; EGFR; PIK3CA; FOS; PIK3CB; PIK3C3; MAPK8; MAPK3; PIK3C2A; RAF1; JAK1; PIK3R1; STAT3; MAP2K1; JUN; PRKCA; SRF; STAT1
Hypoxia Signaling in the Cardiovascular System	EDN1; PTEN; EP300; NQO1; UBE2I; CREB1; ARNT; HIF1A; SLC2A4; NOS3; TP53; LDHA; AKT1; ATM; VEGFA; JUN; ATF4; VHL; HSP90AA1
LPS/IL-1 Mediated Inhibition of RXR Function	IRAK1; MYD88; TRAF6; PPARA; RXRA; ABCA1; MAPK8; ALDH1A1; GSTP1; MAPK9; ABCB1; TRAF2; TLR4; TNF; MAP3K7; NR1H2; SREBF1; JUN; IL1R1
LXR/RXR Activation	FASN; RXRA; NCOR2; ABCA1; NFKB2; IRF3; RELA; NOS2A; TLR4; TNF; RELB; LDLR; NR1H2; NFKB1; SREBF1; IL1R1; CCL2; IL6; MMP9
Amyloid Processing	PRKCE; CSNK1E; MAPK1; CAPNS1; AKT2; CAPN2; CAPN1; MAPK3; MAPK13; MAPT; MAPK14; AKT1; PSEN1; CSNK1A1; GSK3B; AKT3; APP
IL-4 Signaling	AKT2; PIK3CA; PIK3CB; PIK3C3; IRS1; KRAS; SOCS1; PTPN6; NR3C1; PIK3C2A; JAK1; AKT1; JAK2; PIK3R1; FRAP1; AKT3; RPS6KB1
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	EP300; PCAF; BRCA1; GADD45A; PLK1; BTRC; CHEK1; ATR; CHEK2; YWHAZ; TP53; CDKN1A; PRKDC; ATM; SFN; CDKN2A
Nitric Oxide Signaling in the Cardiovascular System	KDR; FLT1; PGF; AKT2; PIK3CA; PIK3CB; PIK3C3; CAV1; PRKCD; NOS3; PIK3C2A; AKT1; PIK3R1; VEGFA; AKT3; HSP90AA1
Purine Metabolism	NME2; SMARCA4; MYH9; RRM2; ADAR; EIF2AK4; PKM2; ENTPD1; RAD51; RRM2B; TJP2; RAD51C; NT5E; POLD1; NME1
cAMP-mediated Signaling	RAP1A; MAPK1; GNAS; CREB1; CAMK2A; MAPK3; SRC; RAF1; MAP2K2; STAT3; MAP2K1; BRAF; ATF4
Mitochondrial Dysfunction	SOD2; MAPK8; CASP8; MAPK10; MAPK9; CASP9; PARK7; PSEN1; PARK2; APP; CASP3
Notch Signaling	HES1; JAG1; NUMB; NOTCH4; ADAM17; NOTCH2; PSEN1; NOTCH3; NOTCH1; DLL4
Endoplasmic Reticulum Stress Pathway	HSPA5; MAPK8; XBP1; TRAF2; ATF6; CASP9; ATF4; EIF2AK3; CASP3
Pyrimidine Metabolism	NME2; AICDA; RRM2; EIF2AK4; ENTPD1; RRM2B; NT5E; POLD1; NME1
Parkinson's Signaling	UCHL1; MAPK8; MAPK13; MAPK14; CASP9; PARK7; PARK2; CASP3
Cardiac & Beta Adrenergic Signaling	GNAS; GNAQ; PPP2R1A; GNB2L1; PPP2CA; PPP1CC; PPP2R5C
Glycolysis/Gluconeogenesis	HK2; GCK; GPI; ALDH1A1; PKM2; LDHA; HK1
Interferon Signaling	IRF1; SOCS1; JAK1; JAK2; IFITM1; STAT1; IFIT3
Sonic Hedgehog Signaling	ARRB2; SMO; GLI2; DYRK1A; GLI1; GSK39; DYRK1B
Glycerophospholipid Metabolism	PLD1; GRN; GPAM; YWHAZ; SPHK1; SPHK2
Phospholipid Degradation	PRDX6; PLD1; GRN; YWHAZ; SPHK1; SPHK2

Tryptophan Metabolism	SIAH2; PRMT5; NEDD4; ALDH1A1; CYP1B1; SIAH1
Lysine Degradation	SUV39H1; EHMT2; NSD1; SETD7; PPP2R5C
Nucleotide Excision Repair Pathway	ERCC5; ERCC4; XPA; XPC; ERCC1
Starch and Sucrose Metabolism	UCHL1; HK2; GCK; GPI; HK1
Aminosugars Metabolism	NQO1; HK2; GCK; HK1
Arachidonic Acid Metabolism	PRDX6; GRN; YWHAZ; CYP1B1
Circadian Rhythm Signaling	CSNK1E; CREB1; ATF4; NR1D1
Coagulation System	BDKRB1; F2R; SERPINE1; F3
Dopamine Receptor Signaling	PPP2R1A; PPP2CA; PPP1CC; PPP2R5C
Glutathione Metabolism	IDH2; GSTP1; ANPEP; IDH1
Glycerolipid Metabolism	ALDH1A1; GPAM; SPHK1; SPHK2
Linoleic Acid Metabolism	PRDX6; GRN; YWHAZ; CYP1B1
Methionine Metabolism	DNMT1; DNMT3B; AHCY; DNMT3A
Pyruvate Metabolism	GLO1; ALDH1A1; PKM2; LDHA
Arginine and Proline Metabolism	ALDH1A1; NOS3; NOS2A
Eicosanoid Signaling	PRDX6; GRN; YWHAZ
Fructose and Mannose Metabolism	HK2; GCK; HK1
Galactose Metabolism	HK2; GCK; HK1
Stilbene, Coumarine and Lignin Biosynthesis	PRDX6; PRDX1; TYR
Antigen Presentation Pathway	CALR; B2M
Biosynthesis of Steroids	NQO1; DHCR7
Butanoate Metabolism	ALDH1A1; NLGN1
Citrate Cycle	IDH2; IDH1
Fatty Acid Metabolism	ALDH1A1; CYP1B1
Glycerophospholipid Metabolism	PRDX6; CHKA
Histidine Metabolism	PRMT5; ALDH1A1
Inositol Metabolism	ERO1L; APEX1
Metabolism of Xenobiotics by Cytochrome p450	GSTP1; CYP1B1
Methane Metabolism	PRDX6; PRDX1
Phenylalanine Metabolism	PRDX6; PRDX1
Propanoate Metabolism	ALDH1A1; LDHA
Selenoamino Acid Metabolism	PRMT5; AHCY
Sphingolipid Metabolism	SPHK1; SPHK2
Aminophosphonate Metabolism	PRMT5
Androgen and Estrogen Metabolism	PRMT5
Ascorbate and Aldarate Metabolism	ALDH1A1
Bile Acid Biosynthesis	ALDH1A1
Cysteine Metabolism	LDHA
Fatty Acid Biosynthesis	FASN

Glutamate Receptor Signaling	GNB2L1
NRF2-mediated Oxidative Stress Response	PRDX1
Pentose Phosphate Pathway	GPI
Pentose and Glucuronate Interconversions	UCHL1
Retinol Metabolism	ALDH1A1
Riboflavin Metabolism	TYR
Tyrosine Metabolism	PRMT5, TYR
Ubiquinone Biosynthesis	PRMT5
Valine, Leucine and Isoleucine Degradation	ALDH1A1
Glycine, Serine and Threonine Metabolism	CHKA
Lysine Degradation	ALDH1A1
Pain/Taste	TRPM5; TRPA1
Pain	TRPM7; TRPC5; TRPC6; TRPC1; Cnr1; cnr2; Grk2; Trpa1; Pomc; Cgrp; Crf; Pka; Era; Nr2b; TRPM5; Prkaca; Prkacb; Prkar1a; Prkar2a
Mitochondrial Function	AIF; CytC; SMAC (Diablo); Aifm-1; Aifm-2
Developmental Neurology	BMP-4; Chordin (Chrd); Noggin (Nog); WNT (Wnt2; Wnt2b, Wnt3a, Wnt4; Wnt5a; Wnt6; Wnt7b; Wnt8b; Wnt9a; Wnt9b; Wnt10a; Wnt10b, Wnt16); beta-catenin; Dkk-1; Frizzled related proteins; Otx-2; Gbx2; FGF-8; Reelin; Dab1; unc-86 (Pou4f1 or Brn3a); Numb; Reln

TABLE 4

INDICATION(S)	THERAPEUTIC PROTEIN
Maple syrup urine disease	3-methyl-2-oxobutanoate dehydrogenase
Medium-chain acyl-CoA dehydrogenase deficiency	Acyl-CoA dehydrogenase
Alpha 1-antitrypsin deficiency	Alpha 1 protease inhibitor
Pompe disease	Alpha glucosidase
Paroxysmal nocturnal hemoglobinuria	Anti-complement factor C5 Mab
Familial dysbetalipoproteinemia	Apolipoprotein E
Argininemia	Arginase
Argininosuccinic acidemia	Argininosuccinate lyase
Citrullinemia, type I	Argininosuccinate synthase
Short-chain acyl-CoA dehydrogenase deficiency	Butyryl-CoA dehydrogenase
Hereditary angioedema	C1 esterase inhibitor
Carbamylphosphate synthetase deficiency	Carbamylphosphate synthetase
Cystic fibrosis	CFTR
Hemophilia B	Factor IX
Hemophilia A, Hemophilia B	Factor VII
Hemophilia A	Factor VIII
Classical galactosemia	Galactose-1-phosphate uridylyltransferase
von Gierke's disease	Glucose-6-phosphatase
Glutaric acidemia, type I	Glutaryl-CoA dehydrogenase

Isovaleric aciduria	Isovaleric acid CoA dehydrogenase deficiency
Homozygous familial hypercholesterolemia	LDL receptor
Long-chain 3-OH acyl-CoA dehydrogenase deficiency	Long-chain-3-hydroxyacyl-CoA dehydrogenase
Very long-chain acyl-CoA dehydrogenase deficiency	Long-chain-acyl-CoA dehydrogenase
Methylmalonyl-CoA mutase deficiency	Methylmalonyl-CoA mutase
Ornithine transcarbamylase deficiency	Ornithine transcarbamylase
Phenylketonuria	Phenylalanine hydroxylase
Acute intermittent porphyria	Porphobilinogen deaminase
Propionic acidemia	Propionyl-CoA carboxylase
Hyperoxaluria, type I	Serine-pyruvate aminotransferase
Crigler-Najjar syndrome	UDP-glucuronosyltransferase
Non-Hodgkin lymphoma	Anti-CD20 mAb
Allergic asthma	Anti-IgE mAb
Psoriasis	Anti-IL-12 & IL-23 mAb
Rheumatoid arthritis	Anti-interleukin-6 (IL-6) mAb
Anemia	Erythropoietin
Rheumatoid arthritis	T-cell costimulation blocker
Rheumatoid arthritis	TNF-alpha inhibitors (including anti-TNF-alpha mAb)
Gout	Urate oxidase
Familial chylomicronemia	Lipoprotein lipase
Melanoma	Anti-CTLA4 mAb
Head and neck cancer, Metastatic colorectal cancer	Anti-EGFr mAb
HER2+ breast cancer, gastric cancer	Anti-HER2 mAb
Metastatic colorectal cancer, NSCLC, others	Anti-VEGF mAb
Blepharospasm, Cervical dystonia, Chronic migraine, more	Botulinum toxin
Female infertility	Follicle stimulating hormone
Type 2 diabetes mellitus	Glucagon-like peptide 1 (GLP-1) agonist
Growth hormone deficiency	Growth hormone 1 / Growth hormone 2
Type 2 diabetes mellitus	Insulin
Hypoparathyroidism	Parathyroid hormone
Asthma	SERCA2
Asthma	FoxP3
Surfactant Deficiency	Pulmonary surfactants (SFTPA1, SFTPB, SFTPC, SFTPD)
Pulmonary Alveolar proteinosis	GM-CSF Receptor (CSF2RA, CSF2RB)
alport syndrome	Col4A5
Stargardt's Disease	ABCA4
Retinitis pigmentosa	Rhodopsins
Adrenoleukodystrophy	ABCD1
Adenosine deaminase deficiency	Adenosine deaminase
Familial adenomatous polyposis	APC
Autosomal recessive polycystic kidney disease	ARPKD
Metachromatic leukodystrophy	Arylsulfatase A
Batten disease	Battenin + others

Beta-thalassemia	Beta globin
X-linked agammaglobulinemia	Bruton's tyrosine kinase
Becker muscular dystrophy	Dystrophin
Duchenne muscular dystrophy	Dystrophin
Marfan syndrome	FBN1
Fragile X syndrome	FMRP
Krabbe disease	Galactocerebrosidase
Sickle cell disease	Hemoglobin
Sanfilippo syndrome, type A (MPS IIIA)	Heparan N-sulfatase
GM2 gangliosidosis	HEXA, HEXB
Hemachromatosis	HFE protein
Huntington disease	Huntingtin
Lesch-Nyhan syndrome	Hypoxanthine phosphoribosyltransferase 1
McArdle disease	Muscle glycogen phosphorylase
Sanfilippo syndrome, type B (MPS IIIB)	N-acetyl-alpha-D-glucosaminidase
Leber's hereditary optic neuropathy	NADH dehydrogenase
Neurofibromatosis, type 1	NF-1
Niemann Pick disease, type C	NPC1
Alpers' disease	POLG
Von Hippel-Lindau disease	pVHL
Paget disease of bone	Sequestosome 1
Carnitine uptake defect	SLC22A5
Cystinuria	SLC7A9
Niemann Pick disease, type A / B	SMPD1
Spinal muscular atrophy	Survival motor neuron protein
Li-Fraumeni syndrome	TP53
Fabry disease	Alpha galactosidase
Alpha-mannosidosis	Alpha-D-mannosidase
Hurler syndrome (MPS I)	Alpha-L iduronidase
Hemolytic uremic syndrome	Anti-complement factor C5 mAb
Morquio syndrome, type B (MPS IVB)	Beta-galactosidase
Multiple carboxylase deficiency	Biotin-methylcrotonoyl-CoA-carboxylase ligase
Homocystinuria	Cystathionine beta-synthase
Cystinosis	Cystinosin
Cystic fibrosis	Deoxyribonuclease I
Erythropoietic protoporphyria	Ferrochelatase
Tyrosinemia, type I	Fumarylacetoacetase
GALK deficiency	Galactokinase
Morquio syndrome, type A (MPS IVA)	Galactose 6-sulfate sulfatase
GALE deficiency	Galactose epimerase
Gaucher disease	Glucocerebrosidase
Alkaptonuria	Homogentisate 1,2-dioxygenase
Hunter syndrome (MPS II)	Iduronate-2-sulfatase
Lysosomal acid lipase deficiency	Lysosomal acid lipase
Hypermethioninemia	Methionine adenosyltransferase
3-Methylcrotonyl-CoA carboxylase deficiency	Methylcrotonoyl-CoA carboxylase
3-Methylglutaconic aciduria	Methylglutaconyl-CoA hydratase

Maroteaux-Lamy syndrome (MPS VI)	N-acetylgalactosamine 4-sulfatase
Familial mediterranean fever	Pyrin (MEFV)
Tetrahydrobiopterin-deficient hyperphenylalaninemia	Tetrahydrobiopterin
Juvenile rheumatoid arthritis	TNF-alpha inhibitors
Psoriatic arthritis	TNF-alpha inhibitors
Hypophosphatasia	TNSALP
Gilbert syndrome	UDP-glucuronosyltransferase
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase
Wilson disease	Wilson disease protein
Systemic lupus erythematosus	Anti-BAFF
Osteoporosis	Anti-RANKL mAb
Multiple sclerosis	Anti-VLA-4 mAb
Neutropenia	G-CSF
Immunoglobulin deficiency	Immunoglobulin
Primary humoral immune deficiencies (e.g., CVID)	Immunoglobulin
Infectious diseases vaccines	Infectious antigen
Hepatitis B, Hepatitis C	Interferon alpha
Multiple sclerosis	Interferon beta
Chronic immune thrombocytopenia	Thrombopoietin
Ehlers-Danlos syndrome, type 1	Proteins encoded by ADAMTS2, B3GALT6, B4GALT7, CHST14, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, DSE, FKBP14, PLOD1, PRDM5, SLC39A13, TNXB, and ZNF469
Stickler syndrome	Proteins encoded by COL11A1, COL11A2, COL2A1, COL9A1, COL9A2, and COL9A3
Hereditary hemorrhagic telangiectasia	Proteins encoded by ACVRL1, ENG, and SMAD4
Hereditary spherocytosis	Proteins encoded by ANK1, EPB42, SLC4A1, SPTA1 and SPTB
Brugada syndrome	Proteins encoded by CACNA1C, CACNA2D1, CACNB2, GPD1L, HCN4, KCND3, KCNE3, KCNE5, KCNJ8, RANGRF, SCN1B, SCN2B, SCN3B, SCN5A, SLMAP, and TRPM4
Osteopetrosis	Proteins encoded by CA2, CLCN7, IKBKG, ITGB3, OSTM1, PLEKHM1, TCIRG1, TNFRSF11A, and TNFSF11
Mitochondrial oxidative phosphorylation disorders	Proteins encoded by FBXL4, and NDUFB9

TABLE 5

INDICATION(S)	THERAPEUTIC PROTEIN	GENE
Achromatopsia type 2	Cyclic nucleotide-gated channel, $\alpha 3$ subunit	CNGA3
Achromatopsia type 3	Cyclic nucleotide-gated channel, $\beta 3$ subunit	CNGB3
Aland Island eye disease	Cav1.4: calcium channel, voltage-gated, L type, $\alpha 1F$ subunit	CACNA1F

Andersen-Tawil syndrome	Kir2.1: potassium channel, inwardly-rectifying, subfamily J, member 2	KCNJ2
Benign familial infantile epilepsy	Nav2.1: sodium channel, voltage-gated, type II, α subunit	SCN2A
	Kv7.2: potassium channel, voltage-gated, KQT-like subfamily, member 2	KCNQ2
	Kv7.3: potassium channel, voltage-gated, KQT-like subfamily, member 3	KCNQ3
Bestrophinopathy, autosomal-recessive	Bestrophin 1	BEST1
Central core disease	RyR1: ryanodine receptor 1	RYR1
Charcot-Marie-Tooth disease type 2C	Transient receptor potential cation channel, subfamily V, member 4	TRPV4
Childhood absence epilepsy	γ -aminobutyric acid A receptor, $\alpha 1$ subunit	GABRA1
	γ -aminobutyric acid A receptor, $\alpha 6$ subunit	GABRA6
	γ -aminobutyric acid A receptor, $\beta 3$ subunit	GABRB3
	γ -aminobutyric acid A receptor, $\gamma 2$ subunit	GABRG2
Cav3.2: calcium channel, voltage-gated, T type, $\alpha 1H$ subunit		CACNA1H
Cognitive impairment with or without cerebellar ataxia	Nav1.6: sodium channel, voltage-gated, type VIII, α subunit	SCN8A
Cone-rod dystrophy, X-linked, type 3	Cav1.4: calcium channel, voltage-gated, L type, $\alpha 1F$ subunit	CACNA1F
Congenital distal spinal muscular atrophy	Transient receptor potential cation channel, subfamily V, member 4	TRPV4
Congenital indifference to pain, autosomal-recessive	Nav1.7: Sodium channel, voltage-gated, type IX, α subunit	SCN9A
Congenital myasthenic syndrome	Cholinergic receptor, muscle nicotinic, $\alpha 1$ subunit	CHRNA1
	Cholinergic receptor, muscle nicotinic, $\beta 1$ subunit	CHRNB1
	Cholinergic receptor, muscle nicotinic, δ subunit	CHRND
	Cholinergic receptor, muscle nicotinic, ϵ subunit	CHRNE
	Nav1.4: sodium channel, voltage-gated, type IV, α subunit	SCN4A
Congenital stationary night blindness type 1C	Transient receptor potential cation channel, subfamily M, member 1	TRPM1
Congenital stationary night blindness type 2A	Cav1.4: calcium channel, voltage-gated, L type, $\alpha 1F$ subunit	CACNA1F
Deafness, autosomal-dominant, type 2A	Kv7.4: potassium channel, voltage-gated, KQT-like subfamily, member 4	KCNQ4
Deafness, autosomal-recessive, type 4, with enlarged vestibular aqueduct	Kir4.1: potassium channel, inwardly-rectifying, subfamily J, member 10	KCNJ10
Dravet syndrome	Nav1.1: sodium channel, voltage-gated, type I, α subunit	SCN1A

	γ -aminobutyric acid A receptor, $\gamma 2$ subunit	GABRG2
Early infantile epileptic encephalopathy type 7	Kv7.2: potassium channel, voltage-gated, KQT-like subfamily, member 2	KCNQ2
Early infantile epileptic encephalopathy type 11	Nav2.1: sodium channel, voltage-gated, type II, α subunit	SCN2A
Early infantile epileptic encephalopathy type 13	Nav1.6: sodium channel, voltage-gated, type VIII, α subunit	SCN8A
Early infantile epileptic encephalopathy type 14	KCa4.1: potassium channel, subfamily T, member 1	KCNT1
EAST/SeSAME syndrome	Kir4.1: potassium channel, inwardly-rectifying, subfamily J, member 10	KCNJ10
Episodic ataxia type 1	Kv1.1: potassium channel, voltage-gated, shaker-related subfamily, member 1	KCNA1
Episodic ataxia type 2	Cav2.1: calcium channel, voltage-gated, P/Q type, $\alpha 1A$ subunit	CACNA1A
Episodic ataxia type 5	Cav $\beta 4$: calcium channel, voltage-gated, $\beta 4$ subunit	CACNB4
Familial episodic pain syndrome	Transient receptor potential cation channel, subfamily A, member 1	TRPA1
Familial hemiplegic migraine type 1	Cav2.1: calcium channel, voltage-gated, P/Q type, $\alpha 1A$ subunit	CACNA1A
Familial hemiplegic migraine type 3	Nav1.1: sodium channel, voltage-gated, type I, α subunit	SCN1A
Generalized epilepsy with febrile seizures plus (GEFS+)	Nav $\beta 1$: sodium channel, voltage-gated, type I, β subunit	SCN1B
	Nav1.1: sodium channel, voltage-gated, type I, α subunit	SCN1A
	γ -aminobutyric acid A receptor, $\gamma 2$ subunit	GABRG2
Generalized epilepsy with paroxysmal dyskinesia	KCa1.1: potassium channel, calcium-activated, large conductance, subfamily M, $\alpha 1$ subunit	KCNMA1
Hereditary hyperekplexia	Glycine receptor, $\alpha 1$ subunit	GLRA1
	Glycine receptor, β subunit	GLRB
Hyperkalemic periodic paralysis	Nav1.4: sodium channel, voltage-gated, type IV, α subunit	SCN4A
Hypokalemic periodic paralysis type 1	Cav1.1: calcium channel, voltage-gated, L type, $\alpha 1S$ subunit	CACNA1S
Hypokalemic periodic paralysis type 2	Nav1.4: sodium channel, voltage-gated, type IV, α subunit	SCN4A
Juvenile macular degeneration	Cyclic nucleotide-gated channel, $\beta 3$ subunit	CNGB3
Juvenile myoclonic epilepsy	γ -aminobutyric acid A receptor, $\alpha 1$ subunit	GABRA1
	Cav $\beta 4$: calcium channel, voltage-gated, $\beta 4$ subunit	CACNB4
Malignant hyperthermia susceptibility	RyR1: ryanodine receptor 1	RYR1
	Cav1.1: calcium channel, voltage-gated, L type, $\alpha 1S$ subunit	CACNA1S
Mucopolidosis type IV	TRPML1/mucolipin 1	MCOLN1

Multiple pterygium syndrome, lethal type	Cholinergic receptor, muscle nicotinic, $\alpha 1$ subunit	CHRNA1
Multiple pterygium syndrome, nonlethal type (Escobar variant)	Cholinergic receptor, muscle nicotinic, δ subunit	CHRND
	Cholinergic receptor, muscle nicotinic, γ subunit	CHRNA4
Myotonia congenita, autosomal-dominant (Thomsen disease)	ClC-1: chloride channel 1, voltage-gated	CLCN1
Myotonia congenita, autosomal-recessive (Becker disease)	ClC-1: chloride channel 1, voltage-gated	CLCN1
Nocturnal frontal lobe epilepsy type 1	Cholinergic receptor, neuronal nicotinic, $\alpha 4$ subunit	CHRNA4
Nocturnal frontal lobe epilepsy type 3	Cholinergic receptor, neuronal nicotinic, $\beta 2$ subunit	CHRNA2
Nocturnal frontal lobe epilepsy type 4	Cholinergic receptor, neuronal nicotinic, $\alpha 2$ subunit	CHRNA2
Nocturnal frontal lobe epilepsy type 5	KCa4.1: potassium channel, subfamily T, member 1	KCNT1
Paramyotonia congenita	Nav1.4: sodium channel, voltage-gated, type IV, α subunit	SCN4A
Paroxysmal extreme pain disorder	Nav1.7: Sodium channel, voltage-gated, type IX, α subunit	SCN9A
Potassium-aggravated myotonia	Nav1.4: sodium channel, voltage-gated, type IV, α subunit	SCN4A
Primary erythralgia	Nav1.7: sodium channel, voltage-gated, type IX, α subunit	SCN9A
Retinitis pigmentosa type 45, autosomal-recessive	Cyclic nucleotide-gated channel, $\beta 1$ subunit	CNGB1
Retinitis pigmentosa type 49, autosomal-recessive	Cyclic nucleotide-gated channel, $\alpha 1$ subunit	CNGA1
Retinitis pigmentosa type 50, autosomal-dominant	Bestrophin 1	BEST1
Scapuloperoneal spinal muscular atrophy	Transient receptor potential cation channel, subfamily V, member 4	TRPV4
Small fiber neuropathy	Nav1.7: sodium channel, voltage-gated, type IX, α subunit	SCN9A
Spinocerebellar ataxia type 6	Cav2.1: calcium channel, voltage-gated, P/Q type, $\alpha 1A$ subunit	CACNA1A
Spinocerebellar ataxia type 13	Kv3.3: potassium channel, voltage-gated, Shaw-related subfamily, member 3	KCNC3
Vitelliform macular dystrophy	Bestrophin 1	BEST1
Vitreoretinopathy	Bestrophin 1	BEST1

TABLE 6 - Secreted Proteins

Uniprot ID	Protein Name	Gene Name
A1E959	Odontogenic ameloblast-associated protein	ODAM
A1KZ92	Peroxidasin-like protein	PXDNL
A1L453	Serine protease 38	PRSS38
A1L4H1	Soluble scavenger receptor cysteine-rich domain-containing protein SSC5D	SSC5D

A2RUU4	Colipase-like protein 1	CLPSL1
A2VDF0	Fucose mutarotase	FUOM
A2VEC9	SCO-spondin	SSPO
A3KMH1	von Willebrand factor A domain-containing protein 8	VWA8
A4D0S4	Laminin subunit beta-4	LAMB4
A4D1T9	Probable inactive serine protease 37	PRSS37
A5D8T8	C-type lectin domain family 18 member A	CLEC18A
A6NC86	phospholipase A2 inhibitor and Ly6/PLAUR domain-containing protein	PINLYP
A6NCI4	von Willebrand factor A domain-containing protein 3A	VWA3A
A6ND01	Probable folate receptor delta	FOLR4
A6NDD2	Beta-defensin 108B-like	
A6NE02	BTB/POZ domain-containing protein 17	BTBD17
A6NEF6	Growth hormone 1	GH1
A6NF02	NPIP-like protein LOC730153	
A6NFB4	HCG1749481, isoform CRA_k	CSH1
A6NFZ4	Protein FAM24A	FAM24A
A6NG13	Glycosyltransferase 54 domain-containing protein	
A6NGN9	IgLON family member 5	IGLON5
A6NHN0	Otolin-1	OTOL1
A6NHN6	Nuclear pore complex-interacting protein-like 2	NPIPL2
A6NI73	Leukocyte immunoglobulin-like receptor subfamily A member 5	LILRA5
A6NIT4	Chorionic somatomammotropin hormone 2 isoform 2	CSH2
A6NJ69	IgA-inducing protein homolog	IGIP
A6NKQ9	Choriogonadotropin subunit beta variant 1	CGB1
A6NMZ7	Collagen alpha-6(VI) chain	COL6A6
A6NNS2	Dehydrogenase/reductase SDR family member 7C	DHRS7C
A6XGL2	Insulin A chain	INS
A8K0G1	Protein Wnt	WNT7B
A8K2U0	Alpha-2-macroglobulin-like protein 1	A2ML1
A8K7I4	Calcium-activated chloride channel regulator 1	CLCA1
A8MTL9	Serpin-like protein HMSD	HMSD
A8MV23	Serpin E3	SERPINE3
A8MZH6	Oocyte-secreted protein 1 homolog	OOSP1
A8TX70	Collagen alpha-5(VI) chain	COL6A5
B0ZBE8	Natriuretic peptide	NPPA
B1A4G9	Somatotropin	GH1
B1A4H2	HCG1749481, isoform CRA_d	CSH1
B1A4H9	Chorionic somatomammotropin hormone	CSH2
B1AJZ6	Protein Wnt	WNT4
B1AKI9	Isthmin-1	ISM1
B2RNN3	Complement C1q and tumor necrosis factor-related protein 9B	C1QTNF9B
B2RUY7	von Willebrand factor C domain-containing protein 2-like	VWC2L

B3GLJ2	Prostate and testis expressed protein 3	PATE3
B4DI03	SEC11-like 3 (<i>S. cerevisiae</i>), isoform CRA_a	SEC11L3
B4DJF9	Protein Wnt	WNT4
B4DUL4	SEC11-like 1 (<i>S. cerevisiae</i>), isoform CRA_d	SEC11L1
B5MCC8	Protein Wnt	WNT10B
B8A595	Protein Wnt	WNT7B
B8A597	Protein Wnt	WNT7B
B8A598	Protein Wnt	WNT7B
B9A064	Immunoglobulin lambda-like polypeptide 5	IGLL5
C9J3H3	Protein Wnt	WNT10B
C9J8I8	Protein Wnt	WNT5A
C9JAF2	Insulin-like growth factor II Ala-25 Del	IGF2
C9JCI2	Protein Wnt	WNT10B
C9JL84	HERV-H LTR-associating protein 1	HLA1
C9JNR5	Insulin A chain	INS
C9JUI2	Protein Wnt	WNT2
D6RF47	Protein Wnt	WNT8A
D6RF94	Protein Wnt	WNT8A
E2RYF7	Protein PBMUCL2	HCG22
E5RFR1	PENK(114-133)	PENK
E7EML9	Serine protease 44	PRSS44
E7EPC3	Protein Wnt	WNT9B
E7EVP0	Nociceptin	PNOC
E9PD02	Insulin-like growth factor I	IGF1
E9PH60	Protein Wnt	WNT16
E9PJL6	Protein Wnt	WNT11
F5GYM2	Protein Wnt	WNT5B
F5H034	Protein Wnt	WNT5B
F5H364	Protein Wnt	WNT5B
F5H7Q6	Protein Wnt	WNT5B
F8WCM5	Protein INS-IGF2	INS-IGF2
F8WDR1	Protein Wnt	WNT2
H0Y663	Protein Wnt	WNT4
H0YK72	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H0YK83	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H0YM39	Chorionic somatomammotropin hormone	CSH2
H0YMT7	Chorionic somatomammotropin hormone	CSH1
H0YN17	Chorionic somatomammotropin hormone	CSH2
H0YNA5	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H0YNG3	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H0YNX5	Signal peptidase complex catalytic subunit SEC11A	SEC11A
H7BZB8	Protein Wnt	WNT10A
H9KV56	Choriogonadotropin subunit beta variant 2	CGB2

I3L0L8	Protein Wnt	WNT9B
J3KNZ1	Choriogonadotropin subunit beta variant 1	CGB1
J3KP00	Choriogonadotropin subunit beta	CGB7
J3QT02	Choriogonadotropin subunit beta variant 1	CGB1
O00175	C-C motif chemokine 24	CCL24
O00182	Galectin-9	LGALS9
O00187	Mannan-binding lectin serine protease 2	MASP2
O00230	Cortistatin	CORT
O00253	Agouti-related protein	AGRP
O00270	12-(S)-hydroxy-5,8,10,14-eicosatetraenoic acid receptor	GPR31
O00292	Left-right determination factor 2	LEFTY2
O00294	Tubby-related protein 1	TULP1
O00295	Tubby-related protein 2	TULP2
O00300	Tumor necrosis factor receptor superfamily member 11B	TNFRSF11B
O00339	Matrilin-2	MATN2
O00391	Sulfhydryl oxidase 1	QSOX1
O00468	Agrin	AGRN
O00515	Ladinin-1	LAD1
O00533	Processed neural cell adhesion molecule L1-like protein	CHL1
O00584	Ribonuclease T2	RNASET2
O00585	C-C motif chemokine 21	CCL21
O00602	Ficolin-1	FCN1
O00622	Protein CYR61	CYR61
O00626	MDC(5-69)	CCL22
O00634	Netrin-3	NTN3
O00744	Protein Wnt-10b	WNT10B
O00755	Protein Wnt-7a	WNT7A
O14498	Immunoglobulin superfamily containing leucine-rich repeat protein	ISLR
O14511	Pro-neuregulin-2, membrane-bound isoform	NRG2
O14594	Neurocan core protein	NCAN
O14625	C-X-C motif chemokine 11	CXCL11
O14638	Ectonucleotide pyrophosphatase/phosphodiesterase family member 3	ENPP3
O14656	Torsin-1A	TOR1A
O14657	Torsin-1B	TOR1B
O14786	Neuropilin-1	NRP1
O14788	Tumor necrosis factor ligand superfamily member 11, membrane form	TNFSF11
O14791	Apolipoprotein L1	APOL1
O14793	Growth/differentiation factor 8	MSTN
O14904	Protein Wnt-9a	WNT9A
O14905	Protein Wnt-9b	WNT9B
O14944	Proepiregulin	EREG
O14960	Leukocyte cell-derived chemotaxin-2	LECT2

O15018	Processed PDZ domain-containing protein 2	PDZD2
O15041	Semaphorin-3E	SEMA3E
O15072	A disintegrin and metalloproteinase with thrombospondin motifs 3	ADAMTS3
O15123	Angiopoietin-2	ANGPT2
O15130	Neuropeptide FF	NPFF
O15197	Ephrin type-B receptor 6	EPHB6
O15204	ADAM DEC1	ADAMDEC1
O15230	Laminin subunit alpha-5	LAMA5
O15232	Matrilin-3	MATN3
O15240	Neuroendocrine regulatory peptide-1	VGF
O15263	Beta-defensin 4A	DEFB4A
O15335	Chondroadherin	CHAD
O15393	Transmembrane protease serine 2 catalytic chain	TMPRSS2
O15444	C-C motif chemokine 25	CCL25
O15467	C-C motif chemokine 16	CCL16
O15496	Group 10 secretory phospholipase A2	PLA2G10
O15520	Fibroblast growth factor 10	FGF10
O15537	Retinoschisin	RS1
O43157	Plexin-B1	PLXNB1
O43184	Disintegrin and metalloproteinase domain-containing protein 12	ADAM12
O43240	Kallikrein-10	KLK10
O43278	Kunitz-type protease inhibitor 1	SPINT1
O43320	Fibroblast growth factor 16	FGF16
O43323	Desert hedgehog protein C-product	DHH
O43405	Cochlin	COCH
O43508	Tumor necrosis factor ligand superfamily member 12, membrane form	TNFSF12
O43555	Progonadoliberin-2	GNRH2
O43557	Tumor necrosis factor ligand superfamily member 14, soluble form	TNFSF14
O43692	Peptidase inhibitor 15	PI15
O43699	Sialic acid-binding Ig-like lectin 6	SIGLEC6
O43820	Hyaluronidase-3	HYAL3
O43827	Angiopoietin-related protein 7	ANGPTL7
O43852	Calumenin	CALU
O43854	EGF-like repeat and discoidin I-like domain-containing protein 3	EDIL3
O43866	CD5 antigen-like	CD5L
O43897	Tolloid-like protein 1	TLL1
O43915	Vascular endothelial growth factor D	FIGF
O43927	C-X-C motif chemokine 13	CXCL13
O60218	Aldo-keto reductase family 1 member B10	AKR1B10
O60235	Transmembrane protease serine 11D	TMPRSS11D

O60258	Fibroblast growth factor 17	FGF17
O60259	Kallikrein-8	KLK8
O60383	Growth/differentiation factor 9	GDF9
O60469	Down syndrome cell adhesion molecule	DSCAM
O60542	Persephin	PSPN
O60565	Gremlin-1	GREM1
O60575	Serine protease inhibitor Kazal-type 4	SPINK4
O60676	Cystatin-8	CST8
O60687	Sushi repeat-containing protein SRPX2	SRPX2
O60844	Zymogen granule membrane protein 16	ZG16
O60882	Matrix metalloproteinase-20	MMP20
O60938	Keratocan	KERA
O75015	Low affinity immunoglobulin gamma Fc region receptor III-B	FCGR3B
O75077	Disintegrin and metalloproteinase domain-containing protein 23	ADAM23
O75093	Slit homolog 1 protein	SLIT1
O75094	Slit homolog 3 protein	SLIT3
O75095	Multiple epidermal growth factor-like domains protein 6	MEGF6
O75173	A disintegrin and metalloproteinase with thrombospondin motifs 4	ADAMTS4
O75200	Nuclear pore complex-interacting protein-like 1	NPIPL1
O75339	Cartilage intermediate layer protein 1 C1	CILP
O75354	Ectonucleoside triphosphate diphosphohydrolase 6	ENTPD6
O75386	Tubby-related protein 3	TULP3
O75398	Deformed epidermal autoregulatory factor 1 homolog	DEAF1
O75443	Alpha-tectorin	TECTA
O75445	Usherin	USH2A
O75462	Cytokine receptor-like factor 1	CRLF1
O75487	Glypican-4	GPC4
O75493	Carbonic anhydrase-related protein 11	CA11
O75594	Peptidoglycan recognition protein 1	PGLYRP1
O75596	C-type lectin domain family 3 member A	CLEC3A
O75610	Left-right determination factor 1	LEFTY1
O75629	Protein CREG1	CREG1
O75636	Ficolin-3	FCN3
O75711	Scrapie-responsive protein 1	SCRG1
O75715	Epididymal secretory glutathione peroxidase	GPX5
O75718	Cartilage-associated protein	CRTAP
O75829	Chondrosurfactant protein	LECT1
O75830	Serpin I2	SERPINI2
O75882	Attractin	ATRIN
O75888	Tumor necrosis factor ligand superfamily member 13	TNFSF13
O75900	Matrix metalloproteinase-23	MMP23A
O75951	Lysozyme-like protein 6	LYZL6

O75973	C1q-related factor	C1QL1
O76038	Secretagogen	SCGN
O76061	Stanniocalcin-2	STC2
O76076	WNT1-inducible-signaling pathway protein 2	WISP2
O76093	Fibroblast growth factor 18	FGF18
O76096	Cystatin-F	CST7
O94769	Extracellular matrix protein 2	ECM2
O94813	Slit homolog 2 protein C-product	SLIT2
O94907	Dickkopf-related protein 1	DKK1
O94919	Endonuclease domain-containing 1 protein	ENDOD1
O94964	N-terminal form	SOGA1
O95025	Semaphorin-3D	SEMA3D
O95084	Serine protease 23	PRSS23
O95150	Tumor necrosis factor ligand superfamily member 15	TNFSF15
O95156	Neurexophilin-2	NXPH2
O95157	Neurexophilin-3	NXPH3
O95158	Neurexophilin-4	NXPH4
O95388	WNT1-inducible-signaling pathway protein 1	WISP1
O95389	WNT1-inducible-signaling pathway protein 3	WISP3
O95390	Growth/differentiation factor 11	GDF11
O95393	Bone morphogenetic protein 10	BMP10
O95399	Urotensin-2	UTS2
O95407	Tumor necrosis factor receptor superfamily member 6B	TNFRSF6B
O95428	Papilin	PAPLN
O95445	Apolipoprotein M	APOM
O95450	A disintegrin and metalloproteinase with thrombospondin motifs 2	ADAMTS2
O95460	Matrilin-4	MATN4
O95467	LHAL tetrapeptide	GNAS
O95631	Netrin-1	NTN1
O95633	Follistatin-related protein 3	FSTL3
O95711	Lymphocyte antigen 86	LY86
O95715	C-X-C motif chemokine 14	CXCL14
O95750	Fibroblast growth factor 19	FGF19
O95760	Interleukin-33	IL33
O95813	Cerberus	CER1
O95841	Angiopoietin-related protein 1	ANGPTL1
O95897	Noelin-2	OLFM2
O95925	Eppin	EPPIN
O95965	Integrin beta-like protein 1	ITGBL1
O95967	EGF-containing fibulin-like extracellular matrix protein 2	EFEMP2
O95968	Secretoglobin family 1D member 1	SCGB1D1
O95969	Secretoglobin family 1D member 2	SCGB1D2
O95970	Leucine-rich glioma-inactivated protein 1	LGI1

O95972	Bone morphogenetic protein 15	BMP15
O95994	Anterior gradient protein 2 homolog	AGR2
O95998	Interleukin-18-binding protein	IL18BP
O96009	Napsin-A	NAPSA
O96014	Protein Wnt-11	WNT11
P00450	Ceruloplasmin	CP
P00451	Factor VIIIa light chain	F8
P00488	Coagulation factor XIII A chain	F13A1
P00533	Epidermal growth factor receptor	EGFR
P00709	Alpha-lactalbumin	LALBA
P00734	Prothrombin	F2
P00738	Haptoglobin beta chain	HP
P00739	Haptoglobin-related protein	HPR
P00740	Coagulation factor IXa heavy chain	F9
P00742	Factor X heavy chain	F10
P00746	Complement factor D	CFD
P00747	Plasmin light chain B	PLG
P00748	Coagulation factor XIIa light chain	F12
P00749	Urokinase-type plasminogen activator long chain A	PLAU
P00750	Tissue-type plasminogen activator	PLAT
P00751	Complement factor B Ba fragment	CFB
P00797	Renin	REN
P00973	2'-5'-oligoadenylate synthase 1	OAS1
P00995	Pancreatic secretory trypsin inhibitor	SPINK1
P01008	Antithrombin-III	SERPINC1
P01009	Alpha-1-antitrypsin	SERPINA1
P01011	Alpha-1-antichymotrypsin His-Pro-less	SERPINA3
P01019	Angiotensin-1	AGT
P01023	Alpha-2-macroglobulin	A2M
P01024	Acylation stimulating protein	C3
P01031	Complement C5 beta chain	C5
P01033	Metalloproteinase inhibitor 1	TIMP1
P01034	Cystatin-C	CST3
P01036	Cystatin-S	CST4
P01037	Cystatin-SN	CST1
P01042	Kininogen-1 light chain	KNG1
P01127	Platelet-derived growth factor subunit B	PDGFB
P01135	Transforming growth factor alpha	TGFA
P01137	Transforming growth factor beta-1	TGFB1
P01138	Beta-nerve growth factor	NGF
P01148	Gonadoliberin-1	GNRH1
P01160	Atrial natriuretic factor	NPPA
P01178	Oxytocin	OXT

P01185	Vasopressin-neurophysin 2-copeptin	AVP
P01189	Corticotropin	POMC
P01210	PENK(237-258)	PENK
P01213	Alpha-neoendorphin	PDYN
P01215	Glycoprotein hormones alpha chain	CGA
P01222	Thyrotropin subunit beta	TSHB
P01225	Follitropin subunit beta	FSHB
P01229	Lutropin subunit beta	LHB
P01233	Choriogonadotropin subunit beta	CGB8
P01236	Prolactin	PRL
P01241	Somatotropin	GH1
P01242	Growth hormone variant	GH2
P01243	Chorionic somatomammotropin hormone	CSH2
P01258	Katacalcin	CALCA
P01266	Thyroglobulin	TG
P01270	Parathyroid hormone	PTH
P01275	Glucagon	GCG
P01282	Intestinal peptide PHM-27	VIP
P01286	Somatoliberin	GHRH
P01298	Pancreatic prohormone	PPY
P01303	C-flanking peptide of NPY	NPY
P01308	Insulin	INS
P01344	Insulin-like growth factor II	IGF2
P01350	Big gastrin	GAST
P01374	Lymphotoxin-alpha	LTA
P01375	C-domain 1	TNF
P01562	Interferon alpha-1/13	IFNA1
P01563	Interferon alpha-2	IFNA2
P01566	Interferon alpha-10	IFNA10
P01567	Interferon alpha-7	IFNA7
P01568	Interferon alpha-21	IFNA21
P01569	Interferon alpha-5	IFNA5
P01570	Interferon alpha-14	IFNA14
P01571	Interferon alpha-17	IFNA17
P01574	Interferon beta	IFNB1
P01579	Interferon gamma	IFNG
P01583	Interleukin-1 alpha	IL1A
P01584	Interleukin-1 beta	IL1B
P01588	Erythropoietin	EPO
P01591	Immunoglobulin J chain	IGJ
P01732	T-cell surface glycoprotein CD8 alpha chain	CD8A
P01833	Polymeric immunoglobulin receptor	PIGR
P01857	Ig gamma-1 chain C region	IGHG1

P01859	Ig gamma-2 chain C region	IGHG2
P01860	Ig gamma-3 chain C region	IGHG3
P01861	Ig gamma-4 chain C region	IGHG4
P01871	Ig mu chain C region	IGHM
P01880	Ig delta chain C region	IGHD
P02452	Collagen alpha-1(I) chain	COL1A1
P02458	Chondrocalcin	COL2A1
P02461	Collagen alpha-1(III) chain	COL3A1
P02462	Collagen alpha-1(IV) chain	COL4A1
P02647	Apolipoprotein A-I	APOA1
P02649	Apolipoprotein E	APOE
P02652	Apolipoprotein A-II	APOA2
P02654	Apolipoprotein C-I	APOC1
P02655	Apolipoprotein C-II	APOC2
P02656	Apolipoprotein C-III	APOC3
P02671	Fibrinogen alpha chain	FGA
P02675	Fibrinopeptide B	FGB
P02679	Fibrinogen gamma chain	FGG
P02741	C-reactive protein	CRP
P02743	Serum amyloid P-component(1-203)	APCS
P02745	Complement C1q subcomponent subunit A	C1QA
P02746	Complement C1q subcomponent subunit B	C1QB
P02747	Complement C1q subcomponent subunit C	C1QC
P02748	Complement component C9b	C9
P02749	Beta-2-glycoprotein 1	APOH
P02750	Leucine-rich alpha-2-glycoprotein	LRG1
P02751	Ugl-Y2	FN1
P02753	Retinol-binding protein 4	RBP4
P02760	Trypstatin	AMBP
P02763	Alpha-1-acid glycoprotein 1	ORM1
P02765	Alpha-2-HS-glycoprotein chain A	AHSG
P02766	Transthyretin	TTR
P02768	Serum albumin	ALB
P02771	Alpha-fetoprotein	AFP
P02774	Vitamin D-binding protein	GC
P02775	Connective tissue-activating peptide III	PPBP
P02776	Platelet factor 4	PF4
P02778	CXCL10(1-73)	CXCL10
P02786	Transferrin receptor protein 1	TFRC
P02787	Serotransferrin	TF
P02788	Lactoferrin-C	LTF
P02790	Hemopexin	HPX
P02808	Statherin	STATH

P02810	Salivary acidic proline-rich phosphoprotein 1/2	PRH2
P02812	Basic salivary proline-rich protein 2	PRB2
P02814	Peptide D1A	SMR3B
P02818	Osteocalcin	BGLAP
P03950	Angiogenin	ANG
P03951	Coagulation factor XIa heavy chain	F11
P03952	Plasma kallikrein	KLKB1
P03956	27 kDa interstitial collagenase	MMP1
P03971	Muellerian-inhibiting factor	AMH
P03973	Antileukoprotease	SLPI
P04003	C4b-binding protein alpha chain	C4BPA
P04004	Somatomedin-B	VTN
P04054	Phospholipase A2	PLA2G1B
P04085	Platelet-derived growth factor subunit A	PDGFA
P04090	Relaxin A chain	RLN2
P04114	Apolipoprotein B-100	APOB
P04118	Colipase	CLPS
P04141	Granulocyte-macrophage colony-stimulating factor	CSF2
P04155	Trefoil factor 1	TFF1
P04180	Phosphatidylcholine-sterol acyltransferase	LCAT
P04196	Histidine-rich glycoprotein	HRG
P04217	Alpha-1B-glycoprotein	A1BG
P04275	von Willebrand antigen 2	VWF
P04278	Sex hormone-binding globulin	SHBG
P04279	Alpha-inhibin-31	SEMG1
P04280	Basic salivary proline-rich protein 1	PRB1
P04628	Proto-oncogene Wnt-1	WNT1
P04745	Alpha-amylase 1	AMY1A
P04746	Pancreatic alpha-amylase	AMY2A
P04808	Prorelaxin H1	RLN1
P05000	Interferon omega-1	IFNW1
P05013	Interferon alpha-6	IFNA6
P05014	Interferon alpha-4	IFNA4
P05015	Interferon alpha-16	IFNA16
P05019	Insulin-like growth factor I	IGF1
P05060	GAWK peptide	CHGB
P05090	Apolipoprotein D	APOD
P05109	Protein S100-A8	S100A8
P05111	Inhibin alpha chain	INHA
P05112	Interleukin-4	IL4
P05113	Interleukin-5	IL5
P05120	Plasminogen activator inhibitor 2	SERPINE2
P05121	Plasminogen activator inhibitor 1	SERPINE1

P05154	Plasma serine protease inhibitor	SERPINA5
P05155	Plasma protease C1 inhibitor	SERPING1
P05156	Complement factor I heavy chain	CFI
P05160	Coagulation factor XIII B chain	F13B
P05161	Ubiquitin-like protein ISG15	ISG15
P05230	Fibroblast growth factor 1	FGF1
P05231	Interleukin-6	IL6
P05305	Big endothelin-1	EDN1
P05408	C-terminal peptide	SCG5
P05451	Lithostathine-1-alpha	REG1A
P05452	Tetranectin	CLEC3B
P05543	Thyroxine-binding globulin	SERPINA7
P05814	Beta-casein	CSN2
P05997	Collagen alpha-2(V) chain	COL5A2
P06276	Cholinesterase	BCHE
P06307	Cholecystokinin-12	CCK
P06396	Gelsolin	GSN
P06681	Complement C2	C2
P06702	Protein S100-A9	S100A9
P06727	Apolipoprotein A-IV	APOA4
P06734	Low affinity immunoglobulin epsilon Fc receptor soluble form	FCER2
P06744	Glucose-6-phosphate isomerase	GPI
P06850	Corticoliberin	CRH
P06858	Lipoprotein lipase	LPL
P06881	Calcitonin gene-related peptide 1	CALCA
P07093	Glia-derived nexin	SERPINE2
P07098	Gastric triacylglycerol lipase	LIPF
P07225	Vitamin K-dependent protein S	PROS1
P07237	Protein disulfide-isomerase	P4HB
P07288	Prostate-specific antigen	KLK3
P07306	Asialoglycoprotein receptor 1	ASGR1
P07355	Annexin A2	ANXA2
P07357	Complement component C8 alpha chain	C8A
P07358	Complement component C8 beta chain	C8B
P07360	Complement component C8 gamma chain	C8G
P07477	Alpha-trypsin chain 2	PRSS1
P07478	Trypsin-2	PRSS2
P07492	Neuromedin-C	GRP
P07498	Kappa-casein	CSN3
P07585	Decorin	DCN
P07911	Uromodulin	UMOD
P07942	Laminin subunit beta-1	LAMB1
P07988	Pulmonary surfactant-associated protein B	SFTPB

P07998	Ribonuclease pancreatic	RNASE1
P08118	Beta-microseminoprotein	MSMB
P08123	Collagen alpha-2(I) chain	COL1A2
P08185	Corticosteroid-binding globulin	SERPINA6
P08217	Chymotrypsin-like elastase family member 2A	CELA2A
P08218	Chymotrypsin-like elastase family member 2B	CELA2B
P08253	72 kDa type IV collagenase	MMP2
P08254	Stromelysin-1	MMP3
P08294	Extracellular superoxide dismutase [Cu-Zn]	SOD3
P08476	Inhibin beta A chain	INHBA
P08493	Matrix Gla protein	MGP
P08572	Collagen alpha-2(IV) chain	COL4A2
P08581	Hepatocyte growth factor receptor	MET
P08603	Complement factor H	CFH
P08620	Fibroblast growth factor 4	FGF4
P08637	Low affinity immunoglobulin gamma Fc region receptor III-A	FCGR3A
P08697	Alpha-2-antiplasmin	SERPINF2
P08700	Interleukin-3	IL3
P08709	Coagulation factor VII	F7
P08833	Insulin-like growth factor-binding protein 1	IGFBP1
P08887	Interleukin-6 receptor subunit alpha	IL6R
P08949	Neuromedin-B-32	NMB
P08F94	Fibrocystin	PKHD1
P09038	Fibroblast growth factor 2	FGF2
P09228	Cystatin-SA	CST2
P09237	Matrilysin	MMP7
P09238	Stromelysin-2	MMP10
P09341	Growth-regulated alpha protein	CXCL1
P09382	Galectin-1	LGALS1
P09466	Glycodelin	PAEP
P09486	SPARC	SPARC
P09529	Inhibin beta B chain	INHBB
P09544	Protein Wnt-2	WNT2
P09603	Processed macrophage colony-stimulating factor 1	CSF1
P09681	Gastric inhibitory polypeptide	GIP
P09683	Secretin	SCT
P09919	Granulocyte colony-stimulating factor	CSF3
P0C091	FRAS1-related extracellular matrix protein 3	FREM3
P0C0L4	C4d-A	C4A
P0C0L5	Complement C4-B alpha chain	C4B
P0C0P6	Neuropeptide S	NPS
P0C7L1	Serine protease inhibitor Kazal-type 8	SPINK8

P0C862	Complement C1q and tumor necrosis factor-related protein 9A	C1QTNF9
P0C8F1	Prostate and testis expressed protein 4	PATE4
P0CG01	Gastrokine-3	GKN3P
P0CG36	Cryptic family protein 1B	CFC1B
P0CG37	Cryptic protein	CFC1
P0CJ68	Humanin-like protein 1	MTRNR2L1
P0CJ69	Humanin-like protein 2	MTRNR2L2
P0CJ70	Humanin-like protein 3	MTRNR2L3
P0CJ71	Humanin-like protein 4	MTRNR2L4
P0CJ72	Humanin-like protein 5	MTRNR2L5
P0CJ73	Humanin-like protein 6	MTRNR2L6
P0CJ74	Humanin-like protein 7	MTRNR2L7
P0CJ75	Humanin-like protein 8	MTRNR2L8
P0CJ76	Humanin-like protein 9	MTRNR2L9
P0CJ77	Humanin-like protein 10	MTRNR2L10
P0DJD7	Pepsin A-4	PGA4
P0DJD8	Pepsin A-3	PGA3
P0DJD9	Pepsin A-5	PGA5
P0DJI8	Amyloid protein A	SAA1
P0DJI9	Serum amyloid A-2 protein	SAA2
P10082	Peptide YY(3-36)	PYY
P10092	Calcitonin gene-related peptide 2	CALCB
P10124	Serglycin	SRGN
P10145	MDNCF-a	IL8
P10147	MIP-1-alpha(4-69)	CCL3
P10163	Peptide P-D	PRB4
P10451	Osteopontin	SPP1
P10599	Thioredoxin	TXN
P10600	Transforming growth factor beta-3	TGFB3
P10643	Complement component C7	C7
P10645	Vasostatin-2	CHGA
P10646	Tissue factor pathway inhibitor	TFPI
P10720	Platelet factor 4 variant(4-74)	PF4V1
P10745	Retinol-binding protein 3	RBP3
P10767	Fibroblast growth factor 6	FGF6
P10909	Clusterin alpha chain	CLU
P10912	Growth hormone receptor	GHR
P10915	Hyaluronan and proteoglycan link protein 1	HAPLN1
P10966	T-cell surface glycoprotein CD8 beta chain	CD8B
P10997	Islet amyloid polypeptide	IAPP
P11047	Laminin subunit gamma-1	LAMC1
P11150	Hepatic triacylglycerol lipase	LIPC
P11226	Mannose-binding protein C	MBL2

P11464	Pregnancy-specific beta-1-glycoprotein 1	PSG1
P11465	Pregnancy-specific beta-1-glycoprotein 2	PSG2
P11487	Fibroblast growth factor 3	FGF3
P11597	Cholesteryl ester transfer protein	CETP
P11684	Uteroglobin	SCGB1A1
P11686	Pulmonary surfactant-associated protein C	SFTPC
P12034	Fibroblast growth factor 5	FGF5
P12107	Collagen alpha-1(XI) chain	COL11A1
P12109	Collagen alpha-1(VI) chain	COL6A1
P12110	Collagen alpha-2(VI) chain	COL6A2
P12111	Collagen alpha-3(VI) chain	COL6A3
P12259	Coagulation factor V	F5
P12272	PTHrP[1-36]	PTHLH
P12273	Prolactin-inducible protein	PIP
P12544	Granzyme A	GZMA
P12643	Bone morphogenetic protein 2	BMP2
P12644	Bone morphogenetic protein 4	BMP4
P12645	Bone morphogenetic protein 3	BMP3
P12724	Eosinophil cationic protein	RNASE3
P12821	Angiotensin-converting enzyme, soluble form	ACE
P12838	Neutrophil defensin 4	DEFA4
P12872	Motilin	MLN
P13232	Interleukin-7	IL7
P13236	C-C motif chemokine 4	CCL4
P13284	Gamma-interferon-inducible lysosomal thiol reductase	IFI30
P13500	C-C motif chemokine 2	CCL2
P13501	C-C motif chemokine 5	CCL5
P13521	Secretogranin-2	SCG2
P13591	Neural cell adhesion molecule 1	NCAM1
P13611	Versican core protein	VCAN
P13671	Complement component C6	C6
P13688	Carcinoembryonic antigen-related cell adhesion molecule 1	CEACAM1
P13725	Oncostatin-M	OSM
P13726	Tissue factor	F3
P13727	Eosinophil granule major basic protein	PRG2
P13942	Collagen alpha-2(XI) chain	COL11A2
P13987	CD59 glycoprotein	CD59
P14138	Endothelin-3	EDN3
P14174	Macrophage migration inhibitory factor	MIF
P14207	Folate receptor beta	FOLR2
P14222	Perforin-1	PRF1
P14543	Nidogen-1	NID1
P14555	Phospholipase A2, membrane associated	PLA2G2A

P14625	Endoplasmin	HSP90B1
P14735	Insulin-degrading enzyme	IDE
P14778	Interleukin-1 receptor type 1, soluble form	IL1R1
P14780	82 kDa matrix metalloproteinase-9	MMP9
P15018	Leukemia inhibitory factor	LIF
P15085	Carboxypeptidase A1	CPA1
P15086	Carboxypeptidase B	CPB1
P15151	Poliovirus receptor	PVR
P15169	Carboxypeptidase N catalytic chain	CPN1
P15248	Interleukin-9	IL9
P15291	N-acetyllactosamine synthase	B4GALT1
P15309	PAPf39	ACPP
P15328	Folate receptor alpha	FOLR1
P15374	Ubiquitin carboxyl-terminal hydrolase isozyme L3	UCHL3
P15502	Elastin	ELN
P15509	Granulocyte-macrophage colony-stimulating factor receptor subunit alpha	CSF2RA
P15515	Histatin-1	HTN1
P15516	His3-(31-51)-peptide	HTN3
P15692	Vascular endothelial growth factor A	VEGFA
P15814	Immunoglobulin lambda-like polypeptide 1	IGLL1
P15907	Beta-galactoside alpha-2,6-sialyltransferase 1	ST6GAL1
P15941	Mucin-1 subunit beta	MUC1
P16035	Metalloproteinase inhibitor 2	TIMP2
P16112	Aggrecan core protein 2	ACAN
P16233	Pancreatic triacylglycerol lipase	PNLIP
P16442	Histo-blood group ABO system transferase	ABO
P16471	Prolactin receptor	PRLR
P16562	Cysteine-rich secretory protein 2	CRISP2
P16619	C-C motif chemokine 3-like 1	CCL3L1
P16860	BNP(3-29)	NPPB
P16870	Carboxypeptidase E	CPE
P16871	Interleukin-7 receptor subunit alpha	IL7R
P17213	Bactericidal permeability-increasing protein	BPI
P17538	Chymotrypsinogen B	CTRB1
P17931	Galectin-3	LGALS3
P17936	Insulin-like growth factor-binding protein 3	IGFBP3
P17948	Vascular endothelial growth factor receptor 1	FLT1
P18065	Insulin-like growth factor-binding protein 2	IGFBP2
P18075	Bone morphogenetic protein 7	BMP7
P18428	Lipopolysaccharide-binding protein	LBP
P18509	PACAP-related peptide	ADCYAP1
P18510	Interleukin-1 receptor antagonist protein	IL1RN
P18827	Syndecan-1	SDC1

P19021	Peptidylglycine alpha-hydroxylating monooxygenase	PAM
P19235	Erythropoietin receptor	EPOR
P19438	Tumor necrosis factor-binding protein 1	TNFRSF1A
P19652	Alpha-1-acid glycoprotein 2	ORM2
P19801	Amiloride-sensitive amine oxidase [copper-containing]	ABP1
P19823	Inter-alpha-trypsin inhibitor heavy chain H2	ITIH2
P19827	Inter-alpha-trypsin inhibitor heavy chain H1	ITIH1
P19835	Bile salt-activated lipase	CEL
P19875	C-X-C motif chemokine 2	CXCL2
P19876	C-X-C motif chemokine 3	CXCL3
P19883	Follistatin	FST
P19957	Elafin	PI3
P19961	Alpha-amylase 2B	AMY2B
P20061	Transcobalamin-1	TCN1
P20062	Transcobalamin-2	TCN2
P20142	Gastricsin	PGC
P20155	Serine protease inhibitor Kazal-type 2	SPINK2
P20231	Tryptase beta-2	TPSB2
P20333	Tumor necrosis factor receptor superfamily member 1B	TNFRSF1B
P20366	Substance P	TAC1
P20382	Melanin-concentrating hormone	PMCH
P20396	Thyroliberin	TRH
P20742	Pregnancy zone protein	PZP
P20774	Mimecan	OGN
P20783	Neurotrophin-3	NTF3
P20800	Endothelin-2	EDN2
P20809	Interleukin-11	IL11
P20827	Ephrin-A1	EFNA1
P20849	Collagen alpha-1(IX) chain	COL9A1
P20851	C4b-binding protein beta chain	C4BPB
P20908	Collagen alpha-1(V) chain	COL5A1
P21128	Poly(U)-specific endoribonuclease	ENDOU
P21246	Pleiotrophin	PTN
P21583	Kit ligand	KITLG
P21741	Midkine	MDK
P21754	Zona pellucida sperm-binding protein 3	ZP3
P21781	Fibroblast growth factor 7	FGF7
P21802	Fibroblast growth factor receptor 2	FGFR2
P21810	Biglycan	BGN
P21815	Bone sialoprotein 2	IBSP
P21860	Receptor tyrosine-protein kinase erbB-3	ERBB3
P21941	Cartilage matrix protein	MATN1
P22003	Bone morphogenetic protein 5	BMP5

P22004	Bone morphogenetic protein 6	BMP6
P22079	Lactoperoxidase	LPO
P22105	Tenascin-X	TNXB
P22301	Interleukin-10	IL10
P22303	Acetylcholinesterase	ACHE
P22352	Glutathione peroxidase 3	GPX3
P22362	C-C motif chemokine 1	CCL1
P22455	Fibroblast growth factor receptor 4	FGFR4
P22466	Galanin message-associated peptide	GAL
P22692	Insulin-like growth factor-binding protein 4	IGFBP4
P22749	Granulysin	GNLY
P22792	Carboxypeptidase N subunit 2	CPN2
P22891	Vitamin K-dependent protein Z	PROZ
P22894	Neutrophil collagenase	MMP8
P23142	Fibulin-1	FBLN1
P23280	Carbonic anhydrase 6	CA6
P23352	Anosmin-1	KAL1
P23435	Cerebellin-1	CBLN1
P23560	Brain-derived neurotrophic factor	BDNF
P23582	C-type natriuretic peptide	NPPC
P23946	Chymase	CMA1
P24043	Laminin subunit alpha-2	LAMA2
P24071	Immunoglobulin alpha Fc receptor	FCAR
P24347	Stromelysin-3	MMP11
P24387	Corticotropin-releasing factor-binding protein	CRHBP
P24592	Insulin-like growth factor-binding protein 6	IGFBP6
P24593	Insulin-like growth factor-binding protein 5	IGFBP5
P24821	Tenascin	TNC
P24855	Deoxyribonuclease-1	DNASE1
P25067	Collagen alpha-2(VIII) chain	COL8A2
P25311	Zinc-alpha-2-glycoprotein	AZGP1
P25391	Laminin subunit alpha-1	LAMA1
P25445	Tumor necrosis factor receptor superfamily member 6	FAS
P25940	Collagen alpha-3(V) chain	COL5A3
P25942	Tumor necrosis factor receptor superfamily member 5	CD40
P26022	Pentraxin-related protein PTX3	PTX3
P26927	Hepatocyte growth factor-like protein beta chain	MST1
P27169	Serum paraoxonase/arylesterase 1	PON1
P27352	Gastric intrinsic factor	GIF
P27487	Dipeptidyl peptidase 4 membrane form	DPP4
P27539	Embryonic growth/differentiation factor 1	GDF1
P27658	Vastatin	COL8A1
P27797	Calreticulin	CALR

P27918	Properdin	CFP
P28039	Acyloxyacyl hydrolase	AOAH
P28300	Protein-lysine 6-oxidase	LOX
P28325	Cystatin-D	CST5
P28799	Granulin-1	GRN
P29122	Proprotein convertase subtilisin/kexin type 6	PCSK6
P29279	Connective tissue growth factor	CTGF
P29320	Ephrin type-A receptor 3	EPHA3
P29400	Collagen alpha-5(IV) chain	COL4A5
P29459	Interleukin-12 subunit alpha	IL12A
P29460	Interleukin-12 subunit beta	IL12B
P29508	Serpin B3	SERPINB3
P29622	Kallistatin	SERPINA4
P29965	CD40 ligand, soluble form	CD40LG
P30990	Neurotensin/neuromedin N	NTS
P31025	Lipocalin-1	LCN1
P31151	Protein S100-A7	S100A7
P31371	Fibroblast growth factor 9	FGF9
P31431	Syndecan-4	SDC4
P31947	14-3-3 protein sigma	SFN
P32455	Interferon-induced guanylate-binding protein 1	GBP1
P32881	Interferon alpha-8	IFNA8
P34096	Ribonuclease 4	RNASE4
P34130	Neurotrophin-4	NTF4
P34820	Bone morphogenetic protein 8B	BMP8B
P35030	Trypsin-3	PRSS3
P35052	Secreted glypican-1	GPC1
P35070	Betacellulin	BTC
P35225	Interleukin-13	IL13
P35247	Pulmonary surfactant-associated protein D	SFTPD
P35318	ADM	ADM
P35542	Serum amyloid A-4 protein	SAA4
P35555	Fibrillin-1	FBN1
P35556	Fibrillin-2	FBN2
P35625	Metalloproteinase inhibitor 3	TIMP3
P35858	Insulin-like growth factor-binding protein complex acid labile subunit	IGFALS
P35916	Vascular endothelial growth factor receptor 3	FLT4
P35968	Vascular endothelial growth factor receptor 2	KDR
P36222	Chitinase-3-like protein 1	CHI3L1
P36952	Serpin B5	SERPINB5
P36955	Pigment epithelium-derived factor	SERPINF1
P36980	Complement factor H-related protein 2	CFHR2
P39059	Collagen alpha-1(XV) chain	COL15A1

P39060	Collagen alpha-1(XVIII) chain	COL18A1
P39877	Calcium-dependent phospholipase A2	PLA2G5
P39900	Macrophage metalloelastase	MMP12
P39905	Glial cell line-derived neurotrophic factor	GNDF
P40225	Thrombopoietin	THPO
P40967	M-alpha	PMEL
P41159	Leptin	LEP
P41221	Protein Wnt-5a	WNT5A
P41222	Prostaglandin-H2 D-isomerase	PTGDS
P41271	Neuroblastoma suppressor of tumorigenicity 1	NBL1
P41439	Folate receptor gamma	FOLR3
P42127	Agouti-signaling protein	ASIP
P42702	Leukemia inhibitory factor receptor	LIFR
P42830	ENA-78(9-78)	CXCL5
P43026	Growth/differentiation factor 5	GDF5
P43251	Biotinidase	BTB
P43652	Afamin	AFM
P45452	Collagenase 3	MMP13
P47710	Casoxin-D	CSN1S1
P47929	Galectin-7	LGALS7B
P47972	Neuronal pentraxin-2	NPTX2
P47989	Xanthine oxidase	XDH
P47992	Lymphotoxin	XCL1
P48023	Tumor necrosis factor ligand superfamily member 6, membrane form	FASLG
P48052	Carboxypeptidase A2	CPA2
P48061	Stromal cell-derived factor 1	CXCL12
P48304	Lithostathine-1-beta	REG1B
P48307	Tissue factor pathway inhibitor 2	TFPI2
P48357	Leptin receptor	LEPR
P48594	Serpin B4	SERPINB4
P48645	Neuromedin-U-25	NMU
P48740	Mannan-binding lectin serine protease 1	MASP1
P48745	Protein NOV homolog	NOV
P48960	CD97 antigen subunit beta	CD97
P49223	Kunitz-type protease inhibitor 3	SPINT3
P49747	Cartilage oligomeric matrix protein	COMP
P49763	Placenta growth factor	PGF
P49765	Vascular endothelial growth factor B	VEGFB
P49767	Vascular endothelial growth factor C	VEGFC
P49771	Fms-related tyrosine kinase 3 ligand	FLT3LG
P49862	Kallikrein-7	KLK7
P49863	Granzyme K	GZMK
P49908	Selenoprotein P	SEPP1

P49913	Antibacterial protein FALL-39	CAMP
P50607	Tubby protein homolog	TUB
P51124	Granzyme M	GZMM
P51512	Matrix metalloproteinase-16	MMP16
P51654	Glypican-3	GPC3
P51671	Eotaxin	CCL11
P51884	Lumican	LUM
P51888	Prolargin	PRELP
P52798	Ephrin-A4	EFNA4
P52823	Stanniocalcin-1	STC1
P53420	Collagen alpha-4(IV) chain	COL4A4
P53621	Coatomer subunit alpha	COPA
P54108	Cysteine-rich secretory protein 3	CRISP3
P54315	Pancreatic lipase-related protein 1	PNLIPRP1
P54317	Pancreatic lipase-related protein 2	PNLIPRP2
P54793	Arylsulfatase F	ARSF
P55000	Secreted Ly-6/uPAR-related protein 1	SLURP1
P55001	Microfibrillar-associated protein 2	MFAP2
P55056	Apolipoprotein C-IV	APOC4
P55058	Phospholipid transfer protein	PLTP
P55075	Fibroblast growth factor 8	FGF8
P55081	Microfibrillar-associated protein 1	MFAP1
P55083	Microfibril-associated glycoprotein 4	MFAP4
P55107	Bone morphogenetic protein 3B	GDF10
P55145	Mesencephalic astrocyte-derived neurotrophic factor	MANF
P55259	Pancreatic secretory granule membrane major glycoprotein GP2	GP2
P55268	Laminin subunit beta-2	LAMB2
P55773	CCL23(30-99)	CCL23
P55774	C-C motif chemokine 18	CCL18
P55789	FAD-linked sulfhydryl oxidase ALR	GFER
P56703	Proto-oncogene Wnt-3	WNT3
P56704	Protein Wnt-3a	WNT3A
P56705	Protein Wnt-4	WNT4
P56706	Protein Wnt-7b	WNT7B
P56730	Neurotrypsin	PRSS12
P56851	Epididymal secretory protein E3-beta	EDDM3B
P56975	Neuregulin-3	NRG3
P58062	Serine protease inhibitor Kazal-type 7	SPINK7
P58215	Lysyl oxidase homolog 3	LOXL3
P58294	Prokineticin-1	PROK1
P58335	Anthrax toxin receptor 2	ANTXR2
P58397	A disintegrin and metalloproteinase with thrombospondin motifs 12	ADAMTS12

P58417	Neurexophilin-1	NXPH1
P58499	Protein FAM3B	FAM3B
P59510	A disintegrin and metalloproteinase with thrombospondin motifs 20	ADAMTS20
P59665	Neutrophil defensin 1	DEFA1B
P59666	Neutrophil defensin 3	DEFA3
P59796	Glutathione peroxidase 6	GPX6
P59826	BPI fold-containing family B member 3	BPIFB3
P59827	BPI fold-containing family B member 4	BPIFB4
P59861	Beta-defensin 131	DEFB131
P60022	Beta-defensin 1	DEFB1
P60153	Inactive ribonuclease-like protein 9	RNASE9
P60827	Complement C1q tumor necrosis factor-related protein 8	C1QTNF8
P60852	Zona pellucida sperm-binding protein 1	ZP1
P60985	Keratinocyte differentiation-associated protein	KRTDAP
P61109	Kidney androgen-regulated protein	KAP
P61278	Somatostatin-14	SST
P61366	Osteocrin	OSTN
P61626	Lysozyme C	LYZ
P61769	Beta-2-microglobulin	B2M
P61812	Transforming growth factor beta-2	TGFB2
P61916	Epididymal secretory protein E1	NPC2
P62502	Epididymal-specific lipocalin-6	LCN6
P62937	Peptidyl-prolyl cis-trans isomerase A	PPIA
P67809	Nuclease-sensitive element-binding protein 1	YBX1
P67812	Signal peptidase complex catalytic subunit SEC11A	SEC11A
P78310	Coxsackievirus and adenovirus receptor	CXADR
P78333	Secreted glypican-5	GPC5
P78380	Oxidized low-density lipoprotein receptor 1	OLR1
P78423	Processed fractalkine	CX3CL1
P78509	Reelin	RELN
P78556	CCL20(2-70)	CCL20
P80075	MCP-2(6-76)	CCL8
P80098	C-C motif chemokine 7	CCL7
P80108	Phosphatidylinositol-glycan-specific phospholipase D	GPLD1
P80162	C-X-C motif chemokine 6	CXCL6
P80188	Neutrophil gelatinase-associated lipocalin	LCN2
P80303	Nucleobindin-2	NUCB2
P80511	Calcitermin	S100A12
P81172	Hepcidin-25	HAMP
P81277	Prolactin-releasing peptide	PRLH
P81534	Beta-defensin 103	DEFB103A
P81605	Dermcidin	DCD
P82279	Protein crumbs homolog 1	CRB1

P82987	ADAMTS-like protein 3	ADAMTSL3
P83105	Serine protease HTRA4	HTRA4
P83110	Serine protease HTRA3	HTRA3
P83859	Orexigenic neuropeptide QRFP	QRFP
P98088	Mucin-5AC	MUC5AC
P98095	Fibulin-2	FBLN2
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein	HSPG2
P98173	Protein FAM3A	FAM3A
Q00604	Norrin	NDP
Q00796	Sorbitol dehydrogenase	SORD
Q00887	Pregnancy-specific beta-1-glycoprotein 9	PSG9
Q00888	Pregnancy-specific beta-1-glycoprotein 4	PSG4
Q00889	Pregnancy-specific beta-1-glycoprotein 6	PSG6
Q01523	HD5(56-94)	DEFA5
Q01524	Defensin-6	DEFA6
Q01955	Collagen alpha-3(IV) chain	COL4A3
Q02297	Pro-neuregulin-1, membrane-bound isoform	NRG1
Q02325	Plasminogen-like protein B	PLGLB1
Q02383	Semenogelin-2	SEMG2
Q02388	Collagen alpha-1(VII) chain	COL7A1
Q02505	Mucin-3A	MUC3A
Q02509	Otoconin-90	OC90
Q02747	Guanylin	GUCA2A
Q02763	Angiopoietin-1 receptor	TEK
Q02817	Mucin-2	MUC2
Q02985	Complement factor H-related protein 3	CFHR3
Q03167	Transforming growth factor beta receptor type 3	TGFB3
Q03403	Trefoil factor 2	TFF2
Q03405	Urokinase plasminogen activator surface receptor	PLAUR
Q03591	Complement factor H-related protein 1	CFHR1
Q03692	Collagen alpha-1(X) chain	COL10A1
Q04118	Basic salivary proline-rich protein 3	PRB3
Q04756	Hepatocyte growth factor activator short chain	HGFAC
Q04900	Sialomucin core protein 24	CD164
Q05315	Eosinophil lysophospholipase	CLC
Q05707	Collagen alpha-1(XIV) chain	COL14A1
Q05996	Processed zona pellucida sperm-binding protein 2	ZP2
Q06033	Inter-alpha-trypsin inhibitor heavy chain H3	ITIH3
Q06141	Regenerating islet-derived protein 3-alpha	REG3A
Q06828	Fibromodulin	FMOD
Q07092	Collagen alpha-1(XVI) chain	COL16A1
Q07325	C-X-C motif chemokine 9	CXCL9
Q07507	Dermatopontin	DPT

Q075Z2	Binder of sperm protein homolog 1	BSPH1
Q07654	Trefoil factor 3	TFF3
Q07699	Sodium channel subunit beta-1	SCN1B
Q08345	Epithelial discoidin domain-containing receptor 1	DDR1
Q08380	Galectin-3-binding protein	LGALS3BP
Q08397	Lysyl oxidase homolog 1	LOXL1
Q08431	Lactadherin	MFGE8
Q08629	Testican-1	SPOCK1
Q08648	Sperm-associated antigen 11B	SPAG11B
Q08830	Fibrinogen-like protein 1	FGL1
Q10471	Polypeptide N-acetylgalactosaminyltransferase 2	GALNT2
Q10472	Polypeptide N-acetylgalactosaminyltransferase 1	GALNT1
Q11201	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1	ST3GAL1
Q11203	CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase	ST3GAL3
Q11206	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 4	ST3GAL4
Q12794	Hyaluronidase-1	HYAL1
Q12805	EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1
Q12836	Zona pellucida sperm-binding protein 4	ZP4
Q12841	Follistatin-related protein 1	FSTL1
Q12904	Aminoacyl tRNA synthase complex-interacting multifunctional protein 1	AIMP1
Q13018	Soluble secretory phospholipase A2 receptor	PLA2R1
Q13072	B melanoma antigen 1	BAGE
Q13093	Platelet-activating factor acetylhydrolase	PLA2G7
Q13103	Secreted phosphoprotein 24	SPP2
Q13162	Peroxiredoxin-4	PRDX4
Q13201	Platelet glycoprotein Ia*	MMRN1
Q13214	Semaphorin-3B	SEMA3B
Q13219	Pappalysin-1	PAPPA
Q13231	Chitotriosidase-1	CHIT1
Q13253	Noggin	NOG
Q13261	Interleukin-15 receptor subunit alpha	IL15RA
Q13275	Semaphorin-3F	SEMA3F
Q13291	Signaling lymphocytic activation molecule	SLAMF1
Q13316	Dentin matrix acidic phosphoprotein 1	DMP1
Q13361	Microfibrillar-associated protein 5	MFAP5
Q13410	Butyrophilin subfamily 1 member A1	BTN1A1
Q13421	Mesothelin, cleaved form	MSLN
Q13429	Insulin-like growth factor I	IGF-I
Q13443	Disintegrin and metalloproteinase domain-containing protein 9	ADAM9
Q13519	Neuropeptide 1	PNOC

Q13751	Laminin subunit beta-3	LAMB3
Q13753	Laminin subunit gamma-2	LAMC2
Q13790	Apolipoprotein F	APOF
Q13822	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	ENPP2
Q14031	Collagen alpha-6(IV) chain	COL4A6
Q14050	Collagen alpha-3(IX) chain	COL9A3
Q14055	Collagen alpha-2(IX) chain	COL9A2
Q14112	Nidogen-2	NID2
Q14114	Low-density lipoprotein receptor-related protein 8	LRP8
Q14118	Dystroglycan	DAG1
Q14314	Fibroleukin	FGL2
Q14393	Growth arrest-specific protein 6	GAS6
Q14406	Chorionic somatomammotropin hormone-like 1	CSHL1
Q14507	Epididymal secretory protein E3-alpha	EDDM3A
Q14508	WAP four-disulfide core domain protein 2	WFDC2
Q14512	Fibroblast growth factor-binding protein 1	FGFBP1
Q14515	SPARC-like protein 1	SPARCL1
Q14520	Hyaluronan-binding protein 2 27 kDa light chain	HABP2
Q14563	Semaphorin-3A	SEMA3A
Q14623	Indian hedgehog protein	IHH
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	ITIH4
Q14667	UPF0378 protein KIAA0100	KIAA0100
Q14703	Membrane-bound transcription factor site-1 protease	MBTPS1
Q14766	Latent-transforming growth factor beta-binding protein 1	LTBP1
Q14767	Latent-transforming growth factor beta-binding protein 2	LTBP2
Q14773	Intercellular adhesion molecule 4	ICAM4
Q14993	Collagen alpha-1(XIX) chain	COL19A1
Q14CN2	Calcium-activated chloride channel regulator 4, 110 kDa form	CLCA4
Q15046	Lysine--tRNA ligase	KARS
Q15063	Periostin	POSTN
Q15109	Advanced glycosylation end product-specific receptor	AGER
Q15113	Procollagen C-endopeptidase enhancer 1	PCOLCE
Q15166	Serum paraoxonase/lactonase 3	PON3
Q15195	Plasminogen-like protein A	PLGLA
Q15198	Platelet-derived growth factor receptor-like protein	PDGFR
Q15223	Poliovirus receptor-related protein 1	PVRL1
Q15238	Pregnancy-specific beta-1-glycoprotein 5	PSG5
Q15363	Transmembrane emp24 domain-containing protein 2	TMED2
Q15375	Ephrin type-A receptor 7	EPHA7
Q15389	Angiopoietin-1	ANGPT1
Q15465	Sonic hedgehog protein	SHH
Q15485	Ficolin-2	FCN2

Q15517	Corneodesmosin	CDSN
Q15582	Transforming growth factor-beta-induced protein ig-h3	TGFBI
Q15661	Tryptase alpha/beta-1	TPSAB1
Q15726	Metastin	KISS1
Q15782	Chitinase-3-like protein 2	CHI3L2
Q15828	Cystatin-M	CST6
Q15846	Clusterin-like protein 1	CLUL1
Q15848	Adiponectin	ADIPOQ
Q16206	Protein disulfide-thiol oxidoreductase	ENOX2
Q16270	Insulin-like growth factor-binding protein 7	IGFBP7
Q16363	Laminin subunit alpha-4	LAMA4
Q16378	Proline-rich protein 4	PRR4
Q16557	Pregnancy-specific beta-1-glycoprotein 3	PSG3
Q16568	CART(42-89)	CARTPT
Q16610	Extracellular matrix protein 1	ECM1
Q16619	Cardiotrophin-1	CTF1
Q16623	Syntaxin-1A	STX1A
Q16627	HCC-1(9-74)	CCL14
Q16651	Prostasin light chain	PRSS8
Q16661	Guanylate cyclase C-activating peptide 2	GUCA2B
Q16663	CCL15(29-92)	CCL15
Q16674	Melanoma-derived growth regulatory protein	MIA
Q16769	GlutaminyI-peptide cyclotransferase	QPCT
Q16787	Laminin subunit alpha-3	LAMA3
Q16842	CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 2	ST3GAL2
Q17RR3	Pancreatic lipase-related protein 3	PNLIPRP3
Q17RW2	Collagen alpha-1(XXIV) chain	COL24A1
Q17RY6	Lymphocyte antigen 6K	LY6K
Q1L6U9	Prostate-associated microseminoprotein	MSMP
Q1W4C9	Serine protease inhibitor Kazal-type 13	SPINK13
Q1ZYL8	Izumo sperm-egg fusion protein 4	IZUMO4
Q29960	HLA class I histocompatibility antigen, Cw-16 alpha chain	HLA-C
Q2I0M5	R-spondin-4	RSPO4
Q2L4Q9	Serine protease 53	PRSS53
Q2MKA7	R-spondin-1	RSPO1
Q2MV58	Tectonic-1	TCTN1
Q2TAL6	Brorin	VWC2
Q2UY09	Collagen alpha-1(XXVIII) chain	COL28A1
Q2VPA4	Complement component receptor 1-like protein	CR1L
Q2WEN9	Carcinoembryonic antigen-related cell adhesion molecule 16	CEACAM16
Q30KP8	Beta-defensin 136	DEFB136
Q30KP9	Beta-defensin 135	DEFB135
Q30KQ1	Beta-defensin 133	DEFB133

Q30KQ2	Beta-defensin 130	DEFB130
Q30KQ4	Beta-defensin 116	DEFB116
Q30KQ5	Beta-defensin 115	DEFB115
Q30KQ6	Beta-defensin 114	DEFB114
Q30KQ7	Beta-defensin 113	DEFB113
Q30KQ8	Beta-defensin 112	DEFB112
Q30KQ9	Beta-defensin 110	DEFB110
Q30KR1	Beta-defensin 109	DEFB109P1
Q32P28	Prolyl 3-hydroxylase 1	LEPRE1
Q3B7J2	Glucose-fructose oxidoreductase domain-containing protein 2	GFOD2
Q3SY79	Protein Wnt	WNT3A
Q3T906	N-acetylglucosamine-1-phosphotransferase subunits alpha/beta	GNPTAB
Q495T6	Membrane metallo-endopeptidase-like 1	MMEL1
Q49AH0	Cerebral dopamine neurotrophic factor	CDNF
Q4G0G5	Secretoglobin family 2B member 2	SCGB2B2
Q4G0M1	Protein FAM132B	FAM132B
Q4LDE5	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1	SVEP1
Q4QY38	Beta-defensin 134	DEFB134
Q4VAJ4	Protein Wnt	WNT10B
Q4W5P6	Protein TMEM155	TMEM155
Q4ZHG4	Fibronectin type III domain-containing protein 1	FNDC1
Q53H76	Phospholipase A1 member A	PLA1A
Q53RD9	Fibulin-7	FBLN7
Q53S33	BolA-like protein 3	BOLA3
Q5BLP8	Neuropeptide-like protein C4orf48	C4orf48
Q5DT21	Serine protease inhibitor Kazal-type 9	SPINK9
Q5EBL8	PDZ domain-containing protein 11	PDZD11
Q5FYB0	Arylsulfatase J	ARSJ
Q5FYB1	Arylsulfatase I	ARSI
Q5GAN3	Ribonuclease-like protein 13	RNASE13
Q5GAN4	Ribonuclease-like protein 12	RNASE12
Q5GAN6	Ribonuclease-like protein 10	RNASE10
Q5GFL6	von Willebrand factor A domain-containing protein 2	VWA2
Q5H8A3	Neuromedin-S	NMS
Q5H8C1	FRAS1-related extracellular matrix protein 1	FREM1
Q5IJ48	Protein crumbs homolog 2	CRB2
Q5J5C9	Beta-defensin 121	DEFB121
Q5JS37	NHL repeat-containing protein 3	NHLRC3
Q5JTB6	Placenta-specific protein 9	PLAC9
Q5JU69	Torsin-2A	TOR2A
Q5JXM2	Methyltransferase-like protein 24	METTL24
Q5JZY3	Ephrin type-A receptor 10	EPHA10

Q5K4E3	Polyserase-2	PRSS36
Q5SRR4	Lymphocyte antigen 6 complex locus protein G5c	LY6G5C
Q5T1H1	Protein eyes shut homolog	EYS
Q5T4F7	Secreted frizzled-related protein 5	SFRP5
Q5T4W7	Artemin	ARTN
Q5T7M4	Protein FAM132A	FAM132A
Q5TEH8	Protein Wnt	WNT2B
Q5TIE3	von Willebrand factor A domain-containing protein 5B1	VWA5B1
Q5UCC4	ER membrane protein complex subunit 10	EMC10
Q5VST6	Abhydrolase domain-containing protein FAM108B1	FAM108B1
Q5VTL7	Fibronectin type III domain-containing protein 7	FNDC7
Q5VUM1	UPF0369 protein C6orf57	C6orf57
Q5VV43	Dyslexia-associated protein KIAA0319	KIAA0319
Q5VWW1	Complement C1q-like protein 3	C1QL3
Q5VXI9	Lipase member N	LIPN
Q5VXJ0	Lipase member K	LIPK
Q5VXM1	CUB domain-containing protein 2	CDCP2
Q5VYX0	Renalase	RNLS
Q5VYY2	Lipase member M	LIPM
Q5W186	Cystatin-9	CST9
Q5W5W9	Regulated endocrine-specific protein 18	RESP18
Q5XG92	Carboxylesterase 4A	CES4A
Q63HQ2	Pikachurin	EGFLAM
Q641Q3	Meteorin-like protein	METRNL
Q66K79	Carboxypeptidase Z	CPZ
Q685J3	Mucin-17	MUC17
Q68BL7	Olfactomedin-like protein 2A	OLFML2A
Q68BL8	Olfactomedin-like protein 2B	OLFML2B
Q68DV7	E3 ubiquitin-protein ligase RNF43	RNF43
Q6B9Z1	Insulin growth factor-like family member 4	IGFL4
Q6BAA4	Fc receptor-like B	FCRLB
Q6E0U4	Dermokine	DMKN
Q6EMK4	Vasorin	VASN
Q6FHJ7	Secreted frizzled-related protein 4	SFRP4
Q6GPI1	Chymotrypsin B2 chain B	CTRB2
Q6GTS8	Probable carboxypeptidase PM20D1	PM20D1
Q6H9L7	Isthmin-2	ISM2
Q6IE36	Ovostatin homolog 2	OVOS2
Q6IE37	Ovostatin homolog 1	OVOS1
Q6IE38	Serine protease inhibitor Kazal-type 14	SPINK14
Q6ISS4	Leukocyte-associated immunoglobulin-like receptor 2	LAIR2
Q6JVE5	Epididymal-specific lipocalin-12	LCN12
Q6JVE6	Epididymal-specific lipocalin-10	LCN10

Q6JVE9	Epididymal-specific lipocalin-8	LCN8
Q6KF10	Growth/differentiation factor 6	GDF6
Q6MZW2	Follistatin-related protein 4	FSTL4
Q6NSX1	Coiled-coil domain-containing protein 70	CCDC70
Q6NT32	Carboxylesterase 5A	CES5A
Q6NT52	Choriogonadotropin subunit beta variant 2	CGB2
Q6NUI6	Chondroadherin-like protein	CHADL
Q6NUJ1	Saposin A-like	PSAPL1
Q6P093	Arylacetamide deacetylase-like 2	AADACL2
Q6P4A8	Phospholipase B-like 1	PLBD1
Q6P5S2	UPF0762 protein C6orf58	C6orf58
Q6P988	Protein notum homolog	NOTUM
Q6PCB0	von Willebrand factor A domain-containing protein 1	VWA1
Q6PDA7	Sperm-associated antigen 11A	SPAG11A
Q6PEW0	Inactive serine protease 54	PRSS54
Q6PEZ8	Podocan-like protein 1	PODNL1
Q6PKH6	Dehydrogenase/reductase SDR family member 4-like 2	DHRS4L2
Q6Q788	Apolipoprotein A-V	APOA5
Q6SPF0	Atherin	SAMD1
Q6UDR6	Kunitz-type protease inhibitor 4	SPINT4
Q6URK8	Testis, prostate and placenta-expressed protein	TEPP
Q6UW01	Cerebellin-3	CBLN3
Q6UW10	Surfactant-associated protein 2	SFTA2
Q6UW15	Regenerating islet-derived protein 3-gamma	REG3G
Q6UW32	Insulin growth factor-like family member 1	IGFL1
Q6UW78	UPF0723 protein C11orf83	C11orf83
Q6UW88	Epigen	EPGN
Q6UWE3	Colipase-like protein 2	CLPSL2
Q6UWF7	NXPE family member 4	NXPE4
Q6UWF9	Protein FAM180A	FAM180A
Q6UWM5	GLIPR1-like protein 1	GLIPR1L1
Q6UWN8	Serine protease inhibitor Kazal-type 6	SPINK6
Q6UWP2	Dehydrogenase/reductase SDR family member 11	DHRS11
Q6UWP8	Suprabasin	SBSN
Q6UWQ5	Lysozyme-like protein 1	LYZL1
Q6UWQ7	Insulin growth factor-like family member 2	IGFL2
Q6UWR7	Ectonucleotide pyrophosphatase/phosphodiesterase family member 6 soluble form	ENPP6
Q6UWT2	Adropin	ENHO
Q6UWU2	Beta-galactosidase-1-like protein	GLB1L
Q6UWW0	Lipocalin-15	LCN15
Q6UWX4	HHIP-like protein 2	HHIPL2
Q6UWY0	Arylsulfatase K	ARSK
Q6UWY2	Serine protease 57	PRSS57

Q6UWY5	Olfactomedin-like protein 1	OLFML1
Q6UX06	Olfactomedin-4	OLFM4
Q6UX07	Dehydrogenase/reductase SDR family member 13	DHRS13
Q6UX39	Amelotin	AMTN
Q6UX46	Protein FAM150B	FAM150B
Q6UX73	UPF0764 protein C16orf89	C16orf89
Q6UXB0	Protein FAM131A	FAM131A
Q6UXB1	Insulin growth factor-like family member 3	IGFL3
Q6UXB2	VEGF co-regulated chemokine 1	CXCL17
Q6UXF7	C-type lectin domain family 18 member B	CLEC18B
Q6UXH0	Hepatocellular carcinoma-associated protein TD26	C19orf80
Q6UXH1	Cysteine-rich with EGF-like domain protein 2	CRELD2
Q6UXH8	Collagen and calcium-binding EGF domain-containing protein 1	CCBE1
Q6UXH9	Inactive serine protease PAMR1	PAMR1
Q6UXI7	Vitrin	VIT
Q6UXI9	Nephronectin	NPNT
Q6UXN2	Trem-like transcript 4 protein	TREML4
Q6UXS0	C-type lectin domain family 19 member A	CLEC19A
Q6UXT8	Protein FAM150A	FAM150A
Q6UXT9	Abhydrolase domain-containing protein 15	ABHD15
Q6UXV4	Apolipoprotein O-like	APOOL
Q6UXX5	Inter-alpha-trypsin inhibitor heavy chain H6	ITIH6
Q6UXX9	R-spondin-2	RSPO2
Q6UY14	ADAMTS-like protein 4	ADAMTSL4
Q6UY27	Prostate and testis expressed protein 2	PATE2
Q6W4X9	Mucin-6	MUC6
Q6WN34	Chordin-like protein 2	CHRD2
Q6WRI0	Immunoglobulin superfamily member 10	IGSF10
Q6X4U4	Sclerostin domain-containing protein 1	SOSTDC1
Q6X784	Zona pellucida-binding protein 2	ZBP2
Q6XE38	Secretoglobin family 1D member 4	SCGB1D4
Q6XPR3	Repetin	RPTN
Q6XZB0	Lipase member I	LIPI
Q6ZMM2	ADAMTS-like protein 5	ADAMTSL5
Q6ZMP0	Thrombospondin type-1 domain-containing protein 4	THSD4
Q6ZNF0	Iron/zinc purple acid phosphatase-like protein	PAPL
Q6ZRI0	Otogelin	OTOG
Q6ZRP7	Sulfhydryl oxidase 2	QSOX2
Q6ZWJ8	Kielin/chordin-like protein	KCP
Q75N90	Fibrillin-3	FBN3
Q765I0	Urotensin-2B	UTS2D
Q76B58	Protein FAM5C	FAM5C

Q76LX8	A disintegrin and metalloproteinase with thrombospondin motifs 13	ADAMTS13
Q76M96	Coiled-coil domain-containing protein 80	CCDC80
Q7L1S5	Carbohydrate sulfotransferase 9	CHST9
Q7L513	Fc receptor-like A	FCRLA
Q7L8A9	Vasohibin-1	VASH1
Q7RTM1	Otopetrin-1	OTOP1
Q7RTW8	Otoancorin	OTOA
Q7RTY5	Serine protease 48	PRSS48
Q7RTY7	Ovochymase-1	OVCH1
Q7RTZ1	Ovochymase-2	OVCH2
Q7Z304	MAM domain-containing protein 2	MAMDC2
Q7Z3S9	Notch homolog 2 N-terminal-like protein	NOTCH2NL
Q7Z4H4	Intermedin-short	ADM2
Q7Z4P5	Growth/differentiation factor 7	GDF7
Q7Z4R8	UPF0669 protein C6orf120	C6orf120
Q7Z4W2	Lysozyme-like protein 2	LYZL2
Q7Z5A4	Serine protease 42	PRSS42
Q7Z5A7	Protein FAM19A5	FAM19A5
Q7Z5A8	Protein FAM19A3	FAM19A3
Q7Z5A9	Protein FAM19A1	FAM19A1
Q7Z5J1	Hydroxysteroid 11-beta-dehydrogenase 1-like protein	HSD11B1L
Q7Z5L0	Vitelline membrane outer layer protein 1 homolog	VMO1
Q7Z5L3	Complement C1q-like protein 2	C1QL2
Q7Z5L7	Podocan	PODN
Q7Z5P4	17-beta-hydroxysteroid dehydrogenase 13	HSD17B13
Q7Z5P9	Mucin-19	MUC19
Q7Z5Y6	Bone morphogenetic protein 8A	BMP8A
Q7Z7B7	Beta-defensin 132	DEFB132
Q7Z7B8	Beta-defensin 128	DEFB128
Q7Z7C8	Transcription initiation factor TFIID subunit 8	TAF8
Q7Z7H5	Transmembrane emp24 domain-containing protein 4	TMED4
Q86SG7	Lysozyme g-like protein 2	LYG2
Q86SI9	Protein CEI	C5orf38
Q86TE4	Leucine zipper protein 2	LUZP2
Q86TH1	ADAMTS-like protein 2	ADAMTSL2
Q86U17	Serpin A11	SERPINA11
Q86UU9	Endokinin-A	TAC4
Q86UW8	Hyaluronan and proteoglycan link protein 4	HAPLN4
Q86UX2	Inter-alpha-trypsin inhibitor heavy chain H5	ITIH5
Q86V24	Adiponectin receptor protein 2	ADIPOR2
Q86VB7	Soluble CD163	CD163
Q86VR8	Four-jointed box protein 1	FJX1
Q86WD7	Serpin A9	SERPINA9

Q86WN2	Interferon epsilon	IFNE
Q86WS3	Placenta-specific 1-like protein	PLAC1L
Q86X52	Chondroitin sulfate synthase 1	CHSY1
Q86XP6	Gastrokine-2	GKN2
Q86XS5	Angiopoietin-related protein 5	ANGPTL5
Q86Y27	B melanoma antigen 5	BAGE5
Q86Y28	B melanoma antigen 4	BAGE4
Q86Y29	B melanoma antigen 3	BAGE3
Q86Y30	B melanoma antigen 2	BAGE2
Q86Y38	Xylosyltransferase 1	XYLT1
Q86Y78	Ly6/PLAUR domain-containing protein 6	LYPD6
Q86YD3	Transmembrane protein 25	TMEM25
Q86YJ6	Threonine synthase-like 2	THNSL2
Q86YW7	Glycoprotein hormone beta-5	GPHB5
Q86Z23	Complement C1q-like protein 4	C1QL4
Q8IU57	Interleukin-28 receptor subunit alpha	IL28RA
Q8IUA0	WAP four-disulfide core domain protein 8	WFDC8
Q8IUB2	WAP four-disulfide core domain protein 3	WFDC3
Q8IUB3	Protein WFDC10B	WFDC10B
Q8IUB5	WAP four-disulfide core domain protein 13	WFDC13
Q8IUH2	Protein CREG2	CREG2
Q8IUK5	Plexin domain-containing protein 1	PLXDC1
Q8IUL8	Cartilage intermediate layer protein 2 C2	CILP2
Q8IUX7	Adipocyte enhancer-binding protein 1	AEBP1
Q8IUX8	Epidermal growth factor-like protein 6	EGFL6
Q8IVL8	Carboxypeptidase O	CPO
Q8IVN8	Somatomedin-B and thrombospondin type-1 domain-containing protein	SBSPON
Q8IVW8	Protein spinster homolog 2	SPNS2
Q8IW75	Serpin A12	SERPINA12
Q8IW92	Beta-galactosidase-1-like protein 2	GLB1L2
Q8IWL1	Pulmonary surfactant-associated protein A2	SFTPA2
Q8IWL2	Pulmonary surfactant-associated protein A1	SFTPA1
Q8IWV2	Contactin-4	CNTN4
Q8IYW4	Signal peptide, CUB and EGF-like domain-containing protein 1	SCUBE1
Q8IX30	Signal peptide, CUB and EGF-like domain-containing protein 3	SCUBE3
Q8IXA5	Sperm acrosome membrane-associated protein 3, membrane form	SPACA3
Q8IXB1	DnaJ homolog subfamily C member 10	DNAJC10
Q8IXL6	Extracellular serine/threonine protein kinase Fam20C	FAM20C
Q8IYD9	Lung adenoma susceptibility protein 2	LAS2
Q8IYP2	Serine protease 58	PRSS58
Q8IYS5	Osteoclast-associated immunoglobulin-like receptor	OSCAR

Q8IZC6	Collagen alpha-1(XXVII) chain	COL27A1
Q8IZJ3	C3 and PZP-like alpha-2-macroglobulin domain-containing protein 8	CPAMD8
Q8IZN7	Beta-defensin 107	DEFB107B
Q8N0V4	Leucine-rich repeat LGI family member 2	LGI2
Q8N104	Beta-defensin 106	DEFB106B
Q8N119	Matrix metalloproteinase-21	MMP21
Q8N129	Protein canopy homolog 4	CNPY4
Q8N135	Leucine-rich repeat LGI family member 4	LGI4
Q8N145	Leucine-rich repeat LGI family member 3	LGI3
Q8N158	Glypican-2	GPC2
Q8N1E2	Lysozyme g-like protein 1	LYG1
Q8N2E2	von Willebrand factor D and EGF domain-containing protein	VWDE
Q8N2E6	Prosalusin	TOR2A
Q8N2S1	Latent-transforming growth factor beta-binding protein 4	LTBP4
Q8N302	Angiogenic factor with G patch and FHA domains 1	AGGF1
Q8N307	Mucin-20	MUC20
Q8N323	NXPE family member 1	NXPE1
Q8N387	Mucin-15	MUC15
Q8N3Z0	Inactive serine protease 35	PRSS35
Q8N436	Inactive carboxypeptidase-like protein X2	CPXM2
Q8N474	Secreted frizzled-related protein 1	SFRP1
Q8N475	Follistatin-related protein 5	FSTL5
Q8N4F0	BPI fold-containing family B member 2	BPIFB2
Q8N4T0	Carboxypeptidase A6	CPA6
Q8N5W8	Protein FAM24B	FAM24B
Q8N687	Beta-defensin 125	DEFB125
Q8N688	Beta-defensin 123	DEFB123
Q8N690	Beta-defensin 119	DEFB119
Q8N6C5	Immunoglobulin superfamily member 1	IGSF1
Q8N6C8	Leukocyte immunoglobulin-like receptor subfamily A member 3	LILRA3
Q8N6G6	ADAMTS-like protein 1	ADAMTSL1
Q8N6Y2	Leucine-rich repeat-containing protein 17	LRRC17
Q8N729	Neuropeptide W-23	NPW
Q8N8U9	BMP-binding endothelial regulator protein	BMPER
Q8N907	DAN domain family member 5	DAND5
Q8NAT1	Glycosyltransferase-like domain-containing protein 2	GTDC2
Q8NAU1	Fibronectin type III domain-containing protein 5	FNDC5
Q8NB37	Parkinson disease 7 domain-containing protein 1	PDDC1
Q8NBI3	Draxin	DRAXIN
Q8NBM8	Prenylcysteine oxidase-like	PCYOX1L
Q8NBP7	Proprotein convertase subtilisin/kexin type 9	PCSK9
Q8NBQ5	Estradiol 17-beta-dehydrogenase 11	HSD17B11

Q8NBV8	Synaptotagmin-8	SYT8
Q8NCC3	Group XV phospholipase A2	PLA2G15
Q8NCF0	C-type lectin domain family 18 member C	CLEC18C
Q8NCW5	NAD(P)H-hydrate epimerase	APOA1BP
Q8NDA2	Hemicentin-2	HMCN2
Q8NDX9	Lymphocyte antigen 6 complex locus protein G5b	LY6G5B
Q8NDZ4	Deleted in autism protein 1	C3orf58
Q8NEB7	Acrosin-binding protein	ACRBP
Q8NES8	Beta-defensin 124	DEFB124
Q8NET1	Beta-defensin 108B	DEFB108B
Q8NEX5	Protein WFDC9	WFDC9
Q8NEX6	Protein WFDC11	WFDC11
Q8NF86	Serine protease 33	PRSS33
Q8NFM7	Interleukin-17 receptor D	IL17RD
Q8NFAQ5	BPI fold-containing family B member 6	BPIFB6
Q8NFAQ6	BPI fold-containing family C protein	BPIFC
Q8NFU4	Follicular dendritic cell secreted peptide	FDCSP
Q8NFW1	Collagen alpha-1(XXII) chain	COL22A1
Q8NG35	Beta-defensin 105	DEFB105B
Q8NG41	Neuropeptide B-23	NPB
Q8NHW6	Otospiralin	OTOS
Q8NI99	Angiopoietin-related protein 6	ANGPTL6
Q8TAA1	Probable ribonuclease 11	RNASE11
Q8TAG5	V-set and transmembrane domain-containing protein 2A	VSTM2A
Q8TAL6	Fin bud initiation factor homolog	FIBIN
Q8TAT2	Fibroblast growth factor-binding protein 3	FGFBP3
Q8TAX7	Mucin-7	MUC7
Q8TB22	Spermatogenesis-associated protein 20	SPATA20
Q8TB73	Protein NDNF	NDNF
Q8TB96	T-cell immunomodulatory protein	ITFG1
Q8TC92	Protein disulfide-thiol oxidoreductase	ENOX1
Q8TCV5	WAP four-disulfide core domain protein 5	WFDC5
Q8TD06	Anterior gradient protein 3 homolog	AGR3
Q8TD33	Secretoglobin family 1C member 1	SCGB1C1
Q8TD46	Cell surface glycoprotein CD200 receptor 1	CD200R1
Q8TDE3	Ribonuclease 8	RNASE8
Q8TDF5	Neuropilin and tolloid-like protein 1	NETO1
Q8TDL5	BPI fold-containing family B member 1	BPIFB1
Q8TE56	A disintegrin and metalloproteinase with thrombospondin motifs 17	ADAMTS17
Q8TE57	A disintegrin and metalloproteinase with thrombospondin motifs 16	ADAMTS16
Q8TE58	A disintegrin and metalloproteinase with thrombospondin motifs 15	ADAMTS15

Q8TE59	A disintegrin and metalloproteinase with thrombospondin motifs 19	ADAMTS19
Q8TE60	A disintegrin and metalloproteinase with thrombospondin motifs 18	ADAMTS18
Q8TE99	Acid phosphatase-like protein 2	ACPL2
Q8TER0	Sushi, nidogen and EGF-like domain-containing protein 1	SNED1
Q8TEU8	WAP, kazal, immunoglobulin, kunitz and NTR domain-containing protein 2	WFIKKN2
Q8WTQ1	Beta-defensin 104	DEFB104B
Q8WTR8	Netrin-5	NTN5
Q8WTU2	Scavenger receptor cysteine-rich domain-containing group B protein	SRCRB4D
Q8WU66	Protein TSPEAR	TSPEAR
Q8WUA8	Tsukushin	TSKU
Q8WUF8	Protein FAM172A	FAM172A
Q8WUJ1	Neuferricin	CYB5D2
Q8WUY1	UPF0670 protein THEM6	THEM6
Q8WVN6	Secreted and transmembrane protein 1	SECTM1
Q8WVQ1	Soluble calcium-activated nucleotidase 1	CANT1
Q8WWA0	Intelectin-1	ITLN1
Q8WWG1	Neuregulin-4	NRG4
Q8WWQ2	Inactive heparanase-2	HPSE2
Q8WWU7	Intelectin-2	ITLN2
Q8WWY7	WAP four-disulfide core domain protein 12	WFDC12
Q8WWY8	Lipase member H	LIPH
Q8WWZ8	Oncoprotein-induced transcript 3 protein	OIT3
Q8WX39	Epididymal-specific lipocalin-9	LCN9
Q8WXA2	Prostate and testis expressed protein 1	PATE1
Q8WXD2	Secretogranin-3	SCG3
Q8WXF3	Relaxin-3 A chain	RLN3
Q8WXI7	Mucin-16	MUC16
Q8WXQ8	Carboxypeptidase A5	CPA5
Q8WXS8	A disintegrin and metalloproteinase with thrombospondin motifs 14	ADAMTS14
Q92484	Acid sphingomyelinase-like phosphodiesterase 3a	SMPDL3A
Q92485	Acid sphingomyelinase-like phosphodiesterase 3b	SMPDL3B
Q92496	Complement factor H-related protein 4	CFHR4
Q92520	Protein FAM3C	FAM3C
Q92563	Testican-2	SPOCK2
Q92583	C-C motif chemokine 17	CCL17
Q92626	Peroxidasin homolog	PXDN
Q92743	Serine protease HTRA1	HTRA1
Q92752	Tenascin-R	TNR
Q92765	Secreted frizzled-related protein 3	FRZB
Q92819	Hyaluronan synthase 2	HAS2

Q92820	Gamma-glutamyl hydrolase	GGH
Q92824	Proprotein convertase subtilisin/kexin type 5	PCSK5
Q92832	Protein kinase C-binding protein NELL1	NELL1
Q92838	Ectodysplasin-A, membrane form	EDA
Q92874	Deoxyribonuclease-1-like 2	DNASE1L2
Q92876	Kallikrein-6	KLK6
Q92913	Fibroblast growth factor 13	FGF13
Q92954	Proteoglycan 4 C-terminal part	PRG4
Q93038	Tumor necrosis factor receptor superfamily member 25	TNFRSF25
Q93091	Ribonuclease K6	RNASE6
Q93097	Protein Wnt-2b	WNT2B
Q93098	Protein Wnt-8b	WNT8B
Q95460	Major histocompatibility complex class I-related gene protein	MR1
Q969D9	Thymic stromal lymphopoietin	TSLP
Q969E1	Liver-expressed antimicrobial peptide 2	LEAP2
Q969H8	UPF0556 protein C19orf10	C19orf10
Q969Y0	NXPE family member 3	NXPE3
Q96A54	Adiponectin receptor protein 1	ADIPOR1
Q96A83	Collagen alpha-1(XXVI) chain	EMID2
Q96A84	EMI domain-containing protein 1	EMID1
Q96A98	Tuberoinfundibular peptide of 39 residues	PTH2
Q96A99	Pentraxin-4	PTX4
Q96BH3	Epididymal sperm-binding protein 1	ELSPBP1
Q96BQ1	Protein FAM3D	FAM3D
Q96CG8	Collagen triple helix repeat-containing protein 1	CTHRC1
Q96DA0	Zymogen granule protein 16 homolog B	ZG16B
Q96DN2	von Willebrand factor C and EGF domain-containing protein	VWCE
Q96DR5	BPI fold-containing family A member 2	BPIFA2
Q96DR8	Mucin-like protein 1	MUCL1
Q96DX4	RING finger and SPRY domain-containing protein 1	RSPRY1
Q96EE4	Coiled-coil domain-containing protein 126	CCDC126
Q96GS6	Abhydrolase domain-containing protein FAM108A1	FAM108A1
Q96GW7	Brevican core protein	BCAN
Q96HF1	Secreted frizzled-related protein 2	SFRP2
Q96I82	Kazal-type serine protease inhibitor domain-containing protein 1	KAZALD1
Q96ID5	Immunoglobulin superfamily member 21	IGSF21
Q96II8	Leucine-rich repeat and calponin homology domain-containing protein 3	LRCH3
Q96IY4	Carboxypeptidase B2	CPB2
Q96JB6	Lysyl oxidase homolog 4	LOXL4
Q96JK4	HHIP-like protein 1	HHIPL1
Q96KN2	Beta-Ala-His dipeptidase	CNDP1
Q96KW9	Protein SPACA7	SPACA7

Q96KX0	Lysozyme-like protein 4	LYZL4
Q96L15	Ecto-ADP-ribosyltransferase 5	ART5
Q96LB8	Peptidoglycan recognition protein 4	PGLYRP4
Q96LB9	Peptidoglycan recognition protein 3	PGLYRP3
Q96LC7	Sialic acid-binding Ig-like lectin 10	SIGLEC10
Q96LR4	Protein FAM19A4	FAM19A4
Q96MK3	Protein FAM20A	FAM20A
Q96MS3	Glycosyltransferase 1 domain-containing protein 1	GLT1D1
Q96NY8	Processed poliovirus receptor-related protein 4	PVRL4
Q96NZ8	WAP, kazal, immunoglobulin, kunitz and NTR domain-containing protein 1	WFIKKN1
Q96NZ9	Proline-rich acidic protein 1	PRAP1
Q96P44	Collagen alpha-1(XI) chain	COL21A1
Q96PB7	Noelin-3	OLFM3
Q96PC5	Melanoma inhibitory activity protein 2	MIA2
Q96PD5	N-acetylmuramoyl-L-alanine amidase	PGLYRP2
Q96PH6	Beta-defensin 118	DEFB118
Q96PL1	Secretoglobin family 3A member 2	SCGB3A2
Q96PL2	Beta-tectorin	TECTB
Q96QH8	Sperm acrosome-associated protein 5	SPACA5
Q96QR1	Secretoglobin family 3A member 1	SCGB3A1
Q96QU1	Protocadherin-15	PCDH15
Q96QV1	Hedgehog-interacting protein	HHIP
Q96RW7	Hemicentin-1	HMCN1
Q96S42	Nodal homolog	NODAL
Q96S86	Hyaluronan and proteoglycan link protein 3	HAPLN3
Q96SL4	Glutathione peroxidase 7	GPX7
Q96SM3	Probable carboxypeptidase X1	CPXM1
Q96T91	Glycoprotein hormone alpha-2	GPHA2
Q99062	Granulocyte colony-stimulating factor receptor	CSF3R
Q99102	Mucin-4 alpha chain	MUC4
Q99217	Amelogenin, X isoform	AMELX
Q99218	Amelogenin, Y isoform	AMELY
Q99435	Protein kinase C-binding protein NELL2	NELL2
Q99470	Stromal cell-derived factor 2	SDF2
Q99542	Matrix metalloproteinase-19	MMP19
Q99574	Neuroserpin	SERPINI1
Q99584	Protein S100-A13	S100A13
Q99616	C-C motif chemokine 13	CCL13
Q99645	Epiphykan	EPYC
Q99674	Cell growth regulator with EF hand domain protein 1	CGREF1
Q99715	Collagen alpha-1(XII) chain	COL12A1
Q99727	Metalloproteinase inhibitor 4	TIMP4
Q99731	C-C motif chemokine 19	CCL19

Q99748	Neurturin	NRTN
Q99935	Proline-rich protein 1	PROL1
Q99942	E3 ubiquitin-protein ligase RNF5	RNF5
Q99944	Epidermal growth factor-like protein 8	EGFL8
Q99954	Submaxillary gland androgen-regulated protein 3A	SMR3A
Q99969	Retinoic acid receptor responder protein 2	RARRES2
Q99972	Myocilin	MYOC
Q99983	Osteomodulin	OMD
Q99985	Semaphorin-3C	SEMA3C
Q99988	Growth/differentiation factor 15	GDF15
Q9BPW4	Apolipoprotein L4	APOL4
Q9BQ08	Resistin-like beta	RETNLB
Q9BQ16	Testican-3	SPOCK3
Q9BQ51	Programmed cell death 1 ligand 2	PDCD1LG2
Q9BQB4	Sclerostin	SOST
Q9BQ14	Coiled-coil domain-containing protein 3	CCDC3
Q9BQP9	BPI fold-containing family A member 3	BPIFA3
Q9BQR3	Serine protease 27	PRSS27
Q9BQY6	WAP four-disulfide core domain protein 6	WFDC6
Q9BRR6	ADP-dependent glucokinase	ADPGK
Q9BS86	Zona pellucida-binding protein 1	ZPBP
Q9BSG0	Protease-associated domain-containing protein 1	PRADC1
Q9BSG5	Retbindin	RTBDN
Q9BT30	Probable alpha-ketoglutarate-dependent dioxygenase ABH7	ALKBH7
Q9BT56	Spexin	C12orf39
Q9BT67	NEDD4 family-interacting protein 1	NDFIP1
Q9BTY2	Plasma alpha-L-fucosidase	FUCA2
Q9BU40	Chordin-like protein 1	CHRD1
Q9BUD6	Spondin-2	SPON2
Q9BUN1	Protein MENT	MENT
Q9BUR5	Apolipoprotein O	APOO
Q9BV94	ER degradation-enhancing alpha-mannosidase-like 2	EDEM2
Q9BWP8	Collectin-11	COLEC11
Q9BWS9	Chitinase domain-containing protein 1	CHID1
Q9BX67	Junctional adhesion molecule C	JAM3
Q9BX93	Group XIIB secretory phospholipase A2-like protein	PLA2G12B
Q9BXI9	Complement C1q tumor necrosis factor-related protein 6	C1QTNF6
Q9BXJ0	Complement C1q tumor necrosis factor-related protein 5	C1QTNF5
Q9BXJ1	Complement C1q tumor necrosis factor-related protein 1	C1QTNF1
Q9BXJ2	Complement C1q tumor necrosis factor-related protein 7	C1QTNF7
Q9BXJ3	Complement C1q tumor necrosis factor-related protein 4	C1QTNF4
Q9BXJ4	Complement C1q tumor necrosis factor-related protein 3	C1QTNF3
Q9BXJ5	Complement C1q tumor necrosis factor-related protein 2	C1QTNF2

Q9BXN1	Asporin	ASPN
Q9BXP8	Pappalysin-2	PAPPA2
Q9BXR6	Complement factor H-related protein 5	CFHR5
Q9BXS0	Collagen alpha-1(XXV) chain	COL25A1
Q9BXX0	EMILIN-2	EMILIN2
Q9BXY4	R-spondin-3	RSPO3
Q9BY15	EGF-like module-containing mucin-like hormone receptor-like 3 subunit beta	EMR3
Q9BY50	Signal peptidase complex catalytic subunit SEC11C	SEC11C
Q9BY76	Angiopoietin-related protein 4	ANGPTL4
Q9BYF1	Processed angiotensin-converting enzyme 2	ACE2
Q9BYJ0	Fibroblast growth factor-binding protein 2	FGFBP2
Q9BYW3	Beta-defensin 126	DEFB126
Q9BYX4	Interferon-induced helicase C domain-containing protein 1	IFIH1
Q9BYZ8	Regenerating islet-derived protein 4	REG4
Q9BZ76	Contactin-associated protein-like 3	CNTNAP3
Q9BZG9	Ly-6/neurotoxin-like protein 1	LYNX1
Q9BZJ3	Tryptase delta	TPSD1
Q9BZM1	Group XIIA secretory phospholipase A2	PLA2G12A
Q9BZM2	Group IIF secretory phospholipase A2	PLA2G2F
Q9BZM5	NKG2D ligand 2	ULBP2
Q9BZP6	Acidic mammalian chitinase	CHIA
Q9BZZ2	Sialoadhesin	SIGLEC1
Q9C0B6	Protein FAM5B	FAM5B
Q9GZM7	Tubulointerstitial nephritis antigen-like	TINAGL1
Q9GZN4	Brain-specific serine protease 4	PRSS22
Q9GZP0	Platelet-derived growth factor D, receptor-binding form	PDGFD
Q9GZT5	Protein Wnt-10a	WNT10A
Q9GZU5	Nyctalopin	NYX
Q9GZV7	Hyaluronan and proteoglycan link protein 2	HAPLN2
Q9GZV9	Fibroblast growth factor 23	FGF23
Q9GZX9	Twisted gastrulation protein homolog 1	TWSG1
Q9GZZ7	GDNF family receptor alpha-4	GFRA4
Q9GZZ8	Extracellular glycoprotein lacritin	LACRT
Q9H0B8	Cysteine-rich secretory protein LCCL domain-containing 2	CRISPLD2
Q9H106	Signal-regulatory protein delta	SIRPD
Q9H114	Cystatin-like 1	CSTL1
Q9H173	Nucleotide exchange factor SIL1	SIL1
Q9H1E1	Ribonuclease 7	RNASE7
Q9H1F0	WAP four-disulfide core domain protein 10A	WFDC10A
Q9H1J5	Protein Wnt-8a	WNT8A
Q9H1J7	Protein Wnt-5b	WNT5B
Q9H1M3	Beta-defensin 129	DEFB129
Q9H1M4	Beta-defensin 127	DEFB127

Q9H1Z8	Augurin	C2orf40
Q9H239	Matrix metalloproteinase-28	MMP28
Q9H2A7	C-X-C motif chemokine 16	CXCL16
Q9H2A9	Carbohydrate sulfotransferase 8	CHST8
Q9H2R5	Kallikrein-15	KLK15
Q9H2X0	Chordin	CHRD
Q9H2X3	C-type lectin domain family 4 member M	CLEC4M
Q9H306	Matrix metalloproteinase-27	MMP27
Q9H324	A disintegrin and metalloproteinase with thrombospondin motifs 10	ADAMTS10
Q9H336	Cysteine-rich secretory protein LCCL domain-containing 1	CRISPLD1
Q9H3E2	Sorting nexin-25	SNX25
Q9H3R2	Mucin-13	MUC13
Q9H3U7	SPARC-related modular calcium-binding protein 2	SMOC2
Q9H3Y0	Peptidase inhibitor R3HDML	R3HDML
Q9H4A4	Aminopeptidase B	RNPEP
Q9H4F8	SPARC-related modular calcium-binding protein 1	SMOC1
Q9H4G1	Cystatin-9-like	CST9L
Q9H5V8	CUB domain-containing protein 1	CDCP1
Q9H6B9	Epoxide hydrolase 3	EPHX3
Q9H6E4	Coiled-coil domain-containing protein 134	CCDC134
Q9H741	UPF0454 protein C12orf49	C12orf49
Q9H772	Gremlin-2	GREM2
Q9H7Y0	Deleted in autism-related protein 1	CXorf36
Q9H8L6	Multimerin-2	MMRN2
Q9H9S5	Fukutin-related protein	FKRP
Q9HAT2	Sialate O-acetyltransferase	SIAE
Q9HB40	Retinoid-inducible serine carboxypeptidase	SCPEP1
Q9HB63	Netrin-4	NTN4
Q9HBJ0	Placenta-specific protein 1	PLAC1
Q9HC23	Prokineticin-2	PROK2
Q9HC57	WAP four-disulfide core domain protein 1	WFDC1
Q9HC73	Cytokine receptor-like factor 2	CRLF2
Q9HC84	Mucin-5B	MUC5B
Q9HCB6	Spondin-1	SPON1
Q9HCQ7	Neuropeptide NPSF	NPVF
Q9HCT0	Fibroblast growth factor 22	FGF22
Q9HD89	Resistin	RETN
Q9NNX1	Tuftelin	TUFT1
Q9NNX6	CD209 antigen	CD209
Q9NP55	BPI fold-containing family A member 1	BPIFA1
Q9NP70	Ameloblastin	AMBN
Q9NP95	Fibroblast growth factor 20	FGF20
Q9NP99	Triggering receptor expressed on myeloid cells 1	TREM1

Q9NPA2	Matrix metalloproteinase-25	MMP25
Q9NPE2	Neugrin	NGRN
Q9NPH0	Lysophosphatidic acid phosphatase type 6	ACP6
Q9NPH6	Odorant-binding protein 2b	OBP2B
Q9NQ30	Endothelial cell-specific molecule 1	ESM1
Q9NQ36	Signal peptide, CUB and EGF-like domain-containing protein 2	SCUBE2
Q9NQ38	Serine protease inhibitor Kazal-type 5	SPINK5
Q9NQ76	Matrix extracellular phosphoglycoprotein	MEPE
Q9NQ79	Cartilage acidic protein 1	CRTAC1
Q9NR16	Scavenger receptor cysteine-rich type 1 protein M160	CD163L1
Q9NR23	Growth/differentiation factor 3	GDF3
Q9NR71	Neutral ceramidase	ASAH2
Q9NR99	Matrix-remodeling-associated protein 5	MXRA5
Q9NRA1	Platelet-derived growth factor C	PDGFC
Q9NRC9	Otoraplin	OTOR
Q9NRE1	Matrix metalloproteinase-26	MMP26
Q9NRJ3	C-C motif chemokine 28	CCL28
Q9NRM1	Enamelin	ENAM
Q9NRN5	Olfactomedin-like protein 3	OLFML3
Q9NRR1	Cytokine-like protein 1	CYTL1
Q9NS15	Latent-transforming growth factor beta-binding protein 3	LTBP3
Q9NS62	Thrombospondin type-1 domain-containing protein 1	THSD1
Q9NS71	Gastrokin-1	GKN1
Q9NS98	Semaphorin-3G	SEMA3G
Q9NSA1	Fibroblast growth factor 21	FGF21
Q9NT22	EMILIN-3	EMILIN3
Q9NTU7	Cerebellin-4	CBLN4
Q9NVR0	Kelch-like protein 11	KLHL11
Q9NWH7	Spermatogenesis-associated protein 6	SPATA6
Q9NXC2	Glucose-fructose oxidoreductase domain-containing protein 1	GFOD1
Q9NY56	Odorant-binding protein 2a	OBP2A
Q9NY84	Vascular non-inflammatory molecule 3	VNN3
Q9NZ20	Group 3 secretory phospholipase A2	PLA2G3
Q9NZC2	Triggering receptor expressed on myeloid cells 2	TREM2
Q9NZK5	Adenosine deaminase CECR1	CECR1
Q9NZK7	Group IIE secretory phospholipase A2	PLA2G2E
Q9NZP8	Complement C1r subcomponent-like protein	C1RL
Q9NZV1	Cysteine-rich motor neuron 1 protein	CRIM1
Q9NZW4	Dentin sialoprotein	DSPP
Q9P0G3	Kallikrein-14	KLK14
Q9P0W0	Interferon kappa	IFNK
Q9P218	Collagen alpha-1(XX) chain	COL20A1
Q9P2C4	Transmembrane protein 181	TMEM181

Q9P2K2	Thioredoxin domain-containing protein 16	TXNDC16
Q9P2N4	A disintegrin and metalloproteinase with thrombospondin motifs 9	ADAMTS9
Q9UBC7	Galanin-like peptide	GALP
Q9UBD3	Cytokine SCM-1 beta	XCL2
Q9UBD9	Cardiotrophin-like cytokine factor 1	CLCF1
Q9UBM4	Opticin	OPTC
Q9UBP4	Dickkopf-related protein 3	DKK3
Q9UBQ6	Exostosin-like 2	EXTL2
Q9UBR5	Chemokine-like factor	CKLF
Q9UBS5	Gamma-aminobutyric acid type B receptor subunit 1	GABBR1
Q9UBT3	Dickkopf-related protein 4 short form	DKK4
Q9UBU2	Dickkopf-related protein 2	DKK2
Q9UBU3	Ghrelin-28	GHRL
Q9UBV4	Protein Wnt-16	WNT16
Q9UBX5	Fibulin-5	FBLN5
Q9UBX7	Kallikrein-11	KLK11
Q9UEF7	Klotho	KL
Q9UFP1	Protein FAM198A	FAM198A
Q9UGM3	Deleted in malignant brain tumors 1 protein	DMBT1
Q9UGM5	Fetuin-B	FETUB
Q9UGP8	Translocation protein SEC63 homolog	SEC63
Q9UHF0	Neurokinin-B	TAC3
Q9UHF1	Epidermal growth factor-like protein 7	EGFL7
Q9UHG2	ProSAAS	PCSK1N
Q9UHI8	A disintegrin and metalloproteinase with thrombospondin motifs 1	ADAMTS1
Q9UHL4	Dipeptidyl peptidase 2	DPP7
Q9UI42	Carboxypeptidase A4	CPA4
Q9UIG4	Psoriasis susceptibility 1 candidate gene 2 protein	PSORS1C2
Q9UIK5	Tomoregulin-2	TMEFF2
Q9UIQ6	Leucyl-cystinyl aminopeptidase, pregnancy serum form	LNPEP
Q9UJA9	Ectonucleotide pyrophosphatase/phosphodiesterase family member 5	ENPP5
Q9UJH8	Meteorin	METRIN
Q9UJJ9	N-acetylglucosamine-1-phosphotransferase subunit gamma	GNPTG
Q9UJW2	Tubulointerstitial nephritis antigen	TINAG
Q9UK05	Growth/differentiation factor 2	GDF2
Q9UK55	Protein Z-dependent protease inhibitor	SERPINA10
Q9UK85	Dickkopf-like protein 1	DKKL1
Q9UKJ1	Paired immunoglobulin-like type 2 receptor alpha	PILRA
Q9UKP4	A disintegrin and metalloproteinase with thrombospondin motifs 7	ADAMTS7
Q9UKP5	A disintegrin and metalloproteinase with thrombospondin motifs 6	ADAMTS6

Q9UKQ2	Disintegrin and metalloproteinase domain-containing protein 28	ADAM28
Q9UKQ9	Kallikrein-9	KLK9
Q9UKR0	Kallikrein-12	KLK12
Q9UKR3	Kallikrein-13	KLK13
Q9UKU9	Angiopoietin-related protein 2	ANGPTL2
Q9UKZ9	Procollagen C-endopeptidase enhancer 2	PCOLCE2
Q9UL52	Transmembrane protease serine 11E non-catalytic chain	TMPRSS11E
Q9ULC0	Endomucin	EMCN
Q9ULI3	Protein HEG homolog 1	HEG1
Q9ULZ1	Apelin-13	APLN
Q9ULZ9	Matrix metalloproteinase-17	MMP17
Q9UM21	Alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase A soluble form	MGAT4A
Q9UM22	Mammalian ependymin-related protein 1	EPDR1
Q9UM73	ALK tyrosine kinase receptor	ALK
Q9UMD9	97 kDa linear IgA disease antigen	COL17A1
Q9UMX5	Neudesin	NENF
Q9UN73	Protocadherin alpha-6	PCDHA6
Q9UNA0	A disintegrin and metalloproteinase with thrombospondin motifs 5	ADAMTS5
Q9UNI1	Chymotrypsin-like elastase family member 1	CELA1
Q9UNK4	Group IID secretory phospholipase A2	PLA2G2D
Q9UP79	A disintegrin and metalloproteinase with thrombospondin motifs 8	ADAMTS8
Q9UPZ6	Thrombospondin type-1 domain-containing protein 7A	THSD7A
Q9UQ72	Pregnancy-specific beta-1-glycoprotein 11	PSG11
Q9UQ74	Pregnancy-specific beta-1-glycoprotein 8	PSG8
Q9UQC9	Calcium-activated chloride channel regulator 2	CLCA2
Q9UQE7	Structural maintenance of chromosomes protein 3	SMC3
Q9UQP3	Tenascin-N	TNN
Q9Y223	UDP-N-acetylglucosamine 2-epimerase	GNE
Q9Y240	C-type lectin domain family 11 member A	CLEC11A
Q9Y251	Heparanase 8 kDa subunit	HPSE
Q9Y258	C-C motif chemokine 26	CCL26
Q9Y264	Angiopoietin-4	ANGPT4
Q9Y275	Tumor necrosis factor ligand superfamily member 13b, membrane form	TNFSF13B
Q9Y287	BRI2 intracellular domain	ITM2B
Q9Y2E5	Epididymis-specific alpha-mannosidase	MAN2B2
Q9Y334	von Willebrand factor A domain-containing protein 7	VWA7
Q9Y337	Kallikrein-5	KLK5
Q9Y3B3	Transmembrane emp24 domain-containing protein 7	TMED7
Q9Y3E2	BolA-like protein 1	BOLA1
Q9Y426	C2 domain-containing protein 2	C2CD2

Q9Y4K0	Lysyl oxidase homolog 2	LOXL2
Q9Y4X3	C-C motif chemokine 27	CCL27
Q9Y5C1	Angiopoietin-related protein 3	ANGPTL3
Q9Y5I2	Protocadherin alpha-10	PCDHA10
Q9Y5I3	Protocadherin alpha-1	PCDHA1
Q9Y5K2	Kallikrein-4	KLK4
Q9Y5L2	Hypoxia-inducible lipid droplet-associated protein	HILPDA
Q9Y5Q5	Atrial natriuretic peptide-converting enzyme	CORIN
Q9Y5R2	Matrix metalloproteinase-24	MMP24
Q9Y5U5	Tumor necrosis factor receptor superfamily member 18	TNFRSF18
Q9Y5W5	Wnt inhibitory factor 1	WIF1
Q9Y5X9	Endothelial lipase	LIPG
Q9Y625	Secreted glypican-6	GPC6
Q9Y646	Carboxypeptidase Q	CPQ
Q9Y6C2	EMILIN-1	EMILIN1
Q9Y6F9	Protein Wnt-6	WNT6
Q9Y6I9	Testis-expressed sequence 264 protein	TEX264
Q9Y6L7	Tolloid-like protein 2	TLL2
Q9Y6N3	Calcium-activated chloride channel regulator family member 3	CLCA3P
Q9Y6N6	Laminin subunit gamma-3	LAMC3
Q9Y6R7	IgGFC-binding protein	FCGBP
Q9Y6Y9	Lymphocyte antigen 96	LY96
Q9Y6Z7	Collectin-10	COLEC10

[0184] In some embodiments, the present invention is useful in treating a disease or disorder listed in Table 1.

[0185] In some embodiments, the present invention is useful in delivering vaccines. Vaccines delivered subcutaneously include vaccines against infectious diseases which include but are not limited to diphtheria, tetanus, pertussis, poliomyelitis, measles, mumps, rubella, haemophilus influenzae type b infections, hepatitis B, influenza, pneumococcal infections, cholera, hepatitis A, meningococcal disease, plague, rabies, bat lyssavirus, yellow fever, Japanese encephalitis, Q fever, tuberculosis, typhoid and varicella-zoster. Vaccines delivered subcutaneously may also include vaccines against cell proliferative disorders such as cancers. In some embodiments, subcutaneously delivered vaccines include cancer vaccines for lymphoproliferative disorders. In some embodiments, the cancer vaccines include subcutaneously delivered mRNA encoding immunogenic agents that direct cellular immune response against cancer cells, using the method of the invention. In some embodiments, a vaccine comprising mRNA encoding MHC-class specific peptides comprising one or more

cancer antigenic epitopes is administered subcutaneously with an mRNA encoding hyaluronidase, which can result in superior systemic delivery of the vaccine and more robust antigenic response.

[0186] In some embodiments, the present invention is useful in treating a liver disease, for example OTC deficiency. Co-injection of mRNA encoding an OTC protein with a hyaluronidase enzyme results in an increased level of OTC enzyme (protein) in a liver cell (e.g., a hepatocyte) of a subject as compared to a baseline level before treatment. Typically, the baseline level is measured before treatment (e.g., up to 12 months prior to the treatment and in some instances, immediately before the treatment). In some embodiments, subcutaneous injection according to the present invention results in an increased OTC protein level in the liver cell by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% as compared to a baseline level before treatment. In some embodiments, subcutaneous injection according to the present invention results in an increased OTC protein level in a liver cell as compared to the OTC protein level a liver cell of subjects who are not treated.

[0187] In some embodiments, subcutaneous injection according to the present invention results in an increased OTC protein level in plasma or serum of subject as compared to a baseline level before treatment. Typically, the baseline level is measured before treatment (e.g., up to 12 months prior to the treatment and in some instances, immediately before the treatment). In some embodiments, administering the provided composition results in an increased OTC protein level in plasma or serum by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% as compared to a baseline level before treatment. In some embodiments, administering the provided composition results in an increased OTC protein level in plasma or serum as compared to an OTC protein level in plasma or serum of subjects who are not treated.

[0188] The compositions and methods of the invention provide for the delivery of mRNA to treat a number of disorders. In particular, the compositions and methods of the present invention are suitable for the treatment of diseases or disorders relating to the deficiency of proteins and/or enzymes that are excreted or secreted in the liver. These include but are not limited to: Phenylalanine hydroxylase (PAH) deficiency (classically known as phenylketonuria, PKU), argininosuccinate synthase 1 (ASS1) deficiency, which causes a liver urea cycle disorder citrullinaemia, erythropoietin (EPO) deficiency, which leads to anemia, erythropoietin being a protein produced both in the kidney and in the liver.

[0189] Disorders for which the present invention are useful include, but are not limited to, disorders such as Fabry disease; hemophilic diseases (such as, e.g., hemophilia B (FIX), hemophilia A (FVIII); SMN1-related spinal muscular atrophy (SMA); amyotrophic lateral

sclerosis (ALS); GALT-related galactosemia; COL4A5-related disorders including Alport syndrome; galactocerebrosidase deficiencies; X-linked adrenoleukodystrophy; Friedreich's ataxia; Pelizaeus-Merzbacher disease; TSC1 and TSC2-related tuberous sclerosis; Sanfilippo B syndrome (MPS IIIB); the FMR1-related disorders which include Fragile X syndrome, Fragile X-Associated Tremor/Ataxia Syndrome and Fragile X Premature Ovarian Failure Syndrome; Prader-Willi syndrome; hereditary hemorrhagic telangiectasia (AT); Niemann-Pick disease Type C1; the neuronal ceroid lipofuscinoses-related diseases including Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), Juvenile Batten disease, Santavuori-Haltia disease, Jansky-Bielschowsky disease, and PTT-1 and TPP1 deficiencies; EIF2B1, EIF2B2, EIF2B3, EIF2B4 and EIF2B5-related childhood ataxia with central nervous system hypomyelination/vanishing white matter; CACNA1A and CACNB4-related Episodic Ataxia Type 2; the MECP2-related disorders including Classic Rett Syndrome, MECP2-related Severe Neonatal Encephalopathy and PPM-X Syndrome; CDKL5-related Atypical Rett Syndrome; Kennedy's disease (SBMA); Notch-3 related cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL); SCN1A and SCN1B-related seizure disorders; the Polymerase G-related disorders which include Alpers-Huttenlocher syndrome, POLG-related sensory ataxic neuropathy, dysarthria, and ophthalmoparesis, and autosomal dominant and recessive progressive external ophthalmoplegia with mitochondrial DNA deletions; X-Linked adrenal hypoplasia; X-linked agammaglobulinemia; and Wilson's disease.

[0190] In some embodiments, the nucleic acids, and in particular mRNA, of the invention may encode functional proteins or enzymes that are secreted into extracellular space. For example, the secreted proteins include clotting factors, components of the complement pathway, cytokines, chemokines, chemoattractants, protein hormones (e.g. EGF, PDF), protein components of serum, antibodies, secretable toll-like receptors, and others. In some embodiments, the compositions of the present invention may include mRNA encoding erythropoietin, α 1-antitrypsin, carboxypeptidase N or human growth hormone.

EXAMPLES

[0191] While certain compounds, compositions and methods of the present invention have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds of the invention and are not intended to limit the same.

Lipid Materials

[0192] The formulations described in the following Examples, unless otherwise specified, contain a multi-component lipid mixture of varying ratios employing one or more cationic lipids, helper lipids (e.g., non-cationic lipids and/or cholesterol lipids) and PEGylated lipids designed to encapsulate various nucleic acid materials. Cationic lipids for the process can include, but are not limited to, cKK-E12 (3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine-2,5-dione), OF-02, Target 23, Target 24, ICE, HGT5000, HGT5001, HGT4003, DOTAP (1,2-dioleoyl-3-trimethylammonium propane), DODAP (1,2-dioleoyl-3-dimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DLinDMA (Heyes, J.; Palmer, L.; Bremner, K.; MacLachlan, I. “Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids” J. Contr. Rel. 2005, 107, 276-287), DLin-KC2-DMA (Semple, S.C. et al. “Rational Design of Cationic Lipids for siRNA Delivery” Nature Biotech. 2010, 28, 172-176), C12-200 (Love, K.T. et al. “Lipid-like materials for low-dose in vivo gene silencing” PNAS 2010, 107, 1864-1869), , dialkylamino-based, imidazole-based, guanidinium-based, etc. Helper lipids can include, but are not limited, to DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine), DOPG (1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)), DOPC (1,2-dioleoyl-sn-glycero-3-phosphatidylcholine), cholesterol, etc. PEGylated lipids can include, but are not limited to, a poly(ethylene) glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C₆-C₂₀ length.

mRNA Materials

[0193] In some embodiments, codon-optimized messenger RNA encoding target protein was synthesized by in vitro transcription from a plasmid DNA template encoding the gene, which was followed by the addition of a 5' cap structure (Cap 1) (Fechter, P.; Brownlee, G.G. “Recognition of mRNA cap structures by viral and cellular proteins” J. Gen. Virology 2005, 86, 1239-1249) and a 3' poly(A). 5' and 3' untranslated regions present in each mRNA product are represented as X and Y, respectively and defined as stated previously.

Example 1. In vivo expression of firefly luciferase protein in mice

[0194] This example illustrates an exemplary method of administering firefly luciferase (FFL) mRNA-loaded LNPs and methods for analyzing firefly luciferase in target tissues *in vivo*.

Wild type mice are treated with LNPs encapsulating mRNA encoding FFL at 20 mg/kg co-formulated with hyaluronidase mRNA at 20 mg/kg by subcutaneous delivery. The luminescence produced by FFL protein is observed at 3, 24 and 48 hours post-subcutaneous administration. Significant luminescence is observed representing the successful production of active FFL protein in the livers of these mice. Further, sustained FFL activity is maintained for at least 24 hours with little to no decrease in intensity.

Example 2. In vivo activity of expressed hOTC in mice

[0195] This example shows a comparison of intravenous administration without hyaluronidase and subcutaneous administration with and without an mRNA encoding hyaluronidase in OTC KO *spf^{ash}* mice and human OTC (hOTC) mRNA-loaded lipid nanoparticles. In this example, hOTC and hyaluronidase mRNAs are present in the same formulation and therefore are administered simultaneously. The hOTC protein is shown to be enzymatically active, as determined by measuring levels of citrulline production using a custom *ex vivo* activity assay. Generally, the production of citrulline can be used to evaluate the activity of the expressed hOTC protein. Citrulline activity of hOTC protein is measured in the liver extracts of mice sacrificed 24 hours after the single dose of the lipid nanoparticles encapsulating hOTC mRNA at 20 mg/kg is delivered subcutaneously with and without hyaluronidase mRNA (20 mg/kg). Citrulline activity in the livers of saline-treated OTC KO mice is also measured. No significant hOTC protein activity is observed after subcutaneous administration of hOTC mRNA without hyaluronidase mRNA co-formulation. hOTC protein activity in those animals is similar to those seen in animals treated with saline. In contrast, hOTC protein activity (as evidenced by citrulline protein levels) is similar in the livers of mice which are administered the hOTC mRNA LNP composition intravenously and those administered the hOTC mRNA LNP composition formulated with hyaluronidase-encoding mRNA subcutaneously. A hyaluronidase mRNA dose dependence on the robustness of OTC mRNA expression can be tested using varying doses of hyaluronidase mRNA in the formulation.

Example 3. In vivo efficiency of CO-hOTC mRNA delivery in mice

[0196] This example shows a comparison of intravenous administration without hyaluronidase versus subcutaneous administration with and without the mRNA encoding hyaluronidase in OTC KO *spf^{ash}* mice using CO-hOTC (codon-optimized human OTC) mRNA-loaded lipid nanoparticles. Subcutaneously delivered CO-hOTC mRNA lipid nanoparticles co-

formulated with hyaluronidase mRNA are more effective than subcutaneously delivered mRNA lipid nanoparticles without the mRNA encoding hyaluronidase.

[0197] Efficiency of administration was determined by comparing CO-hOTC mRNA copy number in the livers of the various treatment groups. CO-hOTC mRNA copy number in the livers of mice is measured 24 hours after a single subcutaneous dose of 20 mg/kg CO-hOTC mRNA and 20 mg/kg hyaluronidase mRNA (SEQ ID NO: 12) LNP formulation. A control set comprise OTC mRNA, without hyaluronidase mRNA. For comparison, CO-hOTC mRNA copy number is also measured in livers of mice 24 hours after a CO-hOTC mRNA LNP solution is injected intravenously at 0.50 mg/kg. mOTC mRNA copy number is measured in the livers of saline-treated wild type (WT) mice, saline-treated OTC KO mice, and OTC KO mice treated intravenously with hOTC LNP solution, subcutaneously with hOTC LNP formulation free of hyaluronidase or subcutaneously with hOTC LNP co-formulated with hyaluronidase.

Example 4. In vivo expression of human erythropoietin (hEPO) in mice

[0198] This example illustrates an exemplary time course of human erythropoietin (hEPO) protein expression following subcutaneous administration of hEPO encoding mRNA using the method disclosed, in comparison with intravenous administration of the same.

[0199] Male CD1 mice are administered either an intravenous dose of hEPO mRNA-loaded lipid nanoparticles at a dosage of 1 mg/kg or a subcutaneous dose of hEPO mRNA-loaded lipid nanoparticles at a dosage of 5 mg/kg co-formulated with 5 mg/kg hyaluronidase mRNA once on day 1. Human EPO protein expression is examined in serum samples by hEPO-specific ELISA for 4 days.

[0200] High level of EPO protein expression is observed in both intravenous-administered and subcutaneous-administered groups of mice at 6 hours after mRNA administration (Day 1) and on Day 2. The expression level is compared to intravenous administration for the same mRNA LNP.

EQUIVALENTS

[0201] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention

described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

We claim:

1. A method for subcutaneous delivery of a messenger RNA (mRNA) to a subject in need thereof, the method comprising: administering subcutaneously to the subject a composition comprising:
 - an mRNA encoding a protein or polypeptide, and
 - an mRNA encoding a hyaluronidase.
2. The method of claim 1 wherein the mRNA encoding a protein or polypeptide is a therapeutic mRNA.
3. The method of claim 1, wherein the hyaluronidase is a mammalian hyaluronidase.
4. The method of claim 3, wherein the mammalian hyaluronidase is a bovine hyaluronidase.
5. The method of claim 3, wherein the mammalian hyaluronidase is a human hyaluronidase.
6. The method of claim 1, wherein the hyaluronidase mRNA has a polynucleotide sequence having at least 80% sequence identity to a sequence selected from any one of SEQ ID NOs: 9, 10, and 12.
7. The method of any one of the preceding claims, wherein the therapeutic mRNA or the hyaluronidase mRNA comprises one or more modified nucleotides.
8. The method of any one of the preceding claims, wherein the therapeutic mRNA or the hyaluronidase mRNA are individually capped and tailed.
9. The method of claim 1 wherein the therapeutic mRNA and the hyaluronidase mRNA are encapsulated in one or more lipid nanoparticles.
10. The method of claim 9, wherein the one or more lipid nanoparticles comprise a cationic lipid.

11. The method of claim 10, wherein the cationic lipid is selected from a group consisting of cKK-E12 (3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine-2,5-dione), OF-02, Target 23, Target 24, ICE, HGT5000, HGT5001, HGT4003, DOTAP (1,2-dioleoyl-3-trimethylammonium propane), DODAP (1,2-dioleoyl-3-dimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DLinDMA, DLin-KC2-DMA, dialkylamino-based, imidazole-based, and guanidinium-based cationic lipids.
12. The method of claim 9, wherein the one or more lipid nanoparticles comprise a PEG-modified lipid.
13. The composition of claim 9, wherein the PEG-modified lipid constitutes at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, or at least 10% of the total lipids in the lipid nanoparticle.
14. The method of any of the above claims where the lipid nanoparticle comprises a liposome.
15. The method of claim 1 wherein the therapeutic mRNA and the hyaluronidase mRNA are encapsulated in a single liposome.
16. The method of claim 1 wherein the therapeutic mRNA is administered in a composition separate from that of the hyaluronidase mRNA composition.
17. The method of claim 1 wherein the therapeutic mRNA and the hyaluronidase mRNA are administered in a single formulation.
18. The method of claim 1 wherein the therapeutic mRNA and the hyaluronidase mRNA are administered in separate formulations.
19. The method of claim 1 wherein the therapeutic mRNA and the hyaluronidase mRNA are administered separately.

20. The method of claim 1 wherein the hyaluronidase mRNA mRNA is administered prior to administering the therapeutic mRNA composition.
21. The method of claim 1 wherein the hyaluronidase-encoding mRNA is administered 0.1 hours, 0.2 hours, 0.3 hours, 0.4 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours or 6 hours prior to administering the therapeutic mRNA composition.
22. The method of claim 1 wherein the hyaluronidase-encoding mRNA is administered 1 hour, 2 hours, 3 hours, 4 hours, 5 hours or 6 hours prior to administering the first mRNA composition.
23. The method of claim 1, wherein the therapeutic mRNA is expressed in the liver.
24. The method of claim 1, wherein the therapeutic mRNA is expressed in the lung.
25. The method of claim 1, wherein the therapeutic mRNA is expressed in the kidney.
26. The method of claim 1, wherein the therapeutic mRNA is expressed in the subcutaneous tissue.
27. The method of claim 1, wherein the therapeutic mRNA is expressed in the serum.
28. The method of claim 1, wherein the administering the mRNA subcutaneously comprises subcutaneous injection.
29. The method of claim 1, wherein the administering the hyaluronidase mRNA subcutaneously comprises topical administration.
30. A method of messenger RNA (mRNA) delivery for *in vivo* protein expression, comprising,
administering via subcutaneous injection to a subject
 - a) an mRNA encoding a protein, and
 - b) an mRNA encoding a hyaluronidase enzyme.

31. A method for treating a disease, disorder or condition in a subject, comprising delivering subcutaneously to the subject a therapeutic mRNA encoding a protein or a polypeptide, and a helper mRNA encoding a hyaluronidase, wherein the therapeutic mRNA-encoded protein or polypeptide is deficient in the subject.
32. The method of claim 31, wherein therapeutic mRNA is expressed in the liver.
33. The method of claim 31, wherein therapeutic mRNA is expressed in the lung.
34. The method of claim 31, wherein the disease, disorder or condition is selected from ornithine transcarbamylase (OTC) deficiency, Phenylalanine hydroxylase (PAH) deficiency (phenylketonuria, PKU), argininosuccinate synthase 1 (ASS1) deficiency, erythropoietin (EPO) deficiency, Fabry disease; hemophilic diseases (such as, e.g., hemophilia B (FIX), hemophilia A (FVIII); SMN1-related spinal muscular atrophy (SMA); amyotrophic lateral sclerosis (ALS); GALT-related galactosemia; COL4A5-related disorders including Alport syndrome; galactocerebrosidase deficiencies; X-linked adrenoleukodystrophy; Friedreich's ataxia; Pelizaeus-Merzbacher disease; TSC1 and TSC2-related tuberous sclerosis; Sanfilippo B syndrome (MPS IIIB); the FMR1-related disorders which include Fragile X syndrome, Fragile X-Associated Tremor/Ataxia Syndrome and Fragile X Premature Ovarian Failure Syndrome; Prader-Willi syndrome; hereditary hemorrhagic telangiectasia (AT); Niemann-Pick disease Type C1; the neuronal ceroid lipofuscinoses-related diseases including Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), Juvenile Batten disease, Santavuori-Haltia disease, Jansky-Bielschowsky disease, and PTT-1 and TPP1 deficiencies; EIF2B1, EIF2B2, EIF2B3, EIF2B4 and EIF2B5-related childhood ataxia with central nervous system hypomyelination/vanishing white matter; CACNA1A and CACNB4-related Episodic Ataxia Type 2; the MECP2-related disorders including Classic Rett Syndrome, MECP2-related Severe Neonatal Encephalopathy and PPM-X Syndrome; CDKL5-related Atypical Rett Syndrome; Kennedy's disease (SBMA); Notch-3 related cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL); SCN1A and SCN1B-related seizure disorders; the Polymerase G-related disorders which include Alpers-Huttenlocher syndrome, POLG-related sensory ataxic neuropathy, dysarthria, and ophthalmoparesis, and autosomal dominant and recessive progressive external

ophthalmoplegia with mitochondrial DNA deletions; X-Linked adrenal hypoplasia; X-linked agammaglobulinemia; and Wilson's disease.

35. The method of claim 31, wherein the disease is ornithine transcarbamylase deficiency.
36. The method of claim 1 or 31, wherein the therapeutic mRNA encodes human ornithine transcarbamylase (OTC).