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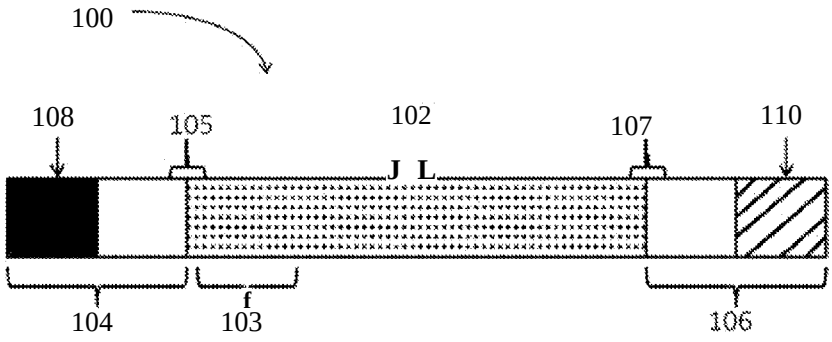
(54) Title: MODIFIED POLYNUCLEOTIDES FOR ALTERING CELL PHENOTYPE

Figure 1



(57) Abstract: The present invention relates to compositions, methods and kits using cell phenotype altering polynucleotides, cell phenotype altering primary transcripts and cell phenotype altering mmRNA molecules.The invention relates to compositions, methods and kits using modified RNA to alter the phenotype of cells. The modified RNA of the invention may encode peptides, polypeptides or multiple proteins. The modified RNA of the invention may also be used to alter the phenotype of cells to produce cell phenotype altering polypeptides of interest.

MODIFIED POLYNUCLEOTIDES FOR ALTERING CELL PHENOTYPE

REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application No 61/736,574, filed December 13, 2012, entitled Modified Polynucleotides for Altering Cell Phenotype, the contents of which is herein incorporated by reference in its entirety.

REFERENCE TO THE SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing file, entitled M033SQLST.txt, was created on December 11, 2013 and is 952,877 bytes in size. The information in electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The invention relates to compositions, methods and kits using modified RNA to alter the phenotype of cells. The modified RNA of the invention may encode peptides, polypeptides or multiple proteins. The modified RNA of the invention may also be used to alter the phenotype of cells to produce cell phenotype altering polypeptides of interest. The cell phenotype altering polypeptides of interest may be used in therapeutics and/or clinical and research settings.

BACKGROUND OF THE INVENTION

[0004] Altering the phenotype of cells in order to express a protein of interest or to change a cell to a different cell phenotype has been used in different clinical, therapeutic and research settings. Altering a phenotype of a cell is currently accomplished by expressing protein from DNA or viral vectors.

[0005] Currently there are studies being done to evaluate the use of human embryonic stem cells as a treatment option for various diseases such as Parkinson's disease and diabetes and injuries such as a spinal cord injury. Embryonic stem cells have the ability to grow indefmitiely while maintaining pluripotency. However, there are ethical difficulties regarding the use of human embryos combined with the problem of tissue rejection following transplantation of the human embryonic stem cells into patients.

[0006] To avoid these ethical and rejection issues, inuced pluripotent stem cells (iPSC) can be generated using the patient's own cells. Induction of iPSC was achieved by Takahashi and Yamanaka *{Cell,* 2006. 126(4):663-76; herein incorporated by reference in its entirety) using viral vectors to express KLF4, c-MYC, OCT4 and SOX2 otherwise collectively known as KMOS. Excisable lentiviral and transposon vectors, repeated application of transient plasmid, episomal and adenovirus vectors have also been used to try to derive iPSC (Chang, C.-W., et al, *Stem Cells,* 2009. 27(5): 1042-1049; Kaji, K., et al, *Nature,* 2009. 458(7239):771-5; Okita, K., et al, *Science,* 2008. 322(5903):949-53; Stadtfeld, M., et al, *Science,* 2008. 322(5903):945-9; Woltjen, K., et al, *Nature,* 2009; Yu, J., et al, *Science,* 2009: 1172482; Fusaki, N., et al, *Proc Jpn Acad Ser B Phys Biol Sci,* 2009. 85(8):348-62; each of which is herein incorporated by reference in its entirety). DNA-free methods to generate human iPSC has also been derived using serial protein transduction with recombinant proteins incorporating cell-penetrating peptide moieties (Kim, D., et al, *Cell Stem Cell,* 2009. 4(6): 472-476; Zhou, H., et al, *Cell Stem Cell,* 2009. 4(5):381-4; each of which is herein incorporated by reference in its entirety), and infectious transgene delivery using the Sendai virus (Fusaki, N., et al, *Proc Jpn Acad Ser B Phys Biol Sci,* 2009. 85(8): p. 348-62; herein incorporated by reference in its entirety).

[0007] However, the clinical application of iPSC is limited by the low efficiency of deriving iPSC and the fact that in order to have cellular cell phenotype altering the genome needs to be modified.

[0008] Therefore, there remains a need in art for cell phenotype altering cell fate using modified RNA encoding various factors related to altering cell fate such as, but not limited to cell phenotype altering factors, transdifferentiation factors, differentiation factors and dedifferentiation factors. The present invention builds upon the aforementioned disclosures and provides compositions, methods and kits using chemically modified messenger RNA (mRNA) encoding proteins which are useful in the field of personal regenerative medicine, cell therapy and therapies for other diseases.

SUMMARY OF THE INVENTION

[0009] Described herein are compositions, methods and kits using modified RNA to modulate cellular function and/or pluripotent cells created by administration of modified RNA encoding factors that alter cell fate.

[0010] In one aspect, a composition comprising at least one cell phenotype altering polynucleotide is provided wherein each of said at least one polynucleotides comprises a first region of linked nucleosides, a first flanking region located at the 5' terminus of the first region, a second flanking region located at the 3' terminus of the first region and a 3' tailing sequence of linked nucleosides. The first region may encode a cell phenotype altering polypeptide such as, but not limited to, SEQ ID NOs: 269-394. Further the first flanking region may include a sequence of linked nucleosides such as, but not limited to, the native 5' untranslated region (UTR) of any of SEQ ID NOs: 269-394, SEQ ID NO: 1 and functional variants thereof. The second flanking region may include a sequence of linked nucleosides such as, but not limited to, the native 3' UTR of any of SEQ ID NOs: 269-394, SEQ ID NOs 2-7 and functional variants thereof.

[0011] The 3' tailing sequence of linked nucleosides may be, but is not limited to a poly-A tail or a Poly A-G quartet. The poly-A tail may be approximately 160 nucleotides in length.

[0012] The first region the cell phenotype altering polynucleotide may include at least a first modified nucleoside. The first region may also comprise a second modified nucleoside. In one aspect, neither the first modified nucleoside or the second modified nucleoside is 5-methylcytosine or pseudouridine. The modified nucleosides may be a purine and/or a pyrimidine nucleoside. The modified nucleosides may be selected from, but not limited to, a modified adenosine, guanosine, cytidine, and uridine. The nucleosides may be modified on the base and/or on the sugar.

[0013] The cell phenotype altering polynucleotide may comprise at least one 5' cap structure. The 5' cap structure may include, but is not limited to, CapO, Capl, ARCA, inosine, Nl-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[0014] Additionally the cell phenotype altering polynucleotide may be purified.

[0015] The cell phenotype altering polynucleotide comprising a first region may encode a cell phenotype altering polypeptide such as, but not limited to, OCT such as OCT4, SOX such as SOX1, SOX2, SOX3, SOX15 and SOX18, NANOG, KLF such as KLF1, KLF2, KLF4 and KLF5, MYC such as c-MYC and n-MYC, REM2, TERT and

LIN28 and variants thereof. The cell phenotype altering polypeptide may have a sequence such as, but not limited to, SEQ ID NO: 269-394.

[0016] The composition of the present invention may comprise at least one, at least two, at least three or at least four cell phenotype altering polynucleotides. In one embodiment, the composition comprises one cell phenotype altering polynucleotide. In another embodiment, the composition comprises two cell phenotype altering polynucleotides. In yet another embodiment, the composition comprises three cell phenotype altering polynucleotides. In yet another embodiment, the composition comprises four cell phenotype altering polynucleotides.

[0017] In one embodiment, the composition of the present invention may comprise a cell phenotype altering polynucleotide encoding OCT4. In another embodiment, the composition of the present invention may comprise a cell phenotype altering polynucleotide encoding SOX2.

[0018] In another embodiment, the composition of the present invention may comprise a cell phenotype altering polynucleotide encoding OCT4 and SOX2. The composition may further comprise a cell phenotype altering polynucleotide encoding NANOG.

[0019] In one embodiment, the composition of the present invention may comprise a cell phenotype altering polynucleotide encoding OCT4, SOX2, KLF4 and c-MYC. In another embodiment, the composition of the present invention may comprise a cell phenotype altering polynucleotide encoding OCT4, SOX2, LIN28 and NANOG.

[0020] Further provided are methods for altering the phenotype of a cell using the compositions and cell phenotype altering polynucleotides, primary constructs and mmRNA of the present invention. The cell may be a human cell or a non-human cell. Further, the cell may be a somatic cell such as, but not limited to, a fibroblast. The methods may provide contacting a cell with the compositions and cell phenotype altering polynucleotides, primary constructs and mmRNA of the present invention at least once. The cell may be contacted once, at least twice and/or a plurality of times.

[0021] The present invention also provides kits comprising the compositions described herein. The kits may comprise at least one of the cell phenotype altering polynucleotides, primary constructs and mmRNA of the present invention. The kits may further comprise packaging and instruction for use thereof, buffers, ligands, lipid or lipid based molecules,

soluble interferon receptors or RNA encoding a soluble interferon receptor (e.g., B18R). Additionally the kits may comprise detectable labels such as but not limited to, radioisotopes, fluorophores, chromophores, enzymes, dyes, metal ions, biotin, avidin, streptavidin, haptens, and quantum dots.

[0022] Further provided are isolated oligonucleotides encoding any of the ell phenotype altering polynucleotides, primary constructs and mmRNA described herein and kits comprising the isolated oligonucleotides.

[0023] Vectors comprising the isolated oligonucleotides encoding any of the ell phenotype altering polynucleotides, primary constructs and mmRNA described herein, kits comprising the vectors and cell comprising the vectors are also described. The kits may comprise vectors containing at least one upstream T7 promoter, a phosphatase and/or apolymerase enzyme and/or a detectable label.

[0024] The details of various embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The foregoing and other objects, features and advantages will be apparent from the following description of particular embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of various embodiments of the invention.

[0026] FIG. 1 is a schematic of a primary construct of the present invention.

[0027] FIG. 2 illustrates lipid structures in the prior art useful in the present invention. Shown are the structures for 98N12-5 (TETA5-LAP), DLin-DMA, DLin-K-DMA (2,2-Dilinoleyl-4-dimethylaminomethyl-[l,3]-dioxolane), DLin-KC2-DMA, DLin-MC3-DMA and C12-200.

DETAILED DESCRIPTION

[0028] The present invention relates to compositions, methods and kits using modified RNA to alter the phenotype of cells. The modified RNA of the invention may encode peptides, polypeptides or multiple proteins. The modified RNA of the invention may also

be used to alter the phenotype of cells to produce cell phenotype altering polypeptides of interest. The cell phenotype altering polypeptides of interest may be used in therapeutics and/or clinical and research settings.

[0029] Human embryonic stem cells have been thought to be useful to treat a host of diseases as they grow indefinitely and maintain their pluripotency and ability to differentiate into cells of all three germ layers. However, the human embryonic stem cells create an ethicial concern and pose a risk for tissue rejection following transplantation. Therefore, there remains a need in the art for compositions, methods and kits for producing induced pluripotent stem (iPS) cells from somatic cells.

[0030] The present invention addresses this need by providing nucleic acid based compounds or polynucleotides which encode a cell phenotype altering cell phenotype altering polypeptide of interest (e.g., modified mRNA or mmRNA) and which have structural and/or chemical features that avoid one or more of the problems in the art, for example, features which are useful for optimizing nucleic acid-based therapeutics while retaining structural and functional integrity, overcoming the threshold of expression, improving expression rates, half life and/or protein concentrations, optimizing protein localization, and avoiding deleterious bio-responses such as the immune response and/or degradation pathways.

[0031] Described herein are compositions, methods and kits of cell phenotype altering polynucleotides encoding one or more cell phenotype altering polypeptides of interest.

[0032] According to the present invention, these polynucleotides are preferably modified as to avoid the deficiencies of other polypeptide-encoding molecules of the art. Hence these polynucleotides are referred to as modified mRNA or mmRNA.

[0033] Provided herein, in part, are cell phenotype altering polynucleotides, primary constructs and/or mmRNA encoding cell phenotype altering polypeptides of interest which have been designed to improve one or more of the stability and/or clearance in tissues, receptor uptake and/or kinetics, cellular access by the compositions, engagement with translational machinery, mRNA half-life, translation efficiency, immune evasion, protein production capacity, secretion efficiency (when applicable), accessibility to circulation, protein half-life and/or modulation of a cell's status, function and/or activity.

[0034] In another aspect, the present disclosure provides chemical modifications located on the sugar moiety of the nucleotide.

[0035] In another aspect, the present disclosure provides chemical modifications located on the phosphate backbone of the cell phenotype altering polynucleotide, primary construct and/or mmRNA.

[0036] In another aspect, the present disclosure provides cell phenotype altering polynucleotides, primary constructs and/or mmRNA that contain chemical modifications, wherein the cell phenotype altering polynucleotide, primary construct and/or mmRNA reduces the cellular innate immune response, as compared to the cellular innate immune induced by a corresponding unmodified nucleic acid.

[0037] In another aspect, the present disclosure provides nucleic acid sequences comprising at least two nucleotides.

[0038] In another aspect, the present disclosure provides compositions comprising a compound as described herein. In some embodiments, the composition is a reaction mixture. In some embodiments, the composition is a pharmaceutical composition. In some embodiments, the composition is a cell culture. In some embodiments, the composition further comprises an RNA polymerase and a cDNA template. In some embodiments, the composition further comprises a nucleotide selected from the group consisting of adenosine, cytosine, guanosine, and uracil.

[0039] In a further aspect, the present disclosure provides methods of making a pharmaceutical formulation comprising a physiologically active secreted protein, comprising transfecting a first population of human cells with the pharmaceutical nucleic acid made by the methods described herein, wherein the secreted protein is active upon a second population of human cells.

[0040] In some embodiments, the secreted protein is capable of interacting with a receptor on the surface of at least one cell present in the second population. Non-limiting examples of secreted proteins include OCT such as OCT 4, SOX such as SOX1, SOX2, SOX3, SOX15 and SOX18, NANOG, KLF such as KLF1, KLF2, KLF4 and KLF5, NR5A2, MYC such as c-MYC and n-MYC, REM2, TERT and LIN28.

[0041] In some embodiments, the second population contains myeloblast cells that express the receptor for the secreted protein.

[0042] In certain embodiments, provided herein are combination therapeutics containing one or more cell phenotype altering cell phenotype altering polynucleotides, primary constructs and/or mmRNA containing translatable regions that encode for a cell phenotype altering protein or proteins which may be used to produce induced pluripotent stem cells from somatic cells.

[0043] In one embodiment, it is intended that the compounds of the present disclosure are stable. It is further appreciated that certain features of the present disclosure, which are, for clarity, described in the context of separate embodiments, can also be provided in combination in a single embodiment. Conversely, various features of the present disclosure which are, for brevity, described in the context of a single embodiment, can also be provided separately or in any suitable subcombination.

I. Compositions of the Invention (mmRNA)

[0044] The present invention provides nucleic acid molecules or polynucleotides, specifically cell phenotype altering polynucleotides, primary constructs and/or mmRNA which encode one or more cell phenotype altering polypeptides of interest. Herein, a cell phenotype altering polynucleotide may also be referred to as a polynucleotide. The term "nucleic acid," in its broadest sense, includes any compound and/or substance that comprise a polymer of nucleotides. These polymers are often referred to as polynucleotides. Exemplary nucleic acids or polynucleotides of the invention include, but are not limited to, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β- D-ribo configuration, a-LNA having an a-L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino- a-LNA having a 2'-amino functionalization) or hybrids thereof.

[0045] In preferred embodiments, the nucleic acid molecule is a messenger RNA (mRNA). As used herein, the term "messenger RNA" (mRNA) refers to any polynucleotide which encodes a cell phenotype altering polypeptide of interest and which is capable of being translated to produce the encoded cell phenotype altering polypeptide of interest *in vitro, in vivo, in situ* or *ex vivo.*

[0046] Traditionally, the basic components of an mRNA molecule include at least a coding region, a 5'UTR, a 3'UTR, a 5' cap and a poly-A tail. Building on this wild type modular structure, the present invention expands the scope of functionality of traditional mRNA molecules by providing cell phenotype altering polynucleotides or cell phenotype altering primary RNA constructs which maintain a modular organization, but which comprise one or more structural and/or chemical modifications or alterations which impart useful properties to the reprogrammming polynucleotides including, in some embodiments, the lack of a substantial induction of the innate immune response of a cell into which the cell phenotype altering polynucleotide is introduced. As such, modified mRNA molecules or modified mRNA of the present invention are termed "mmRNA." As used herein, a "structural" feature or modification is one in which two or more linked nucleotides are inserted, deleted, duplicated, inverted or randomized in a cell phenotype altering polynucleotide, primary construct or mmRNA without significant chemical modification to the nucleotides themselves. Because chemical bonds will necessarily be broken and reformed to effect a structural modification, structural modifications are of a chemical nature and hence are chemical modifications. However, structural modifications will result in a different sequence of nucleotides. For example, the polynucleotide "ATCG" may be chemically modified to "AT-5meC-G". The same polynucleotide may be structurally modified from "ATCG" to "ATCCCG". Here, the dinucleotide "CC" has been inserted, resulting in a structural modification to the polynucleotide.

Cell Phenotype Altering mmRNA Architecture

[0047] The mmRNA of the present invention are distinguished from wild type mRNA in their functional and/or structural design features which serve to, as evidenced herein, overcome existing problems of effective polypeptide production using nucleic acid-based therapeutics.

[0048] Figure 1 shows a representative cell phenotype altering polynucleotide primary construct **100** of the present invention. As used herein, the term "primary construct" or "primary mRNA construct" refers to a polynucleotide transcript which encodes one or more cell phenotype altering polypeptides of interest and which retains sufficient structural and/or chemical features to allow the cell phenotype altering polypeptide of interest encoded therein to be translated. Cell phenotype altering primary

constructs may be cell phenotype altering polynucleotides of the invention. When structurally or chemically modified, the cell phenotype altering primary construct may be referred to as an mmRNA.

[0049] Returning to FIG. **1,** the cell phenotype altering primary construct **100** here contains a first region of linked nucleotides **102** that is flanked by a first flanking region **104** and a second flaking region **106.** As used herein, the "first region" may be referred to as a "coding region" or "region encoding" or simply the "first region." This first region may include, but is not limited to, the encoded cell phenotype altering polypeptide of interest. The cell phenotype altering polypeptide of interest may comprise at its 5' terminus one or more signal sequences encoded by a signal sequence region **103.** The flanking region **104** may comprise a region of linked nucleotides comprising one or more complete or incomplete 5' UTRs sequences. The flanking region **104** may also comprise a 5' terminal cap **108.** The second flanking region **106** may comprise a region of linked nucleotides comprising one or more complete or incomplete 3' UTRs. The flanking region **106** may also comprise a 3' tailing sequence **110.**

[0050] Bridging the 5' terminus of the first region **102** and the first flanking region **104** is a first operational region **105.** Traditionally this operational region comprises a Start codon. The operational region may alternatively comprise any translation initiation sequence or signal including a Start codon.

[0051] Bridging the 3' terminus of the first region **102** and the second flanking region **106** is a second operational region **107.** Traditionally this operational region comprises a Stop codon. The operational region may alternatively comprise any translation initiation sequence or signal including a Stop codon. According to the present invention, multiple serial stop codons may also be used.

[0052] Generally, the shortest length of the first region of the cell phenotype altering primary construct of the present invention can be the length of a nucleic acid sequence that is sufficient to encode for a dipeptide, a tripeptide, a tetrapeptide, a pentapeptide, a hexapeptide, a heptapeptide, an octapeptide, a nonapeptide, or a decapeptide. In another embodiment, the length may be sufficient to encode a peptide of 2-30 amino acids, e.g. 5-30, 10-30, 2-25, 5-25, 10-25, or 10-20 amino acids. The length may be sufficient to encode for a peptide of at least 11, 12, 13, 14, 15, 17, 20, 25 or 30 amino acids, or a

peptide that is no longer than 40 amino acids, e.g. no longer than 35, 30, 25, 20, 17, 15, 14, 13, 12, 11 or 10 amino acids. Examples of dipeptides that the polynucleotide sequences can encode or include, but are not limited to, carnosine and anserine.

[0053] Generally, the length of the first region encoding the cell phenotype altering polypeptide of interest of the present invention is greater than about 30 nucleotides in length (e.g., at least or greater than about 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, and 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000 or up to and including 100,000 nucleotides). As used herein, the "first region" may be referred to as a "coding region" or "region encoding" or simply the "first region."

[0054] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes from about 30 to about 100,000 nucleotides (e.g., from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 1,000, from 30 to 1,500, from 30 to 3,000, from 30 to 5,000, from 30 to 7,000, from 30 to 10,000, from 30 to 25,000, from 30 to 50,000, from 30 to 70,000, from 100 to 250, from 100 to 500, from 100 to 1,000, from 100 to 1,500, from 100 to 3,000, from 100 to 5,000, from 100 to 7,000, from 100 to 10,000, from 100 to 25,000, from 100 to 50,000, from 100 to 70,000, from 100 to 100,000, from 500 to 1,000, from 500 to 1,500, from 500 to 2,000, from 500 to 3,000, from 500 to 5,000, from 500 to 7,000, from 500 to 10,000, from 500 to 25,000, from 500 to 50,000, from 500 to 70,000, from 500 to 100,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 3,000, from 1,000 to 5,000, from 1,000 to 7,000, from 1,000 to 10,000, from 1,000 to 25,000, from 1,000 to 50,000, from 1,000 to 70,000, from 1,000 to 100,000, from 1,500 to 3,000, from 1,500 to 5,000, from 1,500 to 7,000, from 1,500 to 10,000, from 1,500 to 25,000, from 1,500 to 50,000, from 1,500 to 70,000, from 1,500 to 100,000, from 2,000 to 3,000, from 2,000 to 5,000, from 2,000 to 7,000, from 2,000 to 10,000, from 2,000 to 25,000, from 2,000 to 50,000, from 2,000 to 70,000, and from 2,000 to 100,000).

[0055] According to the present invention, the first and second flanking regions may range independently from 15-1,000 nucleotides in length (e.g., greater than 30, 40, 45,

50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, and 900 nucleotides or at least 30, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, and 1,000 nucleotides).

[0056] According to the present invention, the tailing sequence may range from absent to 500 nucleotides in length (e.g., at least 60, 70, 80, 90, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 nucleotides). Where the tailing region is a polyA tail, the length may be determined in units of or as a function of polyA Binding Protein binding. In this embodiment, the polyA tail is long enough to bind at least 4 monomers of PolyA Binding Protein. PolyA Binding Protein monomers bind to stretches of approximately 38 nucleotides. As such, it has been observed that polyA tails of about 80 nucleotides and 160 nucleotides are functional.

[0057] According to the present invention, the capping region may comprise a single cap or a series of nucleotides forming the cap. In this embodiment the capping region may be from 1 to 10, e.g. 2-9, 3-8, 4-7, 1-5, 5-10, or at least 2, or 10 or fewer nucleotides in length. In some embodiments, the cap is absent.

[0058] According to the present invention, the first and second operational regions may range from 3 to 40, e.g., 5-30, 10-20, 15, or at least 4, or 30 or fewer nucleotides in length and may comprise, in addition to a Start and/or Stop codon, one or more signal and/or restriction sequences.

Cyclic Cell Phenotype Altering mmRNA

[0059] According to the present invention, a cell phenotype altering primary construct or mmRNA may be cyclized, or concatemerized, to generate a translation competent molecule to assist interactions between poly-A binding proteins and 5'-end binding proteins. The mechanism of cyclization or concatemerization may occur through at least 3 different routes: 1) chemical, 2) enzymatic, and 3) ribozyme catalyzed. The newly formed 5'-/3'-linkage may be intramolecular or intermolecular.

[0060] In the first route, the 5'-end and the 3'-end of the nucleic acid contain chemically reactive groups that, when close together, form a new covalent linkage between the 5'-end and the 3'-end of the molecule. The 5'-end may contain an NHS-ester reactive group and the 3'-end may contain a 3'-amino-terminated nucleotide such that in an organic solvent the 3'-amino-terminated nucleotide on the 3'-end of a synthetic mRNA

molecule will undergo a nucleophilic attack on the 5'-NHS-ester moiety forming a new 5'-/3'-amide bond.

[0061] In the second route, T4 RNA ligase may be used to enzymatically link a 5'-phosphorylated nucleic acid molecule to the 3'-hydroxyl group of a nucleic acid forming a new phosphorodiester linkage. In an example reaction, 1μg of a nucleic acid molecule is incubated at 37°C for 1 hour with 1-10 units of T4 RNA ligase (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol. The ligation reaction may occur in the presence of a split oligonucleotide capable of base-pairing with both the 5'- and 3'-region in juxtaposition to assist the enzymatic ligation reaction.

[0062] In the third route, either the 5'-or 3'-end of the cDNA template encodes a ligase ribozyme sequence such that during *in vitro* transcription, the resultant nucleic acid molecule can contain an active ribozyme sequence capable of ligating the 5'-end of a nucleic acid molecule to the 3'-end of a nucleic acid molecule. The ligase ribozyme may be derived from the Group I Intron, Group I Intron, Hepatitis Delta Virus, Hairpin ribozyme or may be selected by SELEX (systematic evolution of ligands by exponential enrichment). The ribozyme ligase reaction may take 1 to 24 hours at temperatures between 0 and 37°C.

mmRNA Multimers

[0063] According to the present invention, multiple distinct cell phenotype altering polynucleotides, primary constructs or mmRNA may be linked together through the 3'-end using nucleotides which are modified at the 3'-terminus. Chemical conjugation may be used to control the stoichiometry of delivery into cells. For example, the glyoxylate cycle enzymes, isocitrate lyase and malate synthase, may be supplied into HepG2 cells at a 1:1 ratio to alter cellular fatty acid metabolism. This ratio may be controlled by chemically linking cell phenotype altering polynucleotides, primary constructs or mmRNA using a 3'-azido terminated nucleotide on one cell phenotype altering polynucleotide, primary construct or mmRNA species and a C5-ethynyl or alkynyl-containing nucleotide on the opposite cell phenotype altering polynucleotide, primary construct or mmRNA species. The modified nucleotide is added post-transcriptionally using terminal transferase (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol. After the addition of the 3'-modified nucleotide, the two cell

phenotype altering polynucleotide, primary construct or mmRNA species may be combined in an aqueous solution, in the presence or absence of copper, to form a new covalent linkage via a click chemistry mechanism as described in the literature.

[0064] In another example, more than two cell phenotype altering polynucleotides may be linked together using a functionalized linker molecule. For example, a functionalized saccharide molecule may be chemically modified to contain multiple chemical reactive groups (SH-, NH2-, N3, etc. ..) to react with the cognate moiety on a 3'-functionalized mRNA molecule (i.e., a 3'-maleimide ester, 3'-NHS-ester, alkynyl). The number of reactive groups on the modified saccharide can be controlled in a stoichiometric fashion to directly control the stoichiometric ratio of conjugated cell phenotype altering polynucleotide, primary construct or mmRNA.

Cell Phenotype Altering mmRNA Conjugates and Combinations

[0065] In order to further enhance protein production, cell phenotype altering primary constructs or mmRNA of the present invention can be designed to be conjugated to other polynucleotides, dyes, intercalating agents *(e.g.* acridines), cross-linkers *(e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons *(e.g.*, phenazine, dihydrophenazine), artificial endonucleases *(e.g.* EDTA), alkylating agents, phosphate, amino, mercapto, PEG *(e.g.*, PEG-40K), MPEG, $[MPEG]_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens *(e.g.* biotin), transport/absorption facilitators *(e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases, proteins, e.g., glycoproteins, or peptides, e.g., molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell, hormones and hormone receptors, non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, or a drug.

[0066] Conjugation may result in increased stability and/or half life and may be particularly useful in targeting the cell phenotype altering polynucleotides, primary constructs or mmRNA to specific sites in the cell, tissue or organism.

[0067] According to the present invention, the cell phenotype altering mmRNA or primary constructs may be administered with, or further encode one or more of RNAi agents, siRNAs, shRNAs, miRNAs, miRNA binding sites, antisense RNAs, ribozymes,

catalytic DNA, tRNA, RNAs that induce triple helix formation, aptamers or vectors, and the like.

Bifunctional Cell Phenotype Altering mmRNA

[0068] In one embodiment of the invention are bifunctional polynucleotides (e.g., bifunctional cell phenotype altering primary constructs or bifunctional cell phenotype altering mmRNA). As the name implies, bifunctional polynucleotides are those having or capable of at least two functions. These molecules may also by convention be referred to as multi-functional.

[0069] The multiple functionalities of bifunctional cell phenotype altering polynucleotides may be encoded by the RNA (the function may not manifest until the encoded product is translated) or may be a property of the polynucleotide itself. It may be structural or chemical. Bifunctional modified polynucleotides may comprise a function that is covalently or electrostatically associated with the polynucleotides. Further, the two functions may be provided in the context of a complex of a cell phenotype altering mmRNA and another molecule.

[0070] Bifunctional cell phenotype altering polynucleotides may encode peptides which are anti-proliferative. These peptides may be linear, cyclic, constrained or random coil. They may function as aptamers, signaling molecules, ligands or mimics or mimetics thereof. Anti-proliferative peptides may, as translated, be from 3 to 50 amino acids in length. They may be 5-40, 10-30, or approximately 15 amino acids long. They may be single chain, multichain or branched and may form complexes, aggregates or any multi-unit structure once translated.

Noncoding Cell Phenotype Altering Polynucleotides and Primary Constructs

[0071] As described herein, provided are cell phenotype altering polynucleotides and primary constructs having sequences that are partially or substantially not translatable, e.g., having a noncoding region. Such noncoding region may be the "first region" of the cell phenotype altering primary construct. Alternatively, the noncoding region may be a region other than the first region. Such molecules are generally not translated, but can exert an effect on protein production by one or more of binding to and sequestering one or more translational machinery components such as a ribosomal protein or a transfer RNA (tRNA), thereby effectively reducing protein expression in the cell or modulating

one or more pathways or cascades in a cell which in turn alters protein levels. The cell phenotype altering polynucleotide or primary construct may contain or encode one or more long noncoding RNA (IncRNA, or lincRNA) or portion thereof, a small nucleolar RNA (sno-RNA), micro RNA (miRNA), small interfering RNA (siRNA) or Piwi-interacting RNA (piRNA).

Cell Phenotype Altering Polypeptides of interest

[0072] According to the present invention, the cell phenotype altering primary construct is designed to encode one or more cell phenotype altering polypeptides of interest or fragments thereof. A cell phenotype altering polypeptide of interest may include, but is not limited to, whole polypeptides, a plurality of polypeptides or fragments of polypeptides, which independently may be encoded by one or more nucleic acids, a plurality of nucleic acids, fragments of nucleic acids or variants of any of the aforementioned. As used herein, the term "cell phenotype altering polypeptides of interest" refers to any cell phenotype altering polypeptides which are selected to be encoded in the cell phenotype altering primary construct of the present invention. As used herein, "polypeptide" means a polymer of amino acid residues (natural or unnatural) linked together most often by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. In some instances the polypeptide encoded is smaller than about 50 amino acids and the polypeptide is then termed a peptide. If the polypeptide is a peptide, it will be at least about 2, 3, 4, or at least 5 amino acid residues long. Thus, polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, trimer or tetramer. They may also comprise single chain or multichain polypeptides such as antibodies or insulin and may be associated or linked. Most commonly disulfide linkages are found in multichain polypeptides. The term polypeptide may also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid.

[0073] In one embodiment, a polypeptide of interest may be any of the polypeptides described in U.S. Provisional Patent Application No 61/618,862 filed April 2, 2012,

entitled Modified Polynucleotides for the Production of Biologies, U.S. Provisional Patent Application No 61/681,645 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Biologies, U.S. Provisional Patent Application No 61/737,130, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Biologies, U.S. Provisional Patent Application No 61/618,866, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Antibodies, U.S. Provisional Patent Application No 61/681,647, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Antibodies, U.S. Provisional Patent Application No 61/737,134, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Antibodies, U.S. Provisional Patent Application No 61/618,868, filed April 2, 2013, entitled Modified Polynucleotides for the Production of Vaccines, U.S. Provisional Patent Application No 61/681,648, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Vaccines, U.S. Provisional Patent Application No 61/737,135, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Vaccines, U.S. Provisional Patent Application No 61/618,870, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides, U.S. Provisional Patent Application No 61/681,649, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides, U.S. Provisional Patent Application No 61/737,139, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Therapeutic Proteins and Peptides, U.S. Provisional Patent Application No 61/618,873 filed April 2, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins, U.S. Provisional Patent Application No 61/681,650 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins, U.S. Provisional Patent Application No 61/737,147, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Secreted Proteins, U.S. Provisional Patent Application No 61/618,878 filed April 2, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins, U.S. Provisional Patent Application No 61/681,654 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane Proteins, U.S. Provisional Patent Application No 61/737,152, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Plasma Membrane

Proteins, U.S. Provisional Patent Application No 61/618,885 filed April 2, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins, U.S. Provisional Patent Application No 61/681,658 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins, U.S. Provisional Patent Application No 61/737,155, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Cytoplasmic and Cytoskeletal Proteins, U.S. Provisional Patent Application No 61/618,896, filed April 2, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/668,157, filed July 5, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/681,661, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/737,160, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Intracellular Membrane Bound Proteins, U.S. Provisional Patent Application No 61/618,91 1 filed April 2, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins, U.S. Provisional Patent Application No 61/681,667 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins, U.S. Provisional Patent Application No 61/737,168, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Nuclear Proteins, U.S. Provisional Patent Application No 61/618,922 filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins, U.S. Provisional Patent Application No 61/681,675 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins, U.S. Provisional Patent Application No 61/737,174, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins, U.S. Provisional Patent Application No 61/618,935 filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/681,687 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/737,184, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/618,945 filed April 2, 2012, entitled Modified

Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/681,696 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/737,191, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/618,953 filed April 2, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/681,704 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/737,203, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Proteins Associated with Human Disease, U.S. Provisional Patent Application No 61/681,720, filed August 10, 2012, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides, U.S. Provisional Patent Application No 61/737,213, filed December 14, 2012, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides, U.S. Provisional Patent Application No 61/681,742 filed August 10, 2012, entitled Modified Polynucleotides for the Production of Oncology-Related Proteins and Peptides, International Patent Publication No WO2013151666, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Biologies and Proteins Associated with Human Disease, International Patent Publication No WO2013151667, filed March 9, 2013, entitled Modified Polynucleotides, International Patent Publication No WO2013151668, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Secreted Proteins, International Patent Publication No WO2013 15 1663, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Membrane Proteins, International Patent Publication No WO2013151669, filed March 9, 2013, entitled Modified Polynucleotides for the Produciton of Cytoplasmic and Cytoskeletal Proteins, International Patent Publication No WO2013151670, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Nuclear Proteins, International Patent Publication No WO2013151664, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Proteins, International Patent Publication No WO2013151665, filed March 9, 2013, entitled Modified Polynucleotides for the

Production of Proteins Associated with Human Disease, International Patent Publication No WO2013151671, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Cosmetic Proteins and Peptides, International Patent Publication No WO2013151672, filed March 9, 2013, entitled Modified Polynucleotides for the Production of Oncology-Related Proteins and Peptides and International Patent Publication No WO2013151736, filed March 15, 2013, entitled In Vivo Production of Proteins, the contents of each of which are herein incorporated by reference in its entirety.

[0074] The term "polypeptide variant" refers to molecules which differ in their amino acid sequence from a native or reference sequence. The amino acid sequence variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. Ordinarily, variants will possess at least about 50% identity (homology) to a native or reference sequence, and preferably, they will be at least about 80%, more preferably at least about 90% identical (homologous) to a native or reference sequence.

[0075] In some embodiments "variant mimics" are provided. As used herein, the term "variant mimic" is one which contains one or more amino acids which would mimic an activated sequence. For example, glutamate may serve as a mimic for phosphoro-threonine and/or phosphoro-serine. Alternatively, variant mimics may result in deactivation or in an inactivated product containing the mimic, e.g., phenylalanine may act as an inactivating substitution for tyrosine; or alanine may act as an inactivating substitution for serine.

[0076] "Homology" as it applies to amino acid sequences is defined as the percentage of residues in the candidate amino acid sequence that are identical with the residues in the amino acid sequence of a second sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology. Methods and computer programs for the alignment are well known in the art. It is understood that homology depends on a calculation of percent identity but may differ in value due to gaps and penalties introduced in the calculation.

[0077] By "homologs" as it applies to polypeptide sequences means the corresponding sequence of other species having substantial identity to a second sequence of a second species.

[0078] "Analogs" is meant to include polypeptide variants which differ by one or more amino acid alterations, e.g., substitutions, additions or deletions of amino acid residues that still maintain one or more of the properties of the parent or starting polypeptide.

[0079] The present invention contemplates several types of compositions which are polypeptide based including variants and derivatives. These include substitutional, insertional, deletion and covalent variants and derivatives. The term "derivative" is used synonymously with the term "variant" but generally refers to a molecule that has been modified and/or changed in any way relative to a reference molecule or starting molecule.

[0080] As such, cell phenotype altering mmRNA encoding cell phenotype altering polypeptides containing substitutions, insertions and/or additions, deletions and covalent modifications with respect to reference sequences, in particular the polypeptide sequences disclosed herein, are included within the scope of this invention. For example, sequence tags or amino acids, such as one or more lysines, can be added to the peptide sequences of the invention (e.g., at the N-terminal or C-terminal ends). Sequence tags can be used for peptide purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the amino acid sequence of a peptide or protein may optionally be deleted providing for truncated sequences. Certain amino acids (e.g., C-terminal or N-terminal residues) may alternatively be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

[0081] "Substitutional variants" when referring to polypeptides are those that have at least one amino acid residue in a native or starting sequence removed and a different amino acid inserted in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

[0082] As used herein the term "conservative amino acid substitution" refers to the substitution of an amino acid that is normally present in the sequence with a different amino acid of similar size, charge, or polarity. Examples of conservative substitutions include the substitution of a non-polar (hydrophobic) residue such as isoleucine, valine and leucine for another non-polar residue. Likewise, examples of conservative substitutions include the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, and between glycine and serine. Additionally, the substitution of a basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue such as aspartic acid or glutamic acid for another acidic residue are additional examples of conservative substitutions. Examples of non-conservative substitutions include the substitution of a non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine, alanine, methionine for a polar (hydrophilic) residue such as cysteine, glutamine, glutamic acid or lysine and/or a polar residue for a non-polar residue.

[0083] "Insertional variants" when referring to polypeptides are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a native or starting sequence. "Immediately adjacent" to an amino acid means connected to either the alpha-carboxy or alpha-amino functional group of the amino acid.

[0084] "Deletional variants" when referring to polypeptides are those with one or more amino acids in the native or starting amino acid sequence removed. Ordinarily, deletional variants will have one or more amino acids deleted in a particular region of the molecule.

[0085] "Covalent derivatives" when referring to polypeptides include modifications of a native or starting protein with an organic proteinaceous or non-proteinaceous derivatizing agent, and/or post-translational modifications. Covalent modifications are traditionally introduced by reacting targeted amino acid residues of the protein with an organic derivatizing agent that is capable of reacting with selected side-chains or terminal residues, or by harnessing mechanisms of post-translational modifications that function in selected recombinant host cells. The resultant covalent derivatives are useful in programs directed at identifying residues important for biological activity, for immunoassays, or for the preparation of anti-protein antibodies for immunoaffinity purification of the

recombinant glycoprotein. Such modifications are within the ordinary skill in the art and are performed without undue experimentation.

[0086] Certain post-translational modifications are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues may be present in the cell phenotype altering polypeptides produced in accordance with the present invention.

[0087] Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)).

[0088] "Features" when referring to polypeptides are defined as distinct amino acid sequence-based components of a molecule. Features of the cell phenotype altering polypeptides encoded by the cell phenotype altering mmRNA of the present invention include surface manifestations, local conformational shape, folds, loops, half-loops, domains, half-domains, sites, termini or any combination thereof.

[0089] As used herein when referring to polypeptides the term "surface manifestation" refers to a polypeptide based component of a protein appearing on an outermost surface.

[0090] As used herein when referring to polypeptides the term "local conformational shape" means a polypeptide based structural manifestation of a protein which is located within a definable space of the protein.

[0091] As used herein when referring to polypeptides the term "fold" refers to the resultant conformation of an amino acid sequence upon energy minimization. A fold may occur at the secondary or tertiary level of the folding process. Examples of secondary level folds include beta sheets and alpha helices. Examples of tertiary folds include domains and regions formed due to aggregation or separation of energetic forces. Regions formed in this way include hydrophobic and hydrophilic pockets, and the like.

[0092] As used herein the term "turn" as it relates to protein conformation means a bend which alters the direction of the backbone of a peptide or polypeptide and may involve one, two, three or more amino acid residues.

[0093] As used herein when referring to polypeptides the term "loop" refers to a structural feature of a polypeptide which may serve to reverse the direction of the backbone of a peptide or polypeptide. Where the loop is found in a polypeptide and only alters the direction of the backbone, it may comprise four or more amino acid residues. Oliva et al. have identified at least 5 classes of protein loops (J. Mol Biol 266 (4): 814-830; 1997). Loops may be open or closed. Closed loops or "cyclic" loops may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids between the bridging moieties. Such bridging moieties may comprise a cysteine-cysteine bridge (Cys-Cys) typical in polypeptides having disulfide bridges or alternatively bridging moieties may be non-protein based such as the dibromozylyl agents used herein.

[0094] As used herein when referring to polypeptides the term "half-loop" refers to a portion of an identified loop having at least half the number of amino acid resides as the loop from which it is derived. It is understood that loops may not always contain an even number of amino acid residues. Therefore, in those cases where a loop contains or is identified to comprise an odd number of amino acids, a half-loop of the odd-numbered loop will comprise the whole number portion or next whole number portion of the loop (number of amino acids of the loop/2+/-0.5 amino acids). For example, a loop identified as a 7 amino acid loop could produce half-loops of 3 amino acids or 4 amino acids (7/2=3.5+/-0.5 being 3 or 4).

[0095] As used herein when referring to polypeptides the term "domain" refers to a motif of a polypeptide having one or more identifiable structural or functional characteristics or properties (e.g., binding capacity, serving as a site for protein-protein interactions).

[0096] As used herein when referring to polypeptides the term "half-domain" means a portion of an identified domain having at least half the number of amino acid resides as the domain from which it is derived. It is understood that domains may not always contain an even number of amino acid residues. Therefore, in those cases where a domain contains or is identified to comprise an odd number of amino acids, a half-domain of the

odd-numbered domain will comprise the whole number portion or next whole number portion of the domain (number of amino acids of the domain/2+/-0.5 amino acids). For example, a domain identified as a 7 amino acid domain could produce half-domains of 3 amino acids or 4 amino acids (7/2=3.5+/-0.5 being 3 or 4). It is also understood that sub-domains may be identified within domains or half-domains, these subdomains possessing less than all of the structural or functional properties identified in the domains or half domains from which they were derived. It is also understood that the amino acids that comprise any of the domain types herein need not be contiguous along the backbone of the polypeptide (i.e., nonadjacent amino acids may fold structurally to produce a domain, half-domain or subdomain).

[0097] As used herein when referring to polypeptides the terms "site" as it pertains to amino acid based embodiments is used synonymously with "amino acid residue" and "amino acid side chain." A site represents a position within a peptide or polypeptide that may be modified, manipulated, altered, derivatized or varied within the polypeptide based molecules of the present invention.

[0098] As used herein the terms "termini" or "terminus" when referring to polypeptides refers to an extremity of a peptide or polypeptide. Such extremity is not limited only to the first or final site of the peptide or polypeptide but may include additional amino acids in the terminal regions. The polypeptide based molecules of the present invention may be characterized as having both an N-terminus (terminated by an amino acid with a free amino group (NH2)) and a C-terminus (terminated by an amino acid with a free carboxyl group (COOH)). Proteins of the invention are in some cases made up of multiple polypeptide chains brought together by disulfide bonds or by non-covalent forces (multimers, oligomers). These sorts of proteins will have multiple N- and C-termini. Alternatively, the termini of the polypeptides may be modified such that they begin or end, as the case may be, with a non-polypeptide based moiety such as an organic conjugate.

[0099] Once any of the features have been identified or defined as a desired component of a polypeptide to be encoded by the cell phenotype altering primary construct or mmRNA of the invention, any of several manipulations and/or modifications of these features may be performed by moving, swapping, inverting, deleting,

randomizing or duplicating. Furthermore, it is understood that manipulation of features may result in the same outcome as a modification to the molecules of the invention. For example, a manipulation which involved deleting a domain would result in the alteration of the length of a molecule just as modification of a nucleic acid to encode less than a full length molecule would.

[00100] Modifications and manipulations can be accomplished by methods known in the art such as, but not limited to, site directed mutagenesis. The resulting modified molecules may then be tested for activity using *in vitro* or *in vivo* assays such as those described herein or any other suitable screening assay known in the art.

[00101] According to the present invention, the cell phenotype altering polypeptides may comprise a consensus sequence which is discovered through rounds of experimentation. As used herein a "consensus" sequence is a single sequence which represents a collective population of sequences allowing for variability at one or more sites.

[00102] As recognized by those skilled in the art, protein fragments, functional protein domains, and homologous proteins are also considered to be within the scope of cell phenotype altering polypeptides of interest of this invention. For example, provided herein is any protein fragment (meaning a polypeptide sequence at least one amino acid residue shorter than a reference polypeptide sequence but otherwise identical) of a reference protein 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or greater than 100 amino acids in length. In another example, any protein that includes a stretch of about 20, about 30, about 40, about 50, or about 100 amino acids which are about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or about 100% identical to any of the sequences described herein can be utilized in accordance with the invention. In certain embodiments, a polypeptide to be utilized in accordance with the invention includes 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mutations as shown in any of the sequences provided or referenced herein.

Encoded Cell Phenotype Altering Polypeptides

[00103] The cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention may be designed to encode cell phenotype altering polypeptides of interest such as, but not limited to, those that expression one or more transcription

factors, death receptors, death receptor ligands, Type I or Type II interferon (IFN) genes, reprogramming factors, differentiation factors, de-differentiation factors or developmental potential altering factors.

[00104] In one embodiment cell phenotype altering primary constructs or mmRNA may encode variant polypeptides which have a certain identity with a reference polypeptide sequence. As used herein, a "reference polypeptide sequence" refers to a starting polypeptide sequence. Reference sequences may be wild type sequences or any sequence to which reference is made in the design of another sequence. A "reference polypeptide sequence" may, e.g., be any one of SEQ ID NOs: 269-394 as disclosed herein, e.g., any of SEQ ID NOs 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393 and 394.

[00105] In addition, a "reference polypeptide sequence" may, e.g., be any one of the human transcription factors listed in Table 1, cluster of differentiation molecules in Table 2 or membrane bound receptors in Table 3 of International Publication No. WO 2011130624 or the IFN-signature genes, cell-specific polypeptides, death receptors and death receptor ligands and/or mitogen receptors listed in International Publication No. WO2011130624; herein incorporated by reference in its entirety.

[00106] The term "identity" as known in the art, refers to a relationship between the sequences of two or more peptides, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between peptides, as determined by the number of matches between strings of two or more amino acid residues. Identity measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms"). Identity of related peptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in

Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al, SIAM J. Applied Math. 48, 1073 (1988).

[00107] In some embodiments, the polypeptide variant may have the same or a similar activity as the reference polypeptide. Alternatively, the variant may have an altered activity (e.g., increased or decreased) relative to a reference polypeptide. Generally, variants of a particular polynucleotide or polypeptide of the invention will have at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% but less than 100% sequence identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art. Such tools for alignment include those of the BLAST suite (Stephen F. Altschul, Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.) Other tools are described herein, specifically in the definition of "Identity."

[00108] Default parameters in the BLAST algorithm include, for example, an expect threshold of 10, Word size of 28, Match/Mismatch Scores 1, -2, Gap costs Linear. Any filter can be applied as well as a selection for species specific repeats, e.g., Homo sapiens.

Reprogramming Factors

[00109] The cell phenotype altering polynucleotides, primary constructs or mmRNA disclosed herein, may encode one or more reprogramming factors. As used herein, a "reprogramming factor" is a developmental potential altering factor, such as a protein, RNA or small molecule, the expression of which contributes to the reprogramming of a cell to a less differentiated or undifferentiated state.. As an example, a reprogramming factor may be used to alter the phenotype of a somatic cell, a precursor somatic cell, partially reprogrammed somatic cell, pluripotent cell, multipotent cell, differentiated cell

or an embryonic cell into a pluripotent stem cell or its immediate precursor cell. Reprogramming of a cell may be accomplished by a single transfection or a repeated transfection of a cell-altering polynucleotide, primary construct and/or mmRNA encoding a reprogramming factor.

[00110] The term "reprogramming" refers to a process that reverses the developmental potential of a cell or population of cells. This process includes driving a cell to a state with higher developmental potential. The cell to be reprogrammed may be partially or terminally differentiated prior to undergoing reprogramming.

[00111] A reprogramming factor can be a transcription factor that can reprogram cells to a pluripotent state. Non-limiting examples of reprogramming factors include, OCT such as OCT 4, SOX such as SOXl, SOX2, SOX3, SOX15 and SOX18, NANOG, KLF such as KLF1, KLF2, KLF4 and KLF5, NR5A2, MYC such as c-MYC and n-MYC, REM2, TERT and LIN28.

[00112] As used herein, the term "OCT" refers to the octamer-binding protein family including any variants thereof. The term "OCT4" refers to the ocatmer-binding protein 4 including any variants thereof. OCT4 is also known in the art as POU class 5 homeobox 1 and octamer-binding protein 3 (OCT3). In one embodiment, OCT4 refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 269-294.

[00113] As used herein, the term "SOX" refers to the SRY (sex determining region Y)-box protein family including any variants thereof. The term "SOXl" refers to the protein SRY (sex determining region Y)-box 1 including any variants thereof. In one embodiment, SOXl refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 295. The term "SOX2" refers to the protein SRY (sex determining region Y)-box 2 including any variants thereof. In one embodiment, SOX2 refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 296 and 297. The term "SOX3" refers to the protein SRY (sex determining region Y)-box 3 including any variants thereof. In one embodiment, SOX3 refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 298. The term "SOX15" refers to the protein SRY (sex determining region Y)-box 15 including any variants thereof. In one embodiment, SOX15 refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 299. The term "SOXl 8" refers to the protein SRY (sex determining region Y)-box 18

including any variants thereof. In one embodiment, SOX18 refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 300.

[00114] As used herein, the term "NANOG" refers to the protein Nanog homeobox including any variants thereof. In one embodiment, NANOG refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 301 and 302.

[00115] As used herein, the term "KLF" refers to the kruppel-like factor protein family including any variants thereof. The term "KLFl" refers to the protein kruppel-like factor 1 including any variants thereof. In one embodiment, KLFl refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 303. The term "KLF2" refers to the protein kruppel-like factor 2 including any variants thereof. In one embodiment, KLF2 refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 304. The term "KLF4" refers to the protein kruppel-like factor 4 including any variants thereof. In one embodiment, KLF4 refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 305-308. The term "KLF5" refers to the protein kruppel-like factor 5 including any variants thereof. In one embodiment, KLF5 refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 309-3 11.

[00116] As used herein, the term "NR5A2" refers to the protein nuclear receptor subfamily 5, group A, member 1 including any variants thereof. In one embodiment, NR5A2 refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 312-319.

[00117] As used herein, the term "MYC" refers to the v-myc myelocytomatosis viral oncogene protein family including any variants thereof. The term "c-MYC" refers to the protein v-myc myelocytomatosis viral oncogene homolog (avian) including any variants thereof. In one embodiment, c-MYC refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 320-323. The term "n-MYC"refers to the protein v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian) including any variants thereof. In one embodiment, n-MYC refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 324 and 325.

[001 18] As used herein, the term "REM2" refers to the protein RAS (RAD and GEM)-like GTP binding 2 protein including any variants thereof. In one embodiment, REM2

refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 326 and 327.

[00119] As used herein, the term "TERT" refers to the protein telomerase reverse transcriptase protein including any variants thereof. In one embodiment, TERT refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 328-331

[00120] As used herein, the term "LIN28" refers to the lin-28 homolog protein including any variants thereof. In one embodiment, LIN28 refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 332-334.

[00121] In one embodiment, reprogramming encompasses a complete or partial reversion of the differentiation state. As a non-limiting example, reprogramming can create an increase in the developmental potential of a cell, to that of a cell having a pluripotent state. As another non-limiting example, the partial reversion of the differentiation state of a cell to a state that renders the cell more susceptible to complete reprogramming to a pluripotent state when subject to additional manipulations. Manipulations are described in International Publication No. WO201 1130624, herein incorporated by reference in its entirety. In another embodiment, reprogramming encompasses a partial increase in the developmental potential of a cell such as, but not limited, increasing a somatic cell or a unipotent cell to a multipotent cell.

[00122] In one embodiment, reprogramming encompasses driving a somatic cell to a pluripotent state so that cell has a developmental potential of an embryonic stem cell.

[00123] In one embodiment, the cell phenotype altering polynucleotides, primary constructs or mmRNA described herein cause the cell to assume a pluripotent-like state or an embryonic stem cell phenotype.

Differentiation and De-Differentiation Factors

[00124] The cell phenotype altering polynucleotides, primary constructs or mmRNA disclosed herein, may encode one or more differentiation factors. As used herein, the term "differentiation factor" refers to a developmental potential altering factor such as a protein, RNA or small molecule that can induce a cell to differentiate to a desired cell-type. As used herein, "differentiate" or "differentiating" refers to the process where an uncommitted or less committed cell acquires the features of a committed cell. As a non-limiting example, a committed cell can be a cardiomyocyte, a nerve cell or a skeletal

muscle cell. A cell is "committed" when the cell is far enough into the differentiation pathway where, under normal circumstances, it will continue to differentiate into a specific cell type or subset of cell type instead of into a different cell type or reverting to a lesser differentiated cell type.

[00125] A differentiated cell also encompasses cells that are partially differentiated, such as multipotent cells or cells that are stable, non-pluripotent partially reprogrammed or partially differentiated cells. Further, a differentiated cell can also be a cell of a more specialized cell type derived from a less specialized cell type.

[00126] Non-limiting examples of differentiation factors include, ASCL1, BRN2, MYT1L, MYOD1, CEBP-alpha, PU.1, PRDM16, HNF4-alpha, BDNF, NTF such as NTF3 and NTF4, EGF, CNTF, NGF, Sonic hedgehog, FGF such as FGF-8, and TGF such as TGF-alpha and TGF-beta.

[00127] As used herein, the term "ASCL1" refers to the achaete-scute complex homolog 1 protein including any variants thereof. In one embodiment, ASCL1 refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 335.

[00128] As used herein, the term "BRN2" refers to the POU class 3 homeobox 2 protein including any variants thereof. BRN2 is also known in the art as OTF7 and POU domain class 3, transcription factor 2 (POU3F2). In one embodiment, BRN2 refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 336 and 337.

[00129] As used herein, the term "MYT1L" refers to the myelin transcription factor 1-like protein including any variants thereof. In one embodiment, MYT1L refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 338-341.

[00130] As used herein, the term "MYOD1" refers to the myogenic differentiation 1 protein including any variants thereof. In one embodiment, MYOD1 refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 342

[00131] As used herein, the term "CEBP-alpha" refers to CCAAT/enhancer binding protein (C/EBP), alpha protein including any variants thereof. In one embodiment, CEBP-alpha refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 343.

[00132] As used herein, the term "PU.1" refers to spleen focus forming virus (SFFV) proviral integration oncogene spil protein including any variants thereof. In one

embodiment, PU. 1 refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 334 and 345

[00133] As used herein, the term "PRDM16" refers to PR domain containing 16 protein including any variants thereof. In one embodiment, PRDM16 refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 346-35 1.

[00134] As used herein, the term "FINF4-alpha" refers to hepatocyte nuclear factor 4, alpha protein including any variants thereof. In one embodiment, HNF4-alpha refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 352-357.

[00135] As used herein, the term "BDNF" refers to brain-derived neurotrophic factor protein including any variants thereof. In one embodiment, BDNF refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 358-374.

[00136] As used herein, the term "NTF" refers to the neurotrophin protein family including any variants thereof. The term "NTF3" refers to neurotrophin 3 including any variants thereof. In one embodiment, NTF3 refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 375 and 376. The term "NTF4" refers to neurotrophin 4 including any variants thereof. In one embodiment, NTF4 refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 377.

[00137] As used herein, the term "EGF" refers to epidermal growth factor including any variants thereof. In one embodiment, EGF refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 378-380.

[00138] As used herein, the term "CNTF" refers to ciliary neurotrophic factor including any variants thereof. In one embodiment, CNTF refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 381.

[00139] As used herein, the term "NGF" refers to nerve growth factor protein family including any variants thereof. In one embodiment, NGF refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 382.

[00140] As used herein, the phrase "sonic hedgehog" refers to the sonic hedgehog protein including any variants thereof. In one embodiment, sonic hedgehog refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 383.

[00141] As used herein, the term "FGF" refers to the fibroblast growth factor protein family including any variants thereof. The term "FGF-8" refers to fibroblast growth

factor-8 protein including any variants thereof. In one embodiment, FGF-8 refers to a protein having a sequence such as, but not limited to, SEQ ID NO: 384-387.

[00142] As used herein, the term "TGF" refers to the transforming growth factor protein family including any variants thereof. The term "TGF-alpha" refers to transforming growth factor, alpha protein including any variants thereof. In one embodiment, TGF-alpha refers to a protein having a sequence, such as, but not limited to, SEQ ID NO: 388 and 389. The term "TGF-beta" refers to transforming growth factor, beta protein including any variants thereof. In one embodiment, TGF-beta refers to TGFB1 a protein having a sequence such as, but not limited to, SEQ ID NO: 390, TGFB2 a protein having a sequence such as, but not limited to, SEQ ID NO: 391-392 or TGFB3 a protein having a sequence such as, but not limited to, SEQ ID NO: 393 and 394.

[00143] The cell phenotype altering polynucleotides, primary constructs or mmRNA disclosed herein, may encode one or more de-differentiation factors. As used herein, "de-differentiation" refers to the process of reverting a cell to a less committed position within the lineage of a cell.

[00144] The lineage of a cell defines the heredity or fate of the cell. The differentiation of cells using the cell phenotype altering polynucleotides, primary constructs or mmRNA disclosed herein can be differentiated by one skilled in the art into any cell type or lineage. The cells can be of a lineage such as, but not limited to, endodermal lineage, ecotodermal lineage and mesodermal lineage. Cells of endodermal lineage include, but are not limited to, cells of the gastrointestinal system, cells of the respiratory tract, cells of the endocrine glands, cells of the auditory system, and certain cells of the urinary system, such as the bladder and parts of the urethra. Cells of ectodermal lineage include, but are not limited to, ectodermal lineage cells include, but are not limited to, cells of the epidermis (skin cells, melanocytes), and cells of the neuronal lineage. Cells of mesodermal lineage include, but are not limited to, cells of the circulatory system (cardiac cells and blood vessel cells), cells of the connective tissue, bone cells, dermal cells, myocytes (smooth and skeletal), certain cells of the urinary system, such as kidney cells, splenic cells, mesothelial cells (cells of the peritoneum, pleura, and pericardium), non-germ cells of the reproductive system, and hematopoietic lineage cells.

[00145] The success of differentiation using the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may be monitored by analysis of a variety of criteria known in the art such as, but not limited to, expressed cell markers and characterization of morphological features. Other methods for monitoring the success of differentiation are described in International Publication No. WO201 1130624; herein incorporated by reference in its entirety.

Developmental Potential Altering Factor

[00146] The cell phenotype altering polynucleotides, primary constructs and mmRNA may encode a developmental potential altering factor. As used herein, "developmental potential altering factor" refers to a protein or RNA which can alter the developmental potential of a cell. As a non-limiting example, the cell phenotype altering polynucleotides, primary constructs and mmRNA may encode a developmental potential altering factor that can alter a somatic cell to another developmental state such as a pluripotent state.

[00147] A developmental potential altering factor may include, but is not limited to, a reprogramming factor or a transcription factor.

Transcription Factor

[00148] The cell phenotype altering polynucleotides, primary constructs and mmRNA may encode a transcription factor. As used herein, used herein, the term "transcription factor" refers to a DNA-binding protein that regulates transcription of DNA into RNA, for example, by activation or repression of transcription. Some transcription factors effect regulation of transcription alone, while others act in concert with other proteins. Some transcription factor can both activate and repress transcription under certain conditions. In general, transcription factors bind a specific target sequence or sequences highly similar to a specific consensus sequence in a regulatory region of a target gene. Transcription factors may regulate transcription of a target gene alone or in a complex with other molecules.

Flanking Regions: Untranslated Regions (UTRs)

[00149] Untranslated regions (UTRs) of a gene are transcribed but not translated. The 5'UTR starts at the transcription start site and continues to the start codon but does not include the start codon; whereas, the 3'UTR starts immediately following the stop codon

and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. The regulatory features of a UTR can be incorporated into the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention to enhance the stability of the molecule. The specific features can also be incorporated to ensure controlled down-regulation of the transcript in case they are misdirected to undesired organs sites.

5' UTR and Translation Initiation

[00150] Natural 5'UTRs bear features which play roles in for translation initiation. They harbor signatures like Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5'UTR also have been known to form secondary structures which are involved in elongation factor binding.

[00151] By engineering the features typically found in abundantly expressed genes of specific target organs, one can enhance the stability and protein production of the cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention. For example, introduction of 5' UTR of liver-expressed mRNA, such as albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, or Factor VIII, could be used to enhance expression of a nucleic acid molecule, such as a mmRNA, in hepatic cell lines or liver. Likewise, use of 5' UTR from other tissue-specific mRNA to improve expression in that tissue is possible - for muscle (MyoD, Myosin, Myoglobin, Myogenin, Herculin), for endothelial cells (Tie-1, CD36), for myeloid cells (C/EBP, AML1, G-CSF, GM-CSF, CDl lb, MSR, Fr-1, i-NOS), for leukocytes (CD45, CD 18), for adipose tissue (CD36, GLUT4, ACRP30, adiponectin) and for lung epithelial cells (SP-A/B/C/D).

[00152] Other non-UTR sequences may be incorporated into the 5' (or 3' UTR) UTRs. For example, introns or portions of introns sequences may be incorporated into the flanking regions of the cell phenotype altering polynucleotides, primary constructs or

mmRNA of the invention. Incorporation of intronic sequences may increase protein production as well as mRNA levels.

3' UTR and the AU Rich Elements

[00153] 3'UTRs are known to have stretches of Adenosines and Uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Molecules containing this type of AREs include GM-CSF and TNF-a. Class III ARES are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo.*

[00154] Introduction, removal or modification of 3' UTR AU rich elements (AREs) can be used to modulate the stability of cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention. When engineering specific cell phenotype altering polynucleotides, primary constructs or mmRNA, one or more copies of an ARE can be introduced to make cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein. Transfection experiments can be conducted in relevant cell lines, using cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention and protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different ARE-engineering molecules and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hr, 12 hr, 24 hr, 48 hr, and 7 days post-transfection.

Incorporating microRNA Binding Sites

[00155] microRNAs (or miRNA) are 19-25 nucleotide long noncoding RNAs that bind to the 3'UTR of nucleic acid molecules and down-regulate gene expression either by reducing nucleic acid molecule stability or by inhibiting translation. The polynucleotides, primary constructs or mmRNA of the invention may comprise one or more microRNA target sequences, microRNA seqences, or microRNA seeds. Such sequences may correspond to any known microRNA such as those taught in US Publication US2005/0261218 and US Publication US2005/0059005, the contents of which are incorporated herein by reference in their entirety.

[00156] A microRNA sequence comprises a "seed" region, i.e., a sequence in the region of positions 2-8 of the mature microRNA, which sequence has perfect Watson-Crick complementarity to the miRNA target sequence. A microRNA seed may comprise positions 2-8 or 2-7 of the mature microRNA. In some embodiments, a microRNA seed may comprise 7 nucleotides (e.g., nucleotides 2-8 of the mature microRNA), wherein the seed-complementary site in the corresponding miRNA target is flanked by an adenine (A) opposed to microRNA position 1. In some embodiments, a microRNA seed may comprise 6 nucleotides (e.g., nucleotides 2-7 of the mature microRNA), wherein the seed-complementary site in the corresponding miRNA target is flanked byan adenine (A) opposed to microRNA position 1. See for example, Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Barrel DP; Mol Cell. 2007 Jul 6;27(1):91-105. The bases of the microRNA seed have complete complementarity with the target sequence. By engineering microRNA target sequences into the 3'UTR of cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention one can target the molecule for degradation or reduced translation, provided the microRNA in question is available. This process will reduce the hazard of off target effects upon nucleic acid molecule delivery. Identification of microRNA, microRNA target regions, and their expression patterns and role in biology have been reported (Bonauer et al, Curr Drug Targets 2010 11:943-949; Anand and Cheresh Curr Opin Hematol 201 1 18:171-176; Contreras and Rao Leukemia 2012 26:404-413 (201 1 Dec 20. doi: 10. 1038/leu.201 1.356); Barrel Cell 2009 136:215-233; Landgraf et al, Cell, 2007 129:1401-1414).

[00157] For example, if the nucleic acid molecule is an mRNA and is not intended to be delivered to the liver but ends up there, then miR-122, a microRNA abundant in liver, can inhibit the expression of the gene of interest if one or multiple target sites of miR-122 are engineered into the 3'UTR of the polynucleotides, primary constructs or mmRNA. Introduction of one or multiple binding sites for different microRNA can be engineered to further decrease the longevity, stability, and protein translation of a polynucleotides, primary constructs or mmRNA.

[00158] As used herein, the term "microRNA site" refers to a microRNA target site or a microRNA recognition site, or any nucleotide sequence to which a microRNA binds or associates. It should be understood that "binding" may follow traditional Watson-Crick hybridization rules or may reflect any stable association of the microRNA with the target sequence at or adjacent to the microRNA site.

[00159] Conversely, for the purposes of the cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention, microRNA binding sites can be engineered out of (i.e. removed from) sequences in which they naturally occur in order to increase protein expression in specific tissues. For example, miR-122 binding sites may be removed to improve protein expression in the liver. Regulation of expression in multiple tissues can be accomplished through introduction or removal or one or several microRNA binding sites.

[00160] Examples of tissues where microRNA are known to regulate mRNA, and thereby protein expression, include, but are not limited to, liver (miR-122), muscle (miR-133, miR-206, miR-208), endothelial cells (miR-17-92, miR-126), myeloid cells (miR-142-3p, miR-142-5p, miR-16, miR-21, miR-223, miR-24, miR-27), adipose tissue (let-7, miR-30c), heart (miR-ld, miR-149), kidney (miR-192, miR-194, miR-204), and lung epithelial cells (let-7, miR-133, miR-126). MicroRNA can also regulate complex biological processes such as angiogenesis (miR-132) (Anand and Cheresh Curr Opin Hematol 201 1 18:171-176). In the cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention, binding sites for microRNAs that are involved in such processes may be removed or introduced, in order to tailor the expression of the cell phenotype altering polynucleotides, primary constructs or mmRNA expression to biologically relevant cell types or to the context of relevant biological processes.

[00161] Lastly, through an understanding of the expression patterns of microRNA in different cell types, cell phenotype altering polynucleotides, primary constructs or mmRNA can be engineered for more targeted expression in specific cell types or only under specific biological conditions. Through introduction of tissue-specific microRNA binding sites, cell phenotype altering polynucleotides, primary constructs or mmRNA could be designed that would be optimal for protein expression in a tissue or in the context of a biological condition.

[00162] Transfection experiments can be conducted in relevant cell lines, using engineered cell phenotype altering polynucleotides, primary constructs or mmRNA and protein production can be assayed at various time points post-transfection. For example, cells can be transfected with different microRNA binding site-engineering cell phenotype altering polynucleotides, primary constructs or mmRNA and by using an ELISA kit to the relevant protein and assaying protein produced at 6 hr, 12 hr, 24 hr, 48 hr, 72 hr and 7 days post-transfection. *In vivo* experiments can also be conducted using microRNA-binding site-engineered molecules to examine changes in tissue-specific expression of formulated cell phenotype altering polynucleotides, primary constructs or mmRNA.

5' Capping

[00163] The 5' cap structure of an mRNA is involved in nuclear export, increasing mRNA stability and binds the mRNA Cap Binding Protein (CBP), which is responsibile for mRNA stability in the cell and translation competency through the association of CBP with poly(A) binding protein to form the mature cyclic mRNA species. The cap further assists the removal of 5' proximal introns removal during mRNA splicing.

[00164] Endogenous mRNA molecules may be 5'-end capped generating a 5'-ppp-5'-triphosphate linkage between a terminal guanosine cap residue and the 5'-terminal transcribed sense nucleotide of the mRNA molecule. This 5'-guanylate cap may then be methylated to generate an N7-methyl-guanylate residue. The ribose sugars of the terminal and/or anteterminal transcribed nucleotides of the 5' end of the mRNA may optionally also be 2'-0-methylated. 5'-decapping through hydrolysis and cleavage of the guanylate cap structure may target a nucleic acid molecule, such as an mRNA molecule, for degradation.

[00165] Modifications to the cell phenotype altering polynucleotides, primary constructs, and mmRNA of the present invention may generate a non-hydrolyzable cap structure preventing decapping and thus increasing mRNA half-life. Because cap structure hydrolysis requires cleavage of 5'-ppp-5' phosphorodiester linkages, modified nucleotides may be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, MA) may be used with a-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5'-ppp-5' cap. Additional modified guanosine nucleotides may be used such as a-methyl-phosphonate and seleno-phosphate nucleotides.

[00166] Additional modifications include, but are not limited to, 2'-0-methylation of the ribose sugars of 5'-terminal and/or 5'-anteterminal nucleotides of the mRNA (as mentioned above) on the 2'-hydroxyl group of the sugar ring. Multiple distinct 5'-cap structures can be used to generate the 5'-cap of a nucleic acid molecule, such as an mRNA molecule.

[00167] Cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural (i.e. endogenous, wild-type or physiological) 5'-caps in their chemical structure, while retaining cap function. Cap analogs may be chemically (i.e. non-enzymatically) or enzymatically synthesized and/linked to a nucleic acid molecule.

[00168] For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanines linked by a 5'-5'-triphosphate group, wherein one guanine contains an N7 methyl group as well as a 3'-0-methyl group (i.e., N7,3'-0-dimethyl-guanosine-5'-triphosphate-5'-guanosine (m^7G-3'mppp-G; which may equivaliently be designated 3' O-Me-m7G(5')ppp(5')G). The 3'-0 atom of the other, unmodified, guanine becomes linked to the 5'-terminal nucleotide of the capped nucleic acid molecule (e.g. an mRNA or mmRNA). The N7- and 3'-0-methlyated guanine provides the terminal moiety of the capped nucleic acid molecule (e.g. mRNA or mmRNA).

[00169] Another exemplary cap is mCAP, which is similar to ARCA but has a 2'-0-methyl group on guanosine (i.e., N7,2'-0-dimethyl-guanosine-5'-triphosphate-5'-guanosine, m^7Gm-ppp-G).

[00170] While cap analogs allow for the concomitant capping of a nucleic acid molecule in an in vitro transcription reaction, up to 20% of transcripts remain uncapped. This, as well as the structural differences of a cap analog from an endogenous 5'-cap structure of nucleic acids produced by the endogenous, cellular transcription machinery, may lead to reduced translational competency and reduced cellular stability.

[00171] Cell phenotype altering polynucleotides, primary constructs and mmRNA of the invention may also be capped post-transcriptionally, using enzymes, in order to generate more authentic 5'-cap structures. As used herein, the phrase "more authentic" refers to a feature that closely mirrors or mimics, either structurally or functionally, an endogenous or wild type feature. That is, a "more authentic" feature is better representative of an endogenous, wild-type, natural or physiological cellular function and/or structure as compared to synthetic features or analogs, etc., of the prior art, or which outperforms the corresponding endogenous, wild-type, natural or physiological feature in one or more respects. Non-limiting examples of more authentic 5'cap structures of the present invention are those which, among other things, have enhanced binding of cap binding proteins, increased half life, reduced susceptibility to 5' endonucleases and/or reduced 5'decapping, as compared to synthetic 5'cap structures known in the art (or to a wild-type, natural or physiological 5'cap structure). For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2'-0-methyltransferase enzyme can create a canonical 5'-5'-triphosphate linkage between the 5'-terminal nucleotide of an mRNA and a guanine cap nucleotide wherein the cap guanine contains an N7 methylation and the 5'-terminal nucleotide of the mRNA contains a 2'-0-methyl. Such a structure is termed the Capl structure. This cap results in a higher translational-competency and cellular stability and a reduced activation of cellular pro-inflammatory cytokines, as compared, e.g., to other 5'cap analog structures known in the art. Cap structures include 7mG(5')ppp(5')N,pN2p (cap 0), 7mG(5')ppp(5')NlmpNp (cap 1), and 7mG(5')-ppp(5')NlmpN2mp (cap 2).

[00172] Because the cell phenotype altering polynucleotides, primary constructs or mmRNA may be capped post-transcriptionally, and because this process is more efficient, nearly 100% of the cell phenotype altering polynucleotides, primary constructs

or mmRNA may be capped. This is in contrast to ~80% when a cap analog is linked to an mRNA in the course of an in vitro transcription reaction.

[00173] According to the present invention, 5' terminal caps may include endogenous caps or cap analogs. According to the present invention, a 5' terminal cap may comprise a guanine analog. Useful guanine analogs include inosine, Nl-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

Viral Sequences

[00174] Additional viral sequences such as, but not limited to, the translation enhancer sequence of the barley yellow dwarf virus (BYDV-PAV) can be engineered and inserted in the 3' UTR of the cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention and can stimulate the translation of the construct *in vitro* and *in vivo*. Transfection experiments can be conducted in relevant cell lines at and protein production can be assayed by ELISA at 12hr, 24hr, 48hr, 72 hr and day 7 post-transfection.

IRES Sequences

[00175] Further, provided are cell phenotype altering polynucleotides, primary constructs or mmRNA which may contain an internal ribosome entry site (IRES). First identified as a feature Picorna virus RNA, IRES plays an important role in initiating protein synthesis in absence of the 5' cap structure. An IRES may act as the sole ribosome binding site, or may serve as one of multiple ribosome binding sites of an mRNA. Cell phenotype altering polynucleotides, primary constructs or mmRNA containing more than one functional ribosome binding site may encode several cell phenotype altering peptides or polypeptides that are translated independently by the ribosomes ("multicistronic nucleic acid molecules"). When cell phenotype altering polynucleotides, primary constructs or mmRNA are provided with an IRES, further optionally provided is a second translatable region. Examples of IRES sequences that can be used according to the invention include without limitation, those from picornaviruses (e.g. FMDV), pest viruses (CFFV), polio viruses (PV), encephalomyocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical

swine fever viruses (CSFV), murine leukemia virus (MLV), simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV).

Poly-A tails

[00176] During RNA processing, a long chain of adenine nucleotides (poly-A tail) may be added to a polynucleotide such as an mRNA molecules in order to increase stability. Immediately after transcription, the 3' end of the transcript may be cleaved to free a 3' hydroxyl. Then poly-A polymerase adds a chain of adenine nucleotides to the RNA. The process, called polyadenylation, adds a poly-A tail that can be between 100 and 250 residues long.

[00177] It has been discovered that unique poly-A tail lengths provide certain advantages to the cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention.

[00178] Generally, the length of a poly-A tail of the present invention is greater than 30 nucleotides in length. In another embodiment, the poly-A tail is greater than 35 nucleotides in length (e.g., at least or greater than about 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, and 3,000 nucleotides). In some embodiments, the polynucleotide, primary construct, or mmRNA includes from about 30 to about 3,000 nucleotides (e.g., from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 750, from 30 to 1,000, from 30 to 1,500, from 30 to 2,000, from 30 to 2,500, from 50 to 100, from 50 to 250, from 50 to 500, from 50 to 750, from 50 to 1,000, from 50 to 1,500, from 50 to 2,000, from 50 to 2,500, from 50 to 3,000, from 100 to 500, from 100 to 750, from 100 to 1,000, from 100 to 1,500, from 100 to 2,000, from 100 to 2,500, from 100 to 3,000, from 500 to 750, from 500 to 1,000, from 500 to 1,500, from 500 to 2,000, from 500 to 2,500, from 500 to 3,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 2,500, from 1,000 to 3,000, from 1,500 to 2,000, from 1,500 to 2,500, from 1,500 to 3,000, from 2,000 to 3,000, from 2,000 to 2,500, and from 2,500 to 3,000).

[00179] In one embodiment, the poly-A tail is designed relative to the length of the overall cell phenotype altering polynucleotides, primary constructs or mmRNA. This design may be based on the length of the coding region, the length of a particular feature

or region (such as the first or flanking regions), or based on the length of the ultimate product expressed from the cell phenotype altering polynucleotides, primary constructs or mmRNA.

[00180] In this context the poly-A tail may be 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% greater in length than the cell phenotype altering polynucleotides, primary constructs or mmRNA or feature thereof. The poly-A tail may also be designed as a fraction of cell phenotype altering polynucleotides, primary constructs or mmRNA to which it belongs. In this context, the poly-A tail may be 10, 20, 30, 40, 50, 60, 70, 80, or 90% or more of the total length of the cell phenotype altering construct or the total length of the cell phenotype altering construct minus the poly-A tail. Further, engineered binding sites and conjugation of cell phenotype altering polynucleotides, primary constructs or mmRNA for Poly-A binding protein may enhance expression.

[00181] Additionally, multiple distinct cell phenotype altering polynucleotides, primary constructs or mmRNA may be linked together to the PABP (Poly-A binding protein) through the 3'-end using modified nucleotides at the 3'-terminus of the poly-A tail. Transfection experiments can be conducted in relevant cell lines at and protein production can be assayed by ELISA at 12hr, 24hr, 48hr, 72 hr and day 7 post-transfection.

[00182] In one embodiment, the cell phenotype altering polynucleotide primary constructs of the present invention are designed to include a polyA-G Quartet. The G-quartet is a cyclic hydrogen bonded array of four guanine nucleotides that can be formed by G-rich sequences in both DNA and RNA. In this embodiment, the G-quartet is incorporated at the end of the poly-A tail. The resultant cell phenotype altering mmRNA construct is assayed for stability, protein production and other parameters including half-life at various time points. It has been discovered that the polyA-G quartet results in protein production equivalent to at least 75% of that seen using a poly-A tail of 120 nucleotides alone.

Quantification

[00183] In one embodiment, the cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention may be quantified in exosomes derived from one or more bodily fluid. As used herein "bodily fluids" include peripheral blood,

serum, plasma, ascites, urine, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen, prostatic fluid, cowper's fluid or pre-ejaculatory fluid, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates, blastocyl cavity fluid, and umbilical cord blood. Alternatively, exosomes may be retrieved from an organ selected from the group consisting of lung, heart, pancreas, stomach, intestine, bladder, kidney, ovary, testis, skin, colon, breast, prostate, brain, esophagus, liver, and placenta.

[00184] In the quantification method, a sample of not more than 2mL is obtained from the subject and the exosomes isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfiuidic separation, or combinations thereof. In the analysis, the level or concentration of a cell phenotype altering polynucleotide, primary construct or mmRNA may be an expression level, presence, absence, truncation or alteration of the administered construct. It is advantageous to correlate the level with one or more clinical phenotypes or with an assay for a human disease biomarker. The assay may be performed using construct specific probes, cytometry, qRT-PCR, real-time PCR, PCR, flow cytometry, electrophoresis, mass spectrometry, or combinations thereof while the exosomes may be isolated using immunohistochemical methods such as enzyme linked immunosorbent assay (ELISA) methods. Exosomes may also be isolated by size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfiuidic separation, or combinations thereof.

[00185] These methods afford the investigator the ability to monitor, in real time, the level of cell phenotype altering polynucleotides, primary constructs or mmRNA remaining or delivered. This is possible because the cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention differ from the endogenous forms due to the structural or chemical modifications.

II. Design and synthesis of mmRNA

[00186] Cell phenotype altering polynucleotides, primary constructs or mmRNA for use in accordance with the invention may be prepared according to any available technique including, but not limited to chemical synthesis, enzymatic synthesis, which is generally termed *in vitro* transcription (IVT) or enzymatic or chemical cleavage of a longer precursor, etc. Methods of synthesizing RNAs are known in the art (see, *e.g.*, Gait, M.J. (ed.) *Oligonucleotide synthesis: a practical approach*, Oxford [Oxfordshire], Washington, DC: IRL Press, 1984; and Herdewijn, P. (ed.) *Oligonucleotide synthesis: methods and applications*, Methods in Molecular Biology, v. 288 (Clifton, N.J.) Totowa, N.J.: Humana Press, 2005; both of which are incorporated herein by reference).

[00187] The process of design and synthesis of the cell phenotype altering primary constructs of the invention generally includes the steps of gene construction, mRNA production (either with or without modifications) and purification. In the enzymatic synthesis method, a target cell phenotype altering polynucleotide sequence encoding the cell phenotype altering polypeptide of interest is first selected for incorporation into a vector which will be amplified to produce a cDNA template. Optionally, the target cell phenotype altering polynucleotide sequence and/or any flanking sequences may be codon optimized. The cDNA template is then used to produce mRNA through *in vitro* transcription (IVT). After production, the mRNA may undergo purification and clean-up processes. The steps of which are provided in more detail below.

Gene Construction

[00188] The step of gene construction may include, but is not limited to gene synthesis, vector amplification, plasmid purification, plasmid linearization and clean-up, and cDNA template synthesis and clean-up.

Gene Synthesis

[00189] Once a cell phenotype altering polypeptide of interest, or target, is selected for production, a cell phenotype altering primary construct is designed. Within the cell phenotype altering primary construct, a first region of linked nucleosides encoding the cell phenotype altering polypeptide of interest may be constructed using an open reading frame (ORF) of a selected nucleic acid (DNA or RNA) transcript. The ORF may comprise the wild type ORF, an isoform, variant or a fragment thereof. As used herein,

an "open reading frame" or "ORF" is meant to refer to a nucleic acid sequence (DNA or RNA) which is capable of encoding a cell phenotype altering polypeptide of interest. ORFs often begin with the start codon, ATG and end with a nonsense or termination codon or signal.

[00190] Further, the nucleotide sequence of the first region may be codon optimized. Codon optimization methods are known in the art and may be useful in efforts to achieve one or more of several goals. These goals include to match codon frequencies in target and host organisms to ensure proper folding, bias GC content to increase mRNA stability or reduce secondary structures, minimize tandem repeat codons or base runs that may impair gene construction or expression, customize transcriptional and translational control regions, insert or remove protein trafficking sequences, remove/add post translation modification sites in encoded protein (e.g. glycosylation sites), add, remove or shuffle protein domains, insert or delete restriction sites, modify ribosome binding sites and mRNA degradation sites, to adjust translational rates to allow the various domains of the protein to fold properly, or to reduce or eliminate problem secondary structures within the mRNA. Codon optimization tools, algorithms and services are known in the art, non-limiting examples include services from GeneArt (Life Technologies) and/or DNA2.0 (Menlo Park CA). In one embodiment, the ORF sequence is optimized using optimization algorithms. Codon options for each amino acid are given in Table 1.

Table 1. Codon Options

Amino Acid	Single Letter Code	Codon Options
Isoleucine	I	ATT, ATC, ATA
Leucine	L	CTT, CTC, CTA, CTG, TTA, TTG
Valine	V	GTT, GTC, GTA, GTG
Phenylalanine	F	TTT, TTC
Methionine	M	ATG
Cysteine	C	TGT, TGC
Alanine	A	GCT, GCC, GCA, GCG
Glycine	G	GGT, GGC, GGA, GGG
Proline	P	CCT, CCC, CCA, CCG
Threonine	T	ACT, ACC, ACA, ACG
Serine	S	TCT, TCC, TCA, TCG, AGT, AGC
Tyrosine	Y	TAT, TAC
Tryptophan	W	TGG
Glutamine	Q	CAA, CAG
Asparagine	N	AAT, AAC

Histidine	H	CAT, CAC
Glutamic acid	E	GAA, GAG
Aspartic acid	D	GAT, GAC
Lysine	K	AAA, AAG
Arginine	R	CGT, CGC, CGA, CGG, AGA, AGG
Selenocysteine	Sec	UGA in mRNA in presence of Selenocystein insertion element (SECIS)
Stop codons	Stop	TAA, TAG, TGA

[00191] In one embodiment, after a nucleotide sequence has been codon optimized it may be further evaluated for regions containing restriction sites. At least one nucleotide within the restriction site regions may be replaced with another nucleotide in order to remove the restriction site from the sequence but the replacement of nucleotides does alter the amino acid sequence which is encoded by the codon optimized nucleotide sequence.

[00192] Features, which may be considered beneficial in some embodiments of the present invention, may be encoded by the cell phenotype altering primary construct and may flank the ORF as a first or second flanking region. The flanking regions may be incorporated into the cell phenotype altering primary construct before and/or after optimization of the ORF. It is not required that a cell phenotype altering primary construct contain both a 5' and 3' flanking region. Examples of such features include, but are not limited to, untranslated regions (UTRs), Kozak sequences, an oligo(dT) sequence, and detectable tags and may include multiple cloning sites which may have Xbal recognition.

[00193] In some embodiments, a 5' UTR and/or a 3' UTR may be provided as flanking regions. Multiple 5' or 3' UTRs may be included in the flanking regions and may be the same or of different sequences. Any portion of the flanking regions, including none, may be codon optimized and any may independently contain one or more different structural or chemical modifications, before and/or after codon optimization. Combinations of features may be included in the first and second flanking regions and may be contained within other features. For example, the ORF may be flanked by a 5' UTR which may contain a strong Kozak translational initiation signal and/or a 3' UTR which may include an oligo(dT) sequence for templated addition of a poly-A tail.

[00194] Tables 2 and 3 provide a listing of exemplary UTRs which may be utilized in the cell phenotype altering primary construct of the present invention as flanking regions. Shown in Table 2 is a representative listing of a 5'-untranslated region of the invention. Variants of 5' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G.

Table 2. 5′-Untranslated Regions

5′ UTR Identifier	Name/Description	Sequence	SEQ ID NO.
5UTR-001	Upstream UTR	GGGAAATAAGAGAGAAAAGAAGAGTAAGAAG AAATATAAGAGCCACC	1

[00195] Shown in Table 3 is a representative listing of 3'-untranslated regions of the invention. Variants of 3' UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G.

Table 3. 3′-Untranslated Regions

3′ UTR Identifier	Name/Description	Sequence	SEQ ID NO.
3UTR-001	Creatine Kinase	GCGCCTGCCCACCTGCCACCGACTGCTGGAAC CCAGCCAGTGGGAGGGCCTGGCCCACCAGAGT CCTGCTCCCTCACTCCTCGCCCCGCCCCCTGTC CCAGAGTCCCACCTGGGGGCTCTCTCCACCCTT CTCAGAGTTCCAGTTTCAACCAGAGTTCCAACC AATGGGCTCCATCCTCTGGATTCTGGCCAATGA AATATCTCCCTGGCAGGGTCCTCTTCTTTTCCC AGAGCTCCACCCCAACCAGGAGCTCTAGTTAA TGGAGAGCTCCCAGCACACTCGGAGCTTGTGC TTTGTCTCCACGCAAAGCGATAAATAAAAGCA TTGGTGGCCTTTGGTCTTTGAATAAAGCCTGAG TAGGAAGTCTAGA	2
3UTR-002	Myoglobin	GCCCCTGCCGCTCCCACCCCCACCCATCTGGGC CCCGGGTTCAAGAGAGAGCGGGGTCTGATCTC GTGTAGCCATATAGAGTTTGCTTCTGAGTGTCT GCTTTGTTTAGTAGAGGTGGGCAGGAGGAGCT GAGGGGCTGGGGCTGGGGTGTTGAAGTTGGCT TTGCATGCCCAGCGATGCGCCTCCCTGTGGGAT GTCATCACCCTGGGAACCGGGAGTGGCCCTTG GCTCACTGTGTTCTGCATGGTTTGGATCTGAAT TAATTGTCCTTTCTTCTAAATCCCAACCGAACT TCTTCCAACCTCCAAACTGGCTGTAACCCCAAA TCCAAGCCATTAACTACACCTGACAGTAGCAA TTGTCTGATTAATCACTGGCCCCTTGAAGACAG CAGAATGTCCCTTTGCAATGAGGAGGAGATCT	3

		GGGCTGGGCGGGCCAGCTGGGGAAGCATTTGA CTATCTGGAACTTGTGTGTGCCTCCTCAGGTAT GGCAGTGACTCACCTGGTTTTAATAAAACAAC CTGCAACATCTCATGGTCTTTGAATAAAGCCTG AGTAGGAAGTCTAGA	
3UTR-003	α-actin	ACACACTCCACCTCCAGCACGCGACTTCTCAG GACGACGAATCTTCTCAATGGGGGGGCGGCTG AGCTCCAGCCACCCCGCAGTCACTTTCTTTGTA ACAACTTCCGTTGCTGCCATCGTAAACTGACAC AGTGTTTATAACGTGTACATACATTAACTTATT ACCTCATTTTGTTATTTTTCGAAACAAAGCCCT GTGGAAGAAAATGGAAAACTTGAAGAAGCATT AAAGTCATTCTGTTAAGCTGCGTAAATGGTCTT TGAATAAAGCCTGAGTAGGAAGTCTAGA	4
3UTR-004	Albumin	CATCACATTTAAAAGCATCTCAGCCTACCATG AGAATAAGAGAAAGAAAATGAAGATCAAAAG CTTATTCATCTGTTTTTCTTTTTCGTTGGTGTAA AGCCAACACCCTGTCTAAAAAACATAAATTTC TTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAA TTAATAAAAAATGGAAAGAATCTAATAGAGTG GTACAGCACTGTTATTTTTCAAAGATGTGTTGC TATCCTGAAAATTCTGTAGGTTCTGTGGAAGTT CCAGTGTTCTCTCTTATTCCACTTCGGTAGAGG ATTTCTAGTTTCTTGTGGGCTAATTAAATAAAT CATTAATACTCTTCTAATGGTCTTTGAATAAAG CCTGAGTAGGAAGTCTAGA	5
3UTR-005	α-globin	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATG CCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGG TCTTTGAATAAAGCCTGAGTAGGAAGGCGGCC GCTCGAGCATGCATCTAGA	6
3UTR-006	G-CSF	GCCAAGCCCTCCCCATCCCATGTATTTATCTCT ATTTAATATTTATGTCTATTTAAGCCTCATATTT AAAGACAGGGAAGAGCAGAACGGAGCCCCAG GCCTCTGTGTCCTTCCCTGCATTTCTGAGTTTC ATTCTCCTGCCTGTAGCAGTGAGAAAAAGCTC CTGTCCTCCCATCCCCTGGACTGGGAGGTAGAT AGGTAAATACCAAGTATTTATTACTATGACTGC TCCCCAGCCCTGGCTCTGCAATGGGCACTGGG ATGAGCCGCTGTGAGCCCCTGGTCCTGAGGGT CCCCACCTGGGACCCTTGAGAGTATCAGGTCT CCCACGTGGGAGACAAGAAATCCCTGTTTAAT ATTTAAACAGCAGTGTTCCCCATCTGGGTCCTT GCACCCCTCACTCTGGCCTCAGCCGACTGCAC AGCGGCCCCTGCATCCCCTTGGCTGTGAGGCC CCTGGACAAGCAGAGGTGGCCAGAGCTGGGA GGCATGGCCCTGGGGTCCCACGAATTTGCTGG GGAATCTCGTTTTTCTTCTTAAGACTTTTGGGA CATGGTTTGACTCCCGAACATCACCGACGCGT CTCCTGTTTTTCTGGGTGGCCTCGGGACACCTG	7

		CCCTGCCCCCACGAGGGTCAGGACTGTGACTC TTTTTAGGGCCAGGCAGGTGCCTGGACATTTGC CTTGCTGGACGGGGACTGGGGATGTGGGAGGG AGCAGACAGGAGGAATCATGTCAGGCCTGTGT GTGAAAGGAAGCTCCACTGTCACCCTCCACCT CTTCACCCCCCACTCACCAGTGTCCCCTCCACT GTCACATTGTAACTGAACTTCAGGATAATAAA GTGTTTGCCTCCATGGTCTTTGAATAAAGCCTG AGTAGGAAGGCGGCCGCTCGAGCATGCATCTA GA	

[00196] It should be understood that those listed in the previous tables are examples and that any UTR from any gene may be incorporated into the respective first or second flanking region of the cell phenotype altering primary construct. Furthermore, multiple wild-type UTRs of any known gene may be utilized. It is also within the scope of the present invention to provide artificial UTRs which are not variants of wild type genes. These UTRs or portions thereof may be placed in the same orientation as in the transcript from which they were selected or may be altered in orientation or location. Hence a 5′ or 3' UTR may be inverted, shortened, lengthened, made chimeric with one or more other 5' UTRs or 3' UTRs. As used herein, the term "altered" as it relates to a UTR sequence, means that the UTR has been changed in some way in relation to a reference sequence. For example, a 3' or 5' UTR may be altered relative to a wild type or native UTR by the change in orientation or location as taught above or may be altered by the inclusion of additional nucleotides, deletion of nucleotides, swapping or transposition of nucleotides. Any of these changes producing an "altered" UTR (whether 3' or 5') comprise a variant UTR.

[00197] In one embodiment, the UTRs which may be contemplated by the present invention include the UTRs described in US Patent Application No. 14/043,927, filed October 2, 2013, entitled Terminally Modified RNA, US Provisional Patent Application No 61/775,509, filed March 9, 2013, entitled Heterologous Untranslated Regions for niRNA and US Provisional Patent Application No 61/829,372, filed May 31, 2013, entitled Heterologous Untranslated Regions for mRNA, the contents of each of which is herein incorporated by reference in its entirety. Non-limiting examples of UTRs include the 5'UTRs described in Table 6, Table 38, Table 41, Table 60, Table 62 and the 3'UTRs

described in Table 7 of U.S. Patent Application No. 14/043,927, filed October 2, 2013, entitled Terminally Modified RNA, the 5'UTRs described in Table 2 and Table 21 and the 3'UTRs described in Table 3 of US Provisional Patent Application No 61/775,509, filed March 9, 2013, entitled Heterologous Untranslated Regions for mRNA and the 5'UTRs described in Table 2, Table 21 and Table 22 and the 3'UTRs described in Table 3 of US Provisional Patent Application No 61/829,372, filed May 31, 2013, entitled Heterologous Untranslated Regions for mRNA, the contents of each of which is herein incorporated by reference in its entirety.

[00198] In one embodiment, a flanking region such as a UTR may comprise a terminal modification. Non-limiting examples of terminal modifications include the terminal modifications described in US Patent Application No. 14/043,927, filed October 2, 2013, entitled Terminally Modified RNA, the contents of which is herein incorporated by reference in its entirety, such as the terminal modifications described on pages 35-94 of the specification of US Patent Application No. 14/043,927.

[00199] In one embodiment, a double, triple or quadruple UTR such as a 5' or 3' UTR may be used. As used herein, a "double" UTR is one in which two copies of the same UTR are encoded either in series or substantially in series. For example, a double beta-globin 3' UTR may be used as described in US Patent publication 20100129877, the contents of which are incorporated herein by reference in its entirety.

[00200] It is also within the scope of the present invention to have patterned UTRs. As used herein "patterned UTRs" are those UTRs which reflect a repeating or alternating pattern, such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than 3 times. In these patterns, each letter, A, B, or C represent a different UTR at the nucleotide level.

[00201] In one embodiment, flanking regions are selected from a family of transcripts whose proteins share a common function, structure, feature of property. For example, cell phenotype altering polypeptides of interest may belong to a family of proteins which are expressed in a particular cell, tissue or at some time during development. The UTRs from any of these genes may be swapped for any other UTR of the same or different family of proteins to create a new chimeric primary transcript. As used herein, a "family of proteins" is used in the broadest sense to refer to a group of two or more cell phenotype

altering polypeptides of interest which share at least one function, structure, feature, localization, origin, or expression pattern.

[00202] After optimization (if desired), the cell phenotype altering primary construct components are reconstituted and transformed into a vector such as, but not limited to, plasmids, viruses, cosmids, and artificial chromosomes. For example, the cell phenotype altering optimized construct may be reconstituted and transformed into chemically competent *E. coli,* yeast, neurospora, maize, drosophila, etc. where high copy plasmid-like or chromosome structures occur by methods described herein.

Stop Codons

[00203] In one embodiment, the cell phenotype altering primary constructs of the present invention may include at least two stop codons before the 3' untranslated region (UTR). The stop codon may be selected from TGA, TAA and TAG. In one embodiment, the cell phenotype altering primary constructs of the present invention include the stop codon TGA and one additional stop codon. In a further embodiment the addition stop codon may be TAA.

Vector Amplification

[00204] The vector containing the cell phenotype altering primary construct is then amplified and the plasmid isolated and purified using methods known in the art such as, but not limited to, a maxi prep using the Invitrogen PURELINK™ HiPure Maxiprep Kit (Carlsbad, CA).

Plasmid Linearization

[00205] The plasmid may then be linearized using methods known in the art such as, but not limited to, the use of restriction enzymes and buffers. The linearization reaction may be purified using methods including, for example Invitrogen's PURELINK™ PCR Micro Kit (Carlsbad, CA), and HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC) and Invitrogen's standard PURELINK™ PCR Kit (Carlsbad, CA). The purification method may be modified depending on the size of the linearization reaction which was conducted. The linearized plasmid is then used to generate cDNA for *in vitro* transcription (IVT) reactions.

cDNA Template Synthesis

[00206] A cDNA template may be synthesized by having a linearized plasmid undergo polymerase chain reaction (PCR). Table 4 is a listing of primers and probes that may be usefully in the PCR reactions of the present invention. It should be understood that the listing is not exhaustive and that primer-probe design for any amplification is within the skill of those in the art. Probes may also contain chemically modified bases to increase base-pairing fidelity to the target molecule and base-pairing strength. Such modifications may include 5-methyl-Cytidine, 2, 6-di-amino-purine, 2'-fluoro, phosphoro-thioate, or locked nucleic acids.

Table 4. Primers and Probes

Primer/ Probe Identifier	Sequence (5′-3′)	Hybridization target	SEQ ID NO.
UFP	TTGGACCCTCGTACAGAAGCTAA TACG	cDNA Template	8
URP	T_{x160}CTTCCTACTCAGGCTTTATTC AAAGACCA	cDNA Template	9
GBA1	CCTTGACCTTCTGGAACTTC	Acid glucocerebrosidase	10
GBA2	CCAAGCACTGAAACGGATAT	Acid glucocerebrosidase	11
LUC1	GATGAAAAGTGCTCCAAGGA	Luciferase	12
LUC2	AACCGTGATGAAAAGGTACC	Luciferase	13
LUC3	TCATGCAGATTGGAAAGGTC	Luciferase	14
GCSF1	CTTCTTGGACTGTCCAGAGG	G-CSF	15
GCSF2	GCAGTCCCTGATACAAGAAC	G-CSF	16
GCSF3	GATTGAAGGTGGCTCGCTAC	G-CSF	17

*UFP is universal forward primer; URP is universal reverse primer.

[00207] In one embodiment, the cDNA may be submitted for sequencing analysis before undergoing transcription.

mRNA Production

[00208] The process of mRNA or mmRNA production may include, but is not limited to, *in vitro* transcription, cDNA template removal and RNA clean-up, and mRNA capping and/or tailing reactions.

In Vitro Transcription

[00209] The cDNA produced in the previous step may be transcribed using an *in vitro* transcription (IVT) system. The system typically comprises a transcription buffer, nucleotide triphosphates (NTPs), an RNase inhibitor and a polymerase. The NTPs may be manufactured in house, may be selected from a supplier, or may be synthesized as described herein. The NTPs may be selected from, but are not limited to, those described herein including natural and unnatural (modified) NTPs. The polymerase may be selected from, but is not limited to, T7 RNA polymerase, T3 RNA polymerase and mutant polymerases such as, but not limited to, polymerases able to incorporate modified nucleic acids.

RNA Polymerases

[00210] Any number of RNA polymerases or variants may be used in the design of the cell phenotype altering primary constructs of the present invention.

[00211] RNA polymerases may be modified by inserting or deleting amino acids of the RNA polymerase sequence. As a non-limiting example, the RNA polymerase may be modified to exhibit an increased ability to incorporate a 2'-modified nucleotide triphosphate compared to an unmodified RNA polymerase (see International Publication WO2008078180 and U.S. Patent 8,101,385; herein incorporated by reference in their entireties).

[00212] Variants may be obtained by evolving an RNA polymerase, optimizing the RNA polymerase amino acid and/or nucleic acid sequence and/or by using other methods known in the art. As a non-limiting example, T7 RNA polymerase variants may be evolved using the continuous directed evolution system set out by Esvelt *et al.* (Nature (201 1) 472(7344):499-503; herein incorporated by reference in its entirety) where clones of T7 RNA polymerase may encode at least one mutation such as, but not limited to, lysine at position 93 substituted for threonine (K93T), I4M, A7T, E63V, V64D, A65E, D66Y, T76N, C125R, S128R, A136T, N165S, G175R, H176L, Y178H, F182L, L196F, G198V, D208Y, E222K, S228A, Q239R, T243N, G259D, M267I, G280C, H300R, D351A, A354S, E356D, L360P, A383V, Y385C, D388Y, S397R, M401T, N410S, K450R, P451T, G452V, E484A, H523L, H524N, G542V, E565K, K577E, K577M, N601S, S684Y, L699I, K713E, N748D, Q754R, E775K, A827V, D851N or L864F. As another non-limiting example, T7 RNA polymerase variants may encode at least

mutation as described in U.S. Pub. Nos. 20100120024 and 200701 171 12; herein incorporated by reference in their entireties. Variants of RNA polymerase may also include, but are not limited to, substitutional variants, conservative amino acid substitution, insertional variants, deletional variants and/or covalent derivatives.

[00213] In one embodiment, the cell phenotype altering primary construct may be designed to be recognized by the wild type or variant RNA polymerases. In doing so, the cell phenotype altering primary construct may be modified to contain sites or regions of sequence changes from the wild type or parent cell phenotype altering primary construct.

[00214] In one embodiment, the cell phenotype altering primary construct may be designed to include at least one substitution and/or insertion upstream of an RNA polymerase binding or recognition site, downstream of the RNA polymerase binding or recognition site, upstream of the TATA box sequence, downstream of the TATA box sequence of the cell phenotype altering primary construct but upstream of the coding region of the cell phenotype altering primary construct, within the 5'UTR, before the 5'UTR and/or after the 5'UTR.

[00215] In one embodiment, the 5'UTR of the cell phenotype altering primary construct may be replaced by the insertion of at least one region and/or string of nucleotides of the same base. The region and/or string of nucleotides may include, but is not limited to, at least 3, at least 4, at least 5, at least 6, at least 7 or at least 8 nucleotides and the nucleotides may be natural and/or unnatural. As a non-limiting example, the group of nucleotides may include 5-8 adenine, cytosine, thymine, a string of any of the other nucleotides disclosed herein and/or combinations thereof.

[00216] In one embodiment, the 5'UTR of the cell phenotype altering primary construct may be replaced by the insertion of at least two regions and/or strings of nucleotides of two different bases such as, but not limited to, adenine, cytosine, thymine, any of the other nucleotides disclosed herein and/or combinations thereof. For example, the 5'UTR may be replaced by inserting 5-8 adenine bases followed by the insertion of 5-8 cytosine bases. In another example, the 5'UTR may be replaced by inserting 5-8 cytosine bases followed by the insertion of 5-8 adenine bases.

[00217] In one embodiment, the cell phenotype altering primary construct may include at least one substitution and/or insertion downstream of the transcription start site which

may be recognized by an RNA polymerase. As a non-limiting example, at least one substitution and/or insertion may occur downstream the transcription start site by substituting at least one nucleic acid in the region just downstream of the transcription start site (such as, but not limited to, +1 to +6). Changes to region of nucleotides just downstream of the transcription start site may affect initiation rates, increase apparent nucleotide triphosphate (NTP) reaction constant values, and increase the dissociation of short transcripts from the transcription complex curing initial transcription (Brieba et al, Biochemistry (2002) 41: 5144-5149; herein incorporated by reference in its entirety). The modification, substitution and/or insertion of at least one nucleic acid may cause a silent mutation of the nucleic acid sequence or may cause a mutation in the amino acid sequence.

[00218] In one embodiment, the cell phenotype altering primary construct may include the substitution of 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, at least 10, at least 11, at least 12 or at least 13 guanine bases downstream of the transcription start site.

[00219] In one embodiment, the cell phenotype altering primary construct may include the substitution of at least 1, at least 2, at least 3, at least 4, at least 5 or at least 6 guanine bases in the region just downstream of the transcription start site. As a non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases may be substituted by at least 1, at least 2, at least 3 or at least 4 adenine nucleotides. In another non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases may be substituted by at least 1, at least 2, at least 3 or at least 4 cytosine bases. In another non-limiting example, if the nucleotides in the region are GGGAGA the guanine bases may be substituted by at least 1, at least 2, at least 3 or at least 4 thymine, and/or any of the nucleotides described herein.

[00220] In one embodiment, the cell phenotype altering primary construct may include at least one substitution and/or insertion upstream of the start codon. For the purpose of clarity, one of skill in the art would appreciate that the start codon is the first codon of the protein coding region whereas the transcription start site is the site where transcription begins. The cell phenotype altering primary construct may include, but is not limited to, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7 or at least 8

substitutions and/or insertions of nucleotide bases. The nucleotide bases may be inserted or substituted at 1, at least 1, at least 2, at least 3, at least 4 or at least 5 locations upstream of the start codon. The nucleotides inserted and/or substituted may be the same base (e.g., all A or all C or all T or all G), two different bases (e.g., A and C, A and T, or C and T), three different bases (e.g., A, C and T or A, C and T) or at least four different bases. As a non-limiting example, the guanine base upstream of the coding region in the cell phenotype altering primary construct may be substituted with adenine, cytosine, thymine, or any of the nucleotides described herein. In another non-limiting example the substitution of guanine bases in the cell phenotype altering primary construct may be designed so as to leave one guanine base in the region downstream of the transcription start site and before the start codon (see Esvelt *et al.* Nature (201 1) 472(7344):499-503; herein incorporated by reference in its entirety). As a non-limiting example, at least 5 nucleotides may be inserted at 1 location downstream of the transcription start site but upstream of the start codon and the at least 5 nucleotides may be the same base type.

cDNA Template Removal and Clean-Up

[00221] The cDNA template may be removed using methods known in the art such as, but not limited to, treatment with Deoxyribonuclease I (DNase I). RNA clean-up may also include a purification method such as, but not limited to, AGENCOURT® CLEANSEQ® system from Beckman Coulter (Danvers, MA), HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC) .

Capping and/or Tailing Reactions

[00222] The cell phenotype altering primary construct or mmRNA may also undergo capping and/or tailing reactions. A capping reaction may be performed by methods known in the art to add a 5' cap to the 5' end of the primary construct. Methods for capping include, but are not limited to, using a Vaccinia Capping enzyme (New England Biolabs, Ipswich, MA).

[00223] A poly-A tailing reaction may be performed by methods known in the art, such as, but not limited to, 2' O-methyltransferase and by methods as described herein. If the cell phenotype altering primary construct generated from cDNA does not include a poly-

T, it may be beneficial to perform the poly-A-tailing reaction before the cell phenotype altering primary construct is cleaned.

mRNA Purification

[00224] Cell phenotype altering primary construct or mmRNA purification may include, but is not limited to, mRNA or mmRNA clean-up, quality assurance and quality control. Cell phenotype altering mRNA or mmRNA clean-up may be performed by methods known in the arts such as, but not limited to, AGENCOURT® beads (Beckman Coulter Genomics, Danvers, MA), poly-T beads, LNA™ oligo-T capture probes (EXIQON® Inc, Vedbaek, Denmark) or HPLC based purification methods such as, but not limited to, strong anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC). The term "purified" when used in relation to a polynucleotide such as a "purified mRNA or mmRNA" refers to one that is separated from at least one contaminant. As used herein, a "contaminant" is any substance which makes another unfit, impure or inferior. Thus, a purified polynucleotide (e.g., DNA and RNA) is present in a form or setting different from that in which it is found in nature, or a form or setting different from that which existed prior to subjecting it to a treatment or purification method.

[00225] A quality assurance and/or quality control check may be conducted using methods such as, but not limited to, gel electrophoresis, UV absorbance, or analytical HPLC.

[00226] In another embodiment, the mRNA or mmRNA may be sequenced by methods including, but not limited to reverse-transcriptase-PCR.

[00227] In one embodiment, the cell phenotype altering mRNA or mmRNA may be quantified using methods such as, but not limited to, ultraviolet visible spectroscopy (UV/Vis). A non-limiting example of a UV/Vis spectrometer is a NANODROP® spectrometer (ThermoFisher, Waltham, MA). The quantified cell phenotype altering mRNA or mmRNA may be analyzed in order to determine if the mRNA or mmRNA may be of proper size, check that no degradation of the cell phenotype altering mRNA or mmRNA has occurred. Degradation of the cell phenotype altering mRNA and/or mmRNA may be checked by methods such as, but not limited to, agarose gel electrophoresis, HPLC based purification methods such as, but not limited to, strong

anion exchange HPLC, weak anion exchange HPLC, reverse phase HPLC (RP-HPLC), and hydrophobic interaction HPLC (HIC-HPLC), liquid chromatography-mass spectrometry (LCMS), capillary electrophoresis (CE) and capillary gel electrophoresis (CGE).

Signal Sequences

[00228] The cell phenotype altering primary constructs or mmRNA may also encode additional features which facilitate trafficking of the polypeptides to therapeutically relevant sites. One such feature which aids in protein trafficking is the signal sequence. As used herein, a "signal sequence" or "signal peptide" is a polynucleotide or polypeptide, respectively, which is from about 9 to 200 nucleotides (3-60 amino acids) in length which is incorporated at the 5' (or N-terminus) of the coding region or polypeptide encoded, respectively. Addition of these sequences result in trafficking of the encoded cell phenotype altering polypeptide to the endoplasmic reticulum through one or more secretory pathways. Some signal peptides are cleaved from the protein by signal peptidase after the proteins are transported.

[00229] Table 5 is a representative listing of protein signal sequences which may be incorporated for encoding by the cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention.

Table 5. Signal Sequences

ID	Description	NUCLEOTIDE SEQUENCE (5′-3′)	SEQ ID NO.	ENCODED PEPTIDE	SEQ ID NO.
SS-001	α-1-antitrypsin	ATGATGCCATCCTCAGTCTCATGGGGTATTTTGCTCTTGGCGGGTCTGTGCTGTCTCGTGCCGGTGTCGCTCGCA	18	MMPSSVSWGILLAGLCCLVPVSLA	80
SS-002	G-CSF	ATGGCCGGACCGGCGACTCAGTCGCCCATGAAACTCATGGCCCTGCAGTTGTTGCTTTGGCACTCAGCCCTCTGGACCGTCCAAGAGGCG	19	MAGPATQSPMKLMALQLLLWHSALWTVQEA	81
SS-003	Factor IX	ATGCAGAGAGTGAACATGATTATGGCCGAGTCCCCATCGCTCATCACAATCTGCCTGCTTGGTACCTGCTTTCCGCCGAATGCACTGTCTTTCTGGATCACGAGAATGCGAATAAGATCTTGAACCGACCCAAACGG	20	MQRVNMIMAESPSLITICLLGYLLSAECTVFLDHENANKILNRPKR	82
SS-004	Prolactin	ATGAAAGGATCATTGCTGTTGCTCCTCGTGTCGAACCTTCTGCTTTGCCAGTCCGTAGCCCCC	21	MKGSLLLLLVSNLLLCQSVAP	83

SS-005	Albumin	ATGAAATGGGTGACGTTCATCTCACTGTTGTTTTTGTTCTCGTCCGCCTACTCCAGGGGAGTATTCCGCCGA	22	MKWVTFISLLFLFSSAYSRGVFRR	84
SS-006	HMMSP38	ATGTGGTGGCGGCTCTGGTGGCTGCTCCTGTTGCTCCTCTTGCTGTGGCCCATGGTGTGGGCA	23	MWWRLWWLLLLLLLLPMWA	85
MLS-001	ornithine carbamoyl transferase	TGCTCTTTAACCTCCGCATCCTGTTGAATAACGCTGCGTTCCGAAATGGGCATAACTTCATGGTACGCAACTTCAGATGCGGCCAGCCACTCCAG	24	MLFNLRILLNNAAFRNGHNFMVRNFRCGQPLQ	86
MLS-002	Cytochrome C Oxidase subunit 8A	ATGTCCGTCTTGACACCCCTGCTCTTGAGAGGGCTGACGGGGTCCGCTAGACGCCTGCCGGTACCGCGAGCGAAGATCCACTCCCTG	25	MSVLTPLLLRGLTGSARRLPVPRAKIHSL	87
MLS-003	Cytochrome C Oxidase subunit 8A	ATGAGCGTGCTCACTCCGTTGCTTCTTCGAGGGCTTACGGGATCGGCTCGGAGGTTGCCCGTCCCGAGAGCGAAGATCCATTCGTTG	26	MSVLTPLLLRGLTGSARRLPVPRAKIHSL	88
SS-007	Type III, bacterial	TGACAAAAATAACTTTATCTCCCCAGAATTTTAGAATCCAAAAACAGGAAACCACACTACTAAAAGAAAAATCAACCGAGAAAAATTCTTTAGCAAAAAGTATTCTCGCAGTAAAAATCACTTCATCGAATTAAGGTCAAAATTATCGGAACGTTTTATTTCGCATAAGAACACT	27	MVTKITLSPQNFRIQKQETTLLKEKSTEKNSLAKSILAVKNHFIELRSKLSERFISHKNT	89
SS-008	Viral	ATGCTGAGCTTTGTGGATACCCGCACCCTGCTGCTGCTGGCGGTGACCAGCTGCCTGGCGACCTGCCAG	28	MLSFVDTRTLLLLAVTSCLATCQ	90
SS-009	viral	ATGGGCAGCAGCCAGGCGCCGCGCATGGGCAGCGTGGGCGGCCATGGCCTGATGGCGCTGCTGATGGCGGGCCTGATTCTGCCGGGCATTCTGGCG	29	MGSSQAPRMGSVGGHGLMALLMAGLILPGILA	91
SS-010	Viral	ATGGCGGGCATTTTTTATTTTCTGTTTAGCTTTCTGTTTGGCATTTGCGAT	30	MAGIFYFLFSFLFGICD	92
SS-011	Viral	ATGGAAAACCGCCTGCTGCGCGTGTTTCTGGTGTGGGCGGCGCTGACCATGGATGGCGCGAGCGCG	31	MENRLLRVFLVWAALTMDGASA	93
SS-012	Viral	ATGGCGCGCCAGGGCTGCTTTGGCAGCTATCAGGTGATTAGCCTGTTTACCTTTGCGATTGGCGTGAACCTGTGCCTGGGC	32	MARQGCFGSYQVISLFTFAIGVNLCLG	94
SS-013	*Bacillus*	ATGAGCCGCCTGCCGGTGCTGCTGCTGCTGCAGCTGCTGGTGCGCCCGGGCCTGCAG	33	MSRLPVLLLLQLLVRPGLQ	95
SS-014	*Bacillus*	ATGAAACAGCAGAAACGCCTGTATGCGCGCCTGCTGACCCTGCTGTTTGCGCTGATTTTTCTGCTGCCGCATAGCAGCGCGAGCGCG	34	MKQQKRLYARLLTLLFALIFLLPHSSASA	96

SS-015	Secretion signal	ATGGCGACGCCGCTGCCTCCGCCCTCCCCGCGGCACCTGCGGCTGCTGCGGCTGCTGCTCTCCGCCCTCGTCCTCGGC	35	MATPLPPPSPRHLRLLRLLLSG	97
SS-016	Secretion signal	ATGAAGGCTCCGGGTCGGCTCGTGCTCATCATCCTGTGCTCCGTGGTCTTCTCT	36	MKAPGRLVLIILCSWFS	98
SS-017	Secretion signal	ATGCTTCAGCTTTGGAAACTTGTTCTCCTGTGCGGCGTGCTCACT	37	MLQLWKLLCGVLT	99
SS-018	Secretion signal	ATGCTTTATCTCCAGGGTTGGAGCATGCCTGCTGTGGCA	38	MLYLQGWSMPAVA	100
SS-019	Secretion signal	ATGGATAACGTGCAGCCGAAAATAAAACATCGCCCCTTCTGCTTCAGTGTGAAAGGCCACGTGAAGATGCTGCGGCTGGATATTATCAACTCACTGGTAACAACAGTATTCATGCTCATCGTATCTGTGTTGGCACTGATACCA	39	MDNVQPKIKHRPFCFSVKGHVKMLRLDIINSLVTTVFMLIVSVLALIP	101
SS-020	Secretion signal	ATGCCCTGCCTAGACCAACAGCTCACTGTTCATGCCCTACCCTGCCCTGCCCAGCCCTCCTCTCTGGCCTTCTGCCAAGTGGGGTTCTTAACAGCA	40	MPCLDQQLTVHALPCPAQPSSLAFCQVGFLTA	102
SS-021	Secretion signal	ATGAAAACCTTGTTCAATCCAGCCCCTGCCATTGCTGACCTGGATCCCCAGTTCTACACCCTCTCAGATGTGTTCTGCTGCAATGAAAGTGAGGCTGAGATTTTAACTGGCCTCACGGTGGGCAGCGCTGCAGATGCT	41	MKTLFNPAPAIADLDPQFYTLSDVFCCNESEAEILTGLTVGSAADA	103
SS-022	Secretion signal	ATGAAGCCTCTCCTTGTTGTGTTTGTCTTTCTTTTCCTTTGGGATCCAGTGCTGGCA	42	MKPLLWFVFLFLWDPVLA	104
SS-023	Secretion signal	ATGTCCTGTTCCCTAAAGTTTACTTTGATTGTAATTTTTTTTTACTGTTGGCTTTCATCCAGC	43	MSCSLKFTLIVIFFTCTLSSS	105
SS-024	Secretion signal	ATGGTTCTTACTAAACCTCTTCAAAGAAATGGCAGCATGATGAGCTTTGAAAATGTGAAAGAAAAGAGCAGAGAAGGAGGGCCCCATGCACACACACCCGAAGAAGAATTGTGTTTCGTGGTAACACACTACCCTCAGGTTCAGACCACACTCAACCTGTTTTTCCATATATTCAAGGTTCTTACTCAACCACTTTCCCTTCTGTGGGGT	44	MVLTKPLQRNGSMMSFENVKEKSREGGPHAHTPEEELCFWTHTPQVQTTLNLFFHIFKVLTQPLSLLWG	106
SS-025	Secretion signal	ATGGCCACCCCGCCATTCCGGCTGATAAGGAAGATGTTTTCCTTCAAGGTGAGCAGATGGATGGGGCTTGCCTGCTTCCGGTCCCTGGCGGCATCC	45	MATPPFRLIRKMFSFKVSRWMGLACFRSLAAS	107
SS-026	Secretion signal	ATGAGCTTTTTCCAACTCCTGATGAAAAGGAAGGAACTCATTCCCTTGGTGGTGTTCATGACTGTGGCGGCGGGTGGAGCCTCATCT	46	MSFFQLLMKRKELIPLWFMTVAAGGASS	108
SS-027	Secretion signal	ATGGTCTCAGCTCTGCGGGGAGCACCCCTGATCAGGGTGCACTCAAGC	47	MVSALRGAPLIRVHSSPVSS	109

		CCTGTTTCTTCTCCTTCTGTGAGTG GACCACGGAGGCTGGTGAGCTGC CTGTCATCCCAAAGCTCAGCTCTG AGC		PSVSGPAALV SCLSSQSSAL S	
SS-028	Secretion signal	ATGATGGGGTCCCCAGTGAGTCAT CTGCTGGCCGGCTTCTGTGTGTGG GTCGTCTTGGGC	48	MMGSPVSHL LAGFCVWW LG	110
SS-029	Secretion signal	ATGGCAAGCATGGCTGCCGTGCTC ACCTGGGCTCTGGCTCTTCTTTCA GCGTTTTCGGCCACCCAGGCA	49	MASMAAVLT WALALLSAF SATQA	111
SS-030	Secretion signal	ATGGTGCTCATGTGGACCAGTGGT GACGCCTTCAAGACGGCCTACTTC CTGCTGAAGGGTGCCCCTCTGCAG TTCTCCGTGTGCGGCCTGCTGCAG GTGCTGGTGGACCTGGCCATCCTG GGGCAGGCCTACGCC	50	MVLMWTSG DAFKTAYFLL KGAPLQFSVC GLLQVLVDL AILGQATA	112
SS-031	Secretion signal	ATGGATTTTGTCGCTGGAGCCATC GGAGGCGTCTGCGGTGTTGCTGTG GGCTACCCCCTGGACACGGTGAA GGTCAGGATCCAGACGGAGCCAA AGTACACAGGCATCTGGCACTGC GTCCGGGATACGTATCACCGAGA GCGCGTGTGGG GCTTCTACCGGGGCCTCTCGCTGC CCGTGTGCACGGTGTCCCTGGTAT CTTCC	51	MDFVAGAIG GVCGVAVGY PLDTVKVRIQ TEPLYTGIWH CVRDTYHRE RVWGFYRGL SLPVCTVSLV SS	113
SS-032	Secretion signal	ATGGAGAAGCCCCTCTTCCCATTA GTGCCTTTGCATTGGTTTGGCTTT GGCTACACAGCACTGGTTGTTTCT GGTGGGATCGTTGGCTATGTAAAA ACAGGCAGCGTGCCGTCCCTGGCT GCAGGGCTGCTCTTCGGCAGTCTA GCC	52	MEKPLFPLVP LHWFGFGYT ALWSGGIVG YVKTGSVPSL AAGLLFGSLA	114
SS-033	Secretion signal	ATGGGTCTGCTCCTTCCCCTGGCA CTCTGCATCCTAGTCCTGTGC	53	MGLLLPLAL CILVLC	115
SS-034	Secretion signal	ATGGGGATCCAGACGAGCCCCGT CCTGCTGGCCTCCCTGGGGGTGGG GCTGGTCACTCTGCTCGGCCTGGC TGTGGGC	54	MGIQTSPVLL ASLGVGLVT LLGLAVG	116
SS-035	Secretion signal	ATGTCGGACCTGCTACTACTGGGC CTGATTGGGGGCCTGACTCTCTTA CTGCTGCTGACGCTGCTAGCCTTT GCC	55	MSDLLLLGLI GGLTLLLLLT LLAFA	117
SS-036	Secretion signal	ATGGAGACTGTGGTGATTGTTGCC ATAGGTGTGCTGGCCACCATGTTT CTGGCTTCGTTTGCAGCCTTGGTG CTGGTTTGCAGGCAG	56	METWIVAIG VLATIFLASF AALVLVCRQ	118
SS-037	Secretion signal	ATGCGCGGCTCTGTGGAGTGCACC TGGGGTTGGGGGCACTGTGCCCCC AGCCCCCTGCTCCTTTGGACTCTA CTTCTGTTTGCAGCCCCATTTGGC CTGCTGGGG	57	MAGSVECTW GWGHCAPSP LLLWTLLLFA APFGLLG	119
SS-038	Secretion signal	ATGATGCCGTCCCGTACCAACCTG GCTACTGGAATCCCCAGTAGTAAA	58	MMPSRTNLA TGIPSSKVKY	120

		GTGAAATATTCAAGGCTCTCCAGC ACAGACGATGGCTACATTGACCTT CAGTTTAAGAAAACCCCTCCTAAG ATCCCTTATAAGGCCATCGCACTT GCCACTGTGCTGTTTTTGATTGGC GCC		SRLSSTDDGY IDLQFKKTPP KIPYKAIALA TVLFLIGA	
SS-039	Secretion signal	ATGGCCCTGCCCCAGATGTGTGAC GGGAGCCACTTGGCCTCCACCCTC CGCTATTGCATGACAGTCAGCGGC ACAGTGGTTCTGGTGGCCGGGAC GCTCTGCTTCGCT	59	MALPQMCDG SHLASTLRYC MTVSGTWL VAGTLCFA	121
SS-041	Vrg-6	TGAAAAAGTGGTTCGTTGCTGCCG GCATCGGCGCTGCCGGACTCATGC TCTCCAGCGCCGCCA	60	MKKWFVAA GIGAGLLMLS SAA	122
SS-042	PhoA	ATGAAACAGAGCACCATTGCGCT GGCGCTGCTGCCGCTGCTGTTTAC CCCGGTGACCAAAGCG	61	MKQSTIALAL LPLLFTPVTK A	123
SS-043	OmpA	ATGAAAAAAACCGCGATTGCGAT TGCGGTGGCGCTGGCGGGCTTTGC GACCGTGGCGCAGGCG	62	MKKTAIAIAV ALAGFATVA QA	124
SS-044	STI	ATGAAAAAACTGATGCTGGCGAT TTTTTTTAGCGTGCTGAGCTTTCCG AGCTTTAGCCAGAGC	63	MKKLMLAIF FSVLSFPSFSQ S	125
SS-045	STII	ATGAAAAAAAACATTGCGTTTCTG CTGGCGAGCATGTTTGTGTTTAGC ATTGCGACCAACGCGTATGCG	64	MKKNIAFLL ASMFVFSIAT NAYA	126
SS-046	Amylase	ATGTTTGCGAAACGCTTTAAAACC AGCCTGCTGCCGCTGTTTGCGGGC TTTCTGCTGCTGTTTCATCTGGTGC TGGCGGGCCCGGCGGCGGCGAGC	65	MFAKRFKTS LLPLFAGFLL LFHLVLAGPA AAS	127
SS-047	Alpha Factor	ATGCGCTTTCCGAGCATTTTTACC GCGGTGCTGTTTGCGGCGAGCAGC GCGCTGGCG	66	MRFPSIFTAV LFAASSALA	128
SS-048	Alpha Factor	ATGCGCTTTCCGAGCATTTTTACC ACCGTGCTGTTTGCGGCGAGCAGC GCGCTGGCG	67	MRFPSIFTTV LFAASSALA	129
SS-049	Alpha Factor	ATGCGCTTTCCGAGCATTTTTACC AGCGTGCTGTTTGCGGCGAGCAGC GCGCTGGCG	68	MRFPSIFTSV LFAASSALA	130
SS-050	Alpha Factor	ATGCGCTTTCCGAGCATTTTTACC CATGTGCTGTTTGCGGCGAGCAGC GCGCTGGCG	69	MRFPSIFTHV LFAASSALA	131
SS-051	Alpha Factor	ATGCGCTTTCCGAGCATTTTTACC ATTGTGCTGTTTGCGGCGAGCAGC GCGCTGGCG	70	MRFPSIFTIVL FAASSALA	132
SS-052	Alpha Factor	ATGCGCTTTCCGAGCATTTTTACC TTTGTGCTGTTTGCGGCGAGCAGC GCGCTGGCG	71	MRFPSIFTFV LFAASSALA	133
SS-053	Alpha Factor	ATGCGCTTTCCGAGCATTTTTACC GAAGTGCTGTTTGCGGCGAGCAG CGCGCTGGCG	72	MRFPSIFTEV LFAASSALA	134
SS-054	Alpha Factor	ATGCGCTTTCCGAGCATTTTTACC GGCGTGCTGTTTGCGGCGAGCAGC GCGCTGGCG	73	MRFPSIFTGV LFAASSALA	135

ss-055	Endoglucanase V	ATGCGTTCCTCCCCCCTCCTCCGCTCCGCCGTTGTGGCCGCCCTGCCGGTGTTGGCCCTTGCC	74	MRSSPLLRSAWAALPVLALA	136
SS-056	Secretion signal	ATGGGCGCGGCGGCCGTGCGCTGGCACTTGTGCGTGCTGCTGGCCCTGGGCACACGCGGGCGGCTG	75	MGAAAVRWHLCVLLALGTRGRL	137
ss-057	Fungal	ATGAGGAGCTCCCTTGTGCTGTTCTTTGTCTCTGCGTGGACGGCCTTGGCCAG	76	MRSSLVLFFVSAWTALA	138
SS-058	Fibronectin	ATGCTCAGGGGTCCGGGACCCGGGCGGCTGCTGCTGCTAGCAGTCCTGTGCCTGGGGACATCGGTGCGCTGCACCGAAACCGGGAAGAGCAAGAGG	77	MLRGPGPGRLLLLAVLCLGTSVRCTETGKSKR	139
SS-059	Fibronectin	ATGCTTAGGGGTCCGGGGCCCGGGCTGCTGCTGCTGGCCGTCCAGCTGGGGACAGCGGTGCCCTCCACG	78	MLRGPGPGLLLLAVQCLGTAVPSTGA	140
ss-060	Fibronectin	ATGCGCCGGGGGGCCCTGACCGGGCTGCTCCTGGTCCTGTGCCTGAGTGTTGTGCTACGTGCAGCCCCCTCTGCAACAAGCAAGAAGCGCAGG	79	MRRGALTGLLLVLCLSWLRAAPSATSKKRR	141

[00230] In the table, SS is secretion signal and MLS is mitochondrial leader signal. The cell phenotype altering primary constructs or mmRNA of the present invention may be designed to encode any of the signal sequences of SEQ ID NOs 80-141, or fragments or variants thereof. These sequences may be included at the beginning of the polypeptide coding region, in the middle or at the terminus or alternatively into a flanking region. Further, any of the cell phenotype altering polynucleotide primary constructs of the present invention may also comprise one or more of the sequences defined by SEQ ID NOs 18-79. These may be in the first region or either flanking region.

[00231] Additional signal sequences which may be utilized in the present invention include those taught in, for example, databases such as those found at http://www.signalpeptide.de/ or http://proline.bic.nus.edu.sg/spdb/. Those described in US Patents 8,124,379; 7,413,875 and 7,385,034 are also within the scope of the invention and the contents of each are incorporated herein by reference in their entirety.

Target Selection

[00232] According to the present invention, the cell phenotype altering primary constructs comprise at least a first region of linked nucleosides encoding at least one cell phenotype altering polypeptide of interest. The cell phenotype altering polypeptides of interest or "Targets" of the present invention are listed in Table 6 below, and are

described in Tables 1, 2 and 3 of International Publication No. WO201 1130624 in addition to the IFN-signature genes, cell-specific polypeptides, death receptors and death receptor ligand and mitogen receptors in WO201 1130624; herein incorporated by reference in its entirety. Shown in Table 6, in addition to the name and description of the gene encoding the polypeptide of interest are the ENSEMBL Transcript ID (ENST), the ENSEMBL Protein ID (ENSP) and when available the optimized sequence ID (ORF SEQ ID). For any particular gene there may exist one or more variants or isoforms. Where these exist, they are shown in the table as well. It will be appreciated by those of skill in the art that disclosed in the Table are potential flanking regions. These are encoded in each ENST transcript either to the 5' (upstream) or 3' (downstream) of the ORF or coding region. The coding region is definitively and specifically disclosed by teaching the ENSP sequence. Consequently, the sequences taught flanking that encoding the protein are considered flanking regions. It is also possible to further characterize the 5' and 3' flanking regions by utilizing one or more available databases or algorithms. Databases have annotated the features contained in the flanking regions of the ENST transcripts and these are available in the art.

Table 6. Cell Phenotype Altering Targets

Target No	Gene	Description	ENST	SEQ ID NO	Optimized Trans SEQ ID NO	ENSP	SEQ ID NO
1	OCT4	POU class 5 homeobox 1	448657	142		416165	269
2	OCT4	POU class 5 homeobox 1	259915	143		259915	270
3	OCT4	POU class 5 homeobox 1	550572	144		448254	271
4	OCT4	POU class 5 homeobox 1	376243	145		365419	272
5	OCT4	POU class 5 homeobox 1	383524	146		373016	273
6	OCT4	POU class 5 homeobox 1	553206	147		446757	274
7	OCT4	POU class 5 homeobox 1	412166	148		387646	275
8	OCT4	POU class 5 homeobox 1	434616	149		388842	276
9	OCT4	POU class 5 homeobox 1	550521	150		447969	277
10	OCT4	POU class 5 homeobox 1	553069	151		448231	278
11	OCT4	POU class 5 homeobox 1	549294	152		446561	279
12	OCT4	POU class 5 homeobox 1	433348	153		412665	280
13	OCT4	POU class 5 homeobox 1	546505	154		448154	281
14	OCT4	POU class 5 homeobox 1	454714	155		400047	282
15	OCT4	POU class 5 homeobox 1	451077	156		391507	283
16	OCT4	POU class 5 homeobox 1	429603	157		392877	284
17	OCT4	POU class 5 homeobox 1	547234	158		449442	285
18	OCT4	POU class 5 homeobox 1	547658	159		446962	286

19	OCT4	POU class 5 homeobox 1	548682	160		446815	287
20	OCT4	POU class 5 homeobox 1	550059	161		447874	288
21	OCT4	POU class 5 homeobox 1	433063	162		405041	289
22	OCT4	POU class 5 homeobox 1	419095	163		413622	290
23	OCT4	POU class 5 homeobox 1	548685	164		447156	291
24	OCT4	POU class 5 homeobox 1	429314	165		387619	292
25	OCT4	POU class 5 homeobox 1	437747	166		391681	293
26	OCT4	POU class 5 homeobox 1	547981	167		446531	294
27	SOX1	SRY (sex determining region Y)-box 1	330949	168		330218	295
28	SOX2	SRY (sex determining region Y)-box 2	325404	169		323588	296
29	SOX2	SRY (sex determining region Y)-box 2	431565	170		439111	297
30	SOX3	SRY (sex determining region Y)-box 3	370536	171		359567	298
31	SOX15	SRY (sex determining region Y)-box 15	538513	172		439311	299
32	SOX18	SRY (sex determining region Y)-box 18	340356	173		341815	300
33	NANOG	Nanog homeobox	229307	174		229307	301
34	NANOG	Nanog homeobox	526286	175		435288	302
35	KLF1	Kruppel-like factor 1 (erythroid)	264834	176		264834	303
36	KLF2	Kruppel-like factor 2 (lung)	248071	177		248071	304
37	KLF4	Kruppel-like factor 4 (gut)	411706	178		399921	305
38	KLF4	Kruppel-like factor 4 (gut)	439281	179		396294	306
39	KLF4	Kruppel-like factor 4 (gut)	420475	180		404922	307
40	KLF4	Kruppel-like factor 4 (gut)	374672	181		363804	308
41	KLF5	Kruppel-like factor 5 (intestinal)	377687	182		366915	309
42	KLF5	Kruppel-like factor 5 (intestinal)	539231	183		440407	310
43	KLF5	Kruppel-like factor 5 (intestinal)	545883	184		443600	311
44	NR5A2	nuclear receptor subfamily 5, group A, member 2	537715	185		440930	312
45	NR5A2	nuclear receptor subfamily 5, group A, member 2	367357	186		356326	313
46	NR5A2	nuclear receptor subfamily 5, group A, member 2	367362	187		356331	314
47	NR5A2	nuclear receptor subfamily 5, group A, member 2	447034	188		414888	315
48	NR5A2	nuclear receptor subfamily 5, group A, member 2	544748	189		439116	316
49	NR5A2	nuclear receptor subfamily 5, group A, member 2	235480	190		235480	317
50	NR5A2	nuclear receptor subfamily 5, group A, member 2	236914	191		236914	318
51	NR5A2	nuclear receptor subfamily 5, group A, member 2	542116	192		443477	319

52	c-MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	524013	193		430235	320
53	c-MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	259523	194		259523	321
54	c-MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	377970	195		367207	322
55	c-MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	454617	196		4053 12	323
56	n-MYC	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	42621 1	197		390305	324
57	n-MYC	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	281043	198		281043	325
58	REM2	RAS (RAD and GEM)-like GTP binding 2	267396	199		267396	326
59	REM2	RAS (RAD and GEM)-like GTP binding 2	536884	200		442774	327
60	TERT	telomerase reverse transcriptase	296820	201		296820	328
61	TERT	telomerase reverse transcriptase	334602	202		334346	329
62	TERT	telomerase reverse transcriptase	310581	203		309572	330
63	TERT	telomerase reverse transcriptase	508104	204		426042	331
64	LIN28	lin-28 homolog A (C. elegans)	25423 1	205		25423 1	332
65	LIN28	lin-28 homolog A (C. elegans)	326279	206		3633 14	333
66	LIN28	lin-28 homolog B (C. elegans)	345080	207		344401	334
67	ASCL1	achaete-scute complex homolog 1 (Drosophila)	266744	208		266744	335
68	BRN2	POU class 3 homeobox 2	328345	209		329170	336
69	BRN2	POU class 3 homeobox 2	4251 16	210		390039	337
70	MYT1L	myelin transcription factor 1-like	428368	211		396103	338
71	MYT1L	myelin transcription factor 1-like	295067	212		295067	339
72	MYT1L	myelin transcription factor 1-like	399161	213		3821 14	340
73	MYT1L	myelin transcription factor 1-like	407844	214		384219	341
74	MYOD1	myogenic differentiation 1	250003	215		250003	342
75	CEBP-alpha	CCAAT/enhancer binding protein (C/EBP), alpha	498907	216		4275 14	343

76	PU.l	spleen focus forming virus (SFFV) proviral integration oncogene spil	378538	217		367799	344
77	PU.l	spleen focus forming virus (SFFV) proviral integration oncogene spil	227163	218		227163	345
78	PRDM16	PR domain containing 16	408992	219		386140	346
79	PRDM16	PR domain containing 16	378398	220		367651	347
80	PRDM16	PR domain containing 16	509860	221		425796	348
81	PRDM16	PR domain containing 16	270722	222		270722	349
82	PRDM16	PR domain containing 16	441472	223		407968	350
83	PRDM16	PR domain containing 16	442529	224		405253	351
84	HNF4-alpha	hepatocyte nuclear factor 4, alpha	443598	225		410911	352
85	HNF4-alpha	hepatocyte nuclear factor 4, alpha	316099	226		312987	353
86	HNF4-alpha	hepatocyte nuclear factor 4, alpha	316673	227		315180	354
87	HNF4-alpha	hepatocyte nuclear factor 4, alpha	338692	228		343807	355
88	HNF4-alpha	hepatocyte nuclear factor 4, alpha	457232	229		396216	356
89	HNF4-alpha	hepatocyte nuclear factor 4, alpha	415691	230		412111	357
90	BDNF	brain-derived neurotrophic factor	532997	231		435805	358
91	BDNF	brain-derived neurotrophic factor	525950	232		432035	359
92	BDNF	brain-derived neurotrophic factor	438929	233		414303	360
93	BDNF	brain-derived neurotrophic factor	525528	234		437138	361
94	BDNF	brain-derived neurotrophic factor	533246	235		432376	362
95	BDNF	brain-derived neurotrophic factor	533131	236		432727	363
96	BDNF	brain-derived neurotrophic factor	439476	237		389345	364
97	BDNF	brain-derived neurotrophic factor	395980	238		379304	365
98	BDNF	brain-derived neurotrophic factor	395983	239		379307	366
99	BDNF	brain-derived neurotrophic factor	395981	240		379305	367
100	BDNF	brain-derived neurotrophic factor	395986	241		379309	368
101	BDNF	brain-derived neurotrophic factor	395978	242		379302	369
102	BDNF	brain-derived neurotrophic factor	356660	243		349084	370
103	BDNF	brain-derived neurotrophic factor	530861	244		435564	371
104	BDNF	brain-derived neurotrophic	418212	245		400502	372

		factor					
105	BDNF	brain-derived neurotrophic factor	420794	246		389564	373
106	BDNF	brain-derived neurotrophic factor	314915	247		320002	374
107	NTF3	neurotrophin 3	423158	248		397297	375
108	NTF3	neurotrophin 3	331010	249		328738	376
109	NTF4	neurotrophin 4	30141 1	250		30141 1	377
110	EGF	epidermal growth factor	509793	251		424316	378
111	EGF	epidermal growth factor	265171	252		265171	379
112	EGF	epidermal growth factor	503392	253		421384	380
113	CNTF	ciliary neurotrophic factor	361987	254		355370	381
114	NGF	nerve growth factor (beta polypeptide)	369512	255		358525	382
115		sonic hedgehog	297261	256		297261	383
116	FGF-8	fibroblast growth factor 8 (androgen-induced)	320185	257		321797	384
117	FGF-8	fibroblast growth factor 8 (androgen-induced)	344255	258		340039	385
118	FGF-8	fibroblast growth factor 8 (androgen-induced)	347978	259		321945	386
119	FGF-8	fibroblast growth factor 8 (androgen-induced)	346714	260		344306	387
120	TGF-alpha	transforming growth factor, alpha	295400	261		295400	388
121	TGF-alpha	transforming growth factor, alpha	418333	262		404099	389
122	TGF-beta 1	transforming growth factor, beta 1	221930	263	268	221930	390
123	TGF-beta 2	transforming growth factor, beta 2	366930	264		355897	391
124	TGF-beta 2	transforming growth factor, beta 2	366929	265		355896	392
125	TGF-beta 3	transforming growth factor, beta 3	556285	266		451110	393
126	TGF-beta 3	transforming growth factor, beta 3	238682	267		238682	394

[00233] In one embodiment, the cell phenotype altering primary constructs may comprise at least a first region of linked nucleosides encoding the coding region of at least one cell phenotype altering polypeptide of interest. As a non-limiting example, the first region of linked nucleosides may encode the coding region for c-MYC, KLF4, Lin28, SOX2 or OCT4.

[00234] In one embodiment, the cell phenotype altering primary construct may comprise a first region of linked nucleosides which has been codon optimized.

[00235] In one embodiment, the the cell phenotype altering primary constructs may comprise any of the coding region sequences described in Table 7.

Table 7. Cell Phenotype Altering Coding Regions

Target No	Gene	Description	Sequence	SEQ ID NO
127	c-MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	AUGCCCCUCAACGUUAGCUUCACCAACAGGAACUA UGACCUCGACUACGACUCGGUGCAGCCGUAUUUC UACUGCGACGAGGAGGAGAACUUCUACCAGCAGC AGCAGCAGAGCGAGCUGCAGCCCCCGGCGCCCAGC GAGGAUAUCUGGAAGAAAUUCGAGCUGCUGCCCA CCCCGCCCCUGUCCCCUAGCCGCCGCUCCGGGCUC UGCUCGCCCUCCUACGUUGCGGUCACACCCUUCUC CCUUCGGGGAGACAACGACGGCGGUGGCGGGAGC UUCUCCACGGCCGACCAGCUGGAGAUGGUGACCG AGCUGCUGGGAGGAGACAUGGUGAACCAGAGUUU CAUCUGCGACCCGGACGACGAGACCUUCAUCAAAA ACAUCAUCAUCCAGGACUGUAUGUGGAGCGGCUU CUCGGCCGCCGCCAAGCUCGUCUCAGAGAAGCUGG CCUCCUACCAGGCUGCGCGCAAAGACAGCGGCAGC CCGAACCCCGCCCGCGGCCACAGCGUCUGCUCCAC CUCCAGCUUGUACCUGCAGGAUCUGAGCGCCGCCG CCUCAGAGUGCAUCGACCCCUCGGUGGUCUUCCCC UACCCUCUCAACGACAGCAGCUCGCCCAAGUCCUG CGCCUCGCAAGACUCCAGCGCCUUCUCUCCGUCCU CGGAUUCUCUGCUCUCCUCGACGGAGUCCUCCCCG CAGGGCAGCCCCGAGCCCCUGGUGCUCCAUGAGGA GACACCGCCCACCACCAGCAGCGACUCUGAGGAGG AACAAGAAGAUGAGGAAGAAAUCGAUGUUGUUUC UGUGGAAAAGAGGCAGGCUCCUGGCAAAAGGUCA GAGUCUGGAUCACCUUCUGCUGGAGGCCACAGCA AACCUCCUCACAGCCCACUGGUCCUCAAGAGGUGC CACGUCUCCACACAUCAGCACAACUACGCAGCGCC UCCCUCCACUCGGAAGGACUAUCCUGCUGCCAAGA GGGUCAAGUUGGACAGUGUCAGAGUCCUGAGACA GAUCAGCAACAACCGAAAAUGCACCAGCCCCAGGU CCUCGGACACCGAGGAGAAUGUCAAGAGGCGAAC ACACAACGUCUUGGAGCGCCAGAGGAGGAACGAG CUAAAACGGAGCUUUUUUGCCCUGCGUGACCAGA UCCCGGAGUUGGAAAACAAUGAAAAGGCCCCCAA GGUAGUUAUCCUUAAAAAAGCCACAGCAUACAUC CUGUCCGUCCAAGCAGAGGAGCAAAAGCUCAUUU CUGAAGAGGACUUGUUGCGGAAACGACGAGAACA GUUGAAACACAAACUUGAACAGCUACGGAACUCU UGUGCG	395
128	c-MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	ATGCCCCTCAACGTTAGCTTCACCAACAGGAACTAT GACCTCGACTACGACTCGGTGCAGCCGTATTTCTAC TGCGACGAGGAGGAGAACTTCTACCAGCAGCAGCA GCAGAGCGAGCTGCAGCCCCCGGCGCCCAGCGAGG ATATCTGGAAGAAATTCGAGCTGCTGCCCACCCCGC CCCTGTCCCCTAGCCGCCGCTCCGGGCTCTGCTCGC CCTCCTACGTTGCGGTCACACCCTTCTCCCTTCGGG	396

			GAGACAACGACGGCGGTGGCGGGAGCTTCTCCACG GCCGACCAGCTGGAGATGGTGACCGAGCTGCTGGG AGGAGACATGGTGAACCAGAGTTTCATCTGCGACC CGGACGACGAGACCTTCATCAAAAACATCATCATC CAGGACTGTATGTGGAGCGGCTTCTCGGCCGCCGCC AAGCTCGTCTCAGAGAAGCTGGCCTCCTACCAGGCT GCGCGCAAAGACAGCGGCAGCCCGAACCCCGCCCG CGGCCACAGCGTCTGCTCCACCTCCAGCTTGTACCT GCAGGATCTGAGCGCCGCCGCCTCAGAGTGCATCG ACCCCTCGGTGGTCTTCCCCTACCCTCTCAACGACA GCAGCTCGCCCAAGTCCTGCGCCTCGCAAGACTCCA GCGCCTTCTCTCCGTCCTCGGATTCTCTGCTCTCCTC GACGGAGTCCTCCCCGCAGGGCAGCCCCGAGCCCC TGGTGCTCCATGAGGAGACACCGCCCACCACCAGC AGCGACTCTGAGGAGGAACAAGAAGATGAGGAAG AAATCGATGTTGTTTCTGTGGAAAAGAGGCAGGCTC CTGGCAAAAGGTCAGAGTCTGGATCACCTTCTGCTG GAGGCCACAGCAAACCTCCTCACAGCCCACTGGTC CTCAAGAGGTGCCACGTCTCCACACATCAGCACAA CTACGCAGCGCCTCCCTCCACTCGGAAGGACTATCC TGCTGCCAAGAGGGTCAAGTTGGACAGTGTCAGAG TCCTGAGACAGATCAGCAACAACCGAAAATGCACC AGCCCCAGGTCCTCGGACACCGAGGAGAATGTCAA GAGGCGAACACACAACGTCTTGGAGCGCCAGAGGA GGAACGAGCTAAAACGGAGCTTTTTTGCCCTGCGTG ACCAGATCCCGGAGTTGGAAAACAATGAAAAGGCC CCCAAGGTAGTTATCCTTAAAAAAGCCACAGCATA CATCCTGTCCGTCCAAGCAGAGGAGCAAAAGCTCA TTTCTGAAGAGGACTTGTTGCGGAAACGACGAGAA CAGTTGAAACACAAACTTGAACAGCTACGGAACTC TTGTGCG	
129	KLF4	Kruppel-like factor 4 (gut)	AUGAGGCAGCCACCUGGCGAGUCUGACAUGGCUG UCAGCGACGCGCUGCUCCCAUCUUUCUCCACGUUC GCGUCUGGCCCGGCGGGAAGGGAGAAGACACUGC GUCAAGCAGGUGCCCCGAAUAACCGCUGGCGGGA GGAGCUCUCCCACAUGAAGCGACUUCCCCCAGUGC UUCCCGGCCGCCCCUAUGACCUGGCGGCGGCGACC GUGGCCACAGACCUGGAGAGCGGCGGAGCCGGUG CGGCUUGCGGCGGUAGCAACCUGGCGCCCCUACCU CGGAGAGAGACCGAGGAGUUCAACGAUCUCCUGG ACCUGGACUUUAUUCUCUCCAAUUCGCUGACCCAU CCUCCGGAGUCAGUGGCCGCCACCGUGUCCUCGUC AGCGUCAGCCUCCUCUUCGUCGUCGCCGUCGAGCA GCGGCCCUGCCAGCGCGCCCUCCACCUGCAGCUUC ACCUAUCCGAUCCGGGCCGGGAACGACCCGGGCGU GGCGCCGGGCGGCACGGGCGGAGGCCUCCUCUAUG GCAGGGAGUCCGCUCCCCCUCCGACGGCUCCCUUC AACCUGGCGGACAUCAACGACGUGAGCCCCUCGGG CGGCUUCGUGGCCGAGCUCCUGCGGCCAGAAUUG GACCCGGUGUACAUUCCGCCGCAGCAGCCGCAGCC GCCAGGUGGCGGGCUGAUGGGCAAGUUCGUGCUG AAGGCGUCGCUGAGCGCCCCUGGCAGCGAGUACG GCAGCCCGUCGGUCAUCAGCGUCAGCAAAGGCAGC CCUGACGGCAGCCACCCGGUGGUGGUGGCGCCCUA CAACGGCGGGCCGCCGCGCACGUGCCCCAAGAUCA	397

			AGCAGGAGGCGGUCUCUUCGUGCACCCACUUGGG CGCUGGACCCCCUCUCAGCAAUGGCCACCGGCCGG CUGCACACGACUUCCCCCUGGGGCGGCAGCUCCCC AGCAGGACUACCCCGACCCUGGGUCUUGAGGAAG UGCUGAGCAGCAGGGACUGUCACCCUGCCCUGCCG CUUCCUCCCGGCUUCCAUCCCCACCCGGGGCCCAA UUACCCAUCCUUCCUGCCCGAUCAGAUGCAGCCGC AAGUCCCGCCGCUCCAUUACCAAGAGCUCAUGCCA CCCGGUUCCUGCAUGCCAGAGGAGCCCAAGCCAAA GAGGGGAAGACGAUCGUGGCCCCGGAAAAGGACC GCCACCCACACUUGUGAUUACGCGGGCUGCGGCAA AACCUACACAAAGAGUUCCCAUCUCAAGGCACACC UGCGAACCCACACAGGUGAGAAACCUUACCACUG UGACUGGGACGGCUGUGGAUGGAAAUUCGCCCGC UCAGAUGAACUGACCAGGCACUACCGUAAACACA CGGGGCACCGCCCGUUCCAGUGCCAAAAAUGCGAC CGAGCAUUUUCCAGGUCGGACCACCUCGCCUUACA CAUGAAGAGGCAUUUU	
130	KLF4	Kruppel-like factor 4 (gut)	ATGAGGCAGCCACCTGGCGAGTCTGACATGGCTGT CAGCGACGCGCTGCTCCCATCTTTCTCCACGTTCGC GTCTGGCCCGGCGGGAAGGGAGAAGACACTGCGTC AAGCAGGTGCCCCGAATAACCGCTGGCGGGAGGAG CTCTCCCACATGAAGCGACTTCCCCCAGTGCTTCCC GGCCGCCCCTATGACCTGGCGGCGGCGACCGTGGC CACAGACCTGGAGAGCGGCGGAGCCGGTGCGGCTT GCGGCGGTAGCAACCTGGCGCCCCTACCTCGGAGA GAGACCGAGGAGTTCAACGATCTCCTGGACCTGGA CTTTATTCTCTCCAATTCGCTGACCCATCCTCCGGAG TCAGTGGCCGCCACCGTGTCCTCGTCAGCGTCAGCC TCCTCTTCGTCGTCGCCGTCGAGCAGCGGCCCTGCC AGCGCGCCCTCCACCTGCAGCTTCACCTATCCGATC CGGGCCGGGAACGACCCGGGCGTGGCGCCGGGCGG CACGGGCGGAGGCCTCCTCTATGGCAGGGAGTCCG CTCCCCCTCCGACGGCTCCCTTCAACCTGGCGGACA TCAACGACGTGAGCCCCTCGGGCGGCTTCGTGGCCG AGCTCCTGCGGCCAGAATTGGACCCGGTGTACATTC CGCCGCAGCAGCCGCAGCCGCCAGGTGGCGGGCTG ATGGGCAAGTTCGTGCTGAAGGCGTCGCTGAGCGC CCCTGGCAGCGAGTACGGCAGCCCGTCGGTCATCA GCGTCAGCAAAGGCAGCCCTGACGGCAGCCACCCG GTGGTGGTGGCGCCCTACAACGGCGGGCCGCCGCG CACGTGCCCCAAGATCAAGCAGGAGGCGGTCTCTT CGTGCACCCACTTGGGCGCTGGACCCCCTCTCAGCA ATGGCCACCGGCCGGCTGCACACGACTTCCCCCTGG GGCGGCAGCTCCCCAGCAGGACTACCCCGACCCTG GGTCTTGAGGAAGTGCTGAGCAGCAGGGACTGTCA CCCTGCCCTGCCGCTTCCTCCCGGCTTCCATCCCCA CCCGGGGCCCAATTACCCATCCTTCCTGCCCGATCA GATGCAGCCGCAAGTCCCGCCGCTCCATTACCAAG AGCTCATGCCACCCGGTTCCTGCATGCCAGAGGAGC CCAAGCCAAAGAGGGGAAGACGATCGTGGCCCCGG AAAAGGACCGCCACCCACACTTGTGATTACGCGGG CTGCGGCAAAACCTACACAAAGAGTTCCCATCTCA AGGCACACCTGCGAACCCACACAGGTGAGAAACCT TACCACTGTGACTGGGACGGCTGTGGATGGAAATTC	398

			GCCCGCTCAGATGAACTGACCAGGCACTACCGTAA ACACACGGGGCACCGCCCGTTCCAGTGCCAAAAAT GCGACCGAGCATTTTCCAGGTCGGACCACCTCGCCT TACACATGAAGAGGCATTTT	
131	LIN28	lin-28 homolog A	AUGGGAUCAGUCUCCAACCAACAAUUUGCCGGUG GGUGCGCCAAGGCAGCAGAGGAAGCGCCAGAAGA AGCUCCCGAGGAUGCCGCACGUGCAGCCGAUGAGC CUCAGCUGCUUCAUGGUGCAGGCAUUUGCAAGUG GUUCAAUGUUCGAAUGGGUUUUGGAUUCCUUUCA AUGACCGCAAGAGCAGGAGUGGCCCUUGAUCCAC CCGUGGAUGUGUUUGUGCACCAGUCGAAGCUGCA CAUGGAAGGAUUCCGCUCGCUUAAGGAAGGAGAA GCAGUCGAGUUUACCUUUAAGAAGUCUGCUAAGG GGCUCGAAAGCAUCAGAGUCACGGGACCAGGAGG UGUGUUUUGUAUCGGCUCGGAGCGGAGGCCUAAA GGGAAGUCCAUGCAAAAGCGCAGAUCAAAAGGAG ACAGGUGCUACAACUGUGGUGGUCUGGACCAUCA UGCGAAGGAAUGUAAGCUCCCUCCGCAGCCCAAA AAGUGUCACUUCUGUCAGUCCAUAUCGCAUAUGG UGGCAUCCUGUCCAUUGAAAGCACAGCAAGGCCC UAGCGCACAAGGCAAACCUACUUACUUUCGGGAA GAGGAGGAAGAAAUUCAUAGCCCUACUCUGCUGC CAGAAGCGCAAAAC	399
132	LIN28	lin-28 homolog A	ATGGGATCAGTCTCCAACCAACAATTTGCCGGTGGG TGCGCCAAGGCAGCAGAGGAAGCGCCAGAAGAAG CTCCCGAGGATGCCGCACGTGCAGCCGATGAGCCT CAGCTGCTTCATGGTGCAGGCATTTGCAAGTGGTTC AATGTTCGAATGGGTTTTGGATTCCTTTCAATGACC GCAAGAGCAGGAGTGGCCCTTGATCCACCCGTGGA TGTGTTTGTGCACCAGTCGAAGCTGCACATGGAAGG ATTCCGCTCGCTTAAGGAAGGAGAAGCAGTCGAGT TTACCTTTAAGAAGTCTGCTAAGGGGCTCGAAAGCA TCAGAGTCACGGGACCAGGAGGTGTGTTTTGTATCG GCTCGGAGCGGAGGCCTAAAGGGAAGTCCATGCAA AAGCGCAGATCAAAAGGAGACAGGTGCTACAACTG TGGTGGTCTGGACCATCATGCGAAGGAATGTAAGC TCCCTCCGCAGCCCAAAAAGTGTCACTTCTGTCAGT CCATATCGCATATGGTGGCATCCTGTCCATTGAAAG CACAGCAAGGCCCTAGCGCACAAGGCAAACCTACT TACTTTCGGGAAGAGGAGGAAGAAATTCATAGCCC TACTCTGCTGCCAGAAGCGCAAAAC	400
133	SOX2	SRY (sex determining region Y)-box 2	AUGUACAAUAUGAUGGAAACCGAACUGAAGCCAC CCGGUCCGCAACAGACGUCAGGCGGUGGCGGAGG UAAUUCCACUGCAGCAGCAGCAGGAGGGAAUCAG AAAAACUCUCCUGACAGAGUGAAGCGCCCUAUGA ACGCAUUCAUGGUCUGGUCAAGAGGACAGAGACG GAAGAUGGCACAAGAAAAUCCGAAAAUGCACAAC UCAGAGAUCAGCAAGAGACUUGGCGCUGAAUGGA AACUUCUGUCCGAGACGGAAAAGCGGCCUUUUAU AGACGAAGCAAAGAGGCUUCGCGCACUCCAUAUG AAGGAACAUCCCGAUUACAAGUACCGUCCAAGAC GAAAAACCAAGACUCUUAUGAAGAAGGAUAAGUA CACUCUUCCUGGUGGACUGCUGGCGCCAGGGGGA AAUUCGAUGGCCUCGGGAGUCGGGGUCGGAGCUG	401

			GACUGGGAGCGGGAGUGAACCAACGCAUGGAUUC GUACGCCCAUAUGAACGGUUGGAGCAAUGGCAGC UAUUCCAUGAUGCAAGAUCAACUGGGAUACCCCC AACAUCCCGGUCUUAACGCCCACGGCGCAGCACAA AUGCAGCCUAUGCACCGGUACGAUGUUUCGGCGC UGCAAUACAACUCGAUGACCUCCUCACAGACUUAC AUGAACGGUUCCCCAACCUAUUCGAUGUCAUACU CGCAGCAAGGGACCCCUGGCAUGGCACUCGGUAGC AUGGGAUCAGUGGUGAAAUCCGAAGCAAGCAGCA GCCCUCCAGUGGUCACUUCCAGCUCCCAUUCGCGU GCGCCUUGUCAAGCUGGCGACCUCAGGGACAUGA UUUCGAUGUACCUGCCAGGAGCCGAGGUGCCGGA GCCCGCAGCCCCAUCGCGAUUGCACAUGUCACAGC AUUACCAGUCCGGACCAGUGCCUGGUACCGCCAUU AACGGGACCCUCCCUUUGUCCCAUAUG	
134	SOX2	SRY (sex determining region Y)-box 2	ATGTACAATATGATGGAAACCGAACTGAAGCCACC CGGTCCGCAACAGACGTCAGGCGGTGGCGGAGGTA ATTCCACTGCAGCAGCAGCAGGAGGGAATCAGAAA AACTCTCCTGACAGAGTGAAGCGCCCTATGAACGC ATTCATGGTCTGGTCAAGAGGACAGAGACGGAAGA TGGCACAAGAAAATCCGAAAATGCACAACTCAGAG ATCAGCAAGAGACTTGGCGCTGAATGGAAACTTCT GTCCGAGACGGAAAAGCGGCCTTTTATAGACGAAG CAAAGAGGCTTCGCGCACTCCATATGAAGGAACAT CCCGATTACAAGTACCGTCCAAGACGAAAAACCAA GACTCTTATGAAGAAGGATAAGTACACTCTTCCTGG TGGACTGCTGGCGCCAGGGGGAAATTCGATGGCCT CGGGAGTCGGGGTCGGAGCTGGACTGGGAGCGGGA GTGAACCAACGCATGGATTCGTACGCCCATATGAA CGGTTGGAGCAATGGCAGCTATTCCATGATGCAAG ATCAACTGGGATACCCCCAACATCCCGGTCTTAACG CCCACGGCGCAGCACAAATGCAGCCTATGCACCGG TACGATGTTTCGGCGCTGCAATACAACTCGATGACC TCCTCACAGACTTACATGAACGGTTCCCCAACCTAT TCGATGTCATACTCGCAGCAAGGGACCCCTGGCATG GCACTCGGTAGCATGGGATCAGTGGTGAAATCCGA AGCAAGCAGCAGCCCTCCAGTGGTCACTTCCAGCTC CCATTCGCGTGCGCCTTGTCAAGCTGGCGACCTCAG GGACATGATTTCGATGTACCTGCCAGGAGCCGAGG TGCCGGAGCCCGCAGCCCCATCGCGATTGCACATGT CACAGCATTACCAGTCCGGACCAGTGCCTGGTACCG CCATTAACGGGACCCTCCCTTTGTCCCATATG	402
135	OCT4	POU class 5 homeobox 1	AUGGCAGGACAUCUCGCAUCAGACUUCGCAUUUU CACCACCACCAGGAGGAGGAGGGGACGGACCAGG GGGUCCGGAGCCGGGAUGGGUCGACCCGAGGACU UGGCUGAGCUUCCAAGGCCCGCCUGGCGGACCCGG AAUCGGACCGGGCGUCGGGCCAGGCUCCGAGGUC UGGGGAAUCCCACCUUGCCCUCCGCCAUACGAGUU CUGCGGCGGGAUGGCCUAUUGCGGUCCGCAAGUG GGUGUGGGACUCGUGCCCCAGGGCGGAUUGGAAA CCUCGCAGCCGGAAGGUGAAGCUGGCGUGGGCGU UGAGUCGAACUCCGAUGGAGCCUCCCCGGAGCCUU GCACCGUCACCCCGGGAGCCGUGAAGCUCGAGAAA GAAAAGCUCGAACAGAACCCCGAAGAGAGCCAAG AUAUCAAGGCACUCCAGAAAGAACUCGAACAGUU	403

			UGCGAAGCUGCUGAAGCAGAAGCGGAUCACUCUG GGUUACACCCAGGCCGAUGUGGGACUGACUCUCG GUGUGCUGUUCGGGAAGGUGUUCUCUCAAACGAC UAUCUGUAGAUUCGAGGCCCUGCAGCUGUCGUUC AAGAAUAUGUGUAAACUGCGCCCCCUGCUGCAAA AAUGGGUGGAAGAAGCAGACAACAACGAGAACUU GCAAGAGAUUUGCAAGGCCGAAACCUUGGUGCAA GCCCGCAAGAGGAAGCGGACCAGCAUCGAAAAUC GCGUUAGAGGAAAUCUUGAGAACCUGUUCCUUCA GUGCCCAAAGCCAACGCUGCAGCAAAUUUCACACA UCGCGCAGCAGCUCGGACUGGAGAAAGACGUGGU GCGAGUGUGGUUCUGCAACCGCCGGCAGAAAGGA AAGAGAUCCAGCUCAGAUUACGCGCAGCGGGAGG ACUUUGAAGCUGCCGGAUCCCCCUUUUCGGGGGG ACCGGUCAGCUUCCCACUGGCCCCUGGCCCGCACU UUGGUACCCCGGGAUACGGAUCCCCGCACUUCACU GCUCUGUACUCGUCGGUCCCCUUCCCGGAAGGCGA AGCGUUCCCUCCUGUCUCAGUGACUACUCUUGGA UCGCCGAUGCAUAGCAAU	
136	OCT4	POU class 5 homeobox 1	ATGGCAGGACATCTCGCATCAGACTTCGCATTTTCA CCACCACCAGGAGGAGGAGGGGACGGACCAGGGG GTCCGGAGCCGGGATGGGTCGACCCGAGGACTTGG CTGAGCTTCCAAGGCCCGCCTGGCGGACCCGGAAT CGGACCGGGCGTCGGGCCAGGCTCCGAGGTCTGGG GAATCCCACCTTGCCCTCCGCCATACGAGTTCTGCG GCGGGATGGCCTATTGCGGTCCGCAAGTGGGTGTG GGACTCGTGCCCCAGGGCGGATTGGAAACCTCGCA GCCGGAAGGTGAAGCTGGCGTGGGCGTTGAGTCGA ACTCCGATGGAGCCTCCCCGGAGCCTTGCACCGTCA CCCCGGGAGCCGTGAAGCTCGAGAAAGAAAAGCTC GAACAGAACCCCGAAGAGAGCCAAGATATCAAGGC ACTCCAGAAAGAACTCGAACAGTTTGCGAAGCTGC TGAAGCAGAAGCGGATCACTCTGGGTTACACCCAG GCCGATGTGGGACTGACTCTCGGTGTGCTGTTCGGG AAGGTGTTCTCTCAAACGACTATCTGTAGATTCGAG GCCCTGCAGCTGTCGTTCAAGAATATGTGTAAACTG CGCCCCCTGCTGCAAAAATGGGTGGAAGAAGCAGA CAACAACGAGAACTTGCAAGAGATTTGCAAGGCCG AAACCTTGGTGCAAGCCCGCAAGAGGAAGCGGACC AGCATCGAAAATCGCGTTAGAGGAAATCTTGAGAA CCTGTTCCTTCAGTGCCCAAAGCCAACGCTGCAGCA AATTTCACACATCGCGCAGCAGCTCGGACTGGAGA AAGACGTGGTGCGAGTGTGGTTCTGCAACCGCCGG CAGAAAGGAAAGAGATCCAGCTCAGATTACGCGCA GCGGGAGGACTTTGAAGCTGCCGGATCCCCCTTTTC GGGGGGACCGGTCAGCTTCCCACTGGCCCCTGGCCC GCACTTTGGTACCCCGGGATACGGATCCCCGCACTT CACTGCTCTGTACTCGTCGGTCCCCTTCCCGGAAGG CGAAGCGTTCCCTCCTGTCTCAGTGACTACTCTTGG ATCGCCGATGCATAGCAAT	404

Protein Cleavage Signals and Sites

[00236] In one embodiment, the cell phenotype altering polypeptides of the present invention may include at least one protein cleavage signal containing at least one protein cleavage site. The protein cleavage site may be located at the N-terminus, the C-terminus, at any space between the N- and the C- termini such as, but not limited to, half-way between the N- and C-termini, between the N-terminus and the half way point, between the half way point and the C-terminus, and combinations thereof.

[00237] The cell phenotype altering polypeptides of the present invention may include, but is not limited to, a proprotein convertase (or prohormone convertase), thrombin or Factor Xa protein cleavage signal. Proprotein convertases are a family of nine proteinases, comprising seven basic amino acid-specific subtilisin-like serine proteinases related to yeast kexin, known as prohormone convertase 1/3 (PC 1/3), PC2, furin, PC4, PC5/6, paired basic amino-acid cleaving enzyme 4 (PACE4) and PC7, and two other subtilases that cleave at non-basic residues, called subtilisin kexin isozyme 1 (SKI-1) and proprotein convertase subtilisin kexin 9 (PCSK9). Non-limiting examples of protein cleavage signal amino acid sequences are listed in Table 7 of US Patent Publication No US20130259924, filed March 9, 2013, the contents of which is herein incorporated by reference in its entirety.

[00238] In one embodiment, the cell phenotype altering primary constructs and the cell phenotype altering mmRNA of the present invention may be engineered such that the cell phenotype altering primary construct or mmRNA contains at least one encoded protein cleavage signal. The encoded protein cleavage signal may be located before the start codon, after the start codon, before the coding region, within the coding region such as, but not limited to, half way in the coding region, between the start codon and the half way point, between the half way point and the stop codon, after the coding region, before the stop codon, between two stop codons, after the stop codon and combinations thereof.

[00239] In one embodiment, the cell phenotype altering primary constructs or mmRNA of the present invention may include at least one encoded protein cleavage signal containing at least one protein cleavage site. The encoded protein cleavage signal may include, but is not limited to, a proprotein convertase (or prohormone convertase), thrombin and/or Factor Xa protein cleavage signal. One of skill in the art may use Table 1 above or other known methods to determine the appropriate encoded protein cleavage

signal to include in the primary constructs or mmRNA of the present invention. For example, starting with the protein cleavage site sequences and considering the codons of Table 1 one can design a signal for the cell phenotype altering primary construct which can produce a protein signal in the resulting polypeptide.

[00240] In one embodiment, the cell phenotype altering polypeptides of the present invention include at least one protein cleavage signal and/or site.

[00241] As a non-limiting example, U.S. Pat. No. 7,374,930 and U.S. Pub. No. 20090227660, herein incorporated by reference in their entireties, use a furin cleavage site to cleave the N-terminal methionine of GLP-1 in the expression product from the Golgi apparatus of the cells. In one embodiment, the polypeptides of the present invention include at least one protein cleavage signal and/or site with the proviso that the polypeptide is not GLP-1.

[00242] In one embodiment, the cell phenotype altering primary constructs or mmRNA of the present invention includes at least one encoded protein cleavage signal and/or site.

[00243] In one embodiment, the cell phenotype altering primary constructs or mmRNA of the present invention includes at least one encoded protein cleavage signal and/or site with the proviso that the primary construct or mmRNA does not encode GLP-1 .

[00244] In one embodiment, the cell phenotype alteringprimary constructs or mmRNA of the present invention may include more than one coding region. Where multiple coding regions are present in the cell phenotype alteringprimary construct or mmRNA of the present invention, the multiple coding regions may be separated by encoded protein cleavage sites. As a non-limiting example, the cell phenotype alteringprimary construct or mmRNA may be signed in an ordered pattern. On such pattern follows AXBY form where A and B are coding regions which may be the same or different coding regions and/or may encode the same or different polypeptides, and X and Y are encoded protein cleavage signals which may encode the same or different protein cleavage signals. A second such pattern follows the form AXYBZ where A and B are coding regions which may be the same or different coding regions and/or may encode the same or different polypeptides, and X, Y and Z are encoded protein cleavage signals which may encode the same or different protein cleavage signals. A third pattern follows the form ABXCY where A, B and C are coding regions which may be the same or different coding regions

and/or may encode the same or different polypeptides, and X and Y are encoded protein cleavage signals which may encode the same or different protein cleavage signals.

[00245] In one embodiment, the cell phenotype altering polypeptides, primary constructs and mmRNA can also contain sequences that encode protein cleavage sites so that the cell phenotype altering polypeptides, primary constructs and mmRNA can be released from a carrier region or a fusion partner by treatment with a specific protease for said protein cleavage site.

III. Modifications

[00246] Herein, in a cell phenotype altering polynucleotide (such as a cell phenotype altering primary construct or an mRNA molecule), the terms "modification" or, as appropriate, "modified" refer to modification with respect to A, G, U or C ribonucleotides. Generally, herein, these terms are not intended to refer to the ribonucleotide modifications in naturally occurring 5'-terminal mRNA cap moieties. In a polypeptide, the term "modification" refers to a modification as compared to the canonical set of 20 amino acids, moiety)

[00247] The modifications may be various distinct modifications. In some embodiments, the coding region, the flanking regions and/or the terminal regions may contain one, two, or more (optionally different) nucleoside or nucleotide modifications. In some embodiments, a modified cell phenotype altering polynucleotide, primary construct, or mmRNA introduced to a cell may exhibit reduced degradation in the cell, as compared to an unmodified cell phenotype altering polynucleotide, primary construct, or mmRNA.

[00248] The cell phenotype altering polynucleotides, primary constructs, and mmRNA can include any useful modification, such as to the sugar, the nucleobase, or the internucleoside linkage *(e.g.* to a linking phosphate / to a phosphodiester linkage / to the phosphodiester backbone).. One or more atoms of a pyrimidine nucleobase may be replaced or substituted with optionally substituted amino, optionally substituted thiol, optionally substituted alkyl (e.g., methyl or ethyl), or halo (e.g., chloro or fluoro). In certain embodiments, modifications (e.g., one or more modifications) are present in each of the sugar and the internucleoside linkage. Modifications according to the present invention may be modifications of ribonucleic acids (RNAs) to deoxyribonucleic acids

(DNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs) or hybrids thereof). Additional modifications are described herein.

[00249] As described herein, the cell phenotype altering polynucleotides, primary constructs, and mmRNA of the invention do not substantially induce an innate immune response of a cell into which the mRNA is introduced. Featues of an induced innate immune response include 1) increased expression of pro-inflammatory cytokines, 2) activation of intracellular PRRs (RIG-I, MDA5, etc, and/or 3) termination or reduction in protein translation.

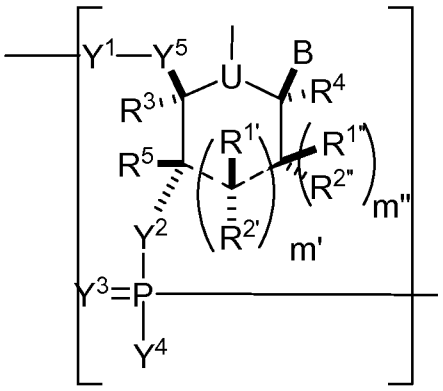
[00250] In certain embodiments, it may desirable to intracellularly degrade a modified nucleic acid molecule introduced into the cell. For example, degradation of a modified nucleic acid molecule may be preferable if precise timing of protein production is desired. Thus, in some embodiments, the invention provides a modified cell phenotype altering nucleic acid molecule containing a degradation domain, which is capable of being acted on in a directed manner within a cell.

[00251] The cell phenotype altering polynucleotides, primary constructs, and mmRNA can optionally include other agents (e.g., RNAi-inducing agents, RNAi agents, siRNAs, shRNAs, miRNAs, antisense RNAs, ribozymes, catalytic DNA, tRNA, RNAs that induce triple helix formation, aptamers, vectors, etc.). In some embodiments, the cell phenotype altering polynucleotides, primary constructs, or mmRNA may include one or more messenger RNAs (mRNAs) and one or more modified nucleoside or nucleotides (e.g., mmRNA molecules). Details for these cell phenotype altering polynucleotides, primary constructs, and mmRNA follow.

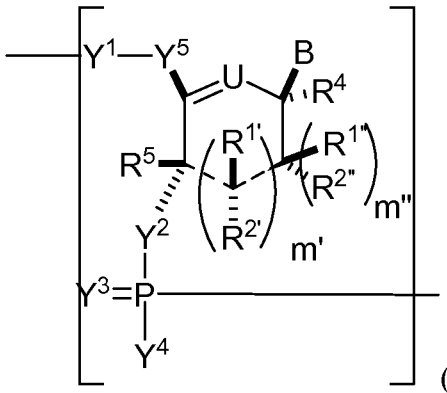
Cell phenotype altering Polynucleotides and Primary Constructs

[00252] The cell phenotype altering polynucleotides, primary constructs, and mmRNA of the invention includes a first region of linked nucleosides encoding a cell phenotype altering polypeptide of interest, a first flanking region located at the 5' terminus of the first region, and a second flanking region located at the 3' terminus of the first region.

[00253] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, first flanking region, or second flanking region) includes n number of linked nucleosides having Formula (la) or Formula (Ia-1):



(Ia)



(Ia-1) or a pharmaceutically acceptable salt or stereoisomer thereof,

[00254] wherein

[00255] U is O, S, $N(R^u)_{n_u}$, or $C(R^u)_{n_u}$, wherein nu is an integer from **0** to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl;

[00256] — is a single bond or absent;

[00257] each of $R^{1'}$, $R^{2'}$, $R^{1''}$, $R^{2''}$, R^1, R^2, R^3, R^4, and R^5 is, independently, if present, H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl,or absent; wherein the combination of R^3 with one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, $R^{2''}$, or R^5 (e.g., the combination of $R^{1'}$ and R^3, the combination of $R^{1''}$ and R^3, the combination of $R^{2'}$ and R^3, the combination of $R^{2''}$ and R^3, or the combination of R^5 and R^3) can join together to form optionally substituted alkylene or optionally substituted heteroalkylene and, taken together with the carbons to which they are attached, provide an optionally substituted heterocyclyl (e.g., a bicyclic, tricyclic, or tetracyclic heterocyclyl); wherein the combination of R^5 with one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, or $R^{2''}$ (e.g., the combination of $R^{1'}$ and R^5, the combination of $R^{1''}$ and R^5, the combination of $R^{2'}$ and R^5, or the combination of $R^{2''}$ and R^5) can join together to form optionally substituted alkylene or optionally substituted heteroalkylene and, taken together with the carbons to which they are attached, provide an optionally substituted heterocyclyl (e.g., a bicyclic, tricyclic, or tetracyclic heterocyclyl); and wherein the combination of R^4 and one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, $R^{2''}$, R^3, or R^5 can join together to form optionally substituted alkylene or optionally

substituted heteroalkylene and, taken together with the carbons to which they are attached, provide an optionally substituted heterocyclyl (e.g., a bicyclic, tricyclic, or tetracyclic heterocyclyl);each of m' and m" is, independently, an integer from 0 to 3 (e.g., from 0 to 2, from 0 to 1, from 1 to 3, or from 1 to 2);

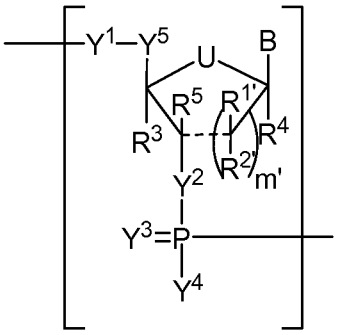
[00258] each of Y^1, Y^2, and Y^3, is, independently, O, S, Se, $-NR^{N1}-$, optionally substituted alkylene, or optionally substituted heteroalkylene, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, or absent;

[00259] each Y^4 is, independently, H, hydroxy, thiol, boranyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted thioalkoxy, optionally substituted alkoxyalkoxy, or optionally substituted amino;

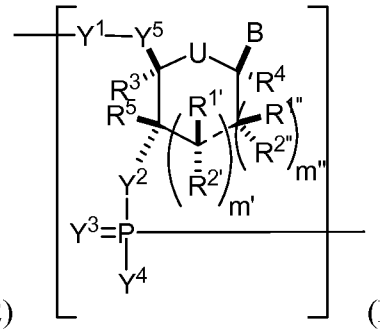
[00260] each Y^5 is, independently, O, S, Se, optionally substituted alkylene (e.g., methylene), or optionally substituted heteroalkylene;

[00261] n is an integer from 1 to 100,000; and

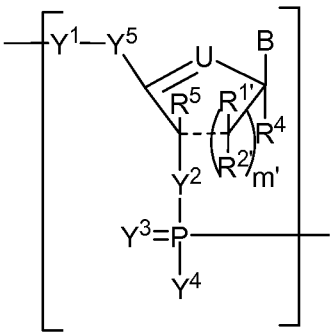
[00262] B is a nucleobase (e.g., a purine, a pyrimidine, or derivatives thereof), wherein the combination of B and $R^{1'}$, the combination of B and $R^{2'}$, the combination of B and $R^{1''}$, or the combination of B and $R^{2''}$ can, taken together with the carbons to which they are attached, optionally form a bicyclic group (e.g., a bicyclic heterocyclyl) or wherein the combination of B, $R^{1''}$, and R^3 or the combination of B, $R^{2''}$, and R^3 can optionally form a tricyclic or tetracyclic group (e.g., a tricyclic or tetracyclic heterocyclyl, such as in Formula (IIo)-(IIp) herein). In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes a modified ribose. In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, the first flanking region, or the second flanking region) includes n number of linked nucleosides having Formula (Ia-2)-(Ia-5) or a pharmaceutically acceptable salt or stereoisomer thereof.



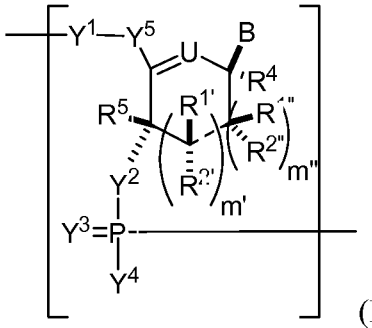
(Ia-2)



(Ia-3)

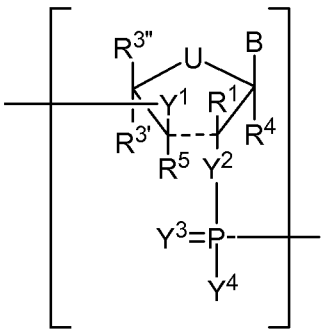


(Ia-4),

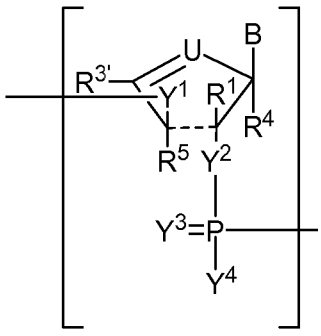


(Ia-5).

[00263] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, the first flanking region, or the second flanking region) includes n number of linked nucleosides having Formula (lb) or Formula (Ib-1):



(Ib),



(Ib-1) or a pharmaceutically acceptable salt or stereoisomer thereof,

[00264] wherein

[00265] U is O, S, $N(R^u)_{n_u}$, or $C(R^u)_{n_u}$, wherein nu is an integer from **0** to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl;

[00266] — is a single bond or absent;

[00267] each of R^1, $R^{3'}$, $R^{3''}$, and R^4 is, independently, H, halo, hydroxy, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally

substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, or absent; and wherein the combination of R^1 and $R^{3'}$ or the combination of R^1 and $R^{3''}$ can be taken together to form optionally substituted alkylene or optionally substituted heteroalkylene (e.g., to produce a locked nucleic acid);

[00268] each R^5 is, independently, H, halo, hydroxy, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, or absent;

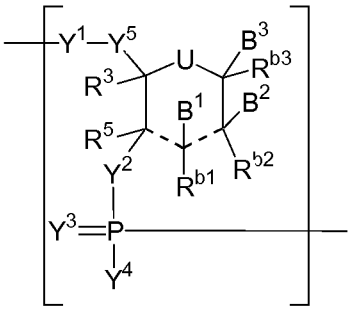
[00269] each of Y^1, Y^2, and Y^3 is, independently, O, S, Se, -NR^{N1}-, optionally substituted alkylene, or optionally substituted heteroalkylene, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted aryl;

[00270] each Y^4 is, independently, H, hydroxy, thiol, boranyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted alkoxyalkoxy, or optionally substituted amino;

[00271] n is an integer from 1 to 100,000; and

[00272] B is a nucleobase.

[00273] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, first flanking region, or second flanking region) includes n number of linked nucleosides having Formula (Ic):



(Ic), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00274] wherein

[00275] U is O, S, $N(R^u)_{n_u}$, or $C(R^u)_{n_u}$, wherein nu is an integer from 0 to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl;

[00276] — is a single bond or absent;

[00277] each of B^1, B^2, and B^3 is, independently, a nucleobase (e.g., a purine, a pyrimidine, or derivatives thereof, as described herein), H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, or optionally substituted aminoalkynyl, wherein one and only one of B^1, B^2, and B^3 is a nucleobase;

[00278] each of R^{b1}, R^{b2}, R^{b3}, R^3, and R^5 is, independently, H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl or optionally substituted aminoalkynyl;

[00279] each of Y^1, Y^2, and Y^3, is, independently, O, S, Se, $-NR^{N1}-$, optionally substituted alkylene, or optionally substituted heteroalkylene, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted aryl;

[00280] each Y^4 is, independently, H, hydroxy, thiol, boranyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted thioalkoxy, optionally substituted alkoxyalkoxy, or optionally substituted amino;

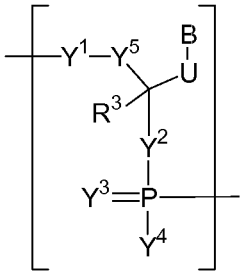
[00281] each Y^5 is, independently, O, S, Se, optionally substituted alkylene (e.g., methylene), or optionally substituted heteroalkylene;

[00282] n is an integer from 1 to 100,000; and

[00283] wherein the ring including U can include one or more double bonds.

[00284] In particular embodiments, the ring including U does not have a double bond between U-CB^3R^{b3} or between CB^3R^{b3}-$C^{B2}R^{b2}$.

[00285] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, first flanking region, or second flanking region) includes n number of linked nucleosides having Formula (Id):



(Id), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00286] wherein

[00287] U is O, S, $N(R^u)_{n_u}$, or $C(R^u)_{n_u}$, wherein nu is an integer from **0** to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl;

[00288] each R^3 is, independently, H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, or optionally substituted aminoalkynyl;

[00289] each of Y^1, Y^2, and Y^3, is, independently, O, S, Se, -NR^{N1}-, optionally substituted alkylene, or optionally substituted heteroalkylene, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted aryl;

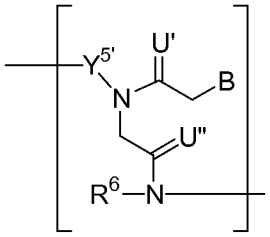
[00290] each Y^4 is, independently, H, hydroxy, thiol, boranyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted thioalkoxy, optionally substituted alkoxyalkoxy, or optionally substituted amino;

[00291] each Y^5 is, independently, O, S, optionally substituted alkylene (e.g., methylene), or optionally substituted heteroalkylene;

[00292] n is an integer from 1 to 100,000; and

[00293] B is a nucleobase (e.g., a purine, a pyrimidine, or derivatives thereof).

[00294] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, first flanking region, or second flanking region) includes n number of linked nucleosides having Formula (Ie):



(Ie), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00295] wherein

[00296] each of U' and U" is, independently, O, S, $N(R^u)_{n_u}$, or $C(R^u)_{n_u}$, wherein nu is an integer from 0 to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl;

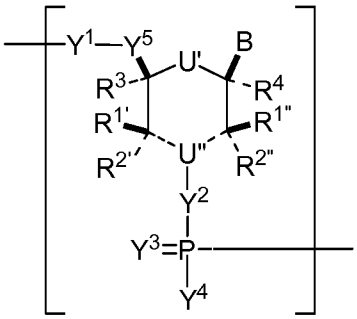
[00297] each R^6 is, independently, H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, or optionally substituted aminoalkynyl;

[00298] each $Y^{5'}$ is, independently, O, S, optionally substituted alkylene (e.g., methylene or ethylene), or optionally substituted heteroalkylene;

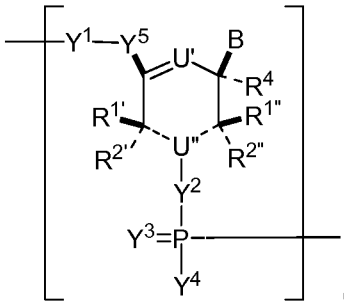
[00299] n is an integer from 1 to 100,000; and

[00300] B is a nucleobase (e.g., a purine, a pyrimidine, or derivatives thereof).

[00301] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, first flanking region, or second flanking region) includes n number of linked nucleosides having Formula (If) or (If-1):



(If),



(If-1), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00302] wherein

[00303] each of U' and U" is, independently, O, S, N, $N(R^u)_{n_u}$, or $C(R^u)_{n_u}$, wherein nu is an integer from 0 to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl (e.g., U' is O and U" is N);

[00304] — is a single bond or absent;

[00305] each of $R^{1'}$, $R^{2'}$, $R^{1"}$, $R^{2"}$, R^3, and R^4 is, independently, H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, or absent; and wherein the combination of $R^{1'}$ and R^3, the combination of $R^{1"}$ and R^3, the combination of $R^{2'}$ and R^3, or the combination of $R^{2"}$ and R^3 can be taken together to form optionally substituted alkylene or optionally substituted heteroalkylene (e.g., to produce a locked nucleic acid);each of m' and m" is, independently, an integer from 0 to 3 (e.g., from 0 to 2, from 0 to 1, from 1 to 3, or from 1 to 2);

[00306] each of Y^1, Y^2, and Y^3, is, independently, O, S, Se, $-NR^{N1}-$, optionally substituted alkylene, or optionally substituted heteroalkylene, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, or absent;

[00307] each Y^4 is, independently, H, hydroxy, thiol, boranyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally

substituted thioalkoxy, optionally substituted alkoxyalkoxy, or optionally substituted amino;

[00308] each Y^5 is, independently, O, S, Se, optionally substituted alkylene (e.g., methylene), or optionally substituted heteroalkylene;

[00309] n is an integer from 1 to 100,000; and

[00310] B is a nucleobase (e.g., a purine, a pyrimidine, or derivatives thereof).

[00311] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (la), (Ia-l)-(Ia-3), (Ib)-(If), and (Ila)-(IIp)), the ring including U has one or two double bonds.

[00312] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), each of R^1, $R^{1'}$, and $R^{1"}$, if present, is H. In further embodiments, each of R^2, $R^{2'}$, and $R^{2"}$, if present, is, independently, H, halo (e.g., fluoro), hydroxy, optionally substituted alkoxy (e.g., methoxy or ethoxy), or optionally substituted alkoxyalkoxy. In particular embodiments, alkoxyalkoxy is -(CH2)s2(OCH2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl). In some embodiments, s2 is 0, si is 1 or 2, s3 is 0 or 1, and R' is Ci-6 alkyl.

[00313] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), each of R^2, $R^{2'}$, and $R^{2"}$, if present, is H. In further embodiments, each of R^1, $R^{1'}$, and $R^{1"}$, if present, is, independently, H, halo (e.g., fluoro), hydroxy, optionally substituted alkoxy (e.g., methoxy or ethoxy), or optionally substituted alkoxyalkoxy. In particular embodiments, alkoxyalkoxy is -(CH2)s2(OCH2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl). In some embodiments, s2 is 0, si is 1 or 2, s3 is 0 or 1, and R' is Ci-6 alkyl.

[00314] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2),

(IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), each of R^3, R^4, and R^5 is, independently, H, halo (e.g., fluoro), hydroxy, optionally substituted alkyl, optionally substituted alkoxy (e.g., methoxy or ethoxy), or optionally substituted alkoxyalkoxy. In particular embodiments, R^3 is H, R^4 is H, R^5 is H, or R^3, R^4, and R^5 are all H. In particular embodiments, R^3 is Ci-6 alkyl, R^4 is Ci-6 alkyl, R^5 is Ci-6 alkyl, or R^3, R^4, and R^5 are all Ci-6 alkyl. In particular embodiments, R^3 and R^4 are both H, and R^5 is Ci-6 alkyl.

[00315] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), R^3 and R^5 join together to form optionally substituted alkylene or optionally substituted heteroalkylene and, taken together with the carbons to which they are attached, provide an optionally substituted heterocyclyl (e.g., a bicyclic, tricyclic, or tetracyclic heterocyclyl, such as trans-3',4' analogs, wherein R^3 and R^5 join together to form heteroalkylene (e.g., -(CH2)biO(CH2)b20(CH2)b3-, wherein each of bl, b2, and b3 are, independently, an integer from 0 to 3).

[00316] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), R^3 and one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, $R^{2''}$, or R^5 join together to form optionally substituted alkylene or optionally substituted heteroalkylene and, taken together with the carbons to which they are attached, provide an optionally substituted heterocyclyl (e.g., a bicyclic, tricyclic, or tetracyclic heterocyclyl, R^3 and one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, $R^{2''}$, or R^5 join together to form heteroalkylene (e.g., -(CH2)biO(CH2)b20(CH2)b3-, wherein each of bl, b2, and b3 are, independently, an integer from 0 to 3).

[00317] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), R^5 and one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, or $R^{2''}$ join together to form optionally substituted alkylene or optionally substituted heteroalkylene and, taken together with the carbons to which they are attached, provide an optionally substituted heterocyclyl (e.g., a bicyclic, tricyclic, or

tetracyclic heterocyclyl, R^5 and one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, or $R^{2''}$ join together to form heteroalkylene (e.g., -(CH2)biO(CH2)b20(CH2)b3-, wherein each of bl, b2, and b3 are, independently, an integer from 0 to 3).

[00318] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), each Y^2 is, independently, O, S, or -NR^{N1}-, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted aryl. In particular embodiments, Y^2 is NR^{N1}-, wherein R^{N1} is H or optionally substituted alkyl (e.g., Ci-6 alkyl, such as methyl, ethyl, isopropyl, or n-propyl).

[00319] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), each Y^3 is, independently, O or S.

[00320] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), R^1 is H; each R^2 is, independently, H, halo (e.g., fluoro), hydroxy, optionally substituted alkoxy (e.g., methoxy or ethoxy), or optionally substituted alkoxyalkoxy (e.g., -(CH2)s2(OCH $_2$CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl, such as wherein s2 is 0, si is 1 or 2, s3 is 0 or 1, and R' is Ci-6 alkyl); each Y^2 is, independently, O or -NR^{N1}-, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted aryl (e.g., wherein R^{N1} is H or optionally substituted alkyl (e.g., Ci-6 alkyl, such as methyl, ethyl, isopropyl, or n-propyl)); and each Y^3 is, independently, O or S (e.g., S). In further embodiments, R^3 is H, halo (e.g., fluoro), hydroxy, optionally substituted alkyl, optionally substituted alkoxy (e.g., methoxy or ethoxy), or optionally substituted alkoxyalkoxy. In yet further embodiments, each Y^1 is , independently, O or -NR^{N1}-, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally

substituted alkynyl, or optionally substituted aryl (e.g., wherein R^{N1} is H or optionally substituted alkyl (e.g., Ci-6 alkyl, such as methyl, ethyl, isopropyl, or n-propyl)); and each Y^4 is, independently, H, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted thioalkoxy, optionally substituted alkoxyalkoxy, or optionally substituted amino.

[00321] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), each R^1 is, independently, H, halo (e.g., fluoro), hydroxy, optionally substituted alkoxy (e.g., methoxy or ethoxy), or optionally substituted alkoxyalkoxy (e.g., -(CH2)s2(OCH2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl, such as wherein s2 is 0, si is 1 or 2, s3 is 0 or 1, and R' is Ci-6 alkyl); R^2 is H; each Y^2 is, independently, O or -NR^{N1}-, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted aryl (e.g., wherein R^{N1} is H or optionally substituted alkyl (e.g., Ci-6 alkyl, such as methyl, ethyl, isopropyl, or n-propyl)); and each Y^3 is, independently, O or S (e.g., S). In further embodiments, R^3 is H, halo (e.g., fluoro), hydroxy, optionally substituted alkyl, optionally substituted alkoxy (e.g., methoxy or ethoxy), or optionally substituted alkoxyalkoxy. In yet further embodiments, each Y^1 is , independently, O or -NR^{N1}-, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted aryl (e.g., wherein R^{N1} is H or optionally substituted alkyl (e.g., Ci-6 alkyl, such as methyl, ethyl, isopropyl, or n-propyl)); and each Y^4 is, independently, H, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted thioalkoxy, optionally substituted alkoxyalkoxy, or optionally substituted amino.

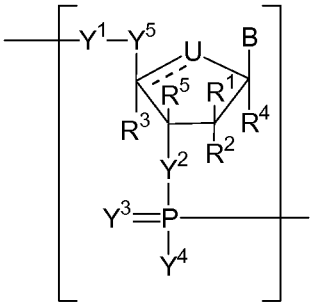
[00322] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), the ring including U is in the β-D (e.g., β-D-ribo) configuration.

[00323] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), the ring including U is in the a-L (e.g., a-L-ribo) configuration.

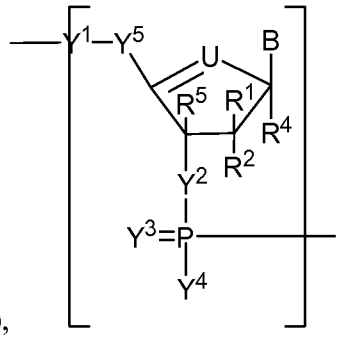
[00324] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), one or more B is not pseudouridine (ψ) or 5-methyl-cytidine (m^5C). In some embodiments, about 10% to about 100% of n number of B nucleobases is not ψ or m^5C (e.g., from 10%> to 20%>, from 10% to 35%, from 10% to 50%, from 10% to 60%, from 10% to 75%, from 10% to 90%, from 10% to 95%, from 10% to 98%, from 10% to 99%, from 20% to 35%, from 20% to 50%, from 20% to 60%, from 20% to 75%, from 20% to 90%, from 20% to 95%, from 20% to 98%, from 20% to 99%, from 20% to 100%, from 50% to 60%, from 50% to 75%, from 50% to 90%, from 50% to 95%, from 50% to 98%, from 50% to 99%, from 50% to 100%, from 75% to 90%, from 75% to 95%, from 75% to 98%, from 75% to 99%, and from 75% to 100% of n number of B is not ψ or m^5C). In some embodiments, B is not ψ or m^5C.

[00325] In some embodiments of the cell phenotype altering polynucleotides, primary constructs, or mmRNA (e.g., Formulas (Ia)-(Ia-5), (Ib)-(If-l), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr)), when B is an unmodified nucleobase selected from cytosine, guanine, uracil and adenine, then at least one of Y^1, Y^2, or Y^3 is not O.

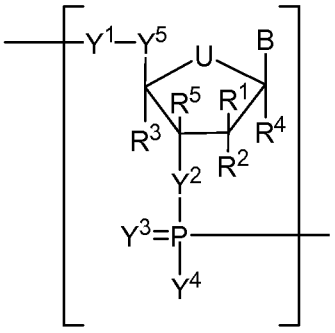
[00326] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes a modified ribose. In some embodiments, the polynucleotide, primary construct, or mmRNA (e.g., the first region, the first flanking region, or the second flanking region) includes n number of linked nucleosides having Formula (Ila)-(IIc):



(IIa),

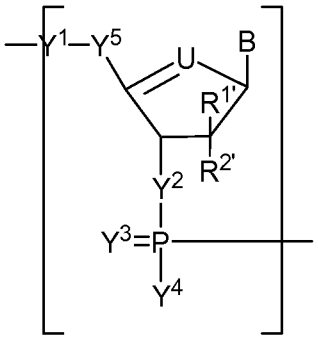


(IIb), or

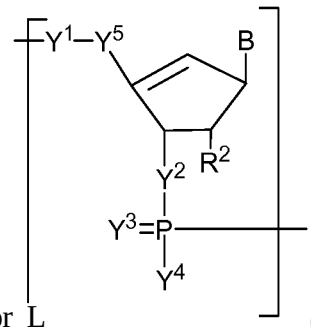


(He), or a pharmaceutically acceptable salt or stereoisomer thereof. In particular embodiments, U is O or $C(R^U)_{n_u}$, wherein nu is an integer from 0 to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl (e.g., U is $-CH_2-$ or -CH-). In other embodiments, each of R^1, R^2, R^3, R^4, and R^5 is, independently, H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, or absent (e.g., each R^1 and R^2 is, independently, H, halo, hydroxy, optionally substituted alkyl, or optionally substituted alkoxy; each R^3 and R^4 is, independently, H or optionally substituted alkyl; and R^5 is H or hydroxy), and --- is a single bond or double bond.

[00327] In particular embodiments, the cell phenotype altering polynucleotides or mmRNA includes n number of linked nucleosides having Formula (lib- 1)-(IIb-2):

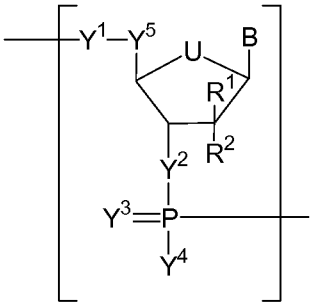


(IIb-l) or L

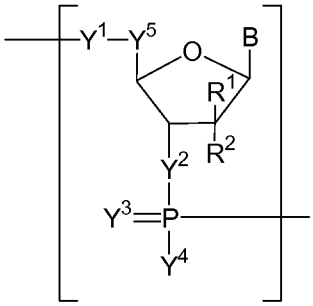


(IIb-2) or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, U is O or $C(R^U)_{n_u}$, wherein nu is an integer from **0** to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl (e.g., U is $-CH_2-$ or -CH-). In other embodiments, each of R^1 and R^2 is, independently, H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, or absent (e.g., each R^1 and R^2 is, independently, H, halo, hydroxy, optionally substituted alkyl, or optionally substituted alkoxy, e.g., H, halo, hydroxy, alkyl, or alkoxy). In particular embodiments, R^2 is hydroxy or optionally substituted alkoxy (e.g., methoxy, ethoxy, or any described herein).

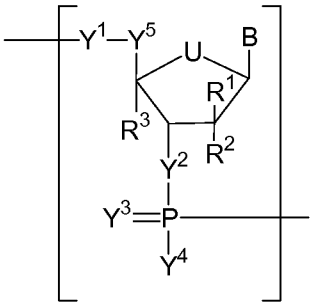
[00328] In particular embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes n number of linked nucleosides having Formula (IIc-l)-(IIc-4):



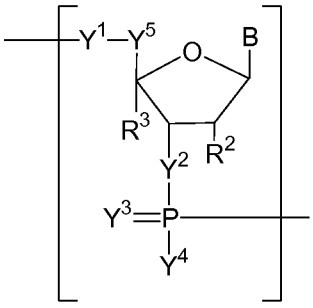
(IIc-1),



(IIc-2),



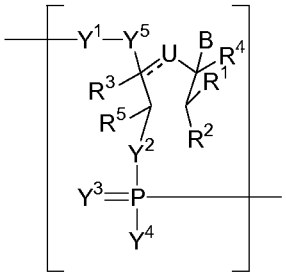
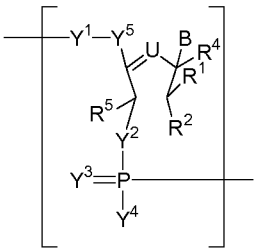
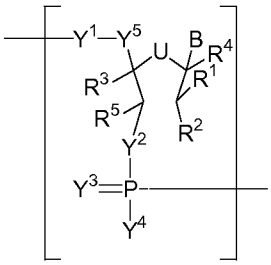
(IIc-3), or



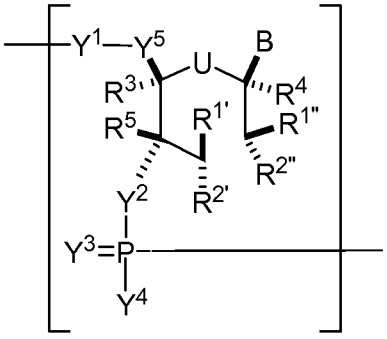
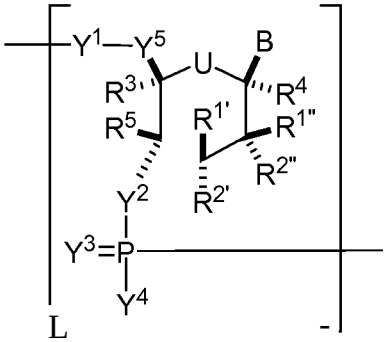
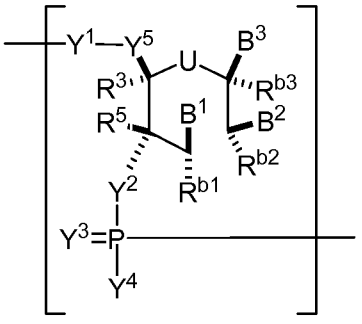
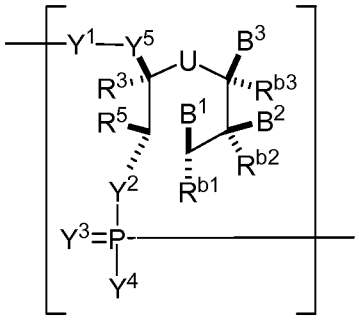
(IIc-4), or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, U is O or $C(R^u)_{nu}$, wherein nu is an integer from 0 to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl (e.g., U is $-CH_2-$ or -CH-). In some embodiments, each of R^1, R^2, and R^3 is, independently, H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, or absent (e.g., each R^1 and R^2 is, independently, H, halo, hydroxy, optionally substituted alkyl, or optionally substituted alkoxy, e.g., H, halo, hydroxy, alkyl, or alkoxy; and each R^3 is, independently, H or optionally substituted alkyl)). In particular embodiments, R^2 is optionally substituted alkoxy (e.g., methoxy or ethoxy, or any described herein). In particular embodiments, R^1 is optionally substituted alkyl, and R^2 is hydroxy. In other embodiments, R^1 is hydroxy, and R^2 is optionally substituted alkyl. In further embodiments, R^3 is optionally substituted alkyl.

[00329] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes an acyclic modified ribose. In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first

region, the first flanking region, or the second flanking region) includes n number of linked nucleosides having Formula (IId)-(IIf):

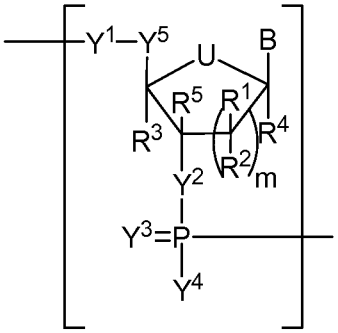

(IId),

(IIe), or

(IIf), or a pharmaceutically acceptable salt or stereoisomer thereof.

[00330] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes an acyclic modified hexitol. In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, the first flanking region, or the second flanking region) includes n number of linked nucleosides Formula (Ilg)-(IIj):

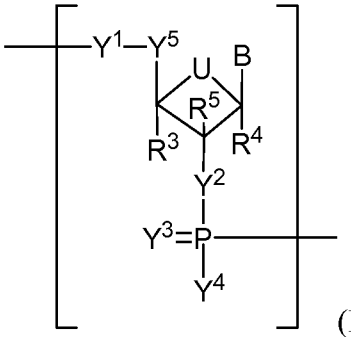

(iig),

(iih),

(IIi), or

(IIj), or a pharmaceutically acceptable salt or stereoisomer thereof.

[00331] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes a sugar moiety having a contracted or an expanded ribose ring. In some embodiments, the cell phenotype altering polynucleotide, primary

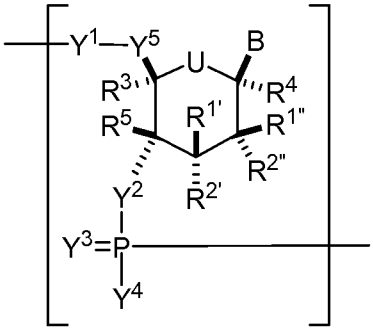
construct, or mmRNA (e.g., the first region, the first flanking region, or the second flanking region) includes n number of linked nucleosides having Formula (Ilk)-(IIm):



(IIk),

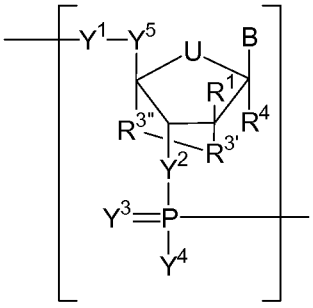


(III), or



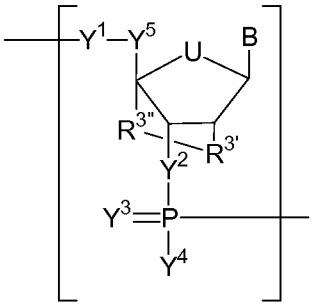
(IIm),or a pharmaceutically acceptable salt or stereoisomer thereof, wherein each of $R^{1'}$, $R^{1"}$, $R^{2'}$, and $R^{2"}$ is, independently, H, halo, hydroxy, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, or absent; and wherein the combination of $R^{2'}$ and R^3 or the combination of $R^{2"}$ and R^3 can be taken together to form optionally substituted alkylene or optionally substituted heteroalkylene.

[00332] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes a locked modified ribose. In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, the first flanking region, or the second flanking region) includes n number of linked nucleosides having Formula (Iln):

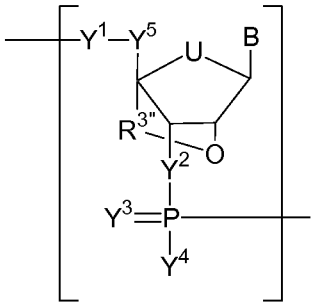


(Iln), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein $R^{3'}$ is O, S, or -NR^{N1}-, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted aryl and $R^{3"}$ is optionally substituted alkylene (e.g., -CH2-, -CH2CH2-, or -CH2CH2CH2-) or optionally substituted heteroalkylene (e.g., -CH2NH-, -CH2CH2NH-, -CH2OCH2-, or -CH2CH20CH2-)(e.g., $R^{3'}$ is O and $R^{3"}$ is optionally substituted alkylene (e.g., -CH2-, -CH2CH2-, or -CH2CH2CH2-)).

[00333] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes n number of linked nucleosides having Formula (**IIn-1)**-(II-n2):



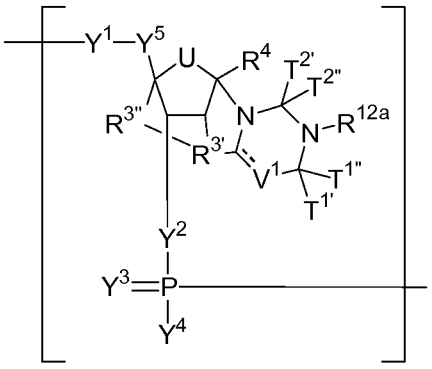
(IIn-1) or



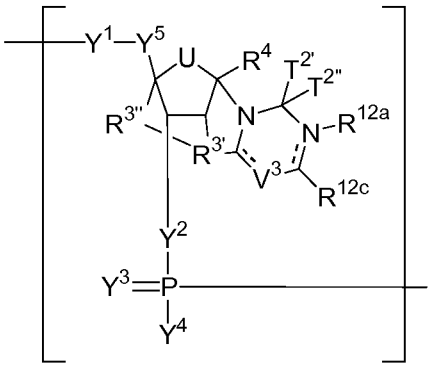
(IIn-2), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein $R^{3'}$ is O, S, or -NR^{N1}-, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted aryl and $R^{3"}$ is optionally substituted alkylene (e.g., -CH2-, -CH2CH2-, or -CH2CH2CH2-) or optionally substituted heteroalkylene (e.g., -CH2NH-, -CH2CH2NH-, -CH2OCH2-, or -CH2CH2OCH2-) (e.g., $R^{3'}$ is O and $R^{3"}$ is optionally substituted alkylene (e.g., -CH2-, -CH2CH2-, or -CH2CH2CH2-))·

[00334] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes a locked modified ribose that forms a tetracyclic

heterocyclyl. In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, the first flanking region, or the second flanking region) includes n number of linked nucleosides having Formula (Ho):



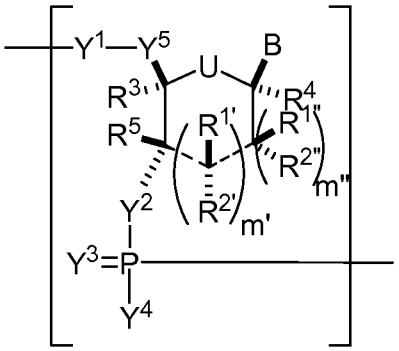
(IIo) or



(IIp), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein R^{12a}, R^{12c}, $T^{1'}$, $T^{1''}$,$T^{2'}$, $T^{2''}$, V^1, and V^3 are as described herein.

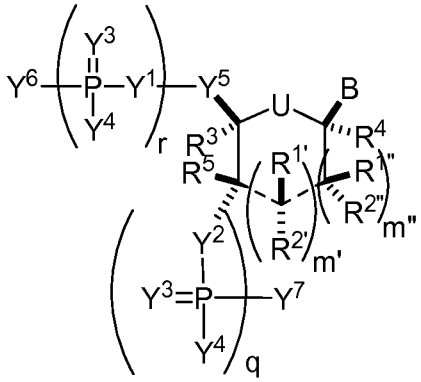
[00335] Any of the formulas for the cell phenotype altering polynucleotides, primary constructs, or mmRNA can include one or more nucleobases described herein (e.g., Formulas (bl)-(b43)).

[00336] In one embodiment, the present invention provides methods of preparing a cell phenotype altering polynucleotide, primary construct, or mmRNA comprising at least one nucleotide, wherein the cell phenotype altering polynucleotide comprises n number of nucleosides having Formula (la), as defined herein:



(la),

the method comprising reacting a compound of Formula (Ilia), as defined herein:

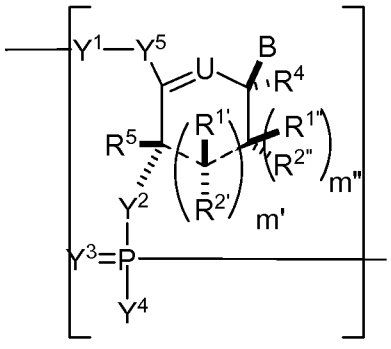


(IIIa),

with an RNA polymerase, and a cDNA template.

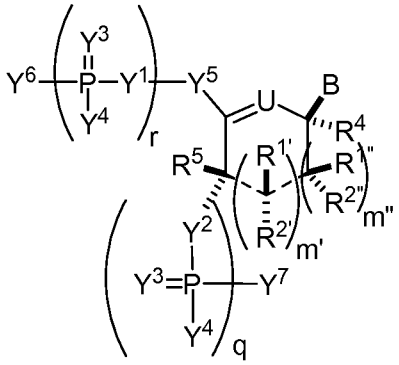
[00337] In a further embodiment, the present invention provides methods of amplifying a cell phenotype altering polynucleotide, primary construct, or mmRNA comprising at least one nucleotide (e.g., mmRNA molecule), the method comprising: reacting a compound of Formula (Ilia), as defined herein, with a primer, a cDNA template, and an RNA polymerase.

[00338] In one embodiment, the present invention provides methods of preparing a cell phenotype altering polynucleotide, primary construct, or mmRNA comprising at least one nucleotide (e.g., mmRNA molecule), wherein the cell phenotype altering polynucleotide comprises n number of nucleosides having Formula (Ia-1), as defined herein:



(Ia-l),

the method comprising reacting a compound of Formula (IIIa-1), as defined herein:

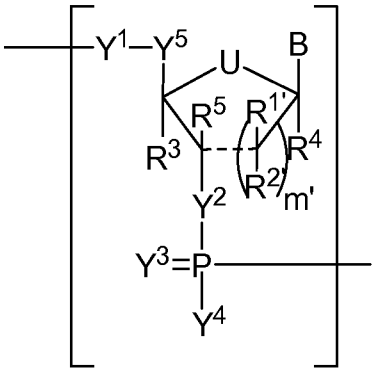


(IIIa-1),

with an RNA polymerase, and a cDNA template.

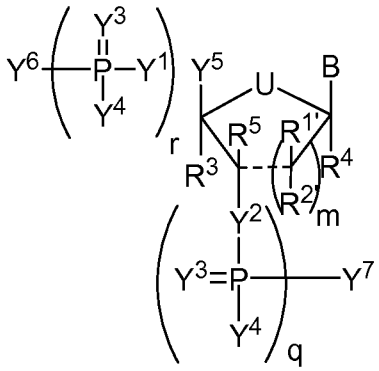
[00339] In a further embodiment, the present invention provides methods of amplifying a cell phenotype altering polynucleotide, primary construct, or mmRNA comprising at least one nucleotide (e.g., mmRNA molecule), the method comprising: reacting a compound of Formula (IIIa-1), as defined herein, with a primer, a cDNA template, and an RNA polymerase.

[00340] In one embodiment, the present invention provides methods of preparing a modified cell phenotype altering mRNA comprising at least one nucleotide (e.g., mmRNA molecule), wherein the polynucleotide comprises n number of nucleosides having Formula (Ia-2), as defined herein:



(Ia-2),

the method comprising reacting a compound of Formula (IIIa-2), as defined herein:



(IIIa-2),

with an RNA polymerase, and a cDNA template.

[00341] In a further embodiment, the present invention provides methods of amplifying a modified cell phenotype altering mRNA comprising at least one nucleotide (e.g., mmRNA molecule), the method comprising: reacting a compound of Formula (IIIa-2), as defined herein, with a primer, a cDNA template, and an RNA polymerase.

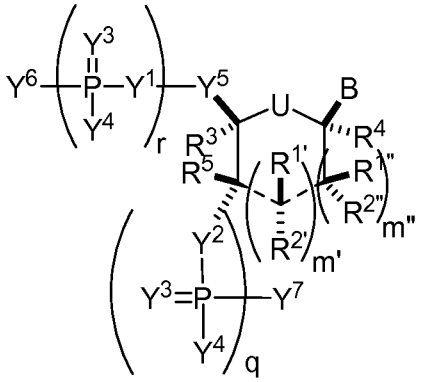
[00342] In some embodiments, the reaction may be repeated from 1 to about 7,000 times. In any of the embodiments herein, B may be a nucleobase of Formula (bl)-(b43).

[00343] The cell phenotype altering polynucleotides, primary constructs, and mmRNA can optionally include 5' and/or 3' flanking regions, which are described herein.

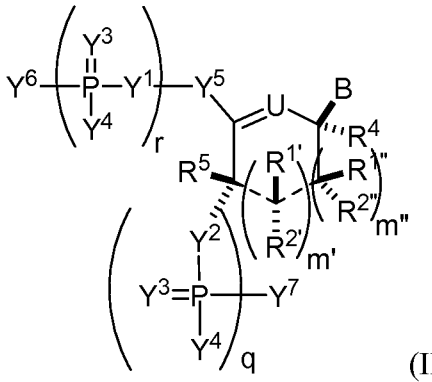
Modified Cell phenotype altering RNA (mmRNA) Molecules

[00344] The present invention also includes building blocks, e.g., modified ribonucleosides, modified ribonucleotides, of modified RNA (mmRNA) molecules. For example, these building blocks can be useful for preparing the cell phenotype altering polynucleotides, primary constructs, or mmRNA of the invention.

[00345] In some embodiments, the building block molecule has Formula (Ilia) or (Ilia-1):

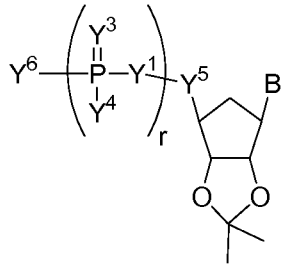


(IIIa),

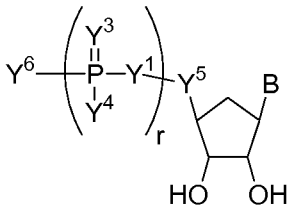


(IIIa-1) or a pharmaceutically acceptable salt or stereoisomer thereof, wherein the substituents are as described herein (e.g., for Formula (la) and (Ia-1)), and wherein when B is an unmodified nucleobase selected from cytosine, guanine, uracil and adenine, then at least one of Y^1, Y^2, or Y^3 is not O.

[00346] In some embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, has Formula (IVa)-(IVb):

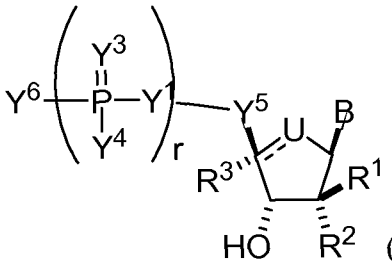


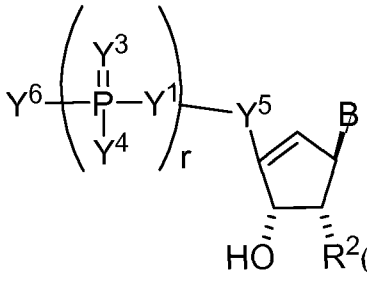
(IVa) or

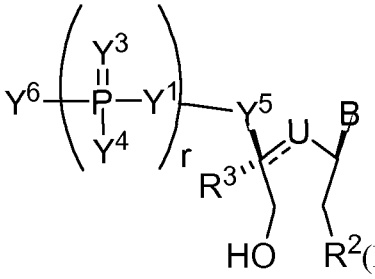


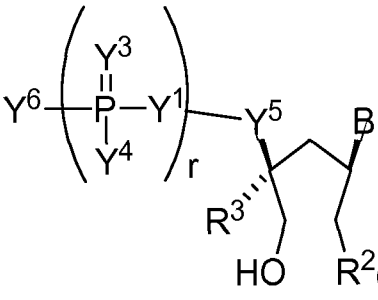
(IVb), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein B is as described herein (e.g., any one of (bl)-(b43)). In particular embodiments, Formula (IVa) or (IVb) is combined with a modified uracil (e.g., any one of formulas (bl)-(b9), (b21)-(b23), and (b28)-(b31), such as formula (bl), (b8), (b28), (b29), or (b30)). In particular embodiments, Formula (IVa) or (IVb) is combined with a modified cytosine (e.g., any one of formulas (M0)-(bl4), (b24), (b25), and (b32)-(b36), such as formula (blO) or (b32)). In particular embodiments, Formula (IVa) or (IVb) is combined with a modified guanine (e.g., any one of formulas (bl5)-(bl7) and (b37)-(b40)). In particular embodiments, Formula (IVa) or (IVb) is combined with a modified adenine (e.g., any one of formulas (M8)-(b20) and (b41)-(b43)).

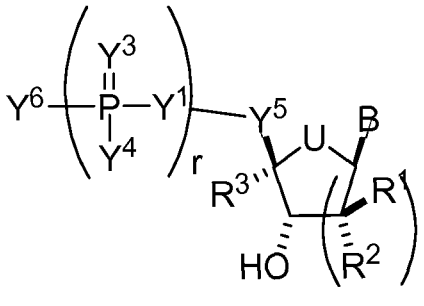
[00347] In some embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, has Formula (IVc)-(IVk):

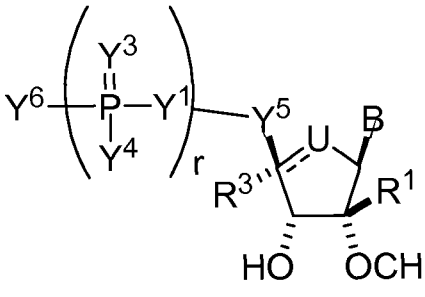

(IVc),

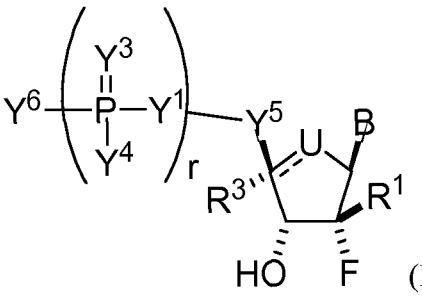

(IVd),

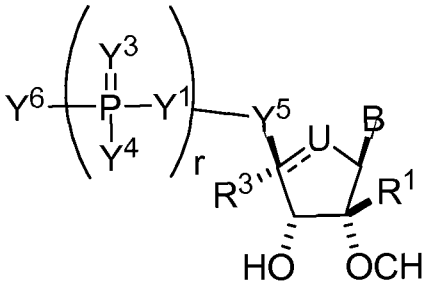

(IVe),

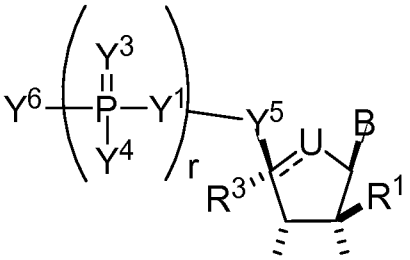

(IVf),

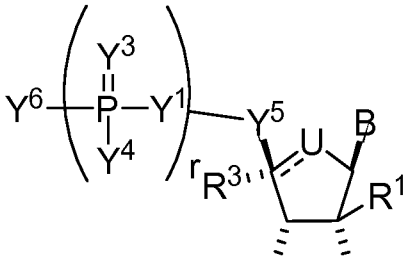

(IVg),


(IVh),


(IVi),

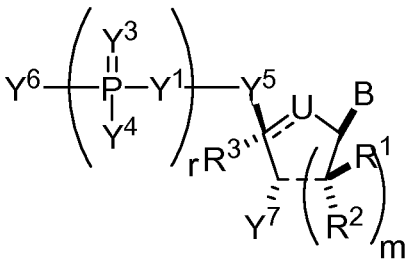

(IVj),

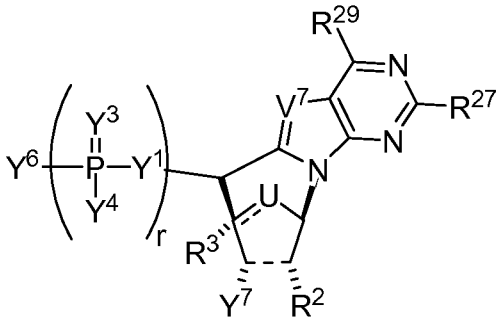




(IVk), or (IVl), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein B is as described herein (e.g., any one of (bl)-(b43)). In particular embodiments, one of Formulas (IVc)-(IVk) is combined with a modified uracil (e.g., any one of formulas (bl)-(b9), (b21)-(b23), and (b28)-(b31), such as formula (bl), (b8), (b28), (b29), or (b30)). In particular embodiments, one of Formulas (IVc)-(IVk) is combined with a modified cytosine (e.g., any one of formulas (M0)-(bl4), (b24), (b25), and (b32)-(b36), such as formula (blO) or (b32)). In particular embodiments, one of Formulas (IVc)-(IVk) is combined with a modified guanine (e.g., any one of formulas (bl5)-(bl7) and (b37)-(b40)). In particular embodiments, one of Formulas (IVc)-(IVk) is combined with a modified adenine (e.g., any one of formulas (M8)-(b20) and (b41)-(b43)).

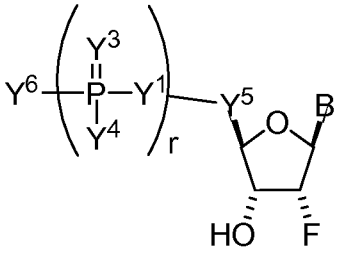
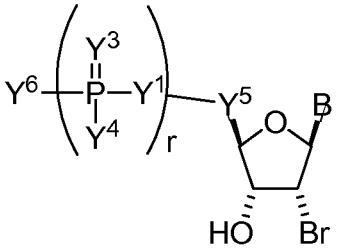
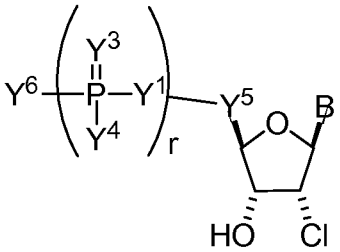
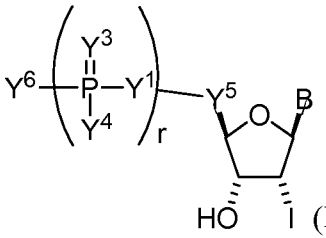
[00348] In other embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, has Formula (Va) or (Vb):



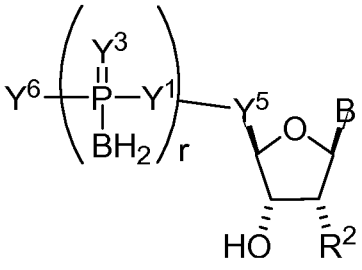


(Va) or (Vb), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein B is as described herein (e.g., any one of (bl)-(b43)).

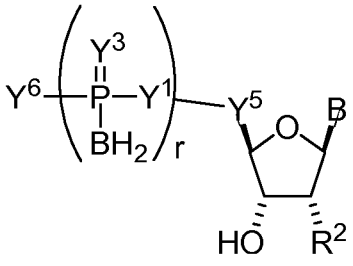
[00349] In other embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, has Formula (IXa)-(IXd):


(IXa),

(IXb),

(IXc), or

(IXd), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein B is as described herein (e.g., any one of (bl)-(b43)). In particular embodiments, one of Formulas (IXa)-(IXd) is combined with a modified uracil (e.g., any one of formulas (bl)-(b9), (b21)-(b23), and (b28)-(b31), such as formula (bl), (b8), (b28), (b29), or (b30)). In particular embodiments, one of Formulas (IXa)-(IXd) is combined with a modified cytosine (e.g., any one of formulas (M0)-(bl4), (b24), (b25), and (b32)-(b36), such as formula (blO) or (b32)). In particular embodiments, one of Formulas (IXa)-(IXd) is combined with a modified guanine (e.g., any one of formulas (bl5)-(bl7) and (b37)-(b40)). In particular embodiments, one of Formulas (IXa)-(IXd) is combined with a modified adenine (e.g., any one of formulas (M8)-(b20) and (b41)-(b43)).

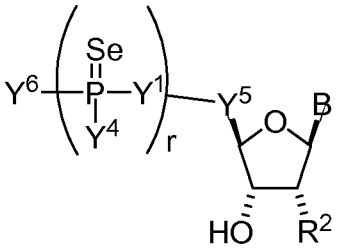
[00350] In other embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, has Formula (IXe)-(IXg):



(IXe),

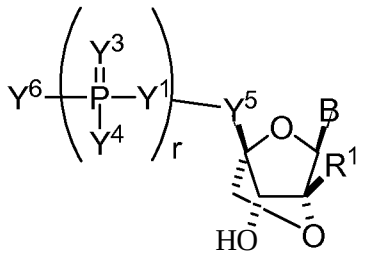


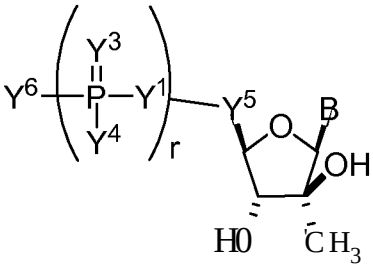
(IXf), or

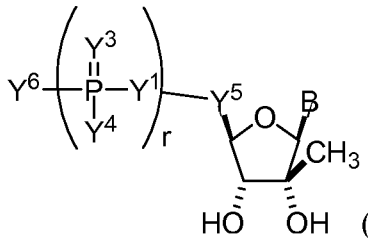


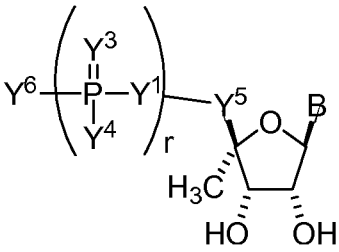
(IXg), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein B is as described herein (e.g., any one of (bl)-(b43)). In particular embodiments, one of Formulas (IXe)-(IXg) is combined with a modified uracil (e.g., any one of formulas (bl)-(b9), (b21)-(b23), and (b28)-(b31), such as formula (bl), (b8), (b28), (b29), or (b30)). In particular embodiments, one of Formulas (IXe)-(IXg) is combined with a modified cytosine (e.g., any one of formulas (M0)-(bl4), (b24), (b25), and (b32)-(b36), such as formula (blO) or (b32)). In particular embodiments, one of Formulas (IXe)-(IXg) is combined with a modified guanine (e.g., any one of formulas (bl5)-(bl7) and (b37)-(b40)). In particular embodiments, one of Formulas (IXe)-(IXg) is combined with a modified adenine (e.g., any one of formulas (M8)-(b20) and (b41)-(b43)).

[00351] In other embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, has Formula (IXh)-(IXk):

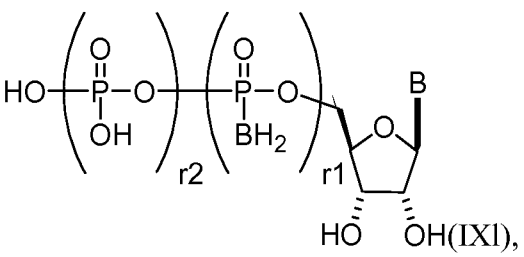

(ixh),


(IXi),

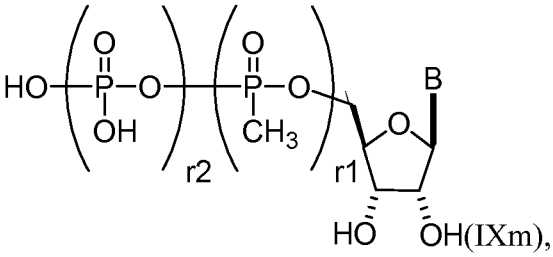

(IXj), or


(IXk), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein B is as described herein (e.g., any one of (bl)-(b43)). In particular embodiments, one of Formulas (IXh)-(IXk) is combined with a modified uracil (e.g., any one of formulas (bl)-(b9), (b21)-(b23), and (b28)-(b31), such as formula (bl), (b8), (b28), (b29), or (b30)). In particular embodiments, one of Formulas (IXh)-(IXk) is combined with a modified cytosine (e.g., any one of formulas (M0)-(bl4), (b24), (b25), and (b32)-(b36), such as formula (blO) or (b32)). In particular embodiments, one of Formulas (IXh)-(IXk) is combined with a modified guanine (e.g., any one of formulas (bl5)-(bl7) and (b37)-(b40)). In particular embodiments, one of Formulas (IXh)-(IXk) is combined with a modified adenine (e.g., any one of formulas (M8)-(b20) and (b41)-(b43)).

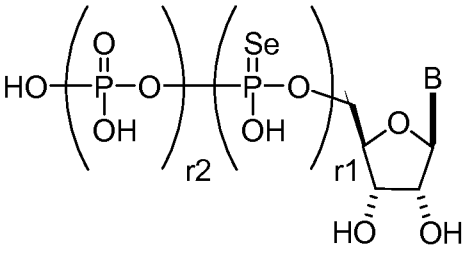
[00352] In other embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, has Formula (IXl)-(IXr):



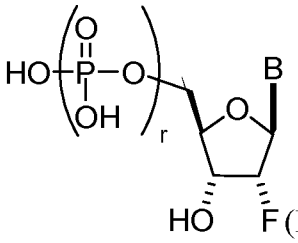
(IXl),



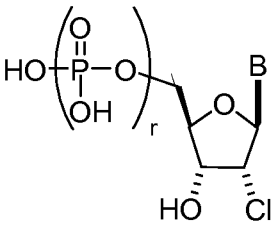
(IXm),



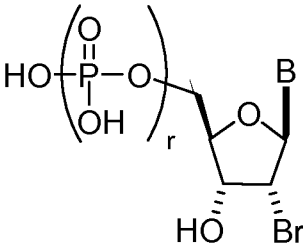
(IXn),



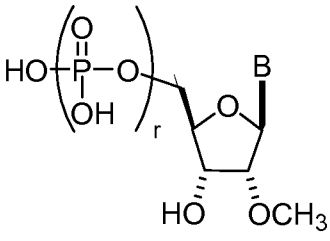
(IXo),



(IXp),

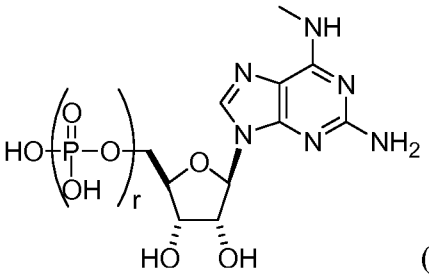


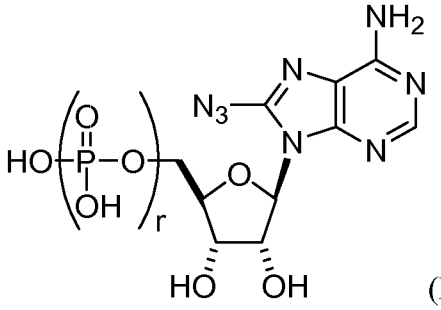
(IXq), or

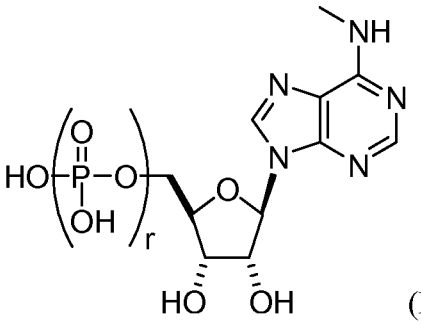


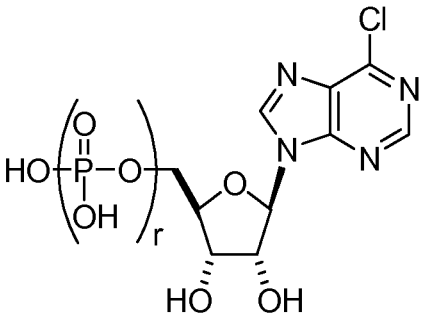
(IXr) or a pharmaceutically acceptable salt or stereoisomer thereof, wherein each r1 and r2 is, independently, an integer from 0 to 5 (e.g., from 0 to 3, from 1 to 3, or from 1 to 5) and B is as described herein (e.g., any one of (bl)-(b43)). In particular embodiments, one of Formulas (IXl)-(IXr) is combined with a modified uracil (e.g., any one of formulas (bl)-(b9), (b21)-(b23), and (b28)-(b31), such as formula (bl), (b8), (b28), (b29), or (b30)). In particular embodiments, one of Formulas (IXl)-(IXr) is combined with a modified cytosine (e.g., any one of formulas (M0)-(bl4), (b24), (b25), and (b32)-(b36), such as formula (blO) or (b32)). In particular embodiments, one of Formulas (IXl)-(IXr) is combined with a modified guanine (e.g., any one of formulas (bl5)-(bl7) and (b37)-(b40)). In particular embodiments, one of Formulas (IXl)-(IXr) is combined with a modified adenine (e.g., any one of formulas (M8)-(b20) and (b41)-(b43)).

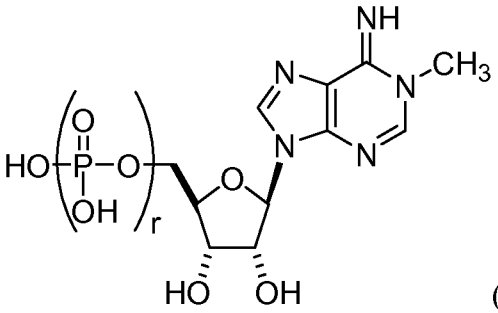
[00353] In some embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, can be selected from the group consisting of:

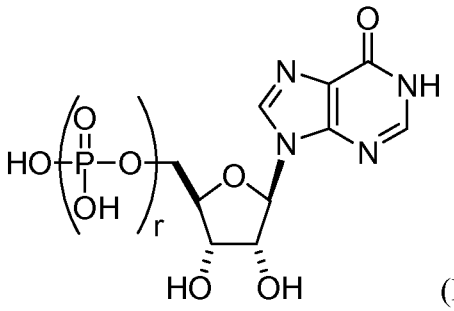

(BB- 1),

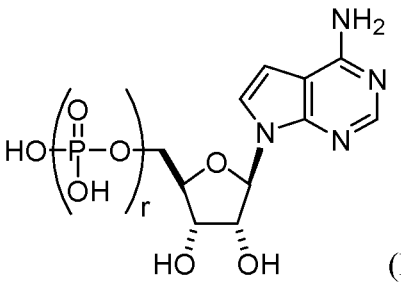

(BB- 2),

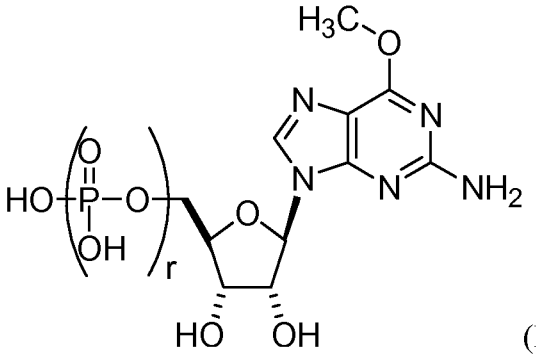

(BB- 3),

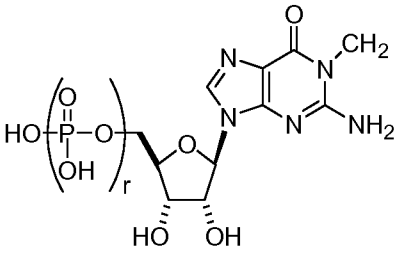
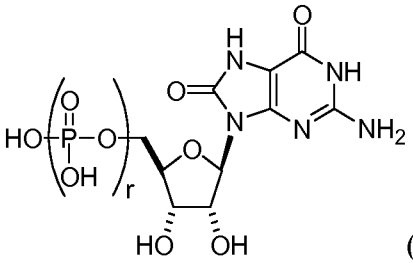
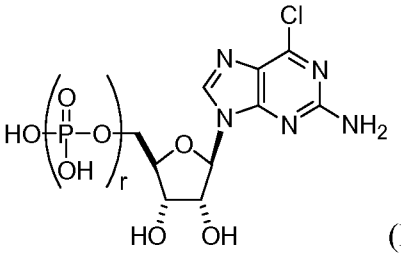
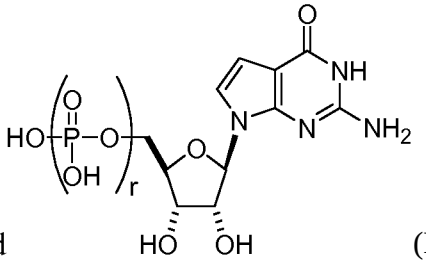

(BB- 4),


(BB- 5),

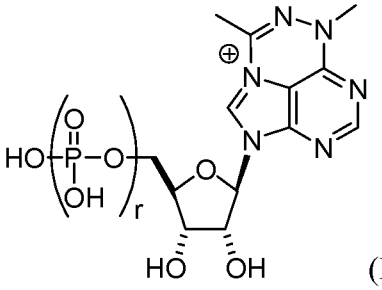

(BB- 6),


(BB- 7),

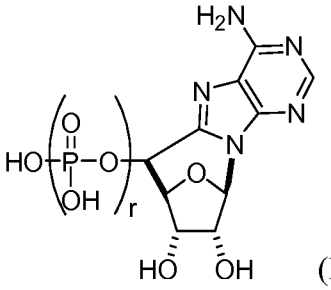

(BB- 8),


(BB- 9),

(BB- 10),

(BB- 11), and

(BB- 12), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein each r is, independently, an integer from 0 to 5 (e.g., from 0 to 3, from 1 to 3, or from 1 to 5).

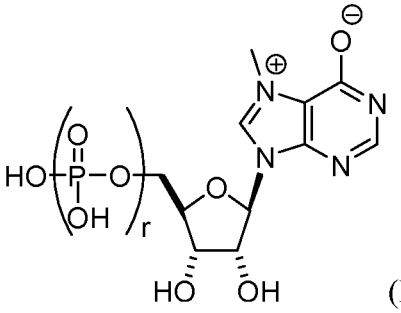
In some embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, can be selected from the group consisting of:



(BB- 13),



(BB- 14),



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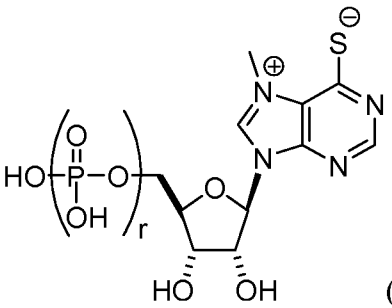

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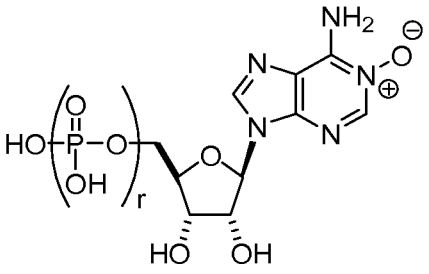

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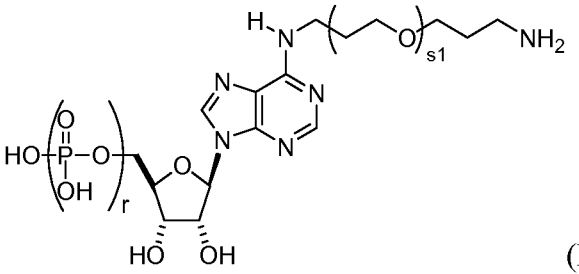

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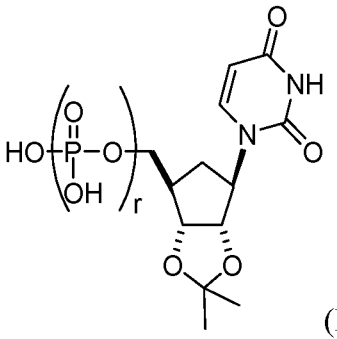

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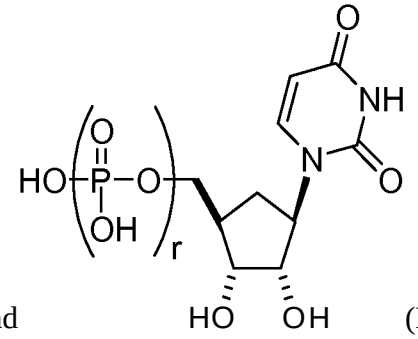
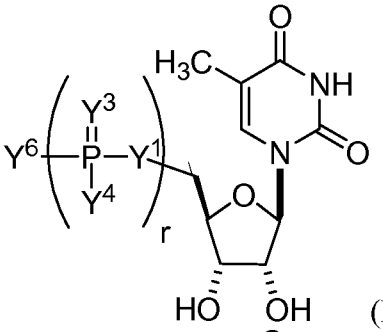


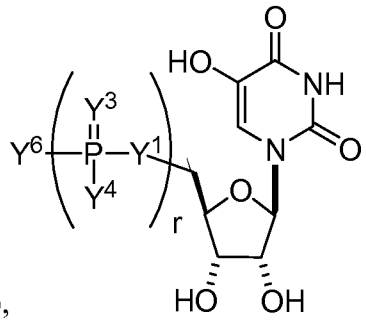
and (BB- 20), or a pharmaceutically acceptable salt or

stereoisomer thereof, wherein each r is, independently, an integer from 0 to 5 (e.g., from 0 to 3, from 1 to 3, or from 1 to 5) and si is as described herein.

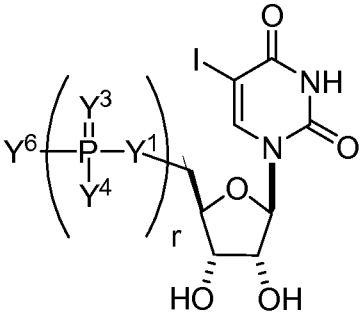
[00354] In some embodiments, the building block molecule, which may be incorporated into a cell phenotype altering nucleic acid (e.g., RNA, mRNA, polynucleotide, primary construct, or mmRNA), is a modified uridine (e.g., selected from the group consisting of:



(BB- 21),



(BB- 22),



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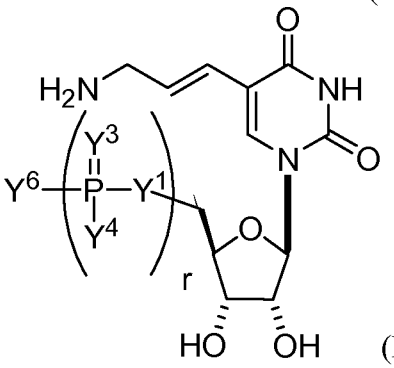

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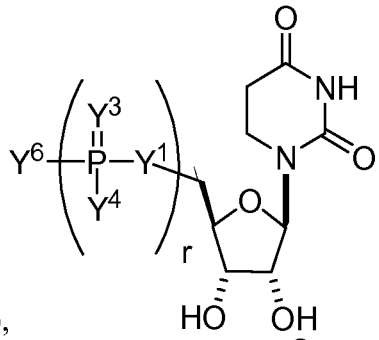

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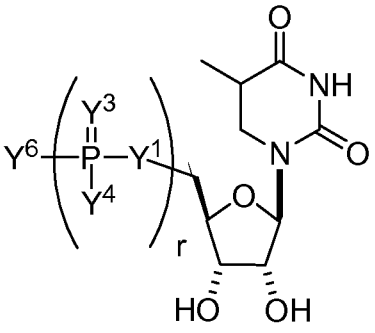
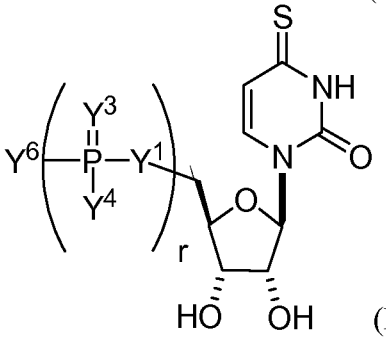


(BB- 26),



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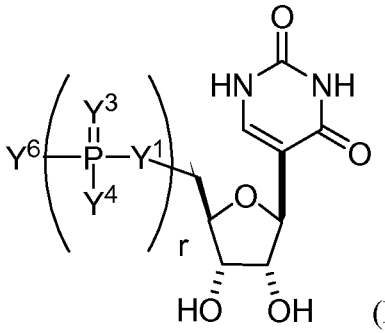

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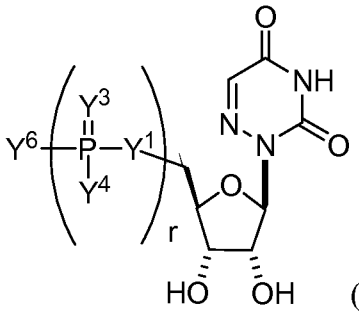

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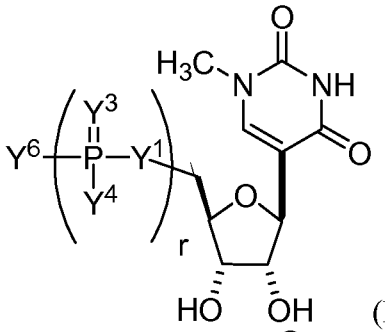

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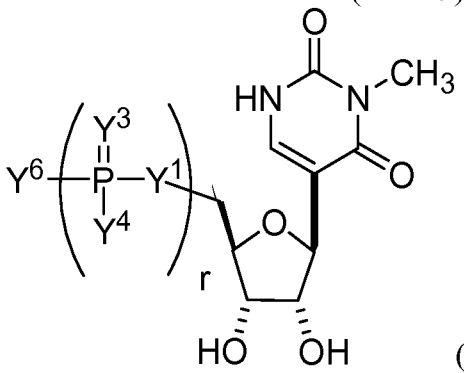

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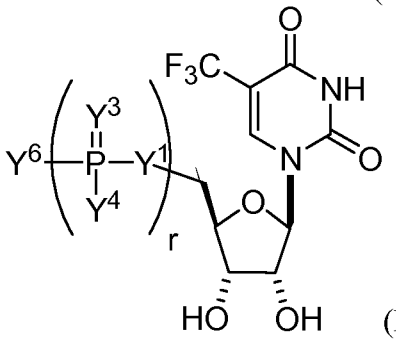

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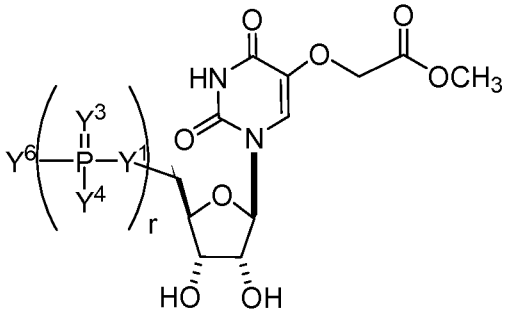

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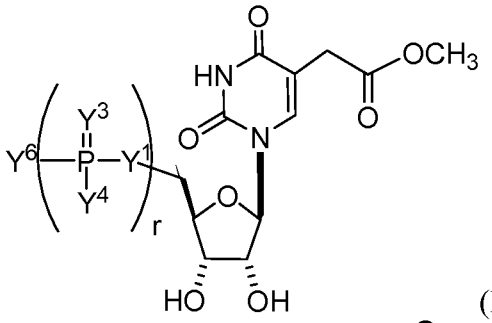

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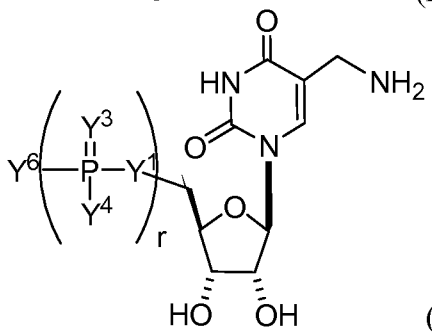

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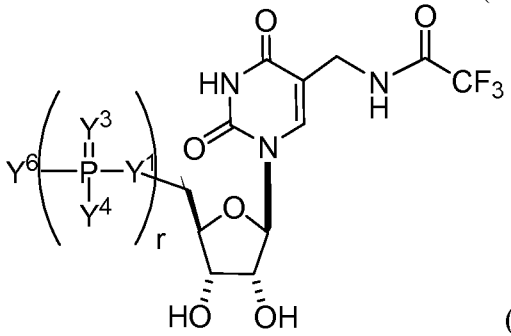

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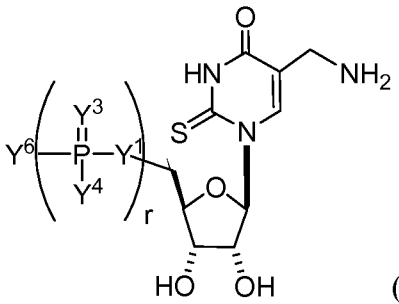

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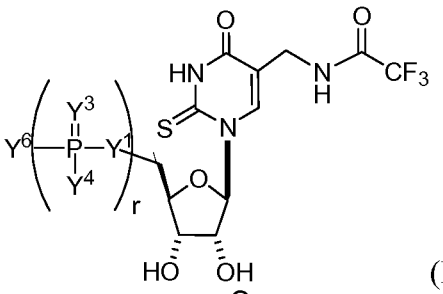

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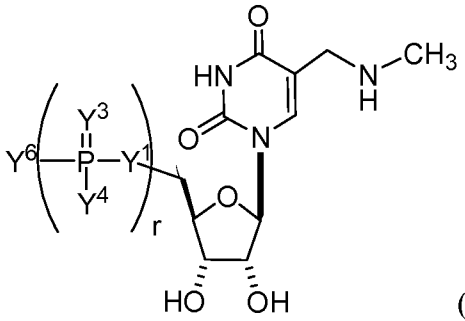

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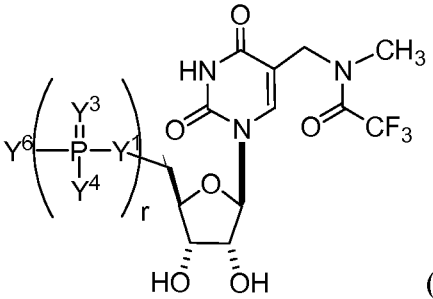

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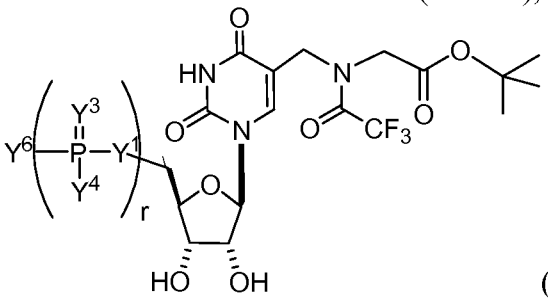

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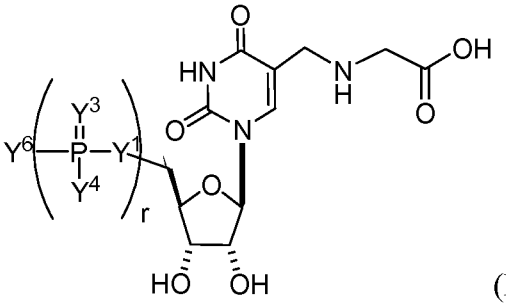

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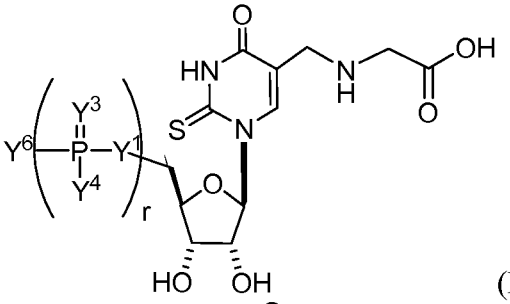

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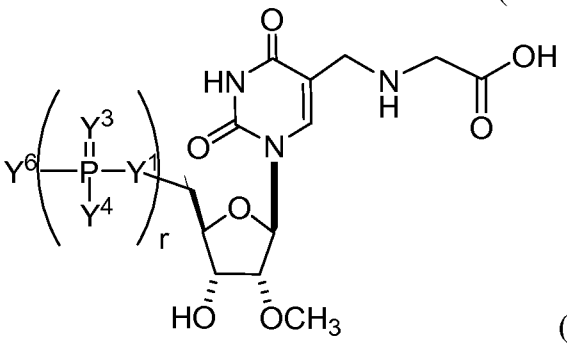

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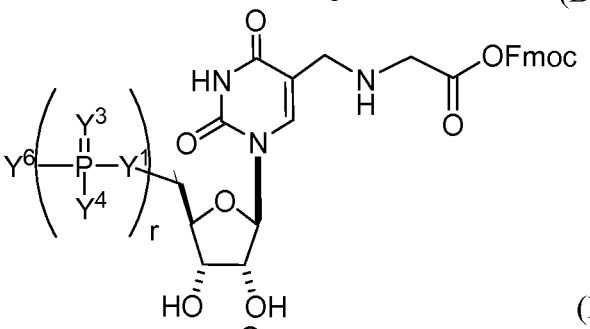

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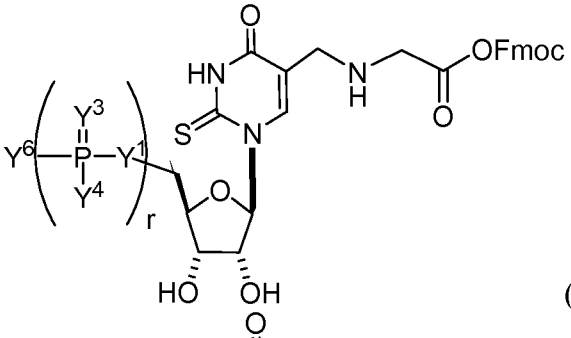

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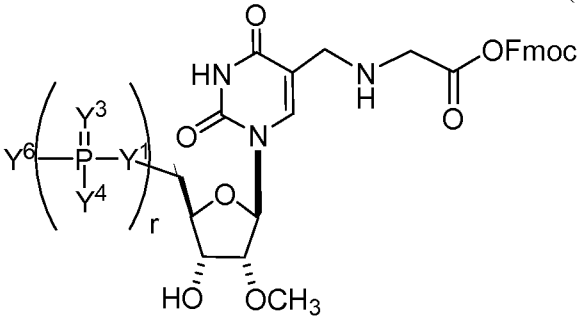

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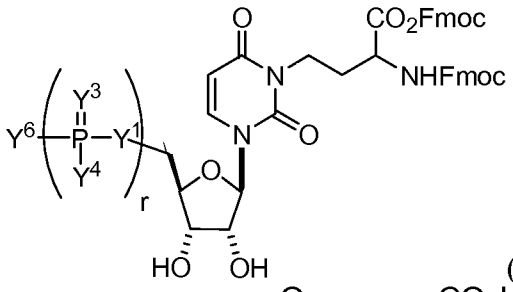

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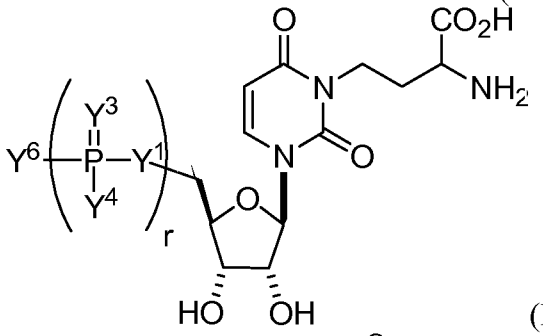

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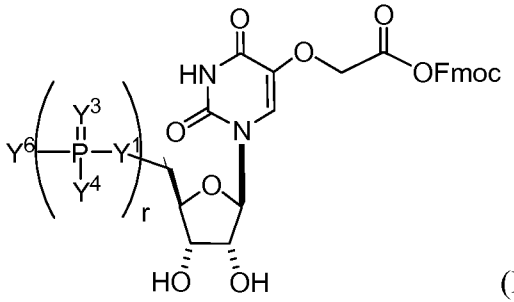

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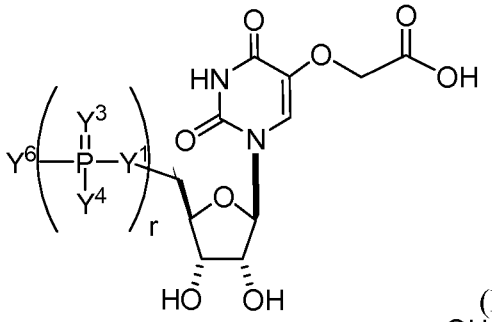

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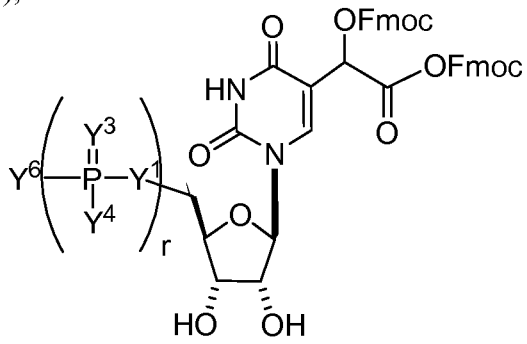
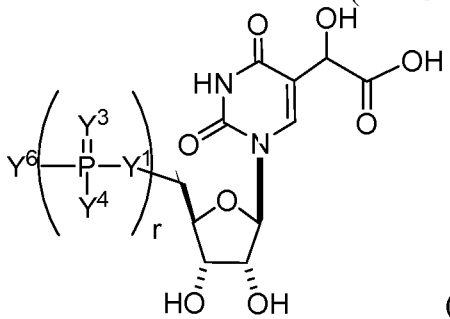
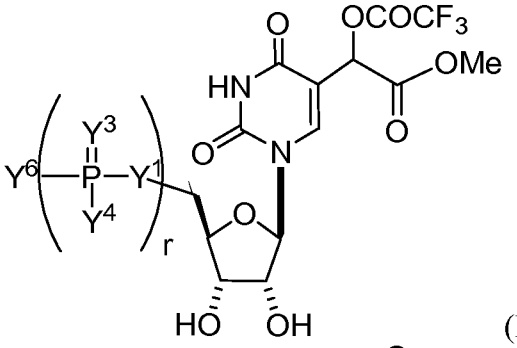




(BB- 52),

(BB- 53),



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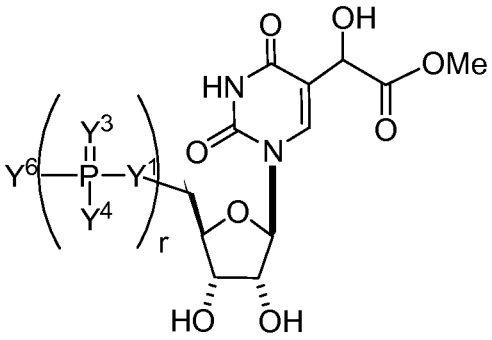
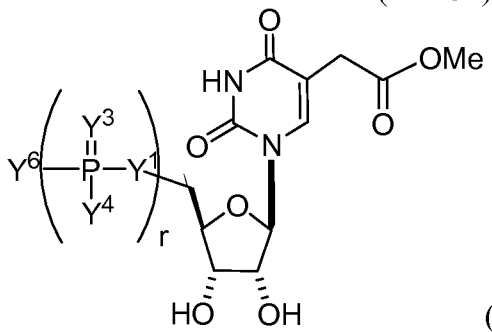
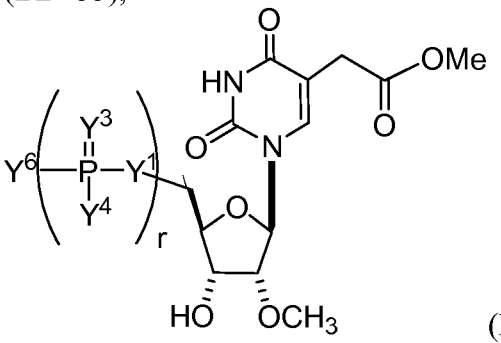




(BB- 55),

(BB-56),



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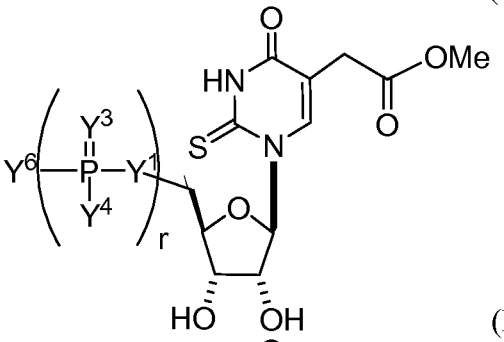

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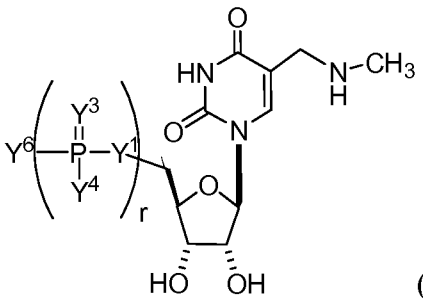

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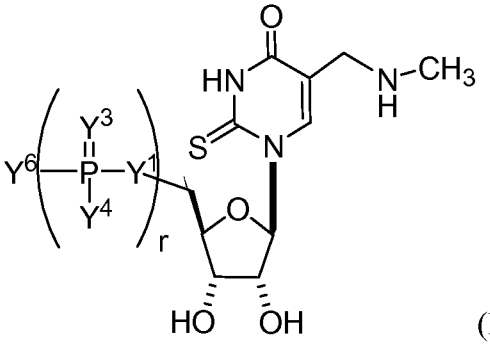

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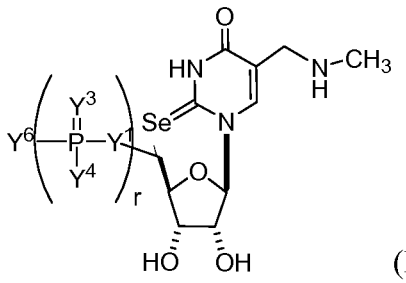

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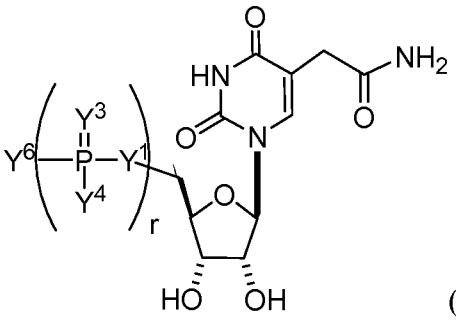

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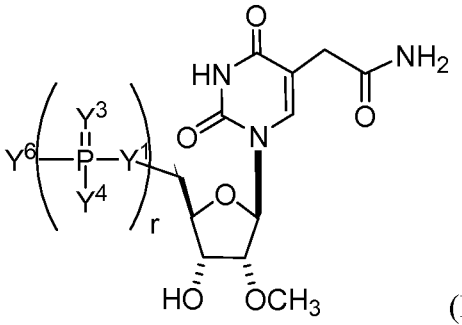

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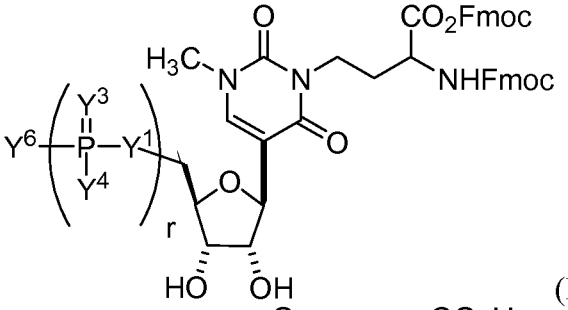

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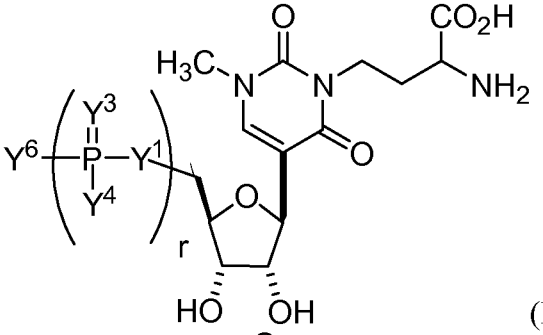

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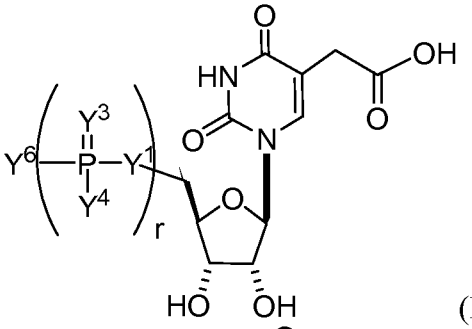

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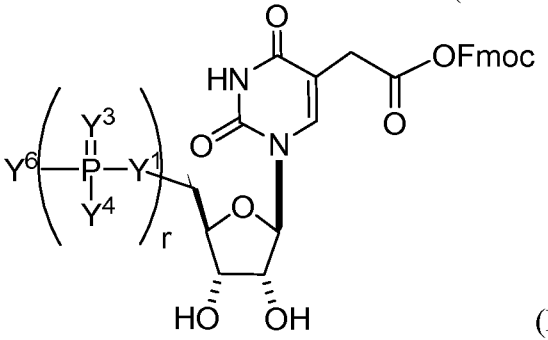

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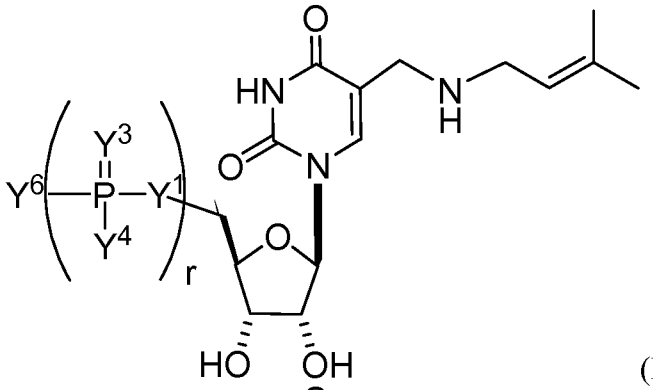

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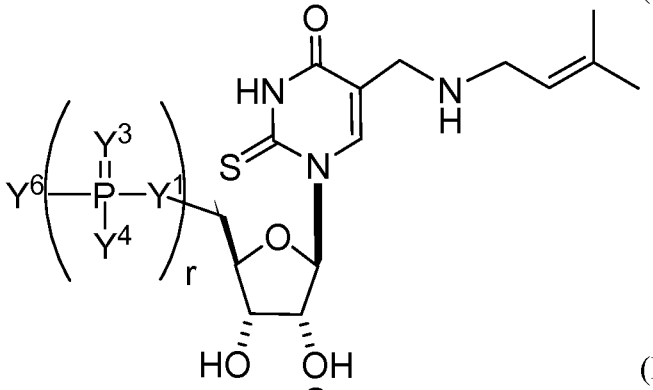

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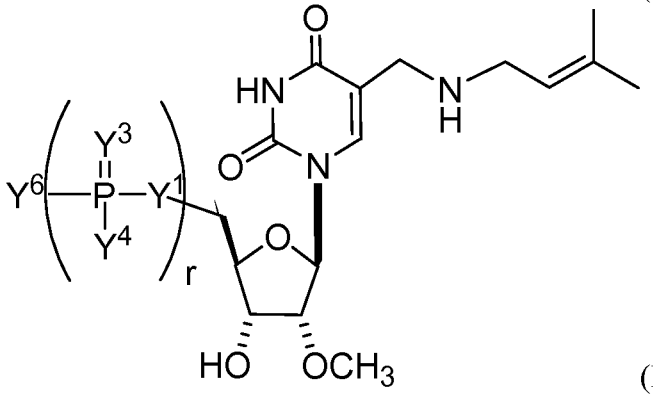

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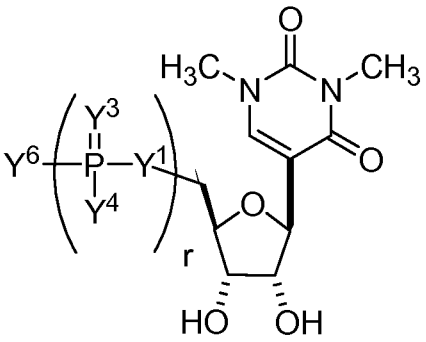

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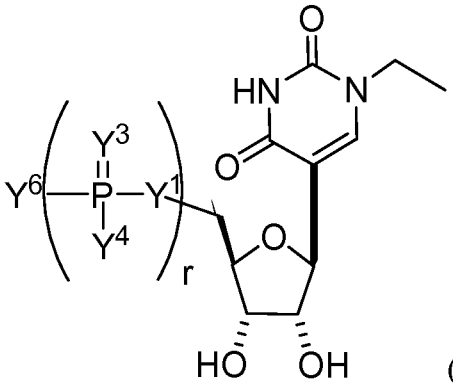

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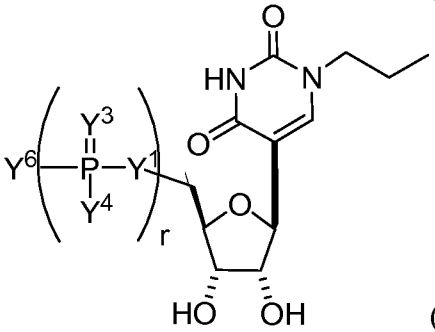

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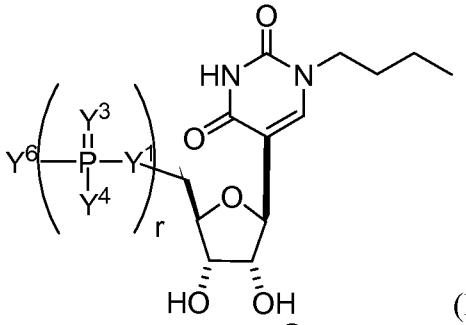

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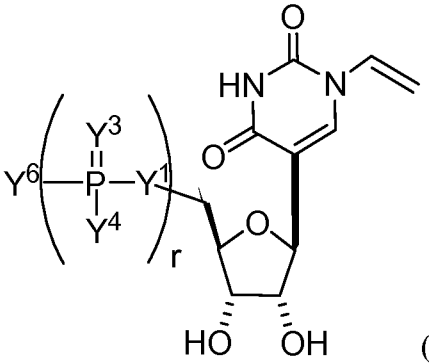

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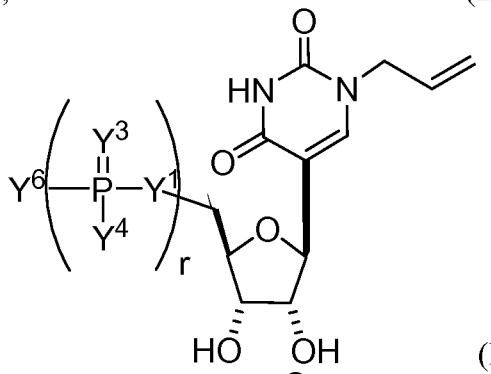

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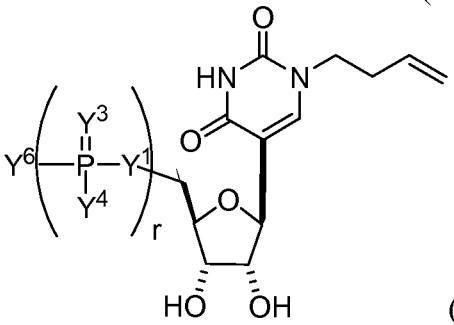

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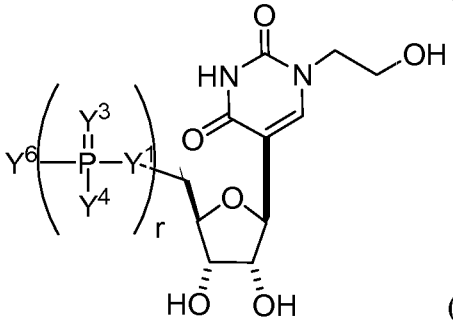

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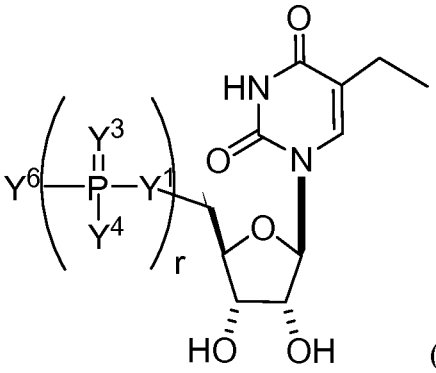
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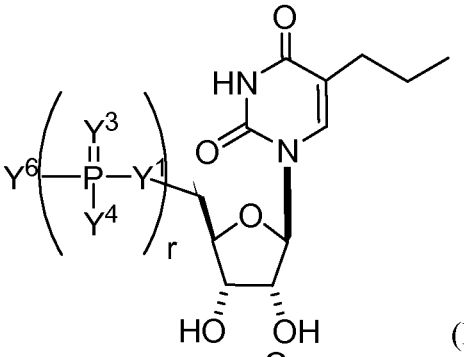
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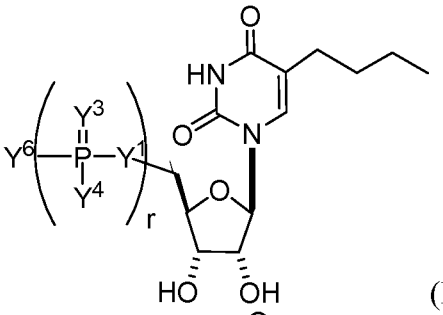
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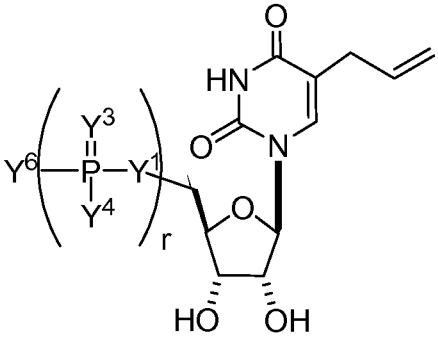
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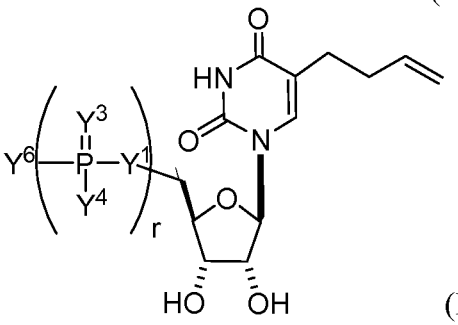
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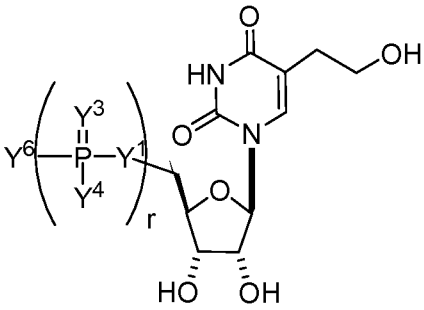
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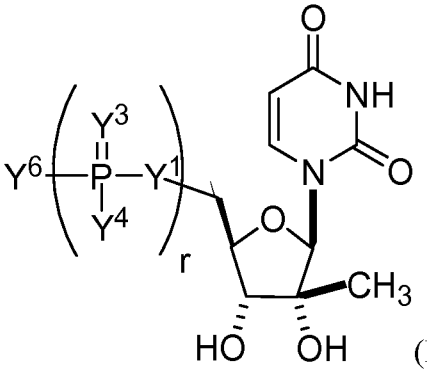
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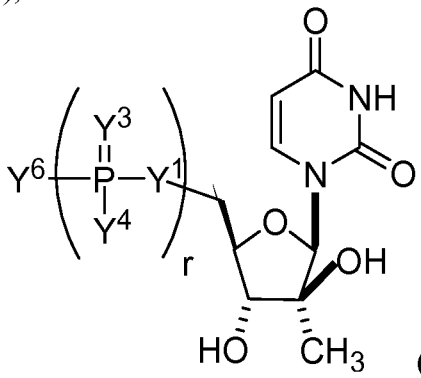
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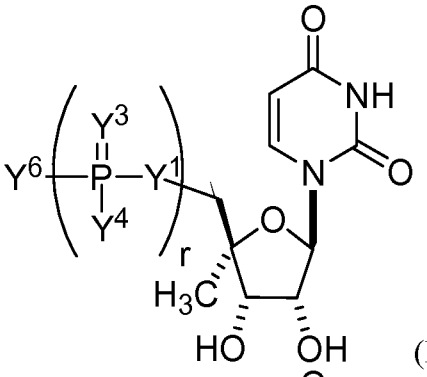
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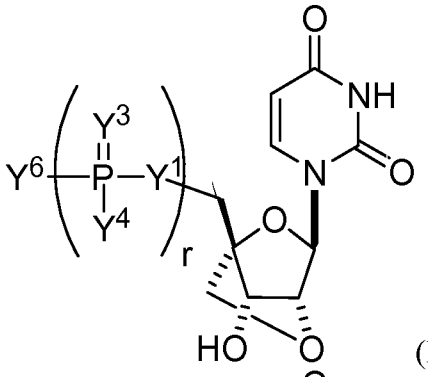
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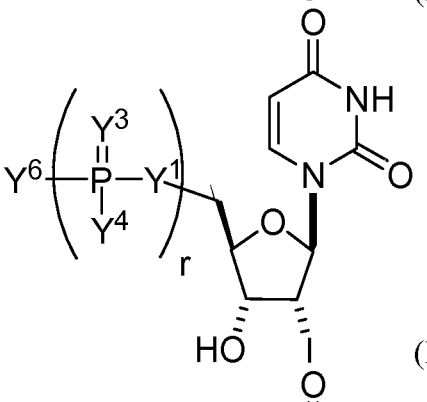
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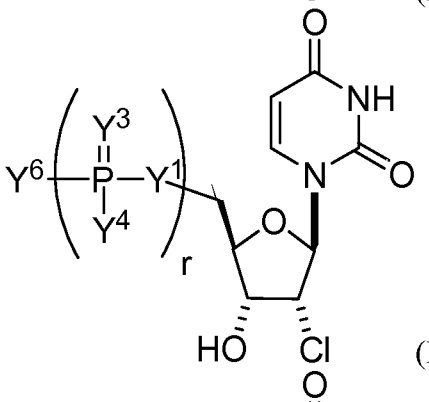
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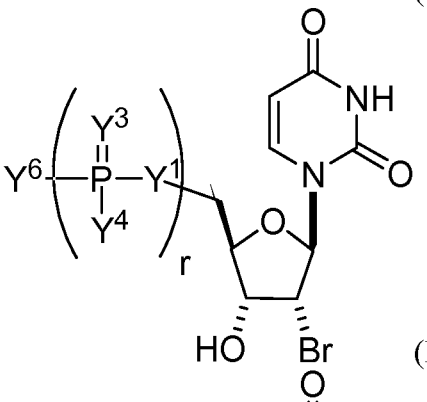
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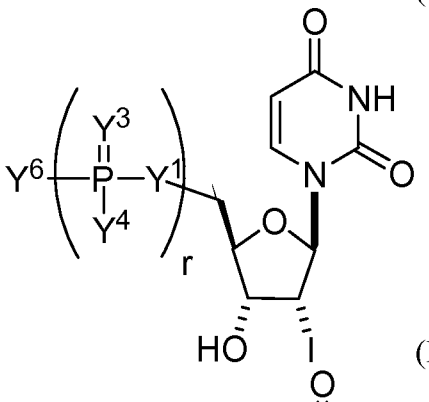
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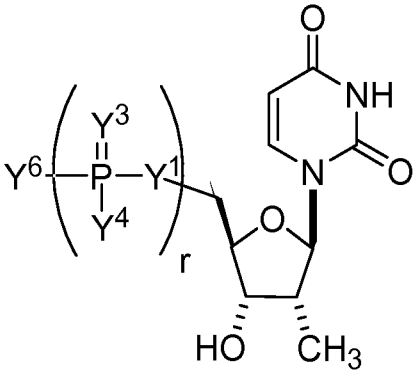
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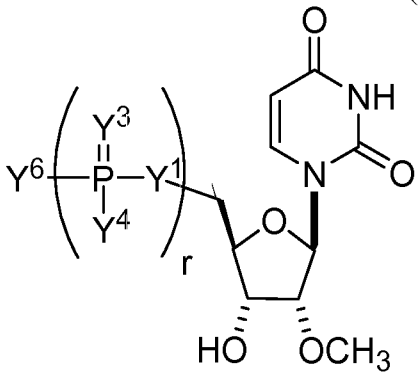
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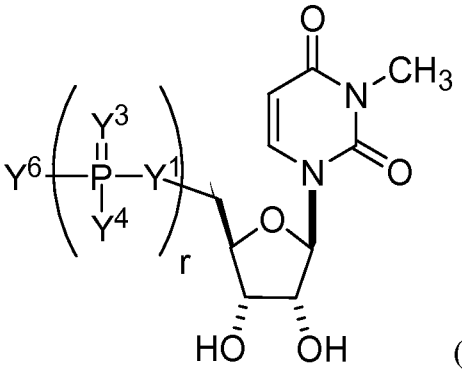

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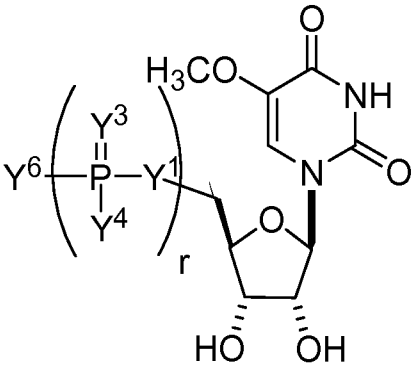

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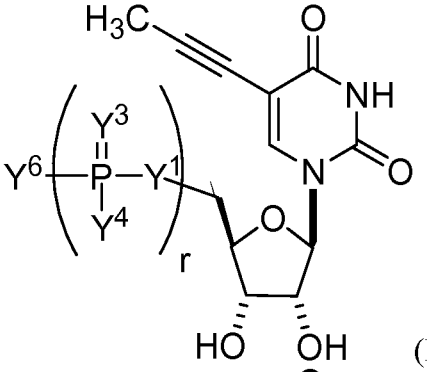

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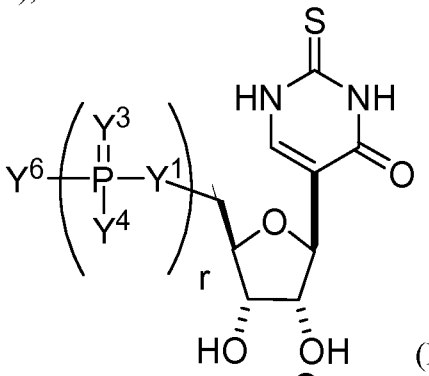

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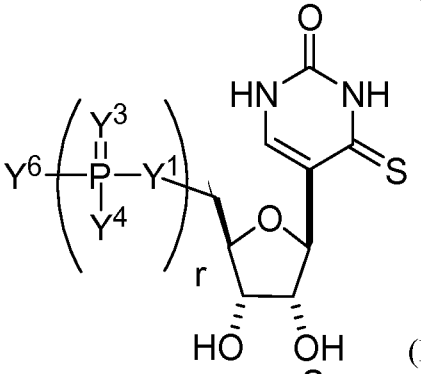

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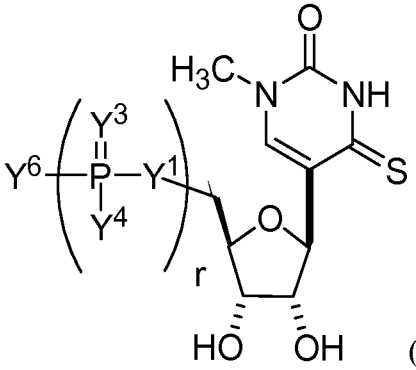

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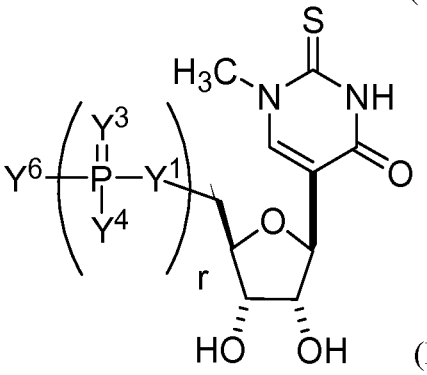

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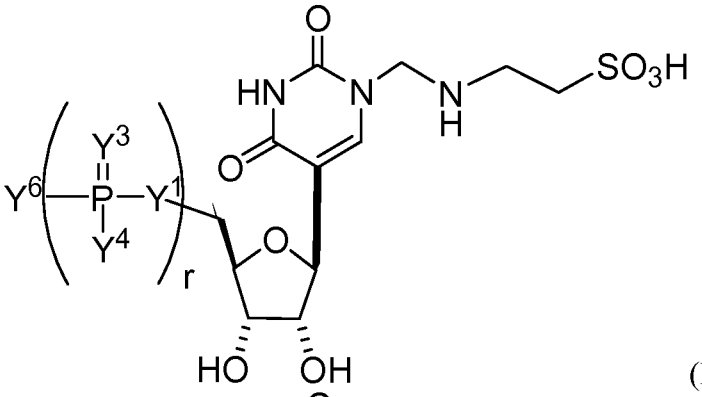

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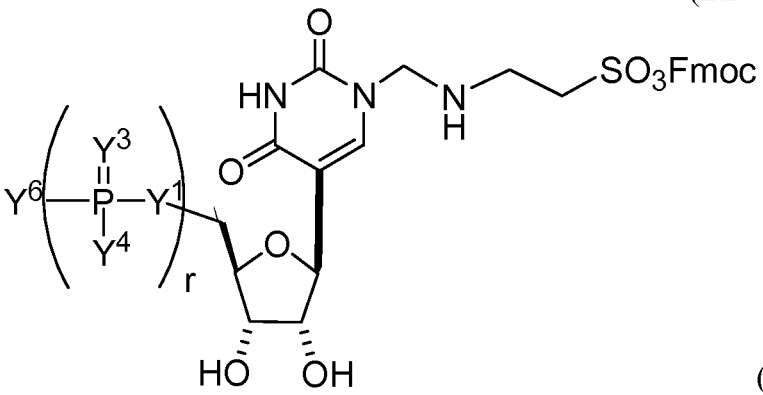

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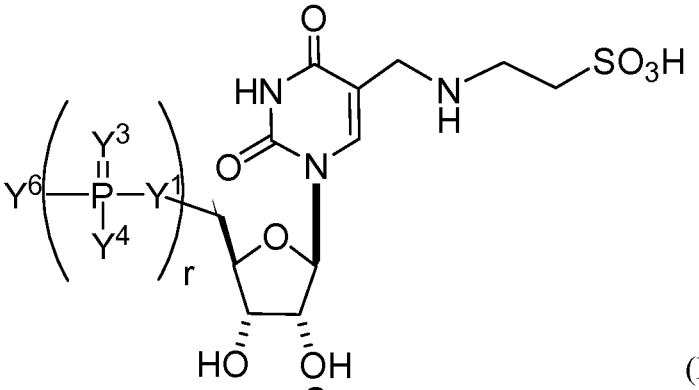

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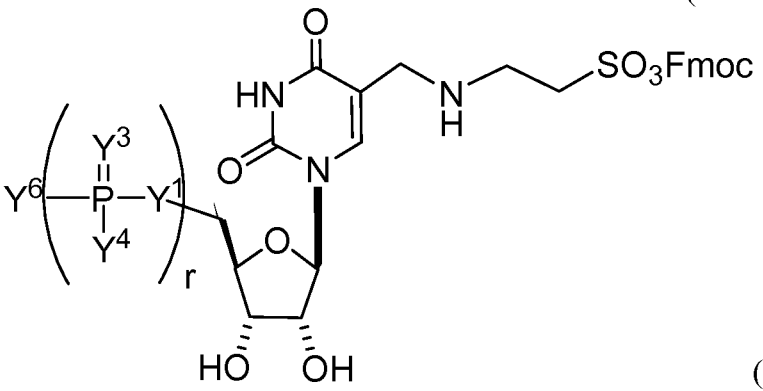

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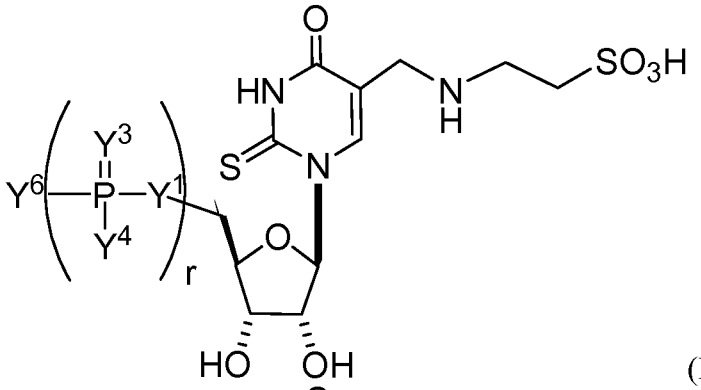

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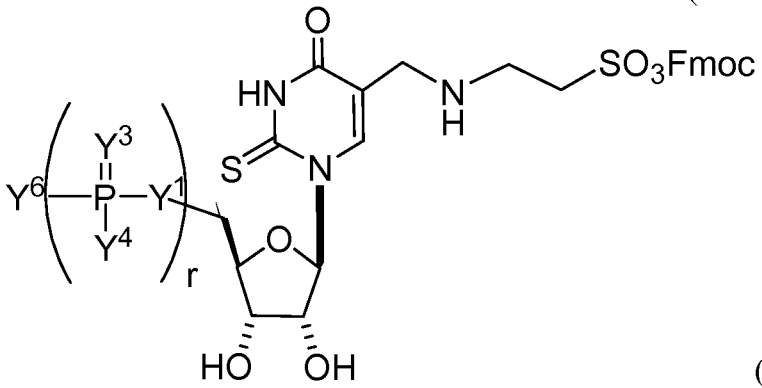

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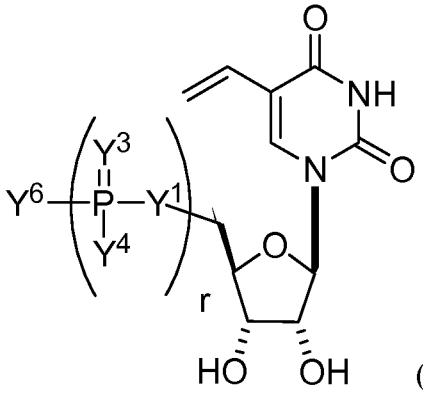

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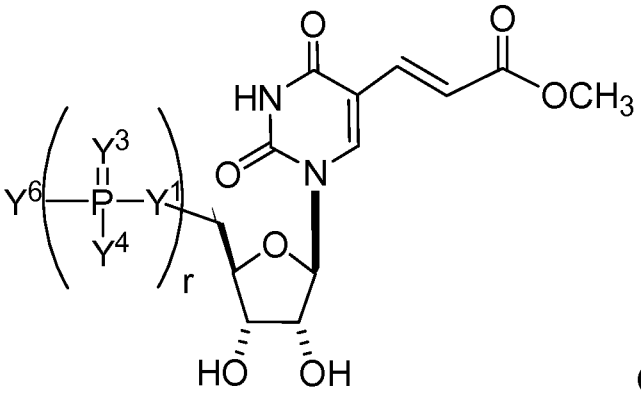

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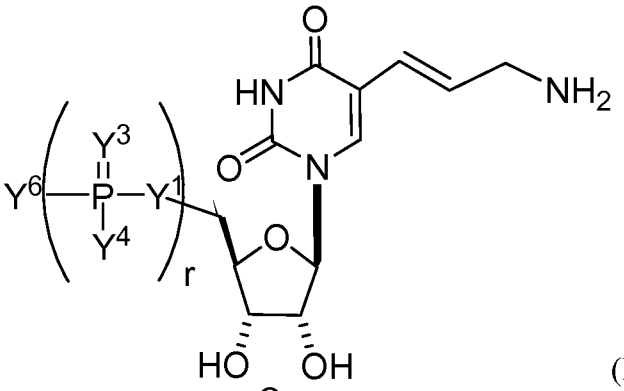

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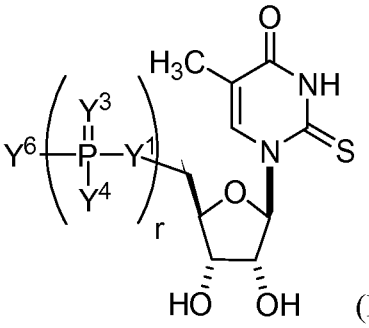

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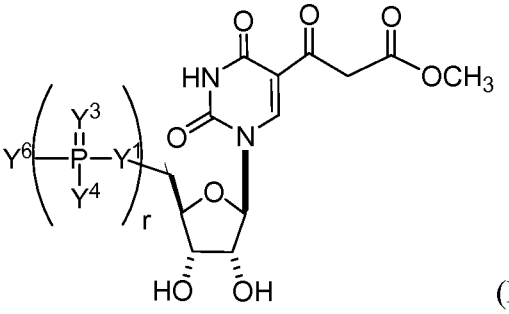

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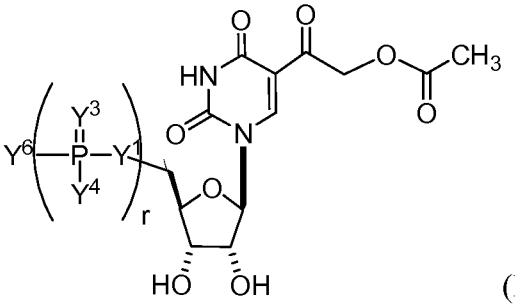

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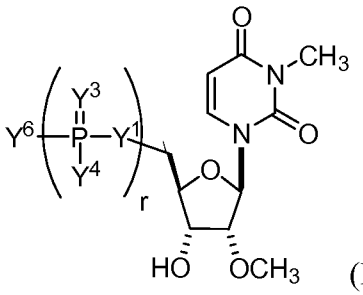

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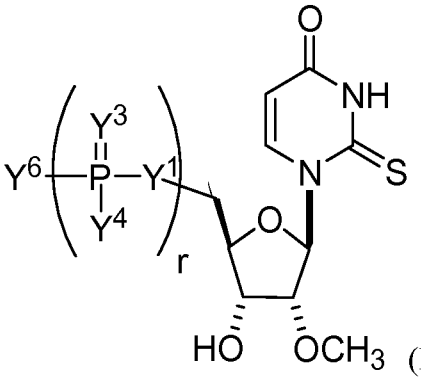
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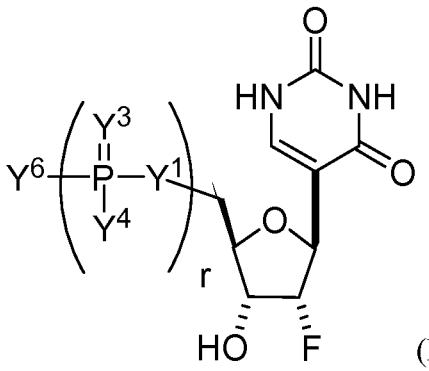
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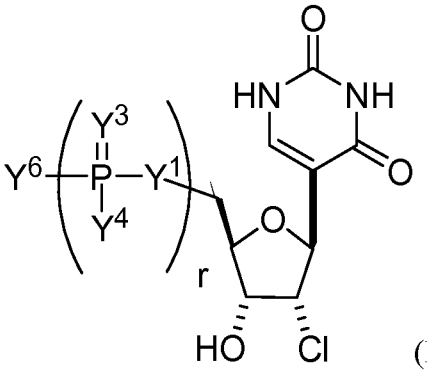
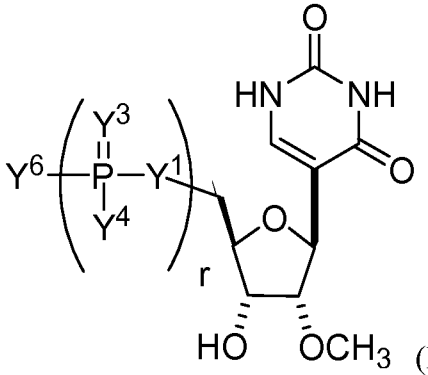

(BB- 117),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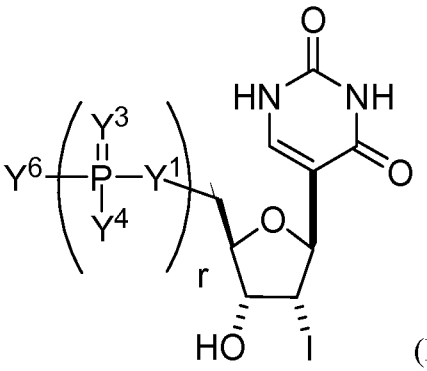
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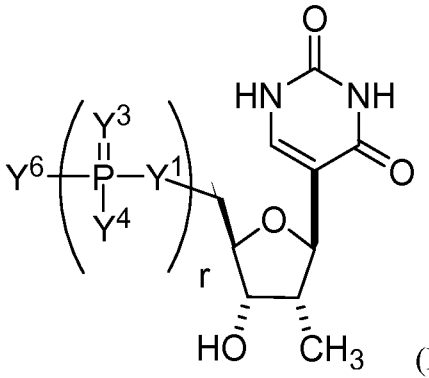
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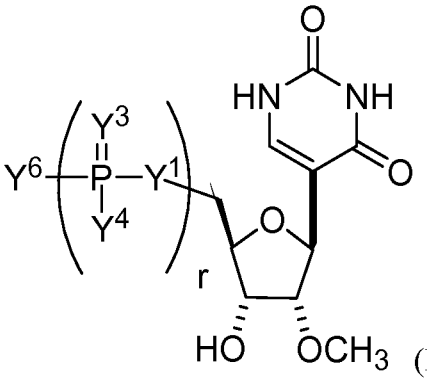
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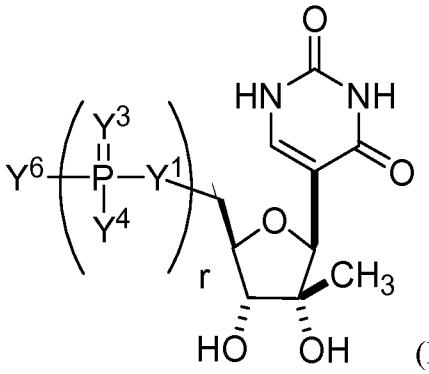
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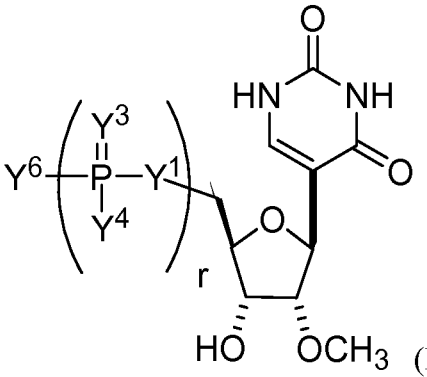

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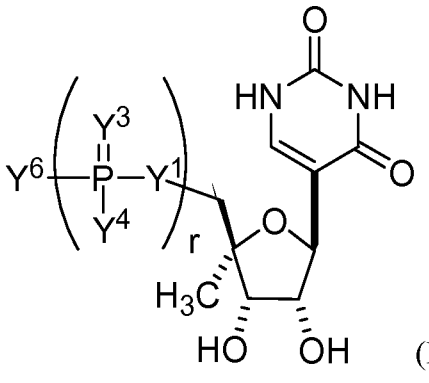

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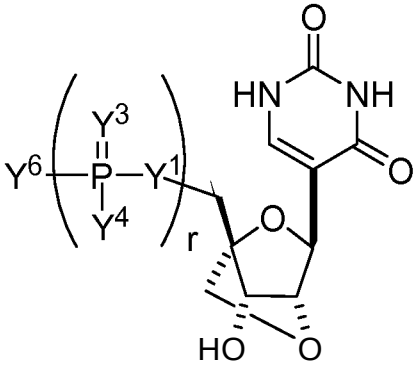
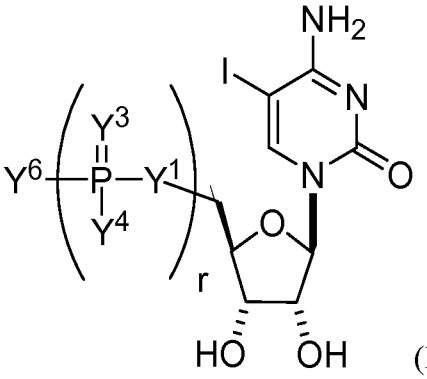
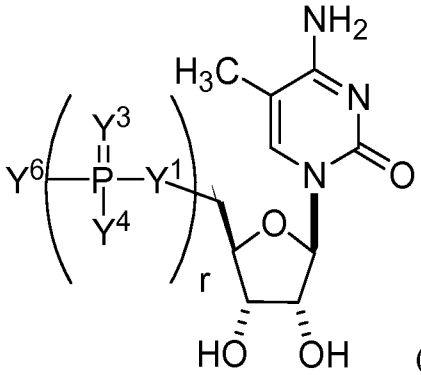


and (BB- 125), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein Y^1, Y^3, Y^4, Y^6, and r are as described herein (e.g., each r is, independently, an integer from 0 to 5, such as from 0 to 3, from 1 to 3, or from 1 to 5)).

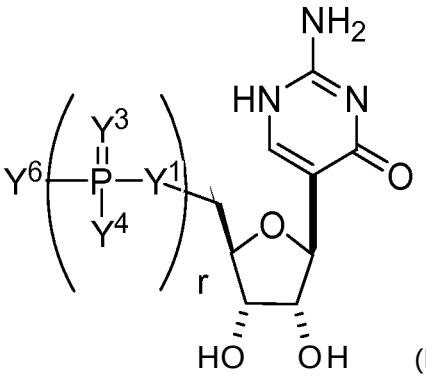
[00355] In some embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, is a modified cytidine (e.g., selected from the group consisting of:



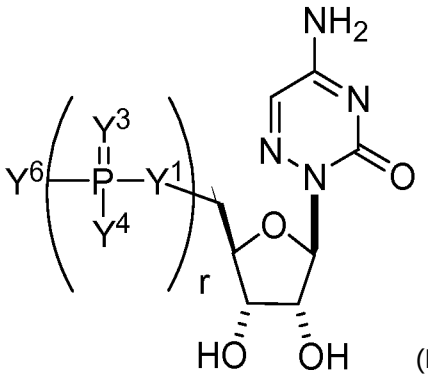
(BB- 126),



(BB- 127),



(BB- 128),



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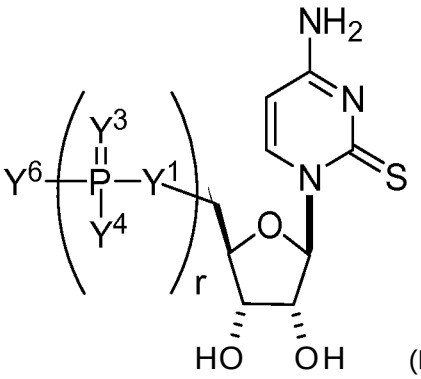
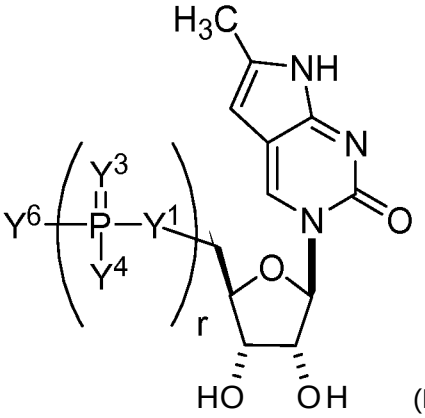
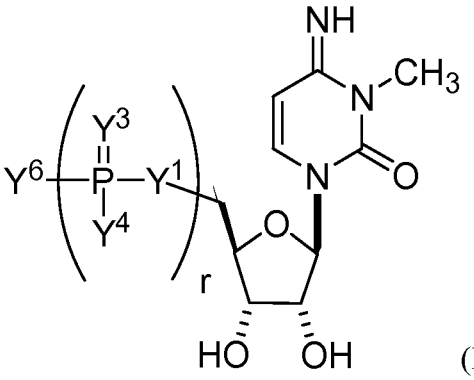


(BB- 130),



(BB- 131),



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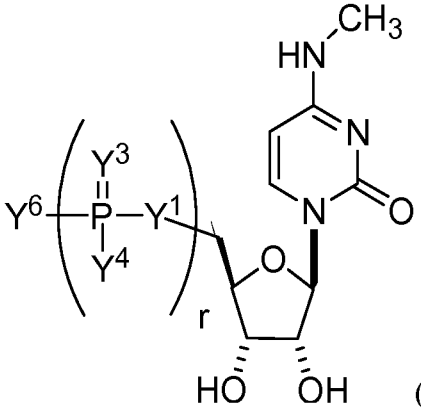
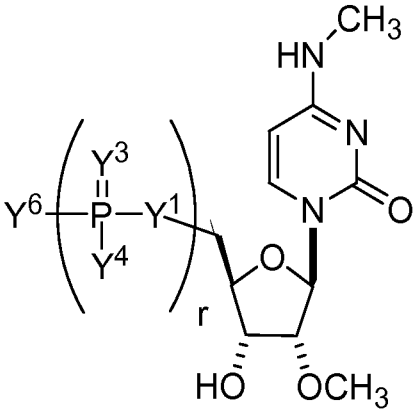


(BB- 133),



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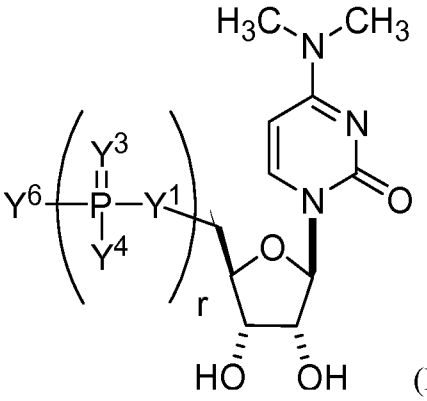

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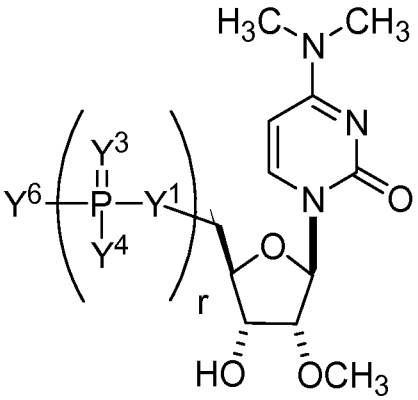

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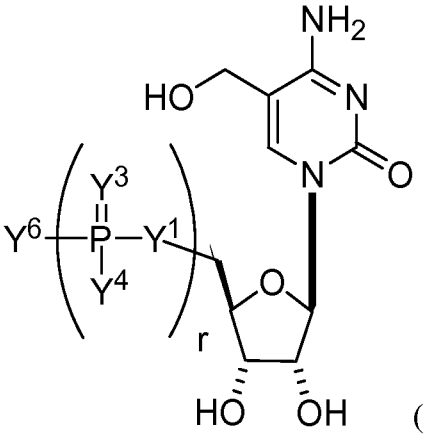
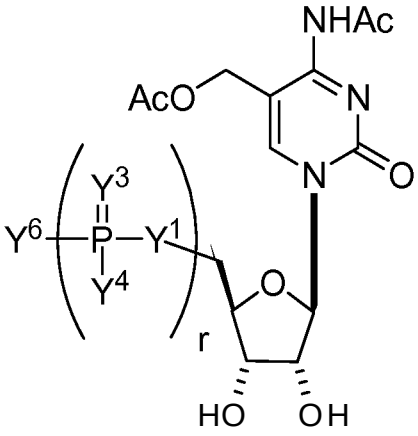


(BB- 137),



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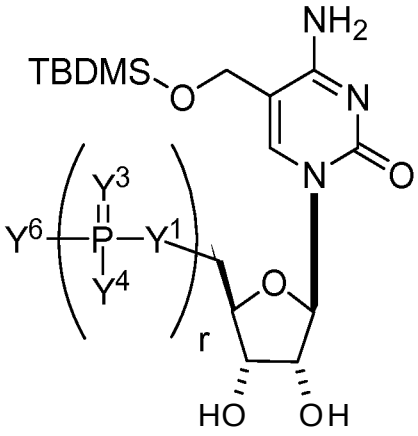

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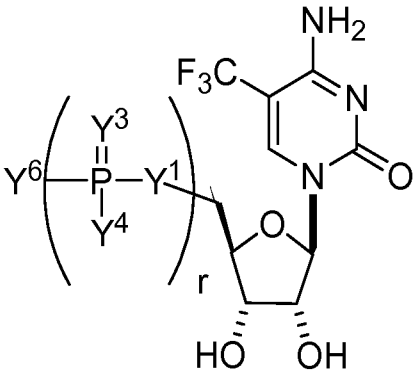
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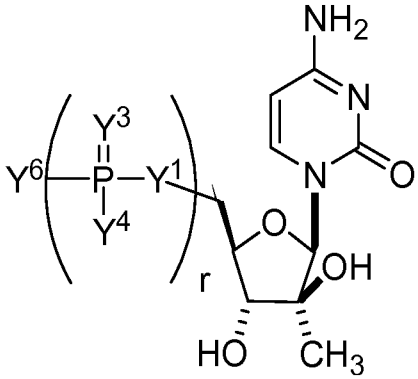
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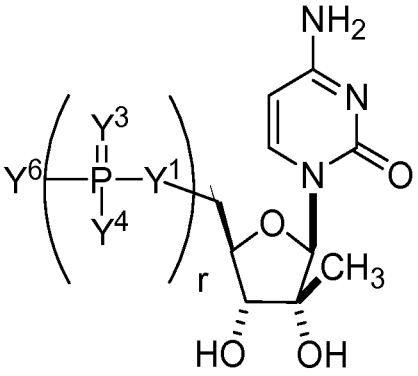
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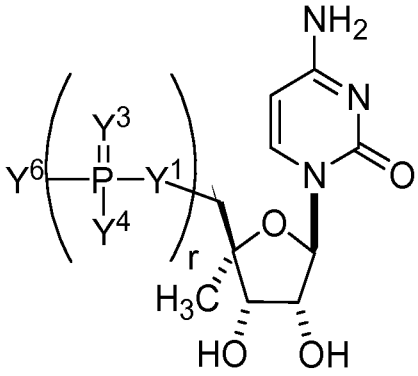
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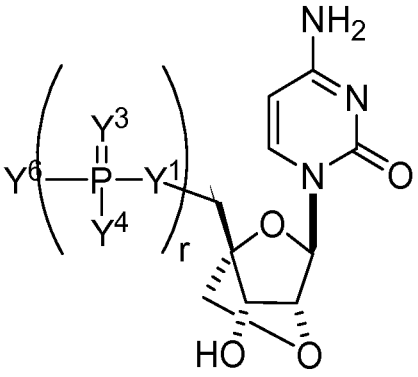
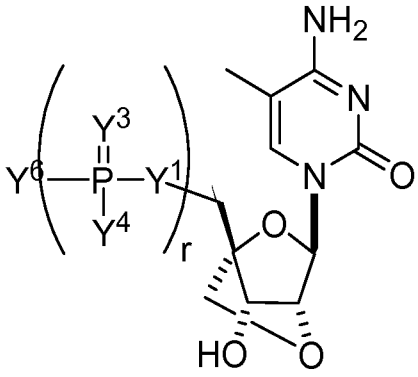
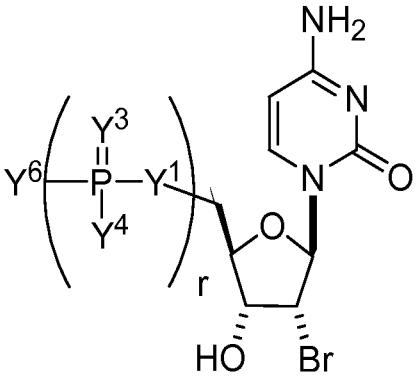

(BB- 144),


(BB- 145),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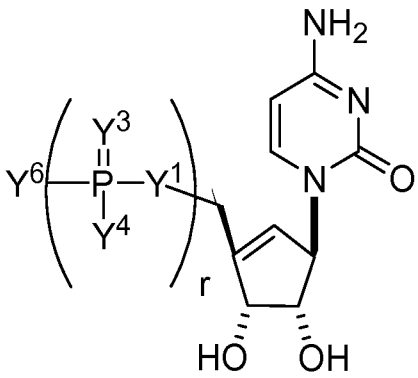
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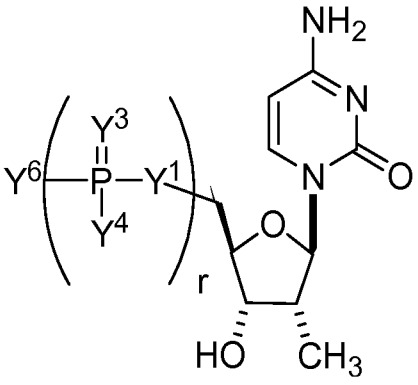
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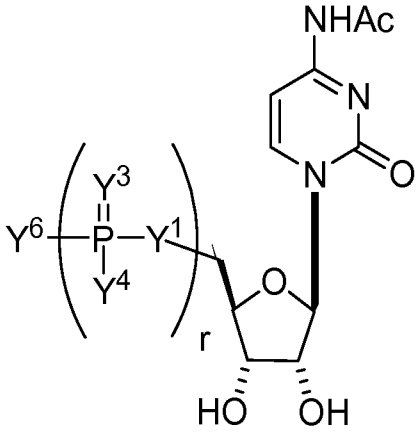
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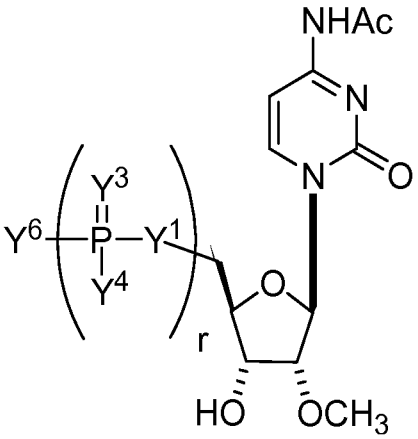
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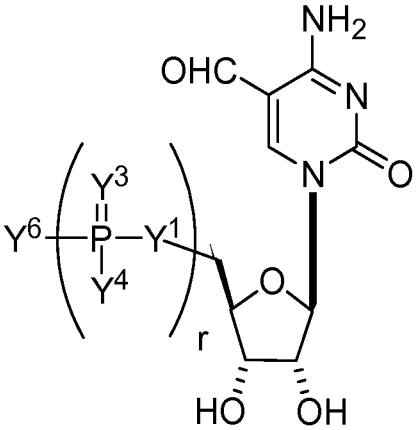
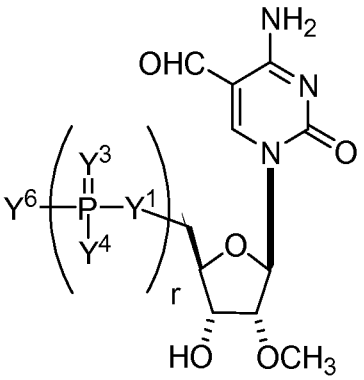

(BB- 151),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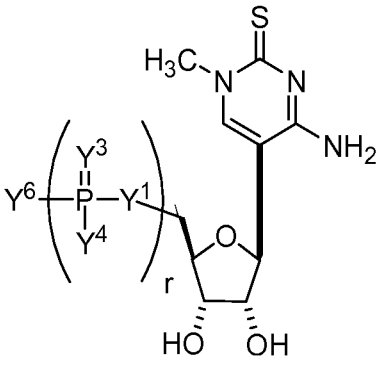
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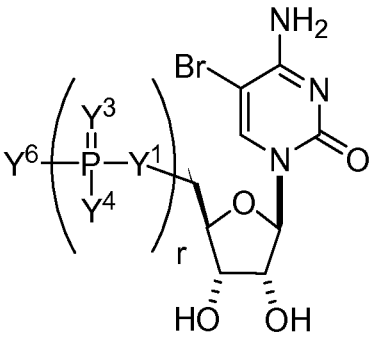
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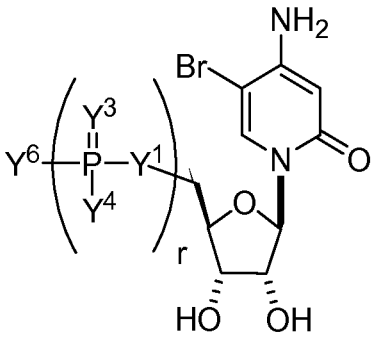
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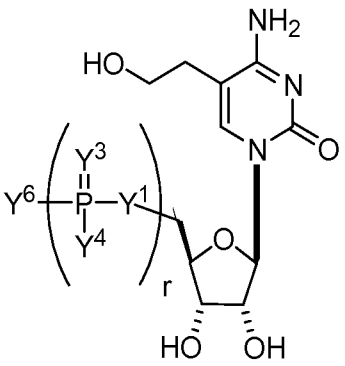

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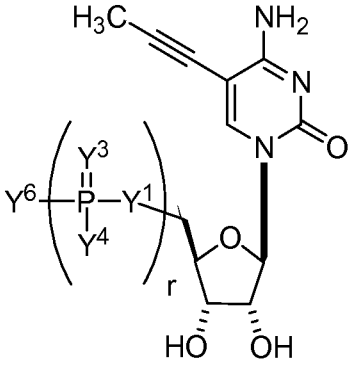

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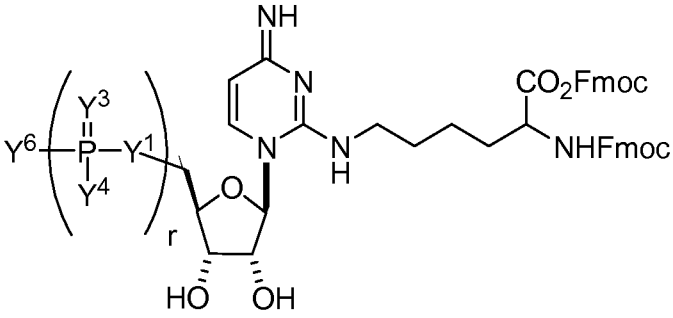
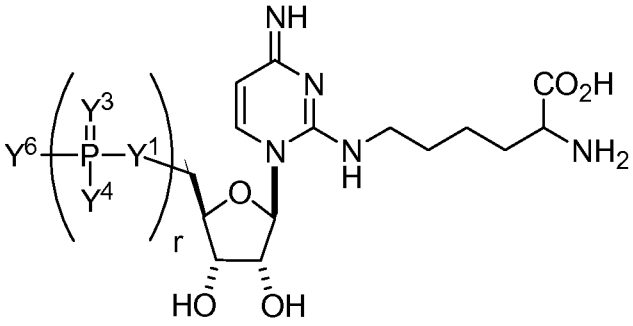
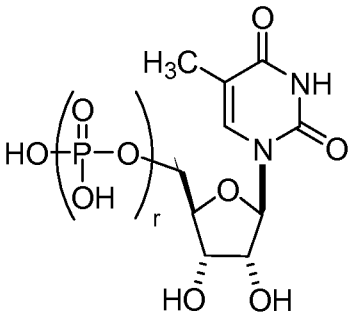


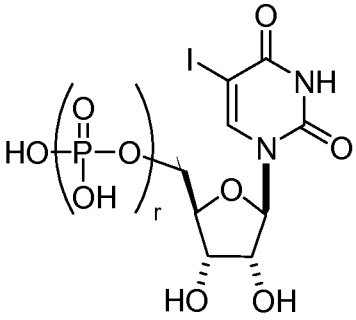
(BB- 158), and



(BB- 159), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein Y^1, Y^3, Y^4, Y^6, and r are as described herein (e.g., each r is, independently, an integer from 0 to 5, such as from 0 to 3, from 1 to 3, or from 1 to 5)). For example, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, can be:

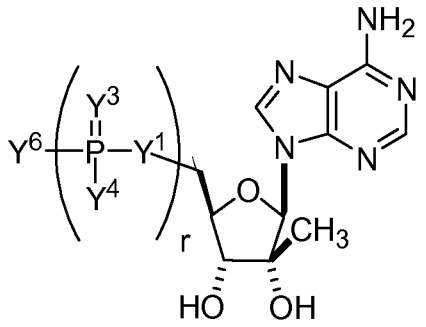


(BB- 160) or

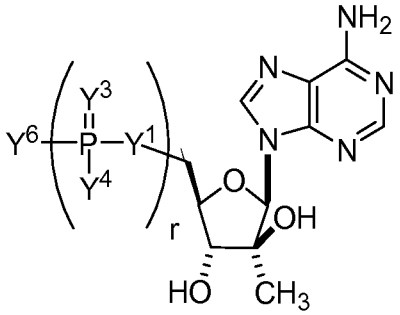


(BB- 161), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein each r is, independently, an integer from 0 to 5 (e.g., from 0 to 3, from 1 to 3, or from 1 to 5).

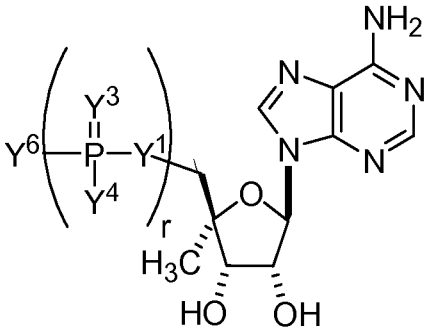
[00356] In some embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, is a modified adenosine (e.g., selected from the group consisting of:



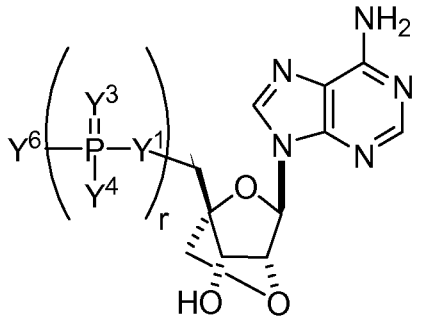
(BB- 162),



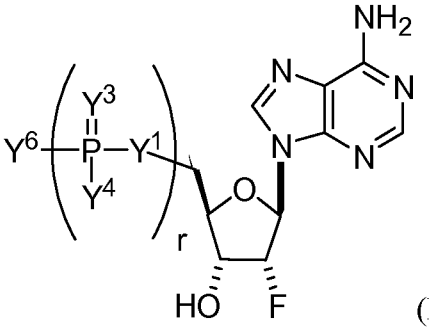
(BB- 163),



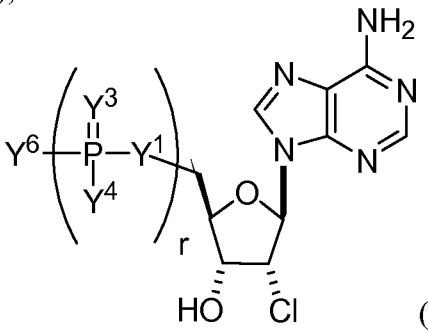
(BB- 164),



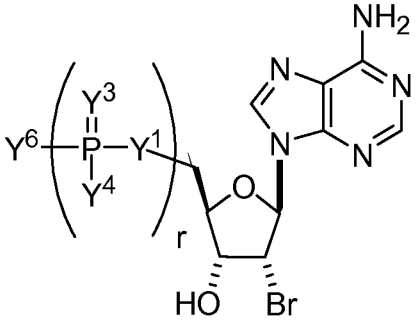
(BB- 165),



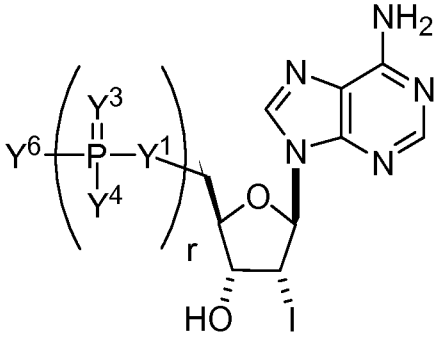
(BB- 166),



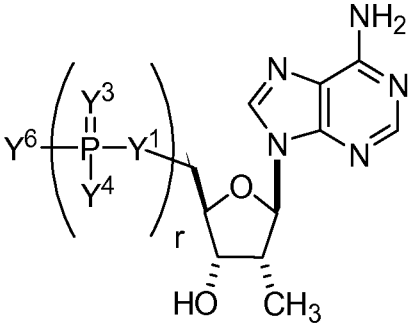
(BB- 167),



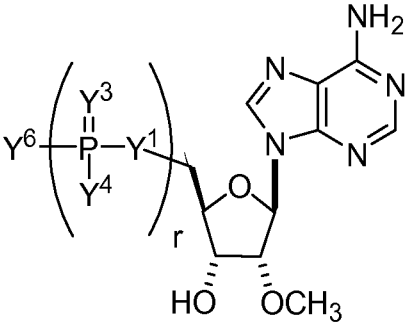
(BB- 168),



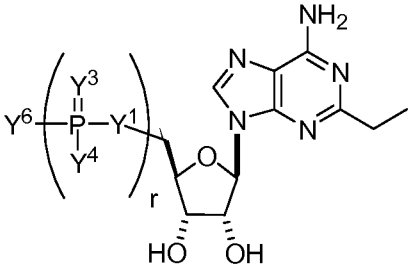
(BB- 169),



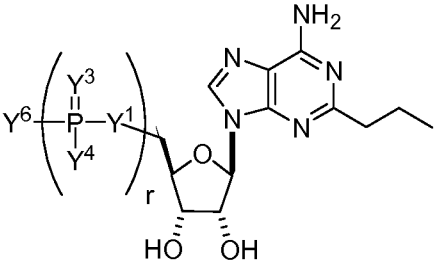
(BB- 170),



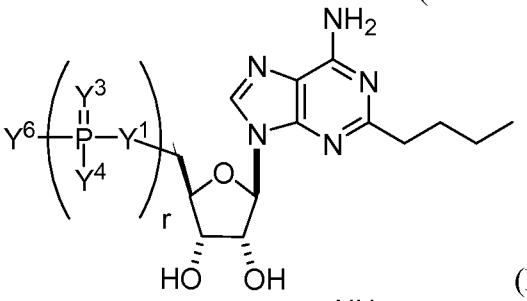
(BB- 171),



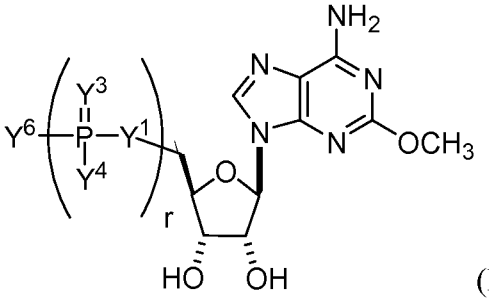
(BB- 172),



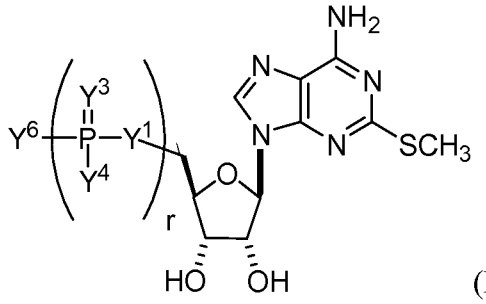
(BB- 173),



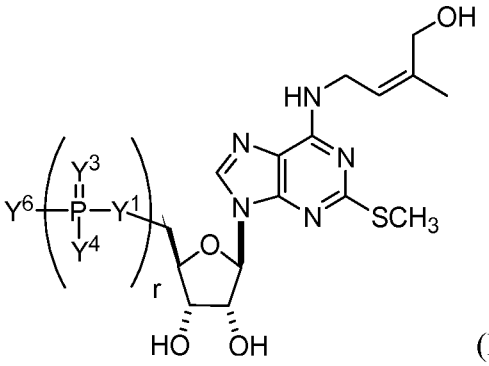
(BB- 174),



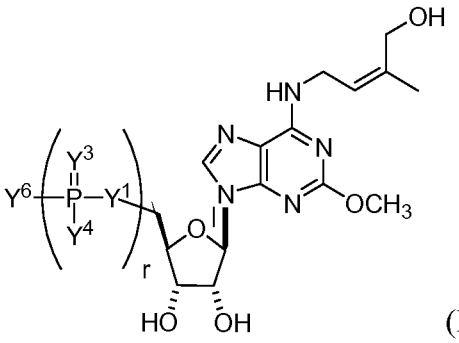
(BB- 175),



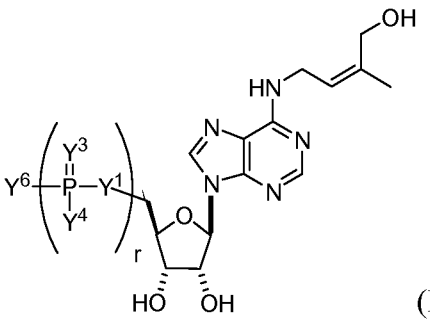
(BB- 176),



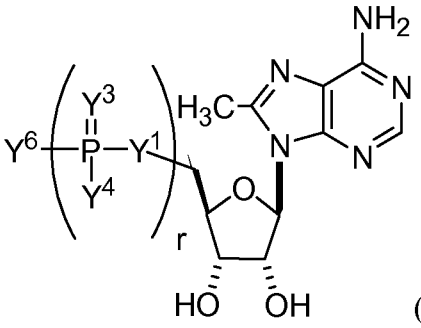
(BB- 177),



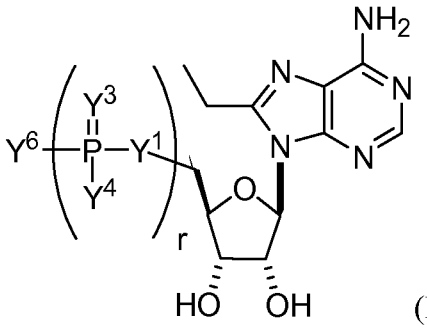
(BB- 178),



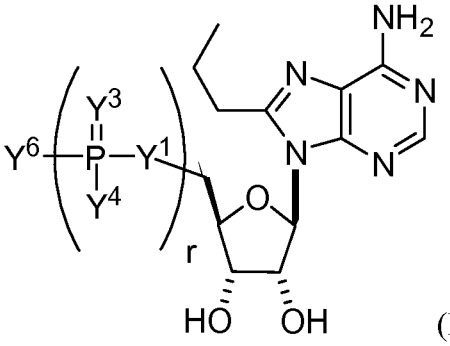
(BB- 179),

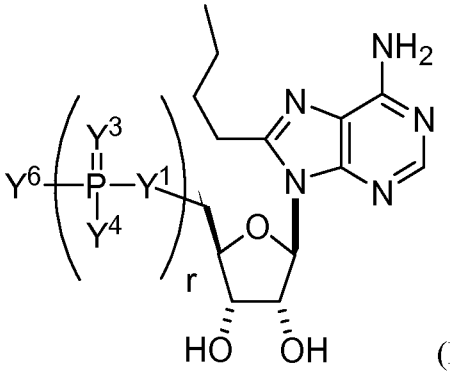


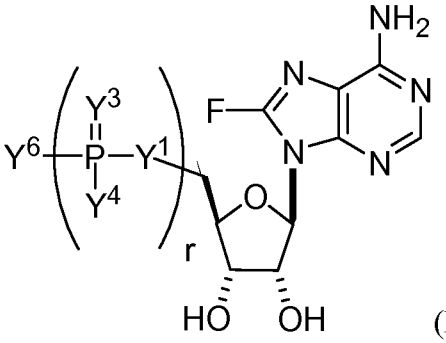
(BB- 180),

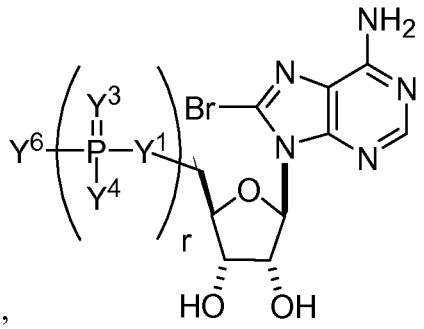


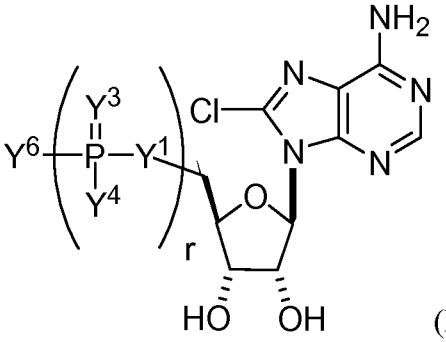
(BB- 181),

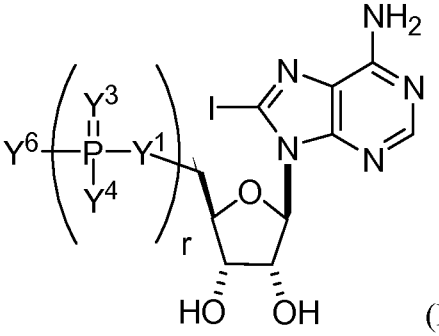

(BB- 182),

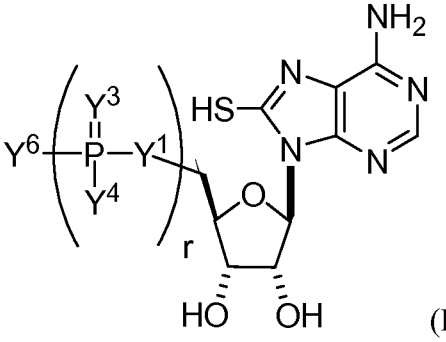

(BB- 183),

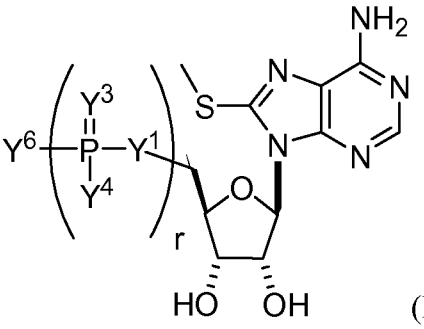

(BB- 184),

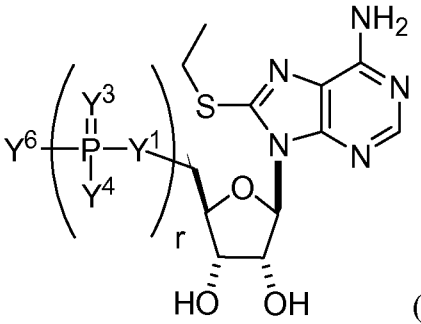

(BB- 185),

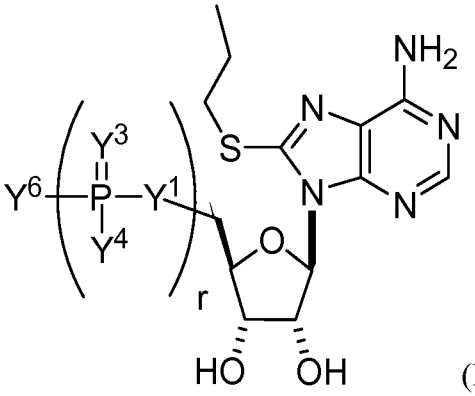

(BB- 186),

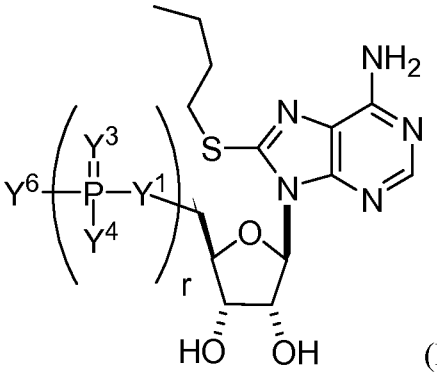

(BB- 187),

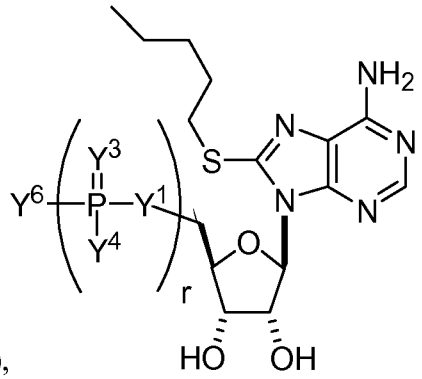

(BB- 188),

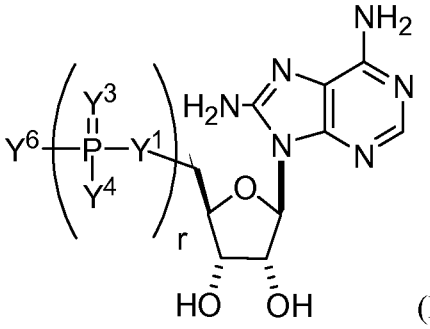

(BB- 189),

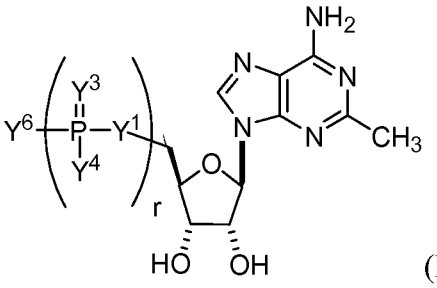

(BB- 190),


(BB- 191),


(BB- 192),


(BB- 193),


(BB- 194),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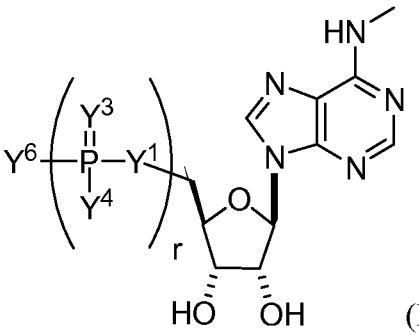
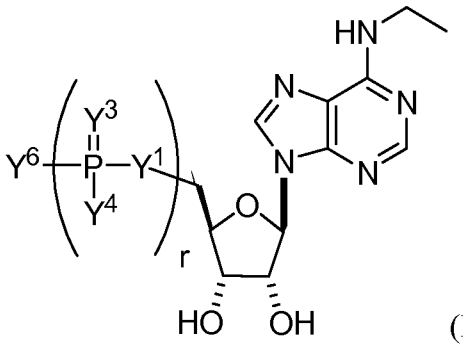
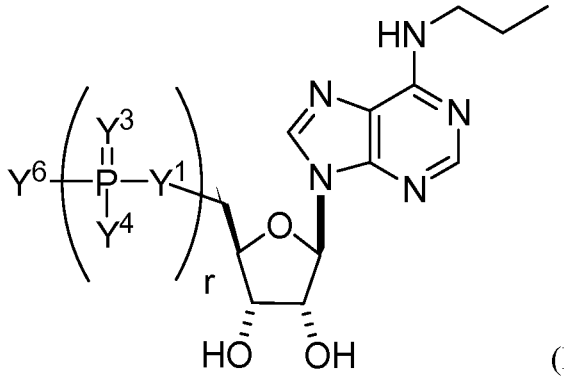
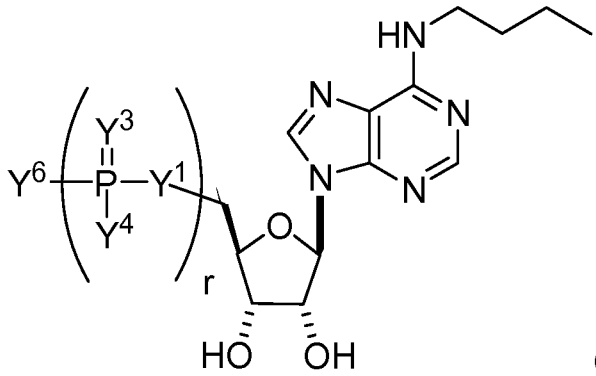

(BB- 196),

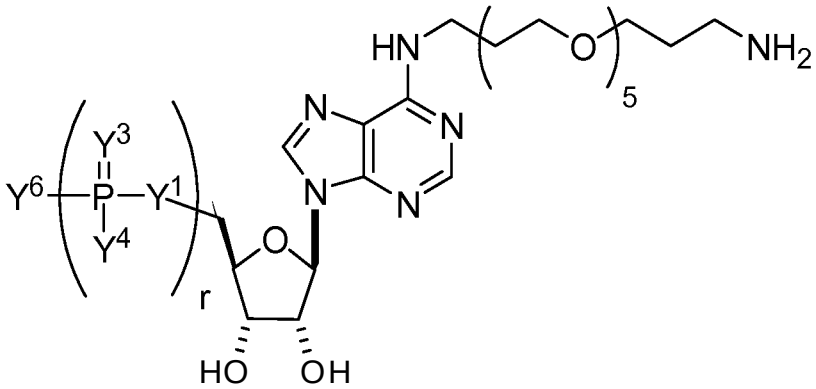

(BB- 197),



(BB- 198),

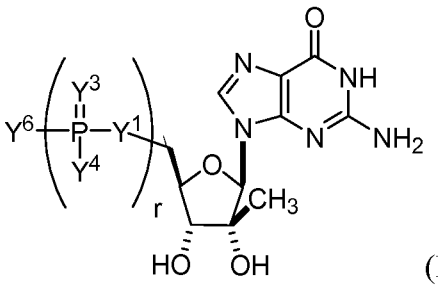


(BB- 199), and

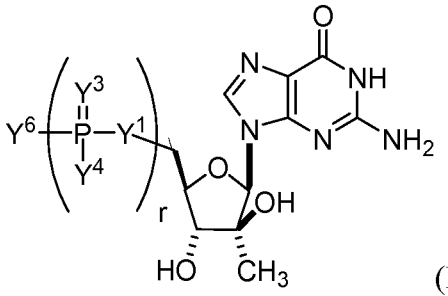


(BB- 200) or a pharmaceutically acceptable salt or stereoisomer thereof, wherein Y^1, Y^3, Y^4, Y^6, and r are as described herein (e.g., each r is, independently, an integer from 0 to 5, such as from 0 to 3, from 1 to 3, or from 1 to 5)).

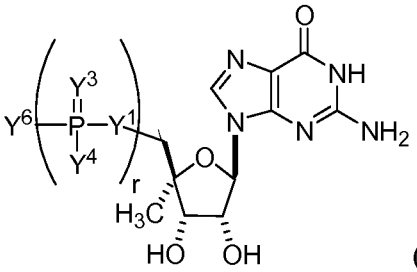
[00357] In some embodiments, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, is a modified guanosine (e.g., selected from the group consisting of:



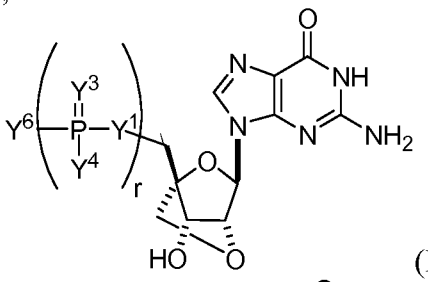
(BB- 201),



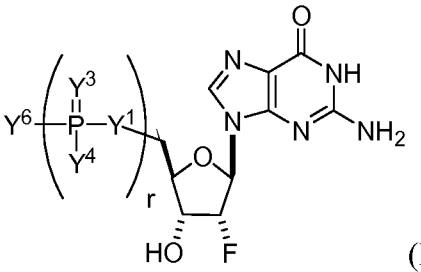
(BB- 202),



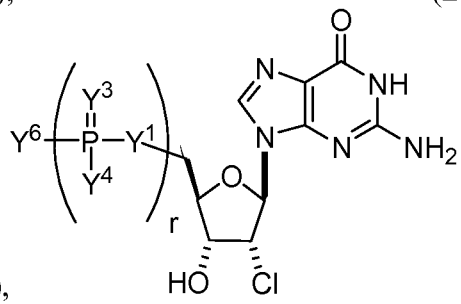
(BB- 203),



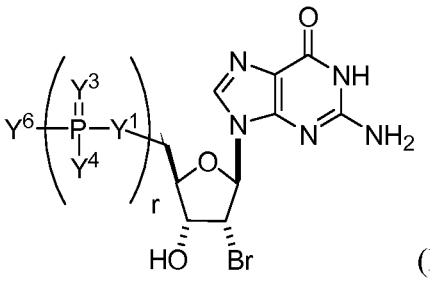
(BB- 204),



(BB- 205),



(BB- 206),



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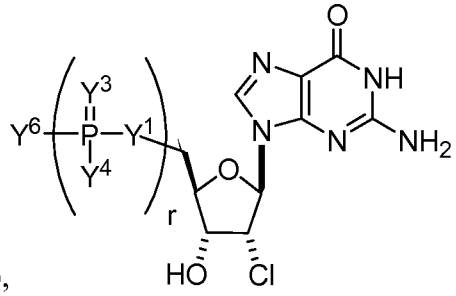

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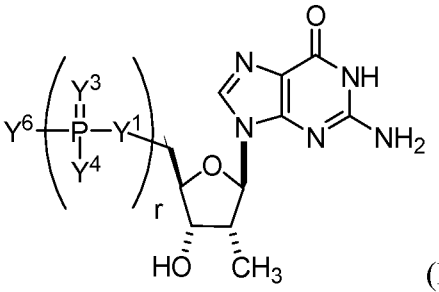
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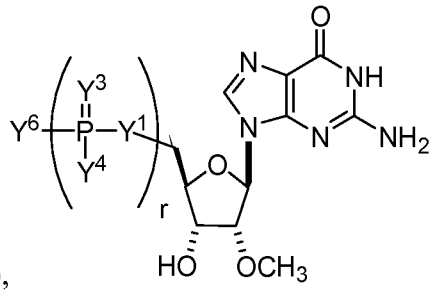
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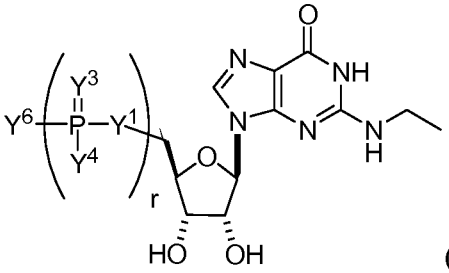
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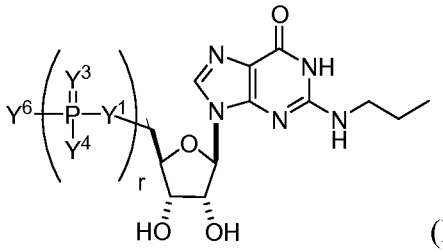
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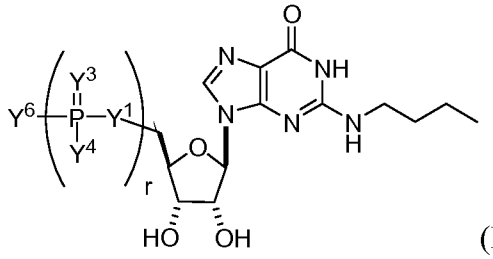
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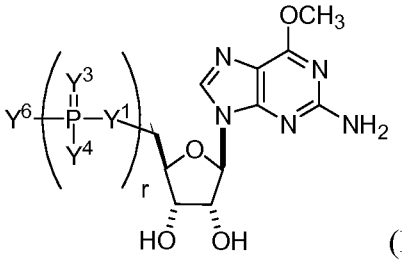
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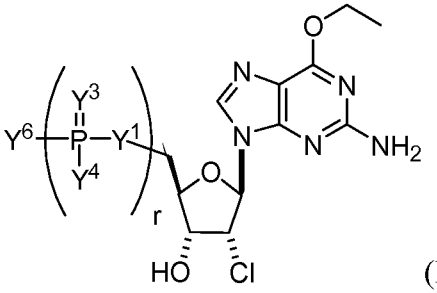
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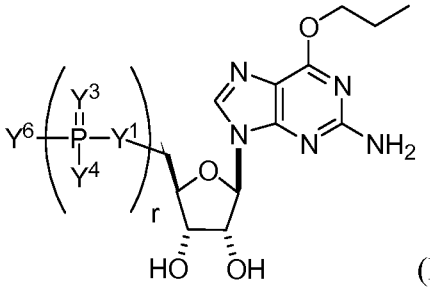
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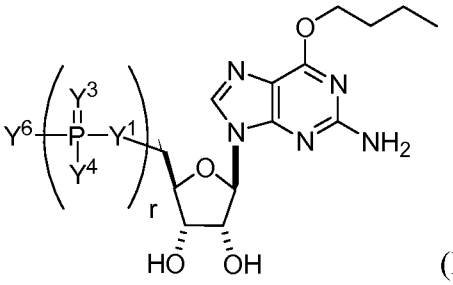
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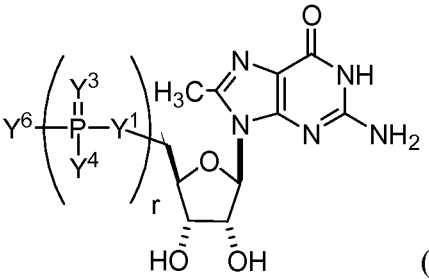
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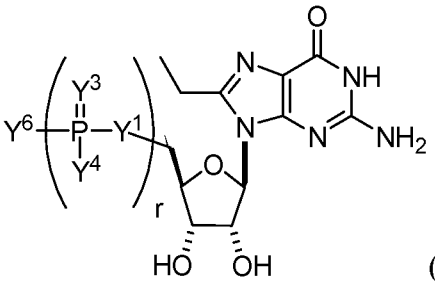

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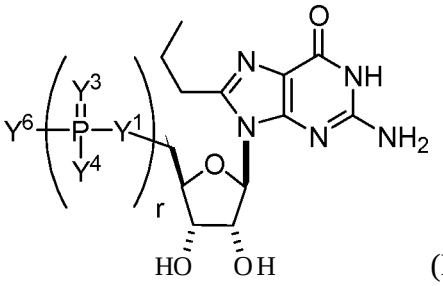

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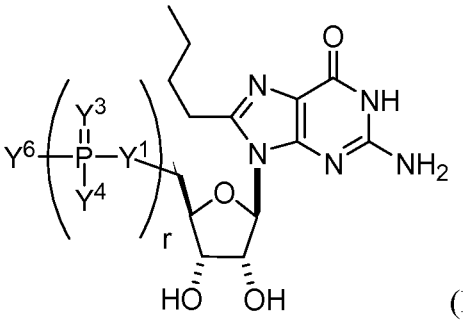

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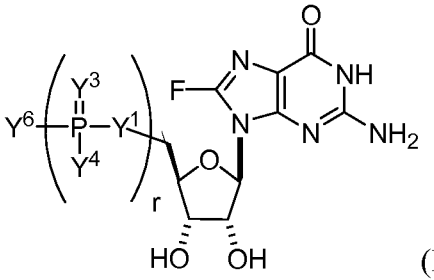

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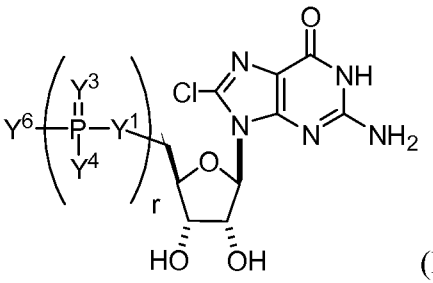

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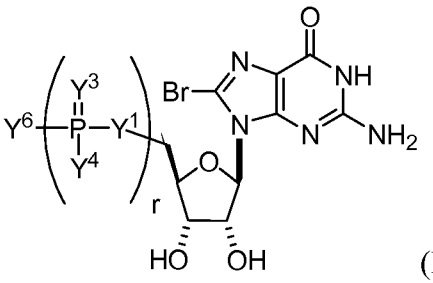

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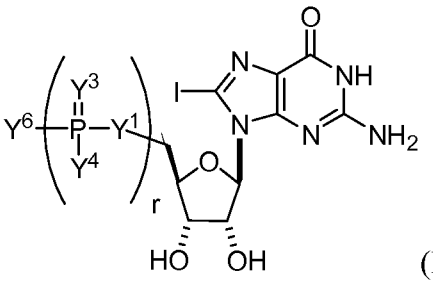

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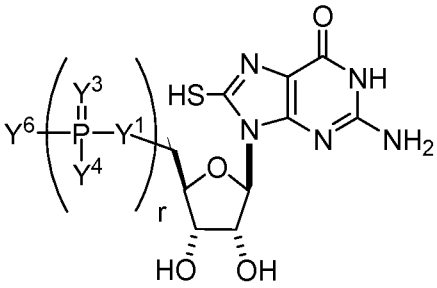

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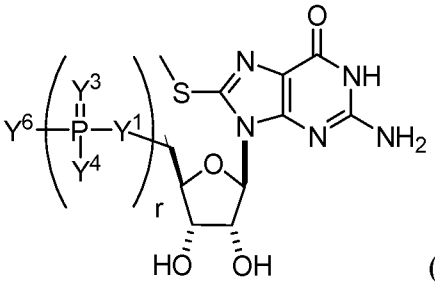

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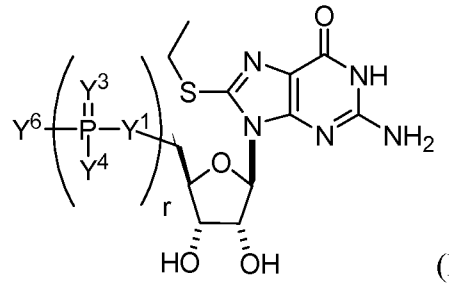

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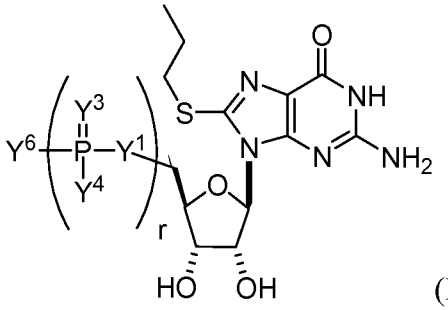

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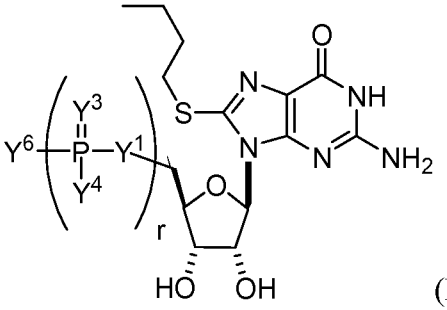

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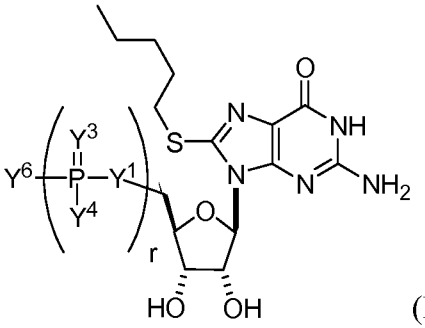

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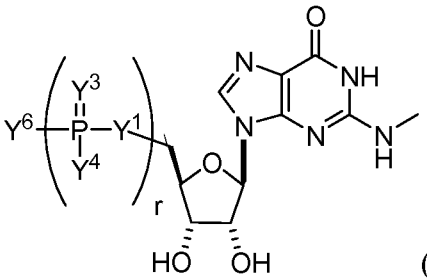
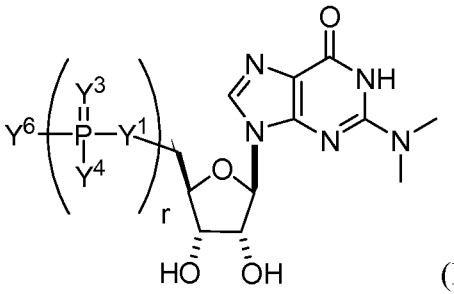


(BB- 232),



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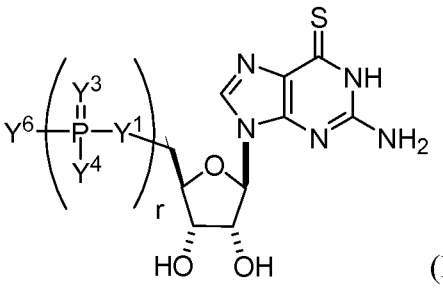

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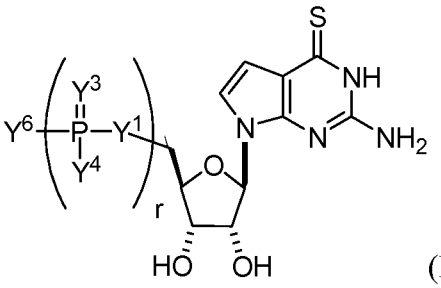

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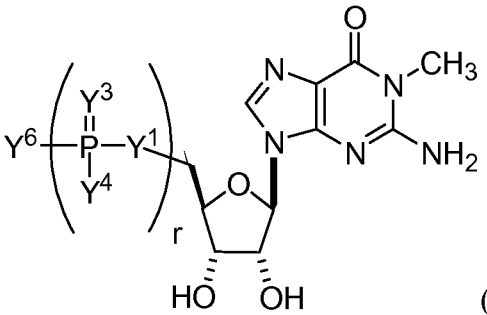
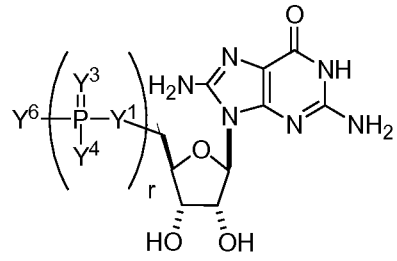
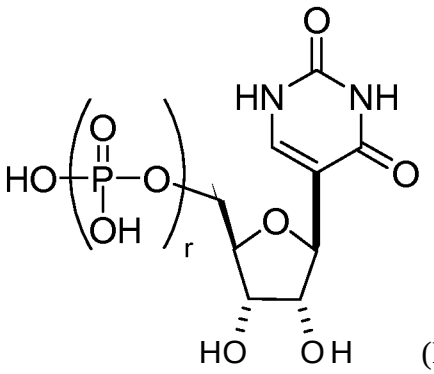


(BB- 236), and

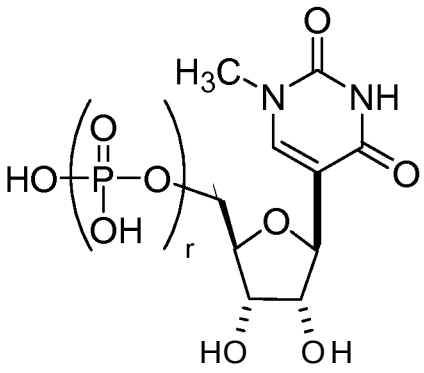


(BB- 237), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein Y^1, Y^3, Y^4, Y^6, and r are as described herein (e.g., each r is, independently, an integer from 0 to 5, such as from 0 to 3, from 1 to 3, or from 1 to 5)).

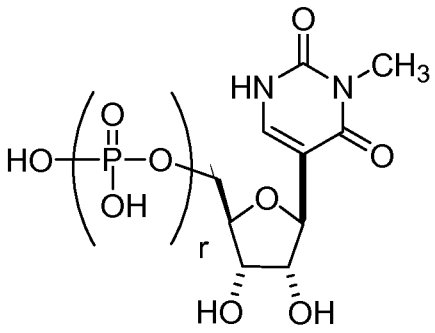
[00358] In some embodiments, the chemical modification can include replacement of C group at C-5 of the ring (e.g., for a pyrimidine nucleoside, such as cytosine or uracil) with N (e.g., replacement of the >CH group at C-5 with $>NR^{N1}$ group, wherein R^{N1} is H or optionally substituted alkyl). For example, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, can be:



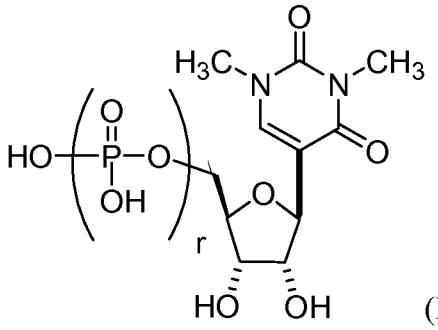
(BB- 238) or



(BB- 239)

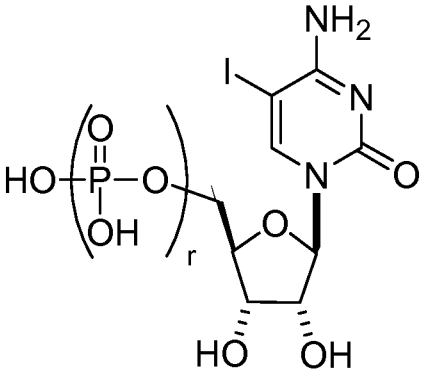


or (BB- 240) or

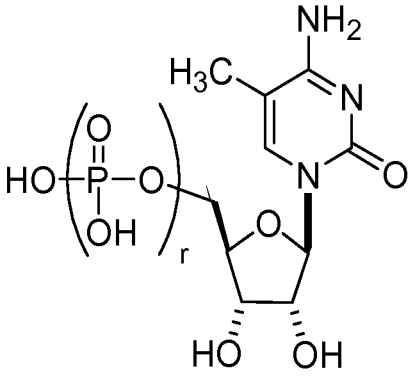


(BB- 241), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein each r is, independently, an integer from 0 to 5 (e.g., from 0 to 3, from 1 to 3, or from 1 to 5).

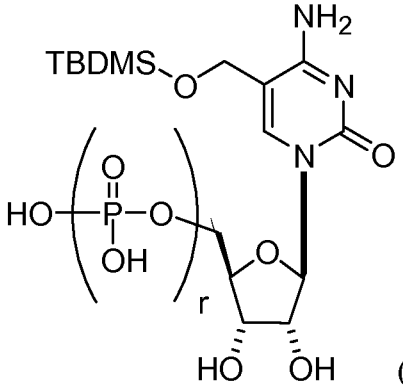
[00359] In another embodiment, the chemical modification can include replacement of the hydrogen at C-5 of cytosine with halo (e.g., Br, CI, F, or I) or optionally substituted alkyl (e.g., methyl). For example, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, can be:



(BB- 242) or

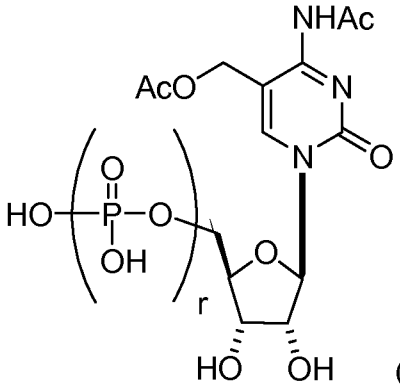


(BB- 243)



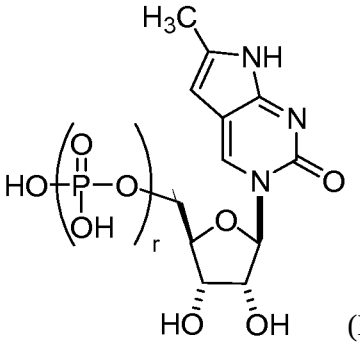
or

(BB- 244) or



(BB- 245), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein each r is, independently, an integer from 0 to 5 (e.g., from 0 to 3, from 1 to 3, or from 1 to 5).

[00360] In yet a further embodiment, the chemical modification can include a fused ring that is formed by the NH2 at the **C-4** position and the carbon atom at the **C-5** position. For example, the building block molecule, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA, can be:



(BB- 246), or a pharmaceutically acceptable salt or stereoisomer thereof, wherein each r is, independently, an integer from 0 to 5 (e.g., from 0 to 3, from 1 to 3, or from 1 to 5).

Modifications on the Sugar

[00361] The modified nucleosides and nucleotides (e.g., building block molecules), which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., RNA or mRNA, as described herein), can be modified on the sugar of the ribonucleic acid. For example, the 2' hydroxyl group (OH) can be modified or replaced with a number of different substituents. Exemplary substitutions at the 2'-position include, but are not limited to, H, halo, optionally substituted **Ci-6** alkyl; optionally substituted **Ci-6** alkoxy; optionally substituted **C_6-io** aryloxy; optionally substituted C3-8 cycloalkyl; optionally substituted C3-8 cycloalkoxy; optionally substituted **C6-io** aryloxy; optionally substituted **C_6-io** aryl-**Ci**-6 alkoxy, optionally substituted **Ci-12** (heterocyclyl)oxy; a sugar (e.g., ribose, pentose, or any described herein); a polyethyleneglycol (PEG), -0(CH2CH $_2$0)nCH2CH $_2$OR, where R is H or optionally substituted alkyl, and n is an integer from 0 to 20 (e.g., from 0 to 4, from 0 to **8,** from 0 to 10, from 0 to 16, from 1 to 4, from 1 to **8,** from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to **8,** from 2 to 10, from 2 to 16, from 2 to 20, from 4 to **8,** from 4 to 10, from 4 to 16, and from 4 to 20); "locked" nucleic acids (LNA) in which the 2'-hydroxyl is connected by a **Ci-6** alkylene or **Ci-6** heteroalkylene bridge to the 4'-carbon of the same ribose sugar, where exemplary bridges included methylene, propylene, ether, or amino

bridges; aminoalkyl, as defined herein; aminoalkoxy, as defined herein; amino as defined herein; and amino acid, as defined herein

[00362] Generally, RNA includes the sugar group ribose, which is a 5-membered ring having an oxygen. Exemplary, non-limiting modified nucleotides include replacement of the oxygen in ribose (e.g., with S, Se, or alkylene, such as methylene or ethylene); addition of a double bond (e.g., to replace ribose with cyclopentenyl or cyclohexenyl); ring contraction of ribose (e.g., to form a 4-membered ring of cyclobutane or oxetane); ring expansion of ribose (e.g., to form a 6- or 7-membered ring having an additional carbon or heteroatom, such as for anhydrohexitol, altritol, mannitol, cyclohexanyl, cyclohexenyl, and morpholino that also has a phosphoramidate backbone); multicyclic forms (e.g., tricyclo; and "unlocked" forms, such as glycol nucleic acid (GNA) (e.g., R-GNA or S-GNA, where ribose is replaced by glycol units attached to phosphodiester bonds), threose nucleic acid (TNA, where ribose is replace with a-L-threofuranosyl-(3'→2')), and peptide nucleic acid (PNA, where 2-amino-ethyl-glycine linkages replace the ribose and phosphodiester backbone). The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a cell phenotype altering polynucleotide, primary construct, or mmRNA molecule can include nucleotides containing, e.g., arabinose, as the sugar.

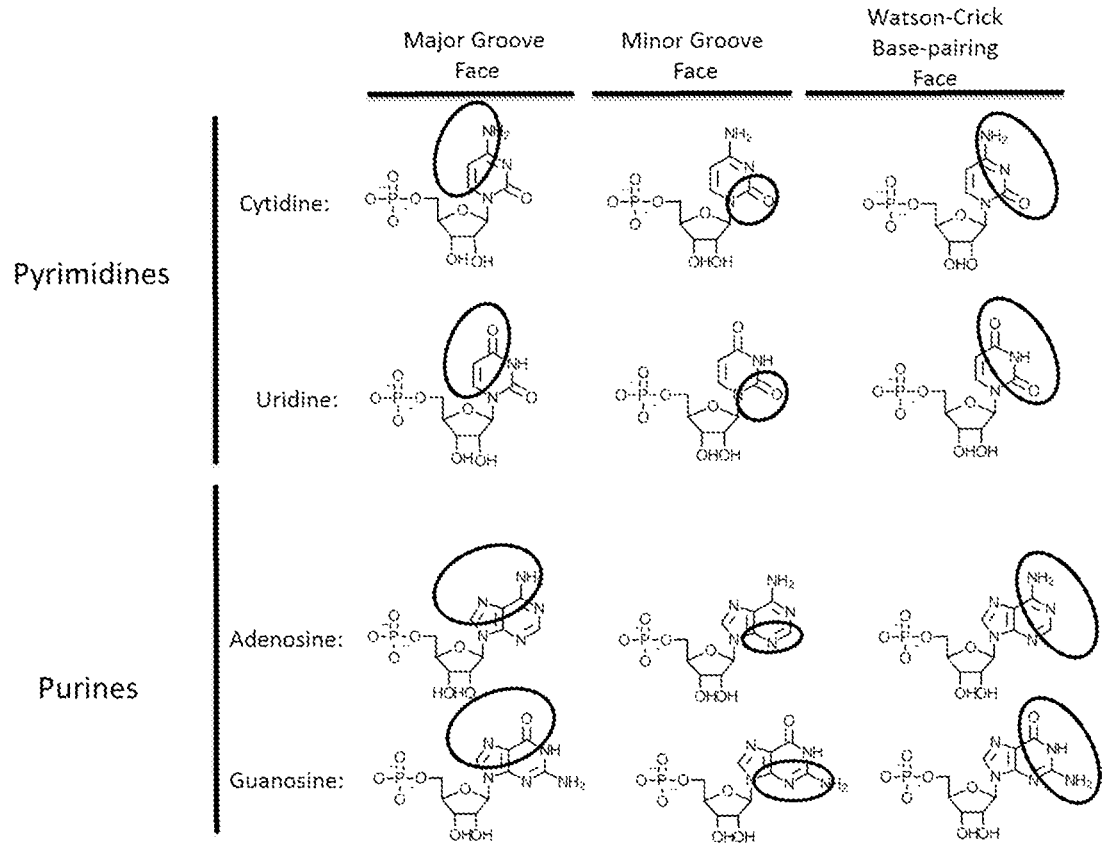
Modifications on the Nucleobase

[00363] The present disclosure provides for modified nucleosides and nucleotides. As described herein "nucleoside" is defined as a compound containing a sugar molecule (e.g., a pentose or ribose) or a derivative thereof in combination with an organic base (e.g., a purine or pyrimidine) or a derivative thereof (also referred to herein as "nucleobase"). As described herein, "nucleotide" is defined as a nucleoside including a phosphate group. In some embodiments, the nucleosides and nucleotides described herein are generally chemically modified. Exemplary non-limiting modifications include an amino group, a thiol group, an alkyl group, a halo group, or any described herein. The modified nucleotides may by synthesized by any useful method, as described herein (e.g., chemically, enzymatically, or recombinantly to include one or more modified or non-natural nucleosides).

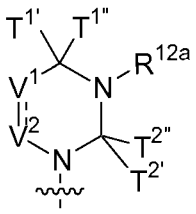
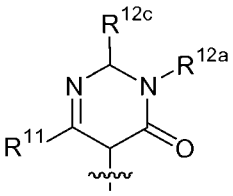
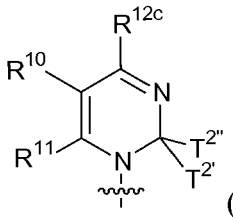
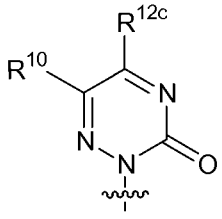
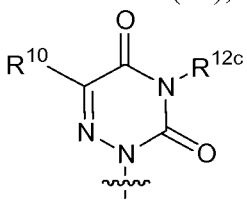
[00364] The modified nucleotide base pairing encompasses not only the standard adenosine-thymine, adenosine-uracil, or guanosine-cytosine base pairs, but also base pairs formed between nucleotides and/or modified nucleotides comprising non-standard or modified bases, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a non-standard base and a standard base or between two complementary non-standard base structures. One example of such non-standard base pairing is the base pairing between the modified nucleotide inosine and adenine, cytosine or uracil.

[00365] The modified nucleosides and nucleotides can include a modified nucleobase. Examples of nucleobases found in RNA include, but are not limited to, adenine, guanine, cytosine, and uracil. Examples of nucleobase found in DNA include, but are not limited to, adenine, guanine, cytosine, and thymine. These nucleobases can be modified or wholly replaced to provide polynucleotides, primary constructs, or mmRNA molecules having enhanced properties, e.g., resistance to nucleases through disruption of the binding of a major groove binding partner.Table 8 below identifies the chemical faces of each canonical nucleotide. Circles identify the atoms comprising the respective chemical regions.

Table 8



[00366] In some embodiments, B is a modified uracil. Exemplary modified uracils include those having Formula (bl)-(b5):


(b1),

(b2),

(b3),

(b4),

or (b5), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00367] wherein

[00368] ⩫ is a single or double bond;

[00369] each of $T^{1'}$, $T^{1''}$, $T^{2'}$, and $T^{2''}$ is, independently, H, optionally substituted alkyl, optionally substituted alkoxy, or optionally substituted thioalkoxy, or the combination of

$T^{1'}$ and $T^{1''}$ or the combination of $T^{2'}$ and $T^{2''}$ join together (e.g., as in T^2) to form **O** (oxo), S (thio), or Se (seleno);

[00370] each of V^1 and V^2 is, independently, **O,** S, **N(**R^{Vb}**)nv,** or C(R^{Vb})$_{nv}$, wherein nv is an integer from **0** to 2 and each R^{Vb} is, independently, H, halo, optionally substituted amino acid, optionally substituted alkyl, optionally substituted haloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted aminoalkyl (e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl), optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, optionally substituted acylaminoalkyl (e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl), optionally substituted alkoxycarbonylalkyl, optionally substituted alkoxycarbonylalkenyl, optionally substituted alkoxycarbonylalkynyl, or optionally substituted alkynyloxy (e.g., optionally substituted with any substituent described herein, such as those selected from (1)-(21) for alkyl);

[00371] **R^{10}** is **H,** halo, optionally substituted amino acid, hydroxy, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aminoalkyl, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, optionally substituted alkoxy, optionally substituted alkoxycarbonylalkyl, optionally substituted alkoxycarbonylalkenyl, optionally substituted alkoxycarbonylalkynyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted carboxyalkoxy, optionally substituted carboxyalkyl, or optionally substituted carbamoylalkyl;

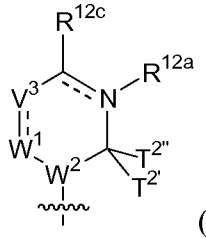
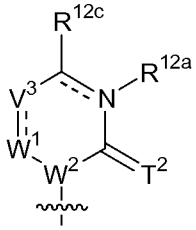
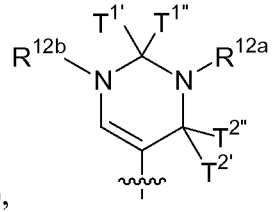
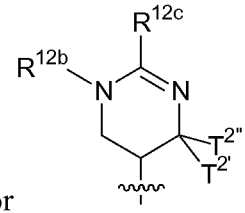
[00372] **R^{11}** is **H** or optionally substituted alkyl;

[00373] **R^{12a}** is **H,** optionally substituted alkyl, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, or optionally substituted aminoalkynyl, optionally substituted carboxyalkyl (e.g., optionally substituted with

hydroxy), optionally substituted carboxyalkoxy, optionally substituted carboxyaminoalkyl, or optionally substituted carbamoylalkyl; and

[00374] R^{12c} is H, halo, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted thioalkoxy, optionally substituted amino, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, or optionally substituted aminoalkynyl.

[00375] Other exemplary modified uracils include those having Formula (b6)-(b9):


(b6),

(b7),

(b8), or


(b9), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00376] wherein

[00377] ⁓ is a single or double bond;

[00378] each of $T^{1'}$, $T^{1''}$, $T^{2'}$, and $T^{2''}$ is, independently, H, optionally substituted alkyl, optionally substituted alkoxy, or optionally substituted thioalkoxy, or the combination of $T^{1'}$ and $T^{1''}$ join together (e.g., as in T^1) or the combination of $T^{2'}$ and $T^{2''}$ join together (e.g., as in T^2) to form O (oxo), S (thio), or Se (seleno), or each T^1 and T^2 is, independently, O (oxo), S (thio), or Se (seleno);

[00379] each of W^1 and W^2 is, independently, $N(R^{Wa})_{n_w}$ or $C(R^{Wa})_{n_w}$, wherein nw is an integer from 0 to 2 and each R^{Wa} is, independently, H, optionally substituted alkyl, or optionally substituted alkoxy;

[00380] each V^3 is, independently, O, S, $N(R^{Va})_{nv}$, or $C(R^{Va})_{nv}$, wherein nv is an integer from 0 to 2 and each R^{Va} is, independently, H, halo, optionally substituted amino acid, optionally substituted alkyl, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclyl, optionally substituted alkheterocyclyl, optionally substituted alkoxy, optionally substituted alkenyloxy, or optionally substituted alkynyloxy , optionally substituted aminoalkyl (e.g., substituted

with an N-protecting group, such as any described herein, e.g., trifluoroacetyl, or sulfoalkyl), optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, optionally substituted acylaminoalkyl (e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl), optionally substituted alkoxycarbonylalkyl, optionally substituted alkoxycarbonylalkenyl, optionally substituted alkoxycarbonylalkynyl, optionally substituted alkoxycarbonylacyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted carboxyalkyl (e.g., optionally substituted with hydroxy and/or an O-protecting group), optionally substituted carboxyalkoxy, optionally substituted carboxyaminoalkyl, or optionally substituted carbamoylalkyl (e.g., optionally substituted with any substituent described herein, such as those selected from (1)-(21) for alkyl), and wherein R^{Va} and R^{12c} taken together with the carbon atoms to which they are attached can form optionally substituted cycloalkyl, optionally substituted aryl, or optionally substituted heterocyclyl (e.g., a 5- or 6-membered ring);

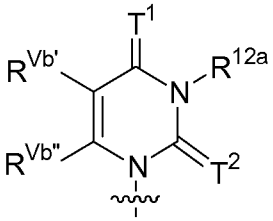
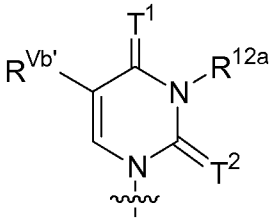
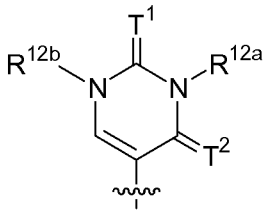
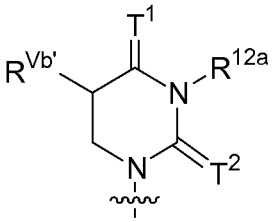
[00381] R^{12a} is H, optionally substituted alkyl, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, optionally substituted carboxyalkyl (e.g., optionally substituted with hydroxy and/or an O-protecting group), optionally substituted carboxyalkoxy, optionally substituted carboxyaminoalkyl, optionally substituted carbamoylalkyl, or absent;

[00382] R^{12b} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, optionally substituted alkaryl, optionally substituted heterocyclyl, optionally substituted alkheterocyclyl, optionally substituted amino acid, optionally substituted alkoxycarbonylacyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted alkoxycarbonylalkyl, optionally substituted alkoxycarbonylalkenyl, optionally substituted alkoxycarbonylalkynyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted carboxyalkyl (e.g., optionally substituted with hydroxy and/or an O-protecting group), optionally substituted carboxyalkoxy, optionally substituted carboxyaminoalkyl, or optionally substituted carbamoylalkyl,

[00383] wherein the combination of R^{12b} and $T^{1'}$ or the combination of R^{12b} and R^{12c} can join together to form optionally substituted heterocyclyl; and

[00384] R^{12c} is H, halo, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted thioalkoxy, optionally substituted amino, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, or optionally substituted aminoalkynyl.

[00385] Further exemplary modified uracils include those having Formula (b28)-(b31):


(b28),

(b29),

(b30), or

(b31), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00386] wherein

[00387] each of T^1 and T^2 is, independently, O (oxo), S (thio), or Se (seleno);

[00388] each $R^{Vb'}$ and $R^{Vb''}$ is, independently, H, halo, optionally substituted amino acid, optionally substituted alkyl, optionally substituted haloalkyl, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkyl (e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl, or sulfoalkyl), optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, optionally substituted acylaminoalkyl (e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl), optionally substituted alkoxycarbonylalkyl, optionally substituted alkoxycarbonylalkenyl, optionally substituted alkoxycarbonylalkynyl, optionally substituted alkoxycarbonylacyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted carboxyalkyl (e.g., optionally substituted with hydroxy and/or an O-protecting

group), optionally substituted carboxyalkoxy, optionally substituted carboxyaminoalkyl, or optionally substituted carbamoylalkyl (e.g., optionally substituted with any substituent described herein, such as those selected from (1)-(21) for alkyl) (e.g., $R^{Vb'}$ is optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted aminoalkyl, e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl, or sulfoalkyl);

[00389] R^{12a} is H, optionally substituted alkyl, optionally substituted carboxyaminoalkyl, optionally substituted aminoalkyl (e.g., e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl, or sulfoalkyl), optionally substituted aminoalkenyl, or optionally substituted aminoalkynyl; and

[00390] R^{12b} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl (e.g., e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl, or sulfoalkyl),

[00391] optionally substituted alkoxycarbonylacyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted alkoxycarbonylalkyl, optionally substituted alkoxycarbonylalkenyl, optionally substituted alkoxycarbonylalkynyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted carboxyalkoxy, optionally substituted carboxyalkyl, or optionally substituted carbamoylalkyl.

[00392] In particular embodiments, T^1 is O (oxo), and T^2 is S (thio) or Se (seleno). In other embodiments, T^1 is S (thio), and T^2 is O (oxo) or Se (seleno). In some embodiments, $R^{Vb'}$ is H, optionally substituted alkyl, or optionally substituted alkoxy.

[00393] In other embodiments, each R^{12a} and R^{12b} is, independently, H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, or optionally substituted hydroxyalkyl. In particular embodiments, R^{12a} is H. In other embodiments, both R^{12a} and R^{12b} are H.

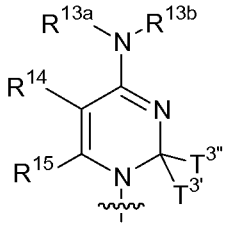
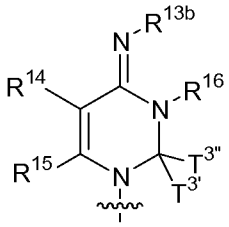
[00394] In some embodiments, each $R^{Vb'}$ of R^{12b} is, independently, optionally substituted aminoalkyl (e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl, or sulfoalkyl), optionally substituted aminoalkenyl,

optionally substituted aminoalkynyl, or optionally substituted acylaminoalkyl (e.g., substituted with an N-protecting group, such as any described herein, e.g., trifluoroacetyl). In some embodiments, the amino and/or alkyl of the optionally substituted aminoalkyl is substituted with one or more of optionally substituted alkyl, optionally substituted alkenyl, optionally substituted sulfoalkyl, optionally substituted carboxy (e.g., substituted with an O-protecting group), optionally substituted hydroxy (e.g., substituted with an O-protecting group), optionally substituted carboxyalkyl (e.g., substituted with an O-protecting group), optionally substituted alkoxycarbonylalkyl (e.g., substituted with an O-protecting group), or *N*-protecting group. In some embodiments, optionally substituted aminoalkyl is substituted with an optionally substituted sulfoalkyl or optionally substituted alkenyl. In particular embodiments, R^{12a} and $R^{Vb"}$ are both H. In particular embodiments, T^1 is O (oxo), and T^2 is S (thio) or Se (seleno).

[00395] In some embodiments, $R^{Vb'}$ is optionally substituted alkoxycarbonylalkyl or optionally substituted carbamoylalkyl.

[00396] In particular embodiments, the optional substituent for R^{12a}, R^{12b}, R^{12c}, or R^{Va} is a polyethylene glycol group (e.g., -(CH2)s2(OCH2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl); or an amino-polyethylene glycol group (e.g., -$NR^{N1}(CH_2)$s2(CH2CH20)si(CH $_2$)s3NR^{N1}, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl).

[00397] In some embodiments, B is a modified cytosine. Exemplary modified cytosines include compounds of Formula (bl0)-(bl4):


(b10),

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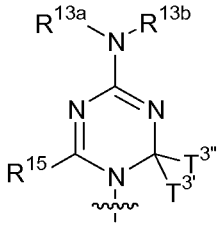
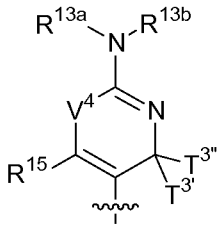
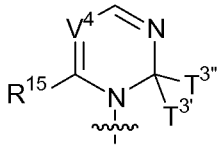

(b12),

(b13), or

(b14), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00398] wherein

[00399] each of $T^{3'}$ and $T^{3''}$ is, independently, H, optionally substituted alkyl, optionally substituted alkoxy, or optionally substituted thioalkoxy, or the combination of $T^{3'}$ and $T^{3''}$ join together (e.g., as in T^3) to form O (oxo), S (thio), or Se (seleno);

[00400] each V^4 is, independently, O, S, **$N(R^{Vc})_{nv}$**, or **$C(R^{Vc})_{nv}$**, wherein nv is an integer from 0 to 2 and each **R^{Vc}** is, independently, **H,** halo, optionally substituted amino acid, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted heterocyclyl, optionally substituted alkheterocyclyl, or optionally substituted alkynyloxy (e.g., optionally substituted with any substituent described herein, such as those selected from (1)-(21) for alkyl), wherein the combination of **R^{13b}** and **R^{Vc}** can be taken together to form optionally substituted heterocyclyl;

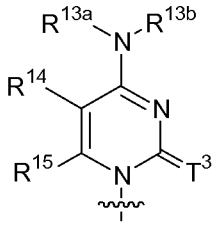
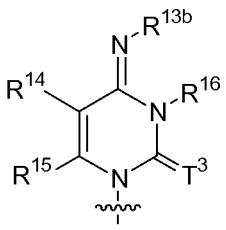
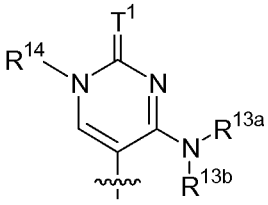
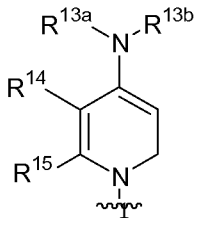
[00401] each V^5 is, independently, **$N(R^{Vd})_{nv}$**, or **$C(R^{Vd})_{nv}$**, wherein nv is an integer from 0 to 2 and each **R^{Vd}** is, independently, **H,** halo, optionally substituted amino acid, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted heterocyclyl, optionally substituted alkheterocyclyl, or optionally substituted alkynyloxy (e.g., optionally substituted with any substituent described herein, such as those selected from (1)-(21) for alkyl) (e.g., V^5 is **-CH** or N);

[00402] each of R^{13a} and R^{13b} is, independently, H, optionally substituted acyl, optionally substituted acyloxyalkyl, optionally substituted alkyl, or optionally substituted alkoxy, wherein the combination of R^{13b} and R^{14} can be taken together to form optionally substituted heterocyclyl;

[00403] each R^{14} is, independently, H, halo, hydroxy, thiol, optionally substituted acyl, optionally substituted amino acid, optionally substituted alkyl, optionally substituted haloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted hydroxyalkyl (e.g., substituted with an O-protecting group), optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted acyloxyalkyl, optionally substituted amino (e.g., -NHR, wherein R is H, alkyl, aryl, or phosphoryl), azido, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted alkheterocyclyl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, or optionally substituted aminoalkyl; and

[00404] each of R^{15} and R^{16} is, independently, H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl.

[00405] Further exemplary modified cytosines include those having Formula (b32)-(b35):


(b32),

(b33),

(b34), or

(b35), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00406] wherein

[00407] each of T^1 and T^3 is, independently, O (oxo), S (thio), or Se (seleno);

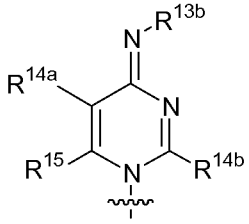
[00408] each of R^{13a} and R^{13b} is, independently, H, optionally substituted acyl, optionally substituted acyloxyalkyl, optionally substituted alkyl, or optionally substituted alkoxy, wherein the combination of R^{13b} and R^{14} can be taken together to form optionally substituted heterocyclyl;

[00409] each R^{14} is, independently, H, halo, hydroxy, thiol, optionally substituted acyl, optionally substituted amino acid, optionally substituted alkyl, optionally substituted haloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted hydroxyalkyl (e.g., substituted with an O-protecting group), optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted acyloxyalkyl, optionally substituted amino (e.g., -NHR, wherein R is H, alkyl, aryl, or phosphoryl), azido, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted alkheterocyclyl, optionally substituted aminoalkyl (e.g., hydroxyalkyl, alkyl, alkenyl, or alkynyl), optionally substituted aminoalkenyl, or optionally substituted aminoalkynyl; and

[00410] each of R^{15} and R^{16} is, independently, H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl (e.g., R^{15} is H, and R^{16} is H or optionally substituted alkyl).

[00411] In some embodiments, R^{15} is H, and R^{16} is H or optionally substituted alkyl. In particular embodiments, R^{14} is H, acyl, or hydroxyalkyl. In some embodiments, R^{14} is halo. In some embodiments, both R^{14} and R^{15} are H. In some embodiments, both R^{15} and R^{16} are H. In some embodiments, each of R^{14} and R^{15} and R^{16} is H. In further embodiments, each of R^{13a} and R^{13b} is independently, H or optionally substituted alkyl.

[00412] Further non-limiting examples of modified cytosines include compounds of Formula (b36):



(b36) or a pharmaceutically acceptable salt or stereoisomer thereof,

[00413] wherein

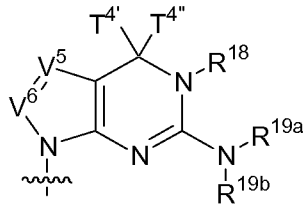
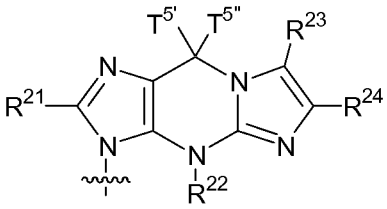
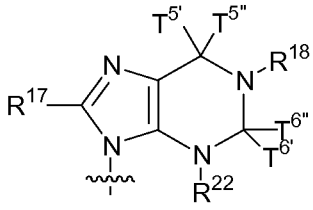
[00414] each R^{13b} is, independently, H, optionally substituted acyl, optionally substituted acyloxyalkyl, optionally substituted alkyl, or optionally substituted alkoxy, wherein the combination of R^{13b} and R^{14b} can be taken together to form optionally substituted heterocyclyl;

[00415] each R^{14a} and R^{14b} is, independently, H, halo, hydroxy, thiol, optionally substituted acyl, optionally substituted amino acid, optionally substituted alkyl, optionally substituted haloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted hydroxyalkyl (e.g., substituted with an O-protecting group), optionally substituted hydroxyalkenyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted acyloxyalkyl, optionally substituted amino (e.g., -NHR, wherein R is H, alkyl, aryl, phosphoryl, optionally substituted aminoalkyl, or optionally substituted carboxyaminoalkyl), azido, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted alkheterocyclyl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, or optionally substituted aminoalkynyl; and

[00416] each of R^{15} is, independently, H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl.

[00417] In particular embodiments, R^{14b} is an optionally substituted amino acid (e.g., optionally substituted lysine). In some embodiments, R^{14a} is H.

[00418] In some embodiments, B is a modified guanine. Exemplary modified guanines include compounds of Formula (bl5)-(bl7):


(b15),

(b16), or

(b17), or a pharmaceutically acceptable salt or stereoisomer thereof,

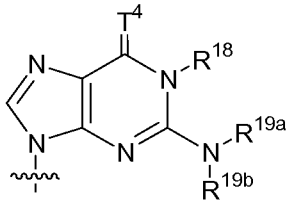
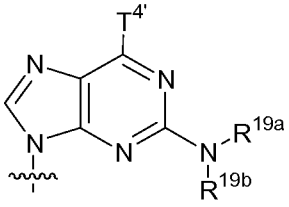
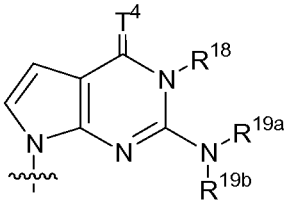
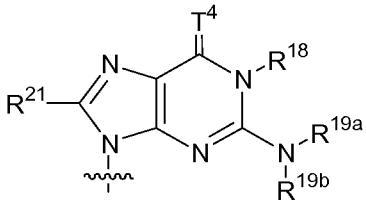
[00419] wherein

[00420] each of $T^{4'}$, $T^{4"}$, $T^{5'}$, $T^{5"}$, $T^{6'}$, and $T^{6"}$ is, independently, H, optionally substituted alkyl, or optionally substituted alkoxy, and wherein the combination of $T^{4'}$ and $T^{4"}$ (e.g., as in T^4) or the combination of $T^{5'}$ and $T^{5"}$ (e.g., as in T^5) or the combination of $T^{6'}$ and $T^{6"}$ (e.g., as in T^6) join together form **O** (oxo), S (thio), or Se (seleno);

[00421] each of V^5 and V^6 is, independently, **O,** S, **N(R^{Vd})nv,** or **C(R^{Vd})nv,** wherein nv is an integer from 0 to 2 and each $\mathbf{R^{Vd}}$ is, independently, **H,** halo, thiol, optionally substituted amino acid, cyano, amidine, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, or optionally substituted alkynyloxy (e.g., optionally substituted with any substituent described herein, such as those selected from (1)-(21) for alkyl) , optionally substituted thioalkoxy, or optionally substituted amino; and

[00422] each of $\mathbf{R^{17}}$, $\mathbf{R^{18}}$, $\mathbf{R^{19a}}$, $\mathbf{R^{19b}}$, $\mathbf{R^{21}}$, $\mathbf{R^{22}}$, $\mathbf{R^{23}}$, and $\mathbf{R^{24}}$ is, independently, **H,** halo, thiol, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted thioalkoxy, optionally substituted amino, or optionally substituted amino acid.

[00423] Exemplary modified guanosines include compounds of Formula (b37)-(b40):


(b37),

(b38),

(b39),

or (b40), or a pharmaceutically acceptable salt or stereoisomer thereof,

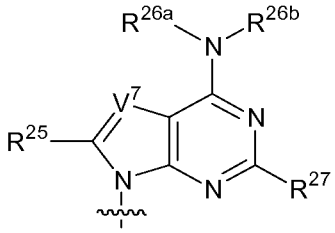
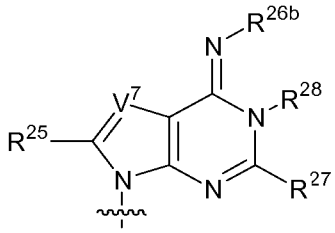
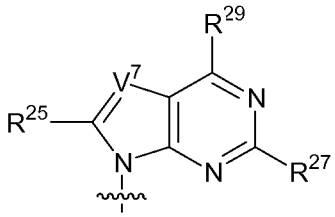
[00424] wherein

[00425] each of $T^{4'}$ is, independently, H, optionally substituted alkyl, or optionally substituted alkoxy, and each T^4 is, independently, O (oxo), S (thio), or Se (seleno);

[00426] each of R^{18}, R^{19a}, R^{19b}, and R^{21} is, independently, H, halo, thiol, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted thioalkoxy, optionally substituted amino, or optionally substituted amino acid.

[00427] In some embodiments, R^{18} is H or optionally substituted alkyl. In further embodiments, T^4 is oxo. In some embodiments, each of R^{19a} and R^{19b} is, independently, H or optionally substituted alkyl.

[00428] In some embodiments, B is a modified adenine. Exemplary modified adenines include compounds of Formula (M8)-(b20):


(b18),

(b19), or

(b20), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00429] wherein

[00430] each V^7 is, independently, O, S, $N(R^{Ve})_{nv}$, or $C(R^{Ve})_{nv}$, wherein nv is an integer from 0 to 2 and each R^{Ve} is, independently, H, halo, optionally substituted amino acid, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, or optionally substituted alkynyloxy (e.g., optionally substituted with any substituent described herein, such as those selected from (1)-(21) for alkyl);

[00431] each R^{25} is, independently, H, halo, thiol, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted thioalkoxy, or optionally substituted amino;

[00432] each of R^{26a} and R^{26b} is, independently, H, optionally substituted acyl, optionally substituted amino acid, optionally substituted carbamoylalkyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted alkoxy, or polyethylene glycol group (e.g., -(CH_2)s2(OCH $_2$CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-$_{20}$ alkyl); or an amino-polyethylene glycol group (e.g., -$NR^{N1}(CH_2)_{s2}(CH_2CH_20)si(CH_2)_S3NR^{N1}$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl);

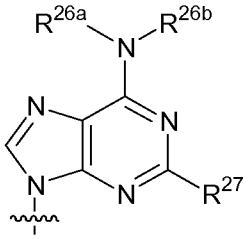
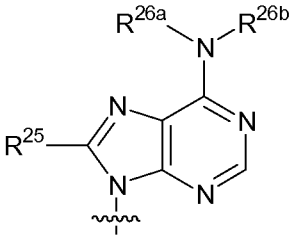
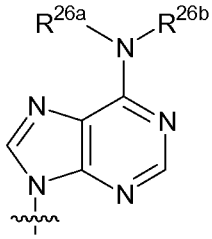
[00433] each R^{27} is, independently, H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted thioalkoxy or optionally substituted amino;

[00434] each R^{28} is, independently, H, optionally substituted alkyl, optionally substituted alkenyl, or optionally substituted alkynyl; and

[00435] each R^{29} is, independently, H, optionally substituted acyl, optionally substituted amino acid, optionally substituted carbamoylalkyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted

hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted alkoxy, or optionally substituted amino.

[00436] Exemplary modified adenines include compounds of Formula (b41)-(b43):


(b41),

(b42), or

(b43), or a pharmaceutically acceptable salt or stereoisomer thereof,

[00437] wherein

[00438] each R^{25} is, independently, H, halo, thiol, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted thioalkoxy, or optionally substituted amino;

[00439] each of R^{26a} and R^{26b} is, independently, H, optionally substituted acyl, optionally substituted amino acid, optionally substituted carbamoylalkyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted hydroxyalkyl, optionally substituted hydroxyalkenyl, optionally substituted hydroxyalkynyl, optionally substituted alkoxy, or polyethylene glycol group (e.g., -(CH_2)s2(OCH $_2$CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-$_{20}$ alkyl); or an amino-polyethylene glycol group (e.g., -$NR^{N1}(CH_2)_{s2}(CH_2CH_20)si(CH_2)_S3NR^{N1}$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl); and

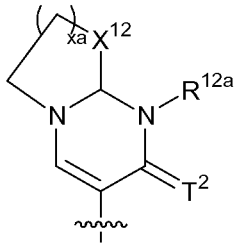
[00440] each R^{27} is, independently, H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted thioalkoxy, or optionally substituted amino.

[00441] In some embodiments, R^{26a} is H, and R^{26b} is optionally substituted alkyl. In some embodiments, each of R^{26a} and R^{26b} is, independently, optionally substituted alkyl.

In particular embodiments, R^{27} is optionally substituted alkyl, optionally substituted alkoxy, or optionally substituted thioalkoxy. In other embodiments, R^{25} is optionally substituted alkyl, optionally substituted alkoxy, or optionally substituted thioalkoxy.

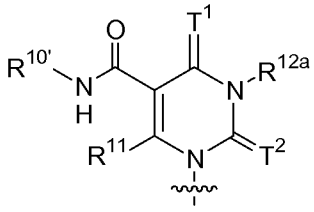
[00442] In particular embodiments, the optional substituent for R^{26a}, R^{26b}, or R^{29} is a polyethylene glycol group (e.g., -(CH2)s2(OCH2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl); or an amino-polyethylene glycol group (e.g., -$NR^{N1}(CH_2)$s2(CH2CH20)si(CH $_2$)s3NR^{N1}, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl).

In some embodiments, B may have Formula (b21):



(b21), wherein X^{12} is, independently, O, S, optionally substituted alkylene (e.g., methylene), or optionally substituted heteroalkylene, xa is an integer from 0 to 3, and R^{12a} and T^2 are as described herein.

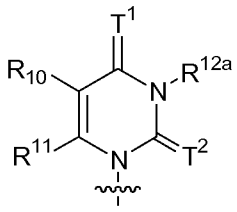
[00443] In some embodiments, B may have Formula (b22):



(b22), wherein $R^{10'}$ is, independently, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, optionally substituted alkoxy, optionally substituted alkoxycarbonylalkyl, optionally substituted alkoxycarbonylalkenyl, optionally substituted alkoxycarbonylalkynyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted carboxyalkoxy, optionally

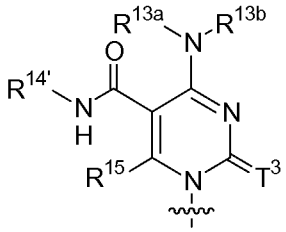
substituted carboxyalkyl, or optionally substituted carbamoylalkyl, and R^{11}, R^{12a}, T^1, and T^2 are as described herein.

[00444] In some embodiments, B may have Formula (b23):



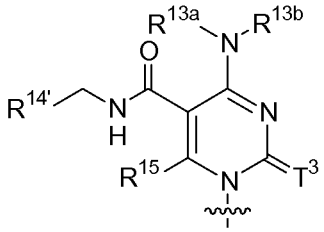
(b23), wherein R^{10} is optionally substituted heterocyclyl (e.g., optionally substituted furyl, optionally substitued thienyl, or optionally substitued pyrrolyl), optionally substituted aryl (e.g., optionally substituted phenyl or optionally substituted naphthyl), or any substituent described herein (e.g., for R^{10}) ;and wherein R^{11} (e.g., H or any substituent described herein), R^{12a} (e.g., H or any substituent described herein), T^1 (e.g., oxo or any substituent described herein), and T^2 (e.g., oxo or any substituent described herein) are as described herein.

In some embodiments, B may have Formula (b24):



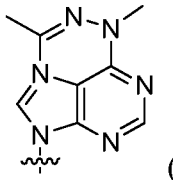
(b24), wherein $R^{14'}$ is, independently, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heterocyclyl, optionally substituted alkaryl, optionally substituted alkheterocyclyl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, optionally substituted alkoxy, optionally substituted alkoxycarbonylalkenyl, optionally substituted alkoxycarbonylalkynyl, optionally substituted alkoxycarbonylalkyl, optionally substituted alkoxycarbonylalkoxy, optionally substituted carboxyalkoxy, optionally substituted carboxyalkyl, or optionally substituted carbamoylalkyl, and R^{13a}, R^{13b}, R^{15}, and T^3 are as described herein.

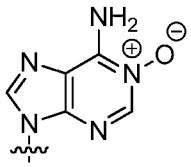
In some embodiments, B may have Formula (b25):



(b25), wherein $R^{14'}$ is optionally substituted heterocyclyl (e.g., optionally substituted furyl, optionally substitued thienyl, or optionally substitued pyrrolyl), optionally substituted aryl (e.g., optionally substituted phenyl or optionally substituted naphthyl), or any substituent described herein (e.g., for R^{14} or $R^{14'}$); and wherein R^{13a} (e.g., H or any substituent described herein), R^{13b} (e.g., H or any substituent described herein), R^{15} (e.g., H or any substituent described herein), and T^3 (e.g., oxo or any substituent described herein) are as described herein.

In some embodiments, B is a nucleobase selected from the group consisting of cytosine, guanine, adenine, and uracil. In some embodiments, B may be:



(b26) or  (b27).

[00445] In some embodiments, the modified nucleobase is a modified uracil. Exemplary nucleobases and nucleosides having a modified uracil include pseudouridine (ψ), pyridin-4-one ribonucleoside, 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s^2U), 4-thio-uridine (s^4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho^5U), 5-aminoallyl-uridine, 5-halo-uridine (e.g., 5-iodo-uridine or 5-bromo-uridine), 3-methyl-uridine (m^3U), 5-methoxy-uridine (mo^5U), uridine 5-oxyacetic acid (cmo^5U), uridine 5-oxyacetic acid methyl ester ($mcmo^5U$), 5-carboxymethyl-uridine (cm^5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm^5U), 5-carboxyhydroxymethyl-uridine methyl ester ($mchm^5U$), 5-methoxycarbonylmethyl-uridine (mcm^5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm^5s^2U), 5-aminomethyl-2-thio-uridine (nm^5s^2U), 5-methylaminomethyl-uridine (mnm^5U), 5-methylaminomethyl-2-thio-uridine (mnm^5s^2U), 5-methylaminomethyl-2-seleno-uridine (mnm^5se^2U), 5-carbamoylmethyl-uridine (ncm^5U), 5-carboxymethylaminomethyl-uridine ($cmnm^5U$), 5-carboxymethylaminomethyl-2-thio-uridine ($cmnm^5s^2U$), 5-propynyl-uridine, 1-propynyl-

pseudouridine, 5-taurinomethyl-uridine (τm^{5}U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine (τm^{5}s^{2}U), 1-taurinomethyl-4-thio-pseudouridine, 5-methyl-uridine (m^{5}U, i.e., having the nucleobase deoxythymine), 1-methyl-pseudouridine (m^{1}ψ), 5-methyl-2-thio-uridine (m^{5}s^{2}U), l-methyl-4-thio-pseudouridine (m^{1}s^{4}ψ), 4-thio-l-methyl-pseudouridine, 3-methyl-pseudouridine (m^{3}ψ), 2-thio-l-methyl-pseudouridine, 1-methyl- 1-deaza-pseudouridine, 2-thio- 1-methyl- 1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m^{5}D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp^{3}U), l-methyl-3-(3-amino-3-carboxypropyl)pseudouridine (acp^{3}ψ), 5-(isopentenylaminomethyl)uridine (inm^{5}U), 5-(isopentenylaminomethyl)-2-thio-uridine (inmVU), a-thio-uridine, 2'-0-methyl-uridine (Um), 5,2'-0-dimethyl-uridine (m^{5}Um), 2'-0-methyl-pseudouridine (ψm), 2-thio-2'-0-methyl-uridine (s^{2}Um), 5-methoxycarbonylmethyl-2'-0-methyl-uridine (mcm^{5}Um), 5-carbamoylmethyl-2'-0-methyl-uridine (ncm^{5}Um), 5-carboxymethylaminomethyl-2'-0-methyl-uridine (cmnm^{5}Um), 3,2'-0-dimethyl-uridine (m^{3}Um), 5-(isopentenylaminomethyl)-2'-0-methyl-uridine (inm^{5}Um), 1-thio-uridine, deoxythymidine, 2'-F-ara-uridine, 2'-F-uridine, 2'-OH-ara-uridine, 5-(2-carbomethoxyvinyl) uridine, and 5-[3-(l-E-propenylamino)uridine.

[00446] In some embodiments, the modified nucleobase is a modified cytosine. Exemplary nucleobases and nucleosides having a modified cytosine include 5-aza-cytidine, 6-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine (m^{3}C), N4-acetyl-cytidine (ac^{4}C), 5-formyl-cytidine *(fC)*, N4-methyl-cytidine (m^{4}C), 5-methyl-cytidine (m^{5}C), 5-halo-cytidine (e.g., 5-iodo-cytidine), 5-hydroxymethyl-cytidine (hm^{5}C), 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine (s^{2}C), 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-l-methyl-pseudoisocytidine, 4-thio- 1-methyl- 1-deaza-pseudoisocytidine, 1-methyl- 1-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, 4-methoxy-l-methyl-pseudoisocytidine, lysidine (k$_2$C), a-thio-

cytidine, 2'-0-methyl-cytidine (Cm), 5,2'-0-dimethyl-cytidine (m^5Cm), N4-acetyl-2'-0-methyl-cytidine (ac^4Cm), N4,2'-0-dimethyl-cytidine (m^4Cm), 5-formyl-2'-0-methyl-cytidine (iCm), N4,N4,2'-0-trimethyl-cytidine (m^4_2Cm), 1-thio-cytidine, 2'-F-ara-cytidine, 2'-F-cytidine, and 2'-OH-ara-cytidine.

[00447] In some embodiments, the modified nucleobase is a modified adenine. Exemplary nucleobases and nucleosides having a modified adenine include 2-amino-purine, 2, 6-diaminopurine, 2-amino-6-halo-purine (e.g., 2-amino-6-chloro-purine), 6-halo-purine (e.g., 6-chloro-purine), 2-amino-6-methyl-purine, 8-azido-adenosine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-amino-purine, 7-deaza-8-aza-2-amino-purine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyl-adenosine (rr^A), 2-methyl-adenine (m^2A), N6-methyl-adenosine (m^6A), 2-methylthio-N6-methyl-adenosine (ms^2m^6A), N6-isopentenyl-adenosine (i^6A), 2-methylthio-N6-isopentenyl-adenosine (ms^2i^6A), N6-(cis-hydroxyisopentenyl)adenosine (io^6A), 2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine (ms^2io^6A), N6-glycinylcarbamoyl-adenosine (g^6A), N6-threonylcarbamoyl-adenosine (t^6A), N6-methyl-N6-threonylcarbamoyl-adenosine (m^6t^6A), 2-methylthio-N6-threonylcarbamoyl-adenosine (ms^2g^6A), N6,N6-dimethyl-adenosine (m^6_2A), N6-hydroxynorvalylcarbamoyl-adenosine (hn^6A), 2-methylthio-N6-hydroxynorvalylcarbamoyl-adenosine (ms^2hn^6A), N6-acetyl-adenosine (ac^6A), 7-methyl-adenine, 2-methylthio-adenine, 2-methoxy-adenine, a-thio-adenosine, 2'-0-methyl-adenosine (Am), N6,2'-0-dimethyl-adenosine (m^6Am), N6,N6,2'-0-trimethyl-adenosine (m^6_2Am), l,2'-0-dimethyl-adenosine (m^m), 2'-0-ribosyladenosine (phosphate) (Ar(p)), 2-amino-N6-methyl-purine, 1-thio-adenosine, 8-azido-adenosine, 2'-F-ara-adenosine, 2'-F-adenosine, 2'-OH-ara-adenosine, and N6-(19-amino-pentaoxanonadecyl)-adenosine.

[00448] In some embodiments, the modified nucleobase is a modified guanine. Exemplary nucleobases and nucleosides having a modified guanine include inosine (I), 1-methyl-inosine ($m^1!$), wyosine (imG), methylwyosine (mimG), 4-demethyl-wyosine (imG-14), isowyosine (imG2), wybutosine (yW), peroxywybutosine (o_2yW), hydroxywybutosine (OHyW), undermodified hydroxywybutosine (OHyW*), 7-deaza-guanosine, queuosine (Q), epoxyqueuosine (oQ), galactosyl-queuosine (galQ), mannosyl-

queuosine (manQ), 7-cyano-7-deaza-guanosine (preQo), 7-aminomethyl-7-deaza-guanosine (preQi), archaeosine (G^+), 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine (m^7G), 6-thio-7-methyl-guanosine, 7-methyl-inosine, 6-methoxy-guanosine, 1-methyl-guanosine (m^1G), N2-methyl-guanosine (m^2G), N2,N2-dimethyl-guanosine (m^22G), N2,7-dimethyl-guanosine ($m^{2,7}G$), N2, N2,7-dimethyl-guanosine ($m^{2,2,7}G$), 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, l-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, N2,N2-dimethyl-6-thio-guanosine, a-thio-guanosine, 2'-0-methyl-guanosine (Gm), N2-methyl-2'-0-methyl-guanosine (m^2Gm), N2,N2-dimethyl-2'-0-methyl-guanosine (m^22Gm), 1-methyl-2'-0-methyl-guanosine (m^1Gm), N2,7-dimethyl-2'-0-methyl-guanosine ($m^{2,7}Gm$), 2'-0-methyl-inosine (Im), l,2'-0-dimethyl-inosine (m^m), and 2'-0-ribosylguanosine (phosphate) (Gr(p)).

[00449] The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine or pyrimidine analog. For example, the nucleobase can each be independently selected from adenine, cytosine, guanine, uracil, or hypoxanthine. In another embodiment, the nucleobase can also include, for example, naturally-occurring and synthetic derivatives of a base, including pyrazolo[3,4-d]pyrimidines, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo *(e.g.,* 8-bromo), 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, deazaguanine, 7-deazaguanine, 3-deazaguanine, deazaadenine, 7-deazaadenine, 3-deazaadenine, pyrazolo[3,4-d]pyrimidine, imidazo[l,5-a]l,3,5 triazinones, 9-deazapurines, imidazo[4,5-d]pyrazines, thiazolo[4,5-d]pyrimidines, pyrazin-2-ones, 1,2,4-triazine, pyridazine; and 1,3,5 triazine. When the nucleotides are depicted using the shorthand A, G, C, T or U, each letter refers to the representative base and/or derivatives thereof, *e.g.,* A includes adenine or adenine analogs, *e.g.,* 7-deaza adenine).

Modifications on the Internucleoside Linkage

[00450] The modified nucleotides, which may be incorporated into a cell phenotype altering polynucleotide, primary construct, or mmRNA molecule, can be modified on the internucleoside linkage (e.g., phosphate backbone). Herein, in the context of the polynucleotide backbone, the phrases "phosphate" and "phosphodiester" are used interchangeably. Backbone phosphate groups can be modified by replacing one or more of the oxygen atoms with a different substituent. Further, the modified nucleosides and nucleotides can include the wholesale replacement of an unmodified phosphate moiety with another internucleoside linkage as described herein. Examples of modified phosphate groups include, but are not limited to, phosphorothioate, phosphoroselenates, boranophosphates,boranophosphate esters, hydrogen phosphonates, phosphoramidates, phosphorodiamidates, alkyl or aryl phosphonates, and phosphotriesters. Phosphorodithioates have both non-linking oxygens replaced by sulfur. The phosphate linker can also be modified by the replacement of a linking oxygen with nitrogen (bridged phosphoramidates), sulfur (bridged phosphorothioates), and carbon (bridged methylene-phosphonates) .

[00451] The a-thio substituted phosphate moiety is provided to confer stability to RNA and DNA polymers through the unnatural phosphorothioate backbone linkages. Phosphorothioate DNA and RNA have increased nuclease resistance and subsequently a longer half-life in a cellular environment. Phosphorothioate linked cell phenotype altering polynucleotides, primary constructs, or mmRNA molecules are expected to also reduce the innate immune response through weaker binding/activation of cellular innate immune molecules.

[00452] In specific embodiments, a modified nucleoside includes an alpha-thio-nucleoside (e.g., 5'-0-(l-thiophosphate)-adenosine, 5'-0-(l-thiophosphate)-cytidine (a-thio-cytidine), 5'-0-(l-thiophosphate)-guanosine, 5'-0-(l-thiophosphate)-uridine, or 5'-0-(1-thiophosphate)-pseudouridine).

[00453] Other internucleoside linkages that may be employed according to the present invention, including internucleoside linkages which do not contain a phosphorous atom, are described herein below.

Combinations of Modified Sugars, Nucleobases, and Internucleoside Linkages

[00454] The cell phenotype altering polynucleotides, primary constructs, and mmRNA of the invention can include a combination of modifications to the sugar, the nucleobase, and/or the internucleoside linkage. These combinations can include any one or more modifications described herein. For examples, any of the nucleotides described herein in Formulas (la), (Ia-l)-(Ia-3), (Ib)-(If), (Ila)-(IIp), (IIb-1), (IIb-2), (IIc-l)-(IIc-2), (IIn-1), (IIn-2), (IVa)-(IVl), and (IXa)-(IXr) can be combined with any of the nucleobases described herein (e.g., in Formulas (bl)-(b43) or any other described herein).

Synthesis of Cell phenotype altering Polypeptides, Primary Constructs, and mmRNA Molecules

[00455] The cell phenotype altering polypeptides, primary constructs, and mmRNA molecules for use in accordance with the invention may be prepared according to any useful technique, as described herein. The modified nucleosides and nucleotides used in the synthesis of cell phenotype altering polynucleotides, primary constructs, and mmRNA molecules disclosed herein can be prepared from readily available starting materials using the following general methods and procedures. Where typical or preferred process conditions (e.g., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are provided, a skilled artisan would be able to optimize and develop additional process conditions. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[00456] The processes described herein can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (e.g., ¾ or ^{13}C) infrared spectroscopy, spectrophotometry (e.g., UV-visible), or mass spectrometry, or by chromatography such as high performance liquid chromatography (HPLC) or thin layer chromatography.

[00457] Preparation of cell phenotype altering polypeptides, primary constructs, and mmRNA molecules of the present invention can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups can be readily determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in Greene, et al, *Protective*

Groups in Organic Synthesis, 2d. Ed., Wiley & Sons, 1991, which is incorporated herein by reference in its entirety.

[00458] The reactions of the processes described herein can be carried out in suitable solvents, which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out, i.e., temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected.

[00459] Resolution of racemic mixtures of modified nucleosides and nucleotides can be carried out by any of numerous methods known in the art. An example method includes fractional recrystallization using a "chiral resolving acid" which is an optically active, salt-forming organic acid. Suitable resolving agents for fractional recrystallization methods are, for example, optically active acids, such as the D and L forms of tartaric acid, diacetyltartaric acid, dibenzoyltartaric acid, mandelic acid, malic acid, lactic acid or the various optically active camphorsulfonic acids. Resolution of racemic mixtures can also be carried out by elution on a column packed with an optically active resolving agent *{e.g.*, dinitrobenzoylphenylglycine). Suitable elution solvent composition can be determined by one skilled in the art.

[00460] Modified nucleosides and nucleotides (e.g., building block molecules) can be prepared according to the synthetic methods described in Ogata et al, J. Org. Chem. 74:2585-2588 (2009); Purmal et al, Nucl. Acids Res. 22(1): 72-78, (1994); Fukuhara et al, Biochemistry, 1(4): 563-568 (1962); and Xu et al, Tetrahedron, 48(9): 1729-1740 (1992), each of which are incorporated by reference in their entirety.

[00461] The cell phenotype altering polypeptides, primary constructs, and mmRNA of the invention may or may not be uniformly modified along the entire length of the molecule. For example, one or more or all types of nucleotide *{e.g.*, purine or pyrimidine, or any one or more or all of A, G, U, C) may or may not be uniformly modified in a cell phenotype altering polynucleotide of the invention, or in a given predetermined sequence region thereof *{e.g.* one or more of the sequence regions

represented in Figure 1). In some embodiments, all nucleotides X in a cell phenotype altering polynucleotide of the invention (or in a given sequence region thereof) are modified, wherein X may any one of nucleotides A, G, U, C, or any one of the combinations A+G, A+U, A+C, G+U, G+C, U+C, A+G+U, A+G+C, G+U+C or A+G+C.

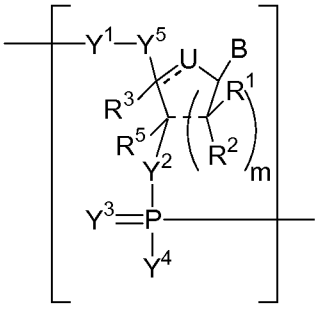
[00462] Different sugar modifications, nucleotide modifications, and/or internucleoside linkages (e.g., backbone structures) may exist at various positions in the cell phenotype altering polynucleotide, primary construct, or mmRNA. One of ordinary skill in the art will appreciate that the nucleotide analogs or other modification(s) may be located at any position(s) of a cell phenotype altering polynucleotide, primary construct, or mmRNA such that the function of the cell phenotype altering polynucleotide, primary construct, or mmRNA is not substantially decreased. A modification may also be a 5′ or 3' terminal modification. The cell phenotype altering polynucleotide, primary construct, or mmRNA may contain from about 1% to about 100% modified nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, i.e. any one or more of A, G, U or C) or any intervening percentage (e.g., from 1% to 20%>, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%).

[00463] In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA includes a modified pyrimidine (e.g., a modified uracil/uridine/U or modified cytosine/cytidine/C). In some embodiments, the uracil or uridine (generally: U) in the cell phenotype altering polynucleotide, primary construct, or mmRNA molecule may be replaced with from about 1% to about 100% of a modified uracil or modified uridine (e.g., from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from

1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100% of a modified uracil or modified uridine). The modified uracil or uridine can be replaced by a compound having a single unique structure or by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures, as described herein).

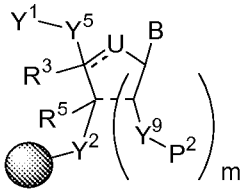
[00464] In some embodiments, the cytosine or cytidine (generally: C) in the cell phenotype altering polynucleotide, primary construct, or mmRNA molecule may be replaced with from about 1% to about 100% of a modified cytosine or modified cytidine (e.g., from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100% of a modified cytosine or modified cytidine). The modified cytosine or cytidine can be replaced by a compound having a single unique structure or by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures, as described herein).

[00465] In some embodiments, the present disclosure provides methods of synthesizing a cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., the first region, first flanking region, or second flanking region) including n number of linked nucleosides having Formula (Ia-1):



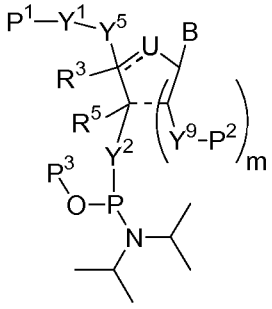
(Ia-1), comprising:

a) reacting a nucleotide of Formula (IV-1):



(IV-1),

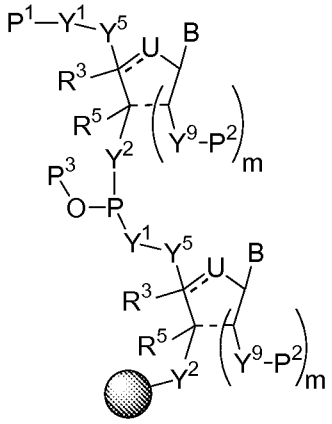
with a phosphoramidite compound of Formula (V-l):



(V-1),

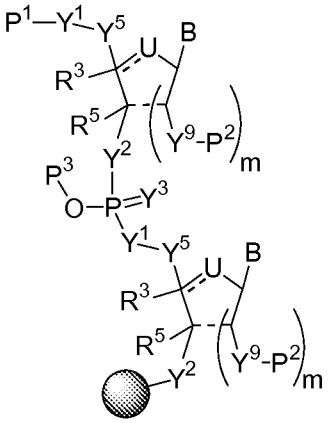
wherein Y^9 is H, hydroxy, phosphoryl, pyrophosphate, sulfate, amino, thiol, optionally substituted amino acid, or a peptide (e.g., including from 2 to 12 amino acids); and each P^1, P^2, and P^3 is, independently, a suitable protecting group; and ● denotes a solid support;

to provide a polynucleotide, primary construct, or mmRNA of Formula (VI-1):



(VI-1), and

b) oxidizing or sulfurizing the polynucleotide, primary construct, or mmRNA of Formula (V) to yield a polynucleotide, primary construct, or mmRNA of Formula (VII-1):



(VII-1), and

c) removing the protecting groups to yield the polynucleotide, primary construct, or mmRNA of Formula (la).

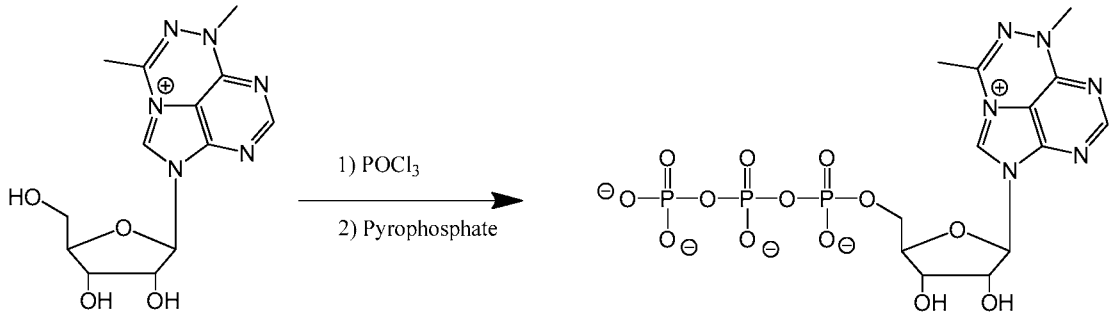
[00466] In some embodiments, steps a) and b) are repeated from 1 to about 10,000 times. In some embodiments, the methods further comprise a nucleotide (e.g., mmRNA molecule) selected from the group consisting of A, C, G and U adenosine, cytosine, guanosine, and uracil. In some embodiments, the nucleobase may be a pyrimidine or derivative thereof. In some embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA is translatable.

[00467] Other components of cell phenotype altering polynucleotides, primary constructs, and mmRNA are optional, and are beneficial in some embodiments. For example, a 5' untranslated region (UTR) and/or a 3'UTR are provided, wherein either or both may independently contain one or more different nucleotide modifications. In such

embodiments, nucleotide modifications may also be present in the translatable region. Also provided are cell phenotype altering polynucleotides, primary constructs, and mmRNA containing a Kozak sequence.

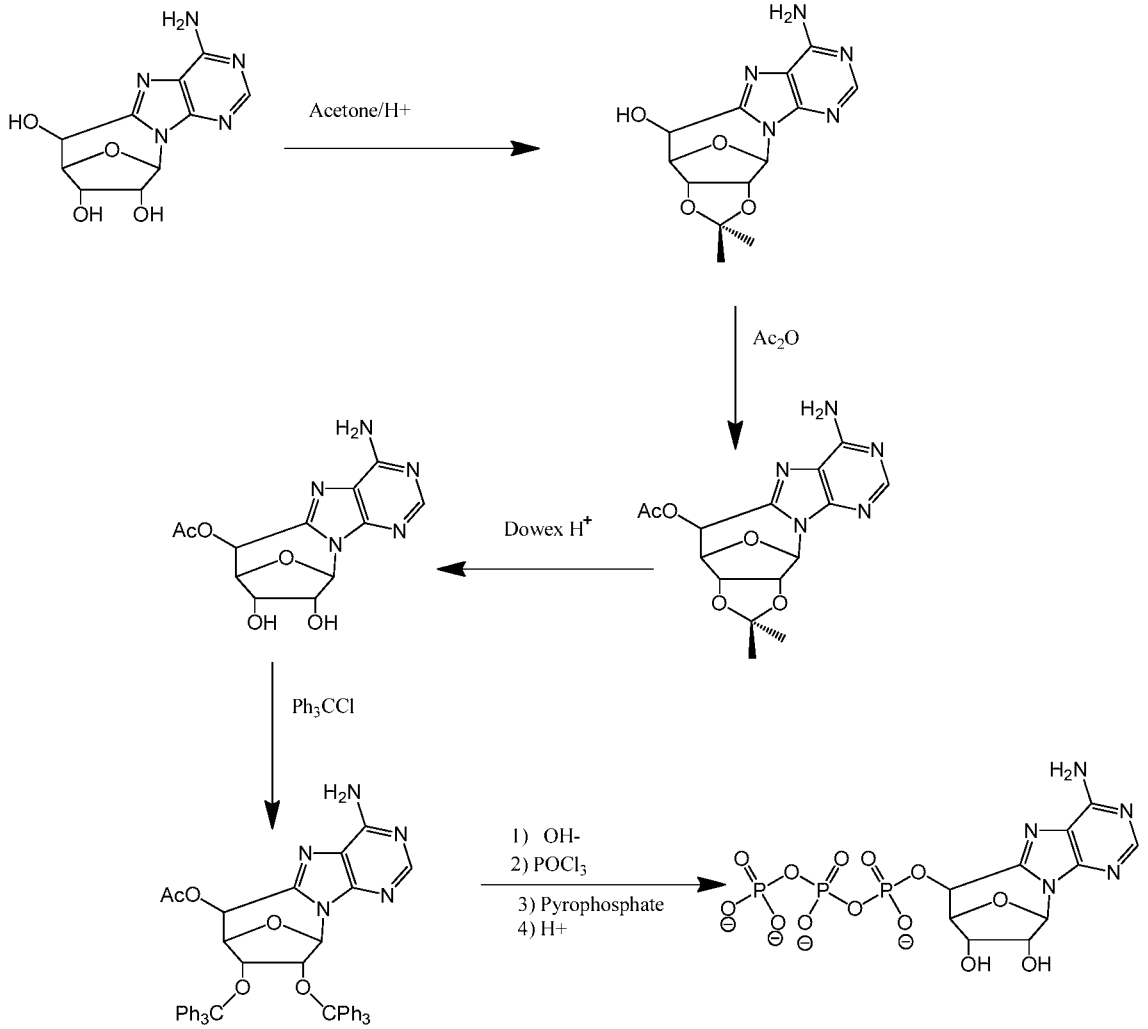
[00468] Exemplary syntheses of modified nucleotides, which are incorporated into a modified cell phenotype altering nucleic acid or mmRNA, e.g., RNA or mRNA, are provided below in Scheme 1 through Scheme 11. Scheme 1 provides a general method for phosphorylation of nucleosides, including modified nucleosides.

Scheme 1



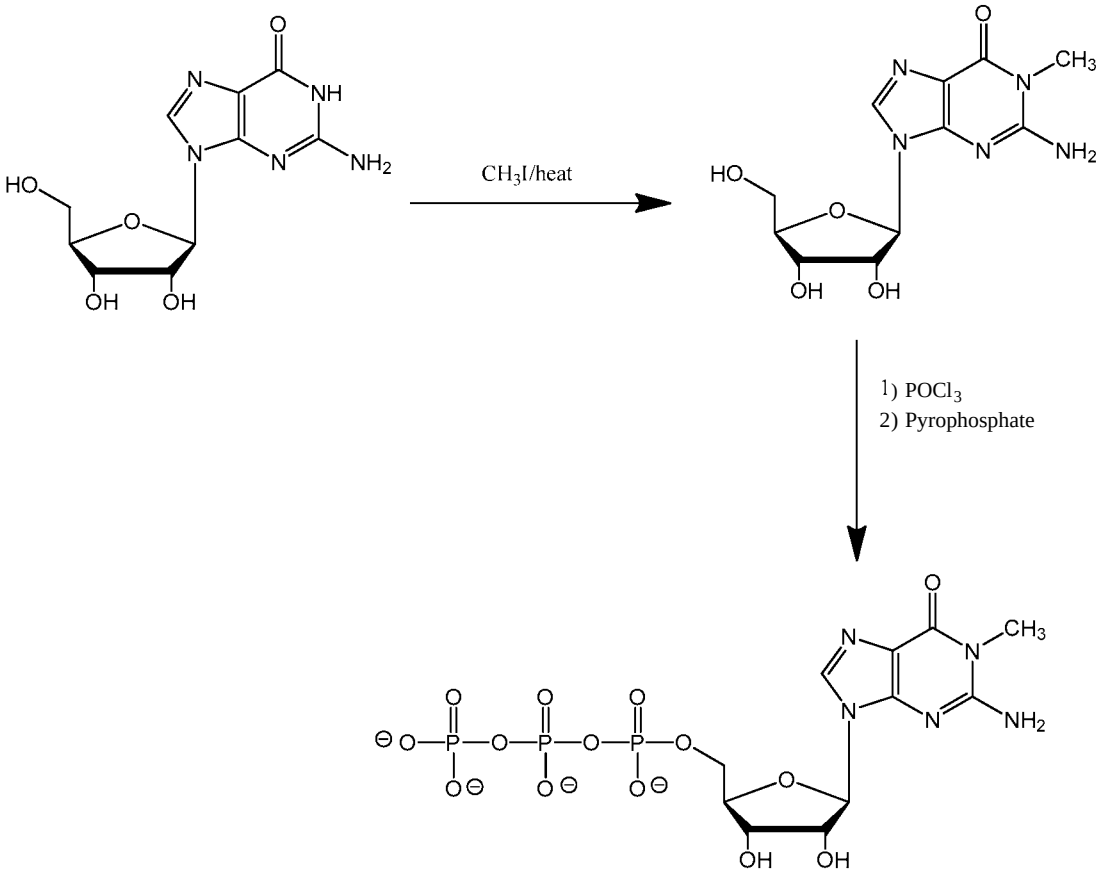
[00469] Various protecting groups may be used to control the reaction. For example, Scheme 2 provides the use of multiple protecting and deprotecting steps to promote phosphorylation at the 5' position of the sugar, rather than the 2' and 3' hydroxyl groups.

Scheme 2

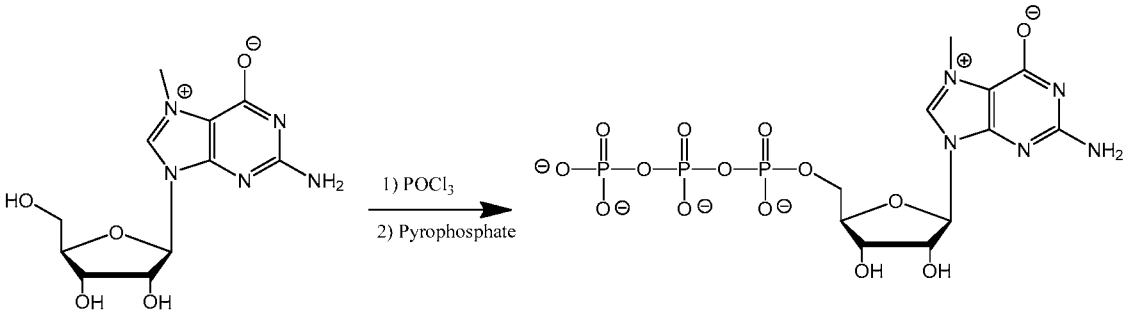


[00470] Modified nucleotides can be synthesized in any useful manner. Schemes 3, 4, and 7 provide exemplary methods for synthesizing modified nucleotides having a modified purine nucleobase; and Schemes 5 and 6 provide exemplary methods for synthesizing modified nucleotides having a modified pseudouridine or pseudoisocytidine, respectively.

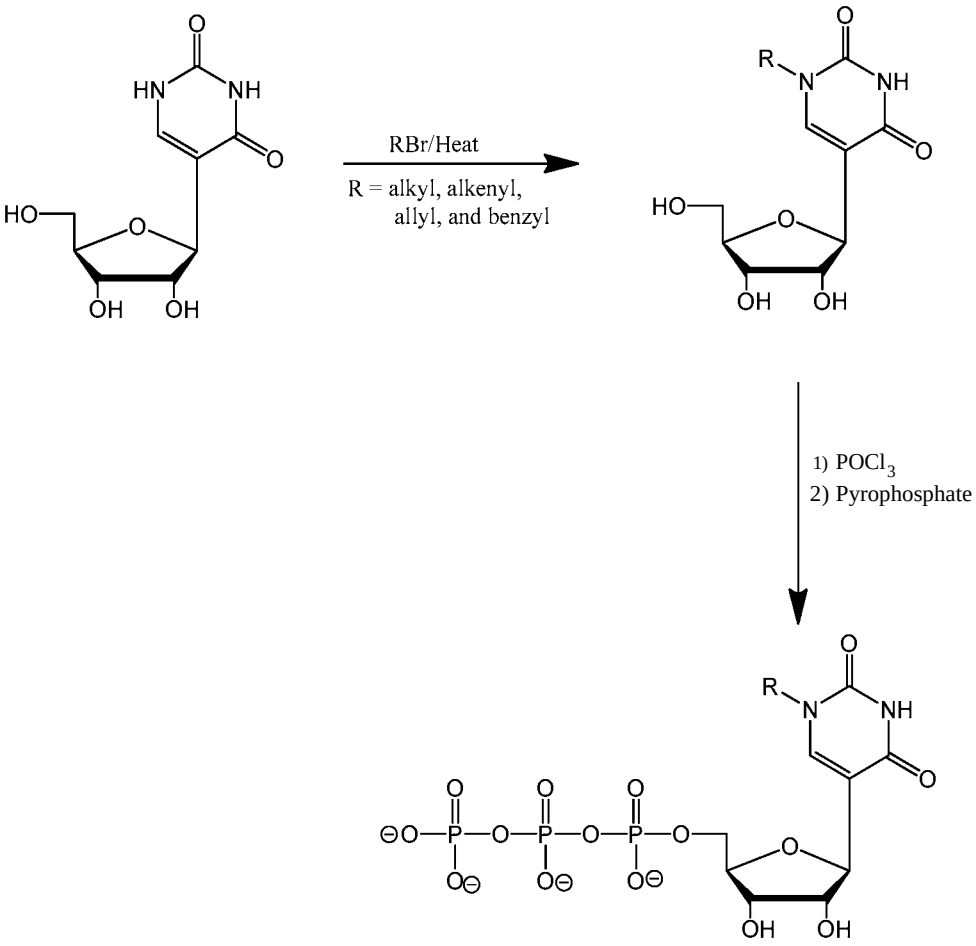
Scheme 3



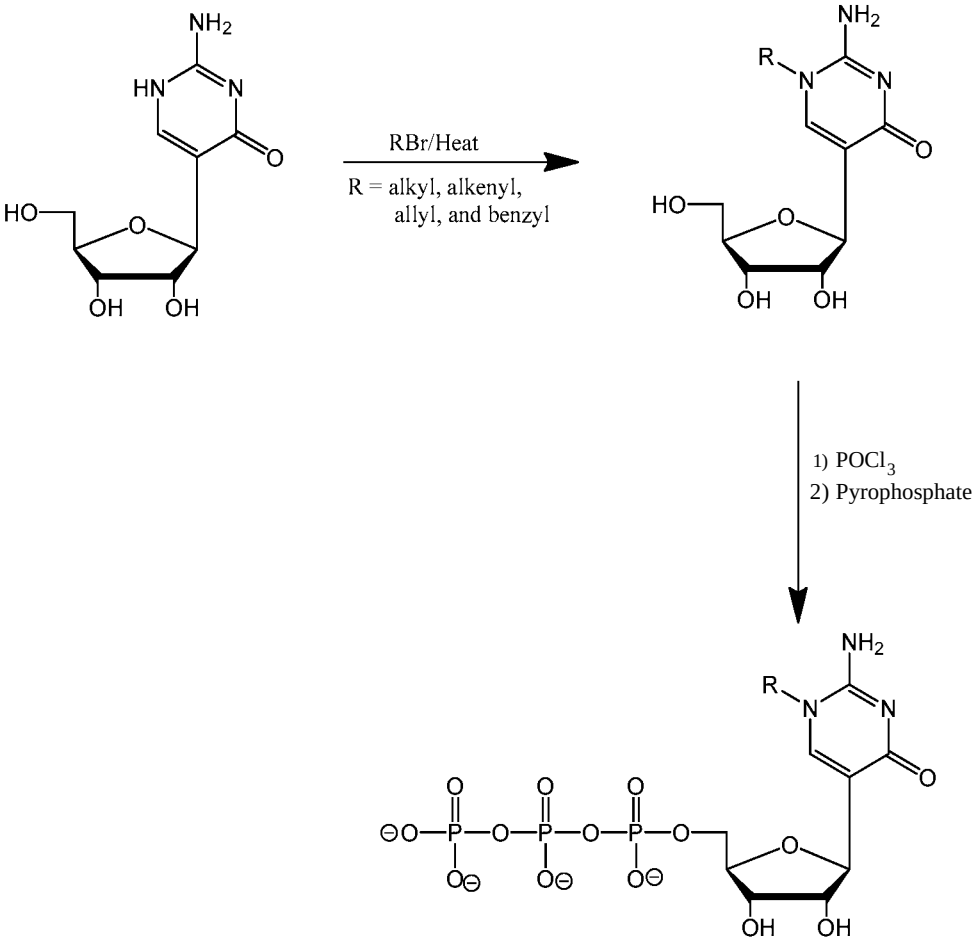
Scheme 4



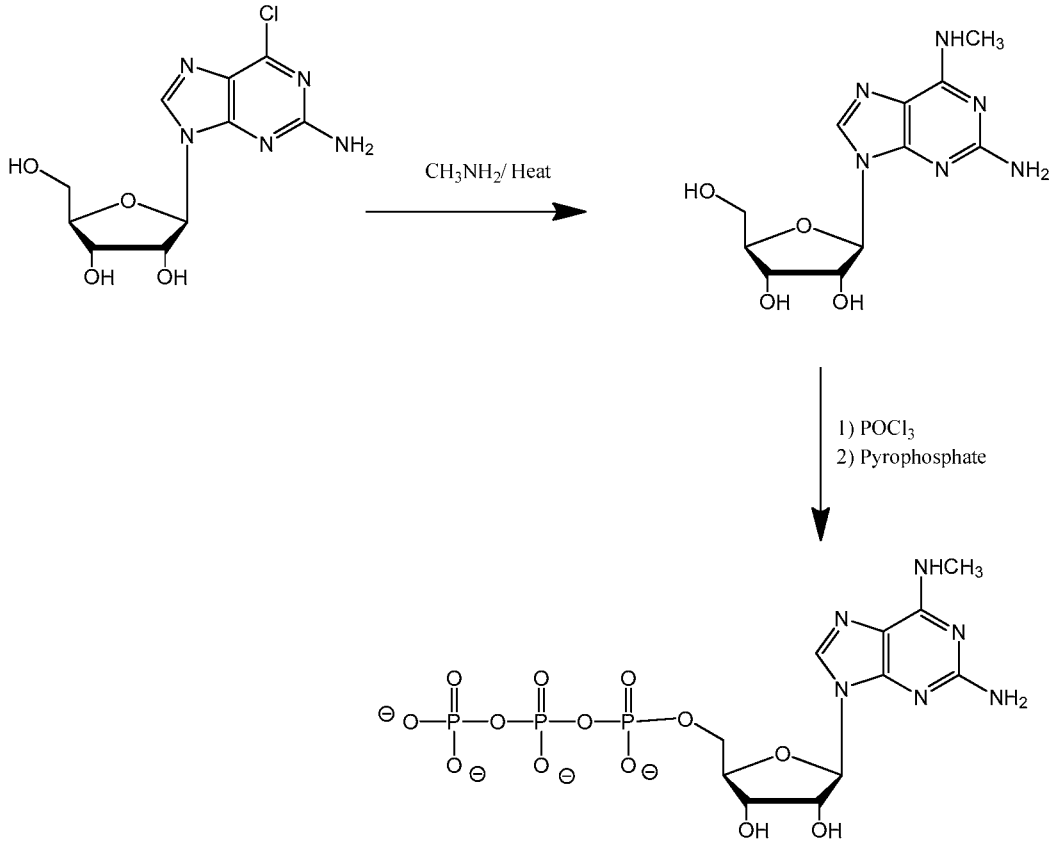
Scheme 5


RBr/Heat
R = alkyl, alkenyl,
allyl, and benzyl
1) POCl3
2) Pyrophosphate

Scheme 6

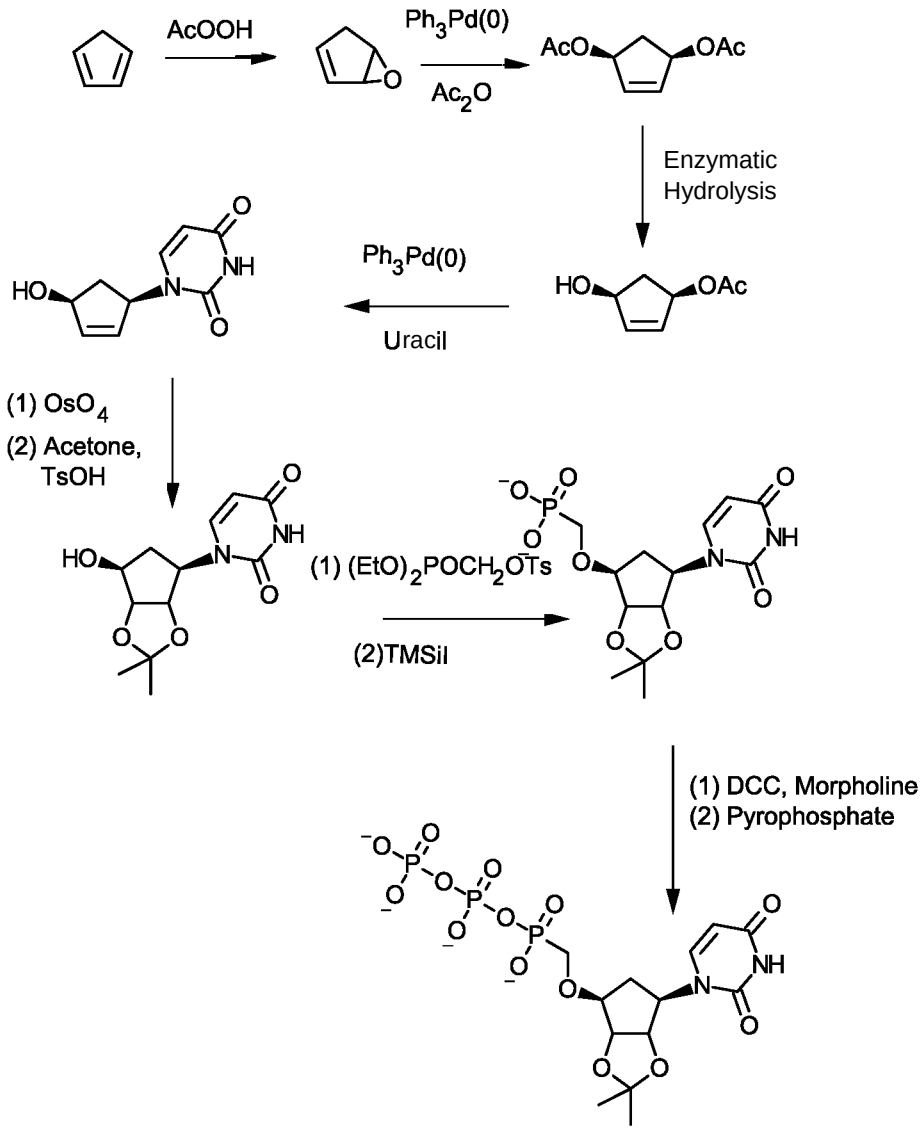

NH2
HN
N
O
HO
O
OH
OH
RBr/Heat
R = alkyl, alkenyl,
allyl, and benzyl
R
1) POCl3
2) Pyrophosphate

Scheme 7

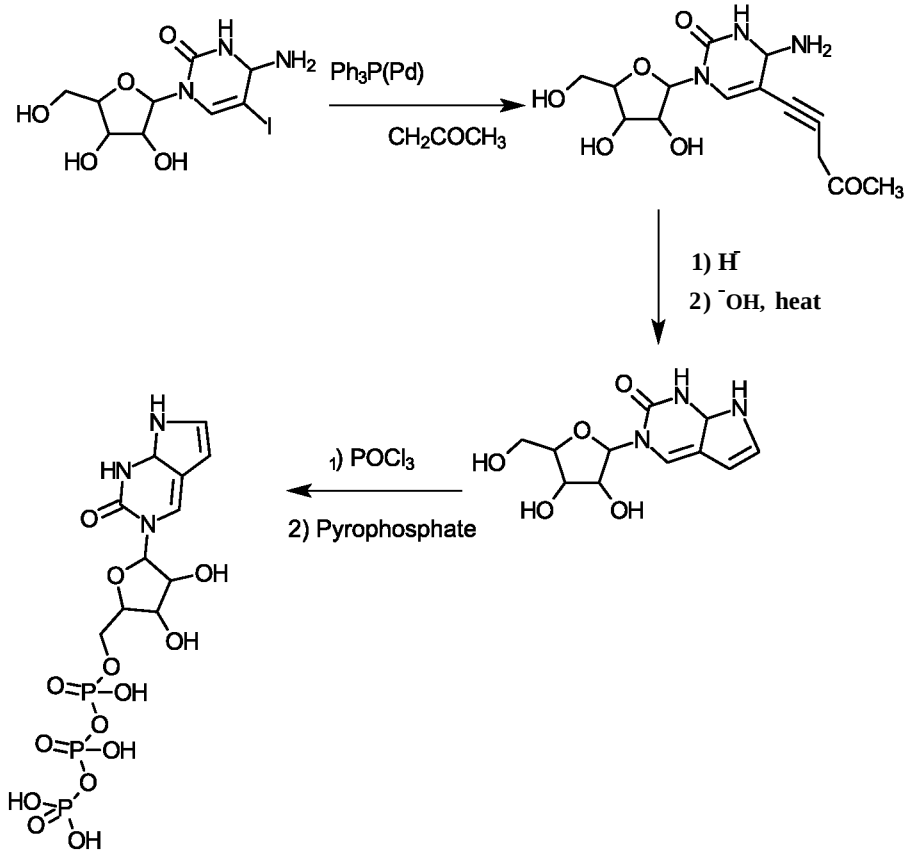


[00471] Schemes 8 and 9 provide exemplary syntheses of modified nucleotides. Scheme 10 provides a non-limiting biocatalytic method for producing nucleotides.

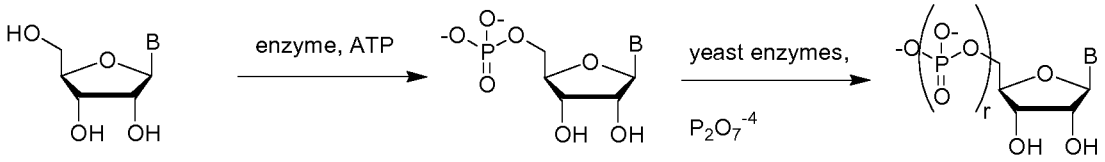
Scheme 8


AcOOH
Ph3Pd(0)
Ac2O
AcO
OAc
Enzymatic
Hydrolysis
HO
OAc
Ph3Pd(0)
Uracil
HO
NH
(1) OsO4
(2) Acetone,
TsOH
(1) (EtO)2POCH2OTs
(2)TMSil
(1) DCC, Morpholine
(2) Pyrophosphate

Scheme 9



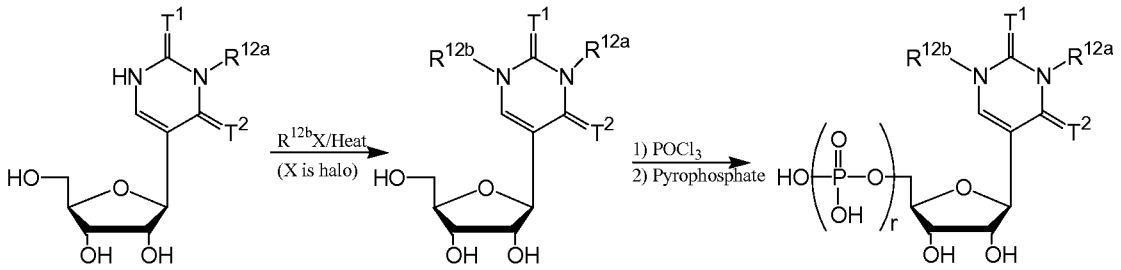
Scheme 10



[00472] Scheme 11 provides an exemplary synthesis of a modified uracil, where the N1 position is modified with R^{12b}, as provided elsewhere, and the 5'-position of ribose is phosphorylated. T^1, T^2, R^{12a}, R^{12b}, and r are as provided herein. This synthesis, as well as optimized versions thereof, can be used to modify other pyrimidine nucleobases and purine nucleobases (see e.g., Formulas (b1)-(b43)) and/or to install one or more phosphate groups (e.g., at the 5' position of the sugar). This alkylating reaction can also be used to include one or more optionally substituted alkyl group at any reactive group

(e.g., amino group) in any nucleobase described herein (e.g., the amino groups in the Watson-Crick base-pairing face for cytosine, uracil, adenine, and guanine).

Scheme 11



Combinations of Nucleotides in mmRNA

[00473] Further examples of modified nucleotides and modified nucleotide combinations are provided below in Table 9. These combinations of modified nucleotides can be used to form the cell phenotype altering polypeptides, primary constructs, or mmRNA of the invention. Unless otherwise noted, the modified nucleotides may be completely substituted for the natural nucleotides of the modified cell phenotype altering nucleic acids or mmRNA of the invention. As a non-limiting example, the natural nucleotide uridine may be substituted with a modified nucleoside described herein. In another non-limiting example, the natural nucleotide uridine may be partially substituted (e.g., about 0.1%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99.9%) with at least one of the modified nucleoside disclosed herein.

Table 9

Modified Nucleotide	Modified Nucleotide Combination
α-thio-cytidine	α-thio-cytidine/5-iodo-uridine
	α-thio-cytidine/N1-methyl-pseudo-uridine
	α-thio-cytidine/α-thio-uridine
	α-thio-cytidine/5-methyl-uridine
	α-thio-cytidine/pseudo-uridine
	about 50% of the cytosines are α-thio-cytidine
pseudoisocytidine	pseudoisocytidine/5-iodo-uridine
	pseudoisocytidine/N1-methyl-pseudouridine
	pseudoisocytidine/α-thio-uridine
	pseudoisocytidine/5-methyl-uridine
	pseudoisocytidine/pseudouridine
	about 25% of cytosines are pseudoisocytidine

	pseudoisocytidine/about 50% of uridines are Nl-methyl-pseudouridine and about 50% of uridines are pseudouridine
	pseudoisocytidine/about 25% of uridines are Nl-methyl-pseudouridine and about 25% of uridines are pseudouridine
pyrrolo-cytidine	pyrrolo-cytidine/5-iodo-uridine
	pyrrolo-cytidine/N 1-methyl-pseudouridine
	pyrrolo-cytidine/ a-thio-uridine
	pyrrolo-cytidine/5-methyl-uridine
	pyrrolo-cytidine/pseudouridine
	about 50% of the cytosines are pyrrolo-cytidine
5-methyl-cytidine	5-methyl-cytidine/5-iodo-uridine
	5-methyl-cytidine/N 1-methyl-pseudouridine
	5-methyl-cytidine/ a-thio-uridine
	5-methyl-cytidine/5-methyl-uridine
	5-methyl-cytidine/pseudouridine
	about 25% of cytosines are 5-methyl-cytidine
	about 50% of cytosines are 5-methyl-cytidine
	5-methyl-cytidine/5-methoxy -uridine
	5-methyl-cytidine/5-bromo-uridine
	5-methyl-cytidine/2-thio-uridine
	5-methyl-cytidine/about 50% of uridines are 2-thio-uridine
	about 50% of uridines are 5-methyl-cytidine/ about 50% of uridines are 2-thio-uridine
N4-acetyl-cytidine	N4-acetyl-cytidine /5-iodo-uridine
	N4-acetyl-cytidine /N 1-methyl-pseudouridine
	N4-acetyl-cytidine /a-thio-uridine
	N4-acetyl-cytidine /5-methyl-uridine
	N4-acetyl-cytidine /pseudouridine
	about 50% of cytosines are N4-acetyl-cytidine
	about 25% of cytosines are N4-acetyl-cytidine
	N4-acety1-cytidine /5-methoxy-uridine
	N4-acetyl-cytidine /5-bromo-uridine
	N4-acetyl-cytidine /2-thio-uridine
	about 50% of cytosines are N4-acetyl-cytidine/ about 50% of uridines are 2-thio-uridine

[00474] Further examples of modified nucleotide combinations are provided below in Table 10. These combinations of modified nucleotides can be used to form the cell phenotype altering polypeptides, primary constructs, or mmRNA of the invention.

Table 10

Modified Nucleotide	**Modified Nucleotide Combination**
modified cytidine having one or more nucleobases of Formula (b10)	modified cytidine with (b10)/pseudouridine
	modified cytidine with (b10)/N1-methyl-pseudouridine
	modified cytidine with (b10)/5-methoxy-uridine
	modified cytidine with (b10)/5-methyl-uridine
	modified cytidine with (b10)/5-bromo-uridine

	modified cytidine with (b10)/2-thio-uridine
	about 50% of cytidine substituted with modified cytidine (blO)/ about 50% of uridines are 2-thio-uridine
modified cytidine having one or more nucleobases of Formula (b32)	modified cytidine with (b32)/pseudouridine
	modified cytidine with (b32)/Nl-methyl-pseudouridine
	modified cytidine with (b32)/5-methoxy-uridine
	modified cytidine with (b32)/5-methyl-uridine
	modified cytidine with (b32)/5-bromo-uridine
	modified cytidine with (b32)/2-thio-uridine
	about 50% of cytidine substituted with modified cytidine (b32)/ about 50% of uridines are 2-thio-uridine
modified uridine having one or more nucleobases of Formula (bl)	modified uridine with (bl)/ N4-acetyl-cytidine
	modified uridine with (bl)/ 5-methyl-cytidine
modified uridine having one or more nucleobases of Formula (b8)	modified uridine with (b8)/ N4-acetyl-cytidine
	modified uridine with (b8)/ 5-methyl-cytidine
modified uridine having one or more nucleobases of Formula (b28)	modified uridine with (b28)/ N4-acetyl-cytidine
	modified uridine with (b28)/ 5-methyl-cytidine
modified uridine having one or more nucleobases of Formula (b29)	modified uridine with (b29)/ N4-acetyl-cytidine
	modified uridine with (b29)/ 5-methyl-cytidine
modified uridine having one or more nucleobases of Formula (b30)	modified uridine with (b30)/ N4-acetyl-cytidine
	modified uridine with (b30)/ 5-methyl-cytidine

[00475] In some embodiments, at least *25%* of the cytosines are replaced by a compound of Formula (M0)-(bl4) (e.g., at least about 30%>, at least about 35%>, at least about 40%, at least about 45%>, at least about 50%>, at least about 55%>, at least about 60%>, at least about 65%>, at least about 70%>, at least about 75%>, at least about 80%>, at least about **85%o,** at least about 90%>, at least about 95%>, or about 100%).

[00476] In some embodiments, at least 25%> of the uracils are replaced by a compound of Formula (bl)-(b9) (e.g., at least about 30%>, at least about 35%>, at least about 40%>, at least about 45%>, at least about 50%>, at least about 55%>, at least about 60%>, at least about **65%o,** at least about 70%>, at least about 75%>, at least about 80%>, at least about 85%>, at least about 90%, at least about 95%, or about 100%).

[00477] In some embodiments, at least 25%> of the cytosines are replaced by a compound of Formula (bl0)-(bl4), and at least 25% of the uracils are replaced by a compound of Formula (bl)-(b9) (e.g., at least about 30%>, at least about 35%>, at least

about 40%, at least about 45%, at least about 50%>, at least about 55%, at least about 60%>, at least about 65%, at least about 70%>, at least about 75%, at least about 80%, at least about 85%o, at least about 90%, at least about 95%, or about 100%).

IV. Pharmaceutical Compositions

Formulation, Administration, Delivery and Dosing

[00478] The present invention provides cell phenotype altering polynucleotides, primary constructs and mmRNA compositions and complexes in combination with one or more pharmaceutically acceptable excipients. Pharmaceutical compositions may optionally comprise one or more additional active substances, e.g. therapeutically and/or prophylactically active substances. General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in *Remington: The Science and Practice of Pharmacy* 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference in its entirety).

[00479] In some embodiments, compositions are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase "active ingredient" generally refers to cell phenotype altering polynucleotides, primary constructs and mmRNA to be delivered as described herein.

[00480] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, *e.g.,* to non-human animals, e.g. non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[00481] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[00482] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00483] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100%, e.g., between .5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient

Formulations

[00484] The cell phenotype altering polynucleotide, primary construct, and mmRNA of the invention can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit the sustained or delayed release (e.g., from a depot formulation of the cell phenotype altering polynucleotide, primary construct, or mmRNA); (4) alter the biodistribution (e.g., target the cell phenotype altering polynucleotide, primary construct, or mmRNA to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo;* and/or (6) alter the release profile of encoded protein *in vivo.* In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients

of the present invention can include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., for transplantation into a subject), hyaluronidase, nanoparticle mimics and combinations thereof. Accordingly, the formulations of the invention can include one or more excipients, each in an amount that together increases the stability of the cell phenotype altering polynucleotide, primary construct, or mmRNA, increases cell transfection by the cell phenotype altering polynucleotide, primary construct, or mmRNA, increases the expression of cell phenotype altering polynucleotide, primary construct, or mmRNA encoded protein, and/or alters the release profile of cell phenotype altering polynucleotide, primary construct, or mmRNA encoded proteins. Further, the primary construct and mmRNA of the present invention may be formulated using self-assembled nucleic acid nanoparticles.

[00485] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of associating the active ingredient with an excipient and/or one or more other accessory ingredients.

[00486] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient may generally be equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage including, but not limited to, one-half or one-third of such a dosage.

[00487] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient.

[00488] In some embodiments, the formulations described herein may contain at least one mmRNA. As a non-limiting example, the formulations may contain 1, 2, 3, 4 or 5 mmRNA. In one embodiment the formulation may contain modified mRNA encoding proteins selected from categories such as, but not limited to, human proteins, veterinary proteins, bacterial proteins, biological proteins, antibodies, immunogenic proteins, therapeutic peptides and proteins, secreted proteins, plasma membrane proteins, cytoplasmic and cytoskeletal proteins, intrancellular membrane bound proteins, nuclear proteins, proteins associated with human disease and/or proteins associated with non-human diseases. In one embodiment, the formulation contains at least three modified mRNA encoding proteins. In one embodiment, the formulation contains at least five modified mRNA encoding proteins.

[00489] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes, but is not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

[00490] In some embodiments, the particle size of the lipid nanoparticle may be increased and/or decreased. The change in particle size may be able to help counter biological reaction such as, but not limited to, inflammation or may increase the biological effect of the modified mRNA delivered to mammals.

[00491] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, surface active agents and/or emulsifiers, preservatives, buffering agents, lubricating agents, and/or oils.

Such excipients may optionally be included in the pharmaceutical formulations of the invention.

Lipidoids

[00492] The synthesis of lipidoids has been extensively described and formulations containing these compounds are particularly suited for delivery of cell phenotype altering polynucleotides, primary constructs or mmRNA (see Mahon et al., Bioconjug Chem. 2010 21:1448-1454; Schroeder et al, J Intern Med. 2010 267:9-21; Akinc et al, Nat Biotechnol. 2008 26:561-569; Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869; Siegwart et al, Proc Natl Acad Sci U S A. 201 1 108:12996-3001; all of which are incorporated herein in their entireties).

[00493] While these lipidoids have been used to effectively deliver double stranded small interfering RNA molecules in rodents and non-human primates (see Akinc et al., Nat Biotechnol. 2008 26:561-569; Frank-Kamenetsky et al, Proc Natl Acad Sci U S A. 2008 105:1 1915-1 1920; Akinc et al, Mol Ther. 2009 17:872-879; Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869; Leuschner et al, Nat Biotechnol. 201 1 29:1005-1010; all of which is incorporated herein in their entirety), the present disclosure describes their formulation and use in delivering single stranded cell phenotype altering polynucleotides, primary constructs, or mmRNA. Complexes, micelles, liposomes or particles can be prepared containing these lipidoids and therefore, can result in an effective delivery of the cell phenotype altering polynucleotide, primary construct, or mmRNA, as judged by the production of an encoded protein, following the injection of a lipidoid formulation via localized and/or systemic routes of administration. Lipidoid complexes of cell phenotype altering polynucleotides, primary constructs, or mmRNA can be administered by various means including, but not limited to, intravenous, intramuscular, or subcutaneous routes.

[00494] *In vivo* delivery of nucleic acids may be affected by many parameters, including, but not limited to, the formulation composition, nature of particle PEGylation, degree of loading, oligonucleotide to lipid ratio, and biophysical parameters such as particle size (Akinc et al., Mol Ther. 2009 17:872-879; herein incorporated by reference in its entirety). As an example, small changes in the anchor chain length of poly(ethylene glycol) (PEG) lipids may result in significant effects on in vivo efficacy. Formulations

with the different lipidoids, including, but not limited to penta[3-(l-laurylaminopropionyl)]-triethylenetetramine hydrochloride (TETA-5LAP; aka 98N12-5, see Murugaiah et al., Analytical Biochemistry, 401:61 (2010)), C12-200 (including derivatives and variants), and MD1, can be tested for *in vivo* activity.

[00495] The lipidoid referred to herein as "98N12-5" is disclosed by Akinc et al, Mol Ther. 2009 17:872-879 and is incorporated by reference in its entirety. (See Figure 2)

[00496] The lipidoid referred to herein as "C12-200" is disclosed by Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869 (see Figure 2) and Liu and Huang, Molecular Therapy. 2010 669-670 (see Figure 2); both of which are herein incorporated by reference in their entirety. The lipidoid formulations can include particles comprising either 3 or 4 or more components in addition to cell phenotype altering polynucleotide, primary construct, or mmRNA. As an example, formulations with certain lipidoids, include, but are not limited to, 98N12-5 and may contain 42% lipidoid, 48% cholesterol and 10% PEG (CI 4 alkyl chain length). As another example, formulations with certain lipidoids, include, but are not limited to, C12-200 and may contain 50%> lipidoid, 10%> disteroylphosphatidyl choline, 38.5% cholesterol, and 1.5% PEG-DMG.

[00497] In one embodiment, a cell phenotype altering polynucleotide, primary construct, or mmRNA formulated with a lipidoid for systemic intravenous administration can target the liver. For example, a final optimized intravenous formulation using cell phenotype altering polynucleotide, primary construct, or mmRNA, and comprising a lipid molar composition of 42% 98N12-5, 48% cholesterol, and 10% PEG-lipid with a final weight ratio of about 7.5 to 1 total lipid to cell phenotype altering polynucleotide, primary construct, or mmRNA, and a C14 alkyl chain length on the PEG lipid, with a mean particle size of roughly 50-60 nm, can result in the distribution of the formulation to be greater than 90% to the liver (see, Akinc et al, Mol Ther. 2009 17:872-879; herein incorporated in its entirety). In another example, an intravenous formulation using a C12-200 (see US provisional application 61/175,770 and published international application WO20 10 129709, each of which is herein incorporated by reference in their entirety) lipidoid may have a molar ratio of 50/10/38.5/1.5 of C12-200/disteroylphosphatidyl choline/cholesterol/PEG-DMG, with a weight ratio of 7 to 1 total lipid to polynucleotide, primary construct, or mmRNA, and a mean particle size of 80 nm may be effective to

deliver cell phenotype altering polynucleotide, primary construct, or mmRNA to hepatocytes (see, Love et al, Proc Natl Acad Sci U S A. 2010 107:1864-1869 herein incorporated by reference). In another embodiment, an MD1 lipidoid-containing formulation may be used to effectively deliver polynucleotide, primary construct, or mmRNA to hepatocytes *in vivo.* The characteristics of optimized lipidoid formulations for intramuscular or subcutaneous routes may vary significantly depending on the target cell type and the ability of formulations to diffuse through the extracellular matrix into the blood stream. While a particle size of less than 150 nm may be desired for effective hepatocyte delivery due to the size of the endothelial fenestrae (see, Akinc et al., Mol Ther. 2009 17:872-879 herein incorporated by reference), use of a lipidoid-formulated cell phenotype altering polynucleotide, primary construct, or mmRNA to deliver the formulation to other cells types including, but not limited to, endothelial cells, myeloid cells, and muscle cells may not be similarly size-limited. Use of lipidoid formulations to deliver siRNA in vivo to other non-hepatocyte cells such as myeloid cells and endothelium has been reported (see Akinc et al., Nat Biotechnol. 2008 26:561-569; Leuschner et al, Nat Biotechnol. 201 1 29:1005-1010; Cho et al. Adv. Funct. Mater. 2009 19:31 12-31 18; 8th International Judah Folkman Conference, Cambridge, MA October 8-9, 2010 herein incorporated by reference in its entirety). Effective delivery to myeloid cells, such as monocytes, lipidoid formulations may have a similar component molar ratio. Different ratios of lipidoids and other components including, but not limited to, disteroylphosphatidyl choline, cholesterol and PEG-DMG, may be used to optimize the formulation of the cell phenotype altering polynucleotide, primary construct, or mmRNA for delivery to different cell types including, but not limited to, hepatocytes, myeloid cells, muscle cells, etc. For example, the component molar ratio may include, but is not limited to, 50% C12-200, 10% disteroylphosphatidyl choline, 38.5% cholesterol, and %1.5 PEG-DMG (see Leuschner et al, Nat Biotechnol 201 1 29:1005-1010; herein incorporated by reference in its entirety). The use of lipidoid formulations for the localized delivery of nucleic acids to cells (such as, but not limited to, adipose cells and muscle cells) via either subcutaneous or intramuscular delivery, may not require all of the formulation components desired for systemic delivery, and as such may comprise only

the lipidoid and the cell phenotype altering polynucleotide, primary construct, or mmRNA.

[00498] Combinations of different lipidoids may be used to improve the efficacy of cell phenotype altering polynucleotide, primary construct, or mmRNA directed protein production as the lipidoids may be able to increase cell transfection by the cell phenotype altering polynucleotide, primary construct, or mmRNA; and/or increase the translation of encoded protein (see Whitehead et al, Mol. Ther. 201 1, 19:1688-1694, herein incorporated by reference in its entirety).

Liposomes, Lipoplexes, and Lipid Nanoparticles

[00499] The cell phenotype altering polynucleotide, primary construct, and mmRNA of the invention can be formulated using one or more liposomes, lipoplexes, or lipid nanoparticles. In one embodiment, pharmaceutical compositions of cell phenotype altering polynucleotide, primary construct, or mmRNA include liposomes. Liposomes are artificially-prepared vesicles which may primarily be composed of a lipid bilayer and may be used as a delivery vehicle for the administration of nutrients and pharmaceutical formulations. Liposomes can be of different sizes such as, but not limited to, a multilamellar vesicle (MLV) which may be hundreds of nanometers in diameter and may contain a series of concentric bilayers separated by narrow aqueous compartments, a small unicellular vesicle (SUV) which may be smaller than 50 nm in diameter, and a large unilamellar vesicle (LUV) which may be between 50 and 500 nm in diameter. Liposome design may include, but is not limited to, opsonins or ligands in order to improve the attachment of liposomes to unhealthy tissue or to activate events such as, but not limited to, endocytosis. Liposomes may contain a low or a high pH in order to improve the delivery of the pharmaceutical formulations.

[00500] The formation of liposomes may depend on the physicochemical characteristics such as, but not limited to, the pharmaceutical formulation entrapped and the liposomal ingredients , the nature of the medium in which the lipid vesicles are dispersed, the effective concentration of the entrapped substance and its potential toxicity, any additional processes involved during the application and/or delivery of the vesicles, the optimization size, polydispersity and the shelf-life of the vesicles for the

intended application, and the batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

[00501] In one embodiment, pharmaceutical compositions described herein may include, without limitation, liposomes such as those formed from l,2-dioleyloxy -*N*,*N*-dimethylaminopropane (DODMA) liposomes, DiLa2 liposomes from Marina Biotech (Bothell, WA), l,2-dilinoleyloxy-3-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[l,3]-dioxolane (DLin-KC2-DMA), and MC3 (US20 100324 120; herein incorporated by reference in its entirety) and liposomes which may deliver small molecule drugs such as, but not limited to, DOXIL® from Janssen Biotech, Inc. (Horsham, PA).

[00502] In one embodiment, pharmaceutical compositions described herein may include, without limitation, liposomes such as those formed from the synthesis of stabilized plasmid-lipid particles (SPLP) or stabilized nucleic acid lipid particle (SNALP) that have been previously described and shown to be suitable for oligonucleotide delivery in vitro and in vivo (see Wheeler et al. Gene Therapy. 1999 6:271-281; Zhang et al. Gene Therapy. 1999 6:1438-1447; Jeffs et al. Pharm Res. 2005 22:362-372; Morrissey et al, Nat Biotechnol. 2005 2:1002-1007; Zimmermann et al, Nature. 2006 441:1 11-1 14; Heyes et al. J Contr Rel. 2005 107:276-287; Semple et al. Nature Biotech. 2010 28:172-176; Judge et al. J Clin Invest. 2009 119:661-673; deFougerolles *Hum Gene Ther*. 2008 19: 125-132; all of which are incorporated herein in their entireties). The original manufacture method by Wheeler et al. was a detergent dialysis method, which was later improved by Jeffs et al. and is referred to as the spontaneous vesicle formation method. The liposome formulations are composed of 3 to 4 lipid components in addition to the cell phenotype altering polynucleotide, primary construct, or mmRNA. As an example a liposome can contain, but is not limited to, 55% cholesterol, 20% disteroylphosphatidyl choline (DSPC), 10% PEG-S-DSG, and 15% l,2-dioleyloxy -*N*,*N*-dimethylaminopropane (DODMA), as described by Jeffs et al. As another example, certain liposome formulations may contain, but are not limited to, 48% cholesterol, 20% DSPC, 2% PEG-c-DMA, and 30% cationic lipid, where the cationic lipid can be 1,2-distearloxy -*N*,*N*-dimethylaminopropane (DSDMA), DODMA, DLin-DMA, or l,2-dilinolenyloxy-3-dimethylaminopropane (DLenDMA), as described by Heyes et al.

[00503] In one embodiment, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may be formulated in a lipid vesicle which may have crosslinks between functionalized lipid bilayers.

[00504] In one embodiment, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may be formulated in a lipid-polycation complex. The formation of the lipid-polycation complex may be accomplished by methods known in the art and/or as described in U.S. Pub. No. 20120178702, herein incorporated by reference in its entirety. As a non-limiting example, the polycation may include a cationic peptide or a polypeptide such as, but not limited to, polylysine, polyornithine and/or polyarginine. In another embodiment, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may be formulated in a lipid-polycation complex which may further include a neutral lipid such as, but not limited to, cholesterol or dioleoyl phosphatidylethanolamine (DOPE).

[00505] The liposome formulation may be influenced by, but not limited to, the selection of the cationic lipid component, the degree of cationic lipid saturation, the nature of the PEGylation, ratio of all components and biophysical parameters such as size. In one example by Semple et al. (Semple et al. Nature Biotech. 2010 28:172-176), the liposome formulation was composed of 57.1 % cationic lipid, 7.1% dipalmitoylphosphatidylcholme, 34.3 % cholesterol, and 1.4% PEG-c-DMA. As another example, changing the composition of the cationic lipid could more effectively deliver siRNA to various antigen presenting cells (Basha et al. Mol Ther. 201 1 19:2186-2200; herein incorporated by reference in its entirety).

[00506] In some embodiments, the ratio of PEG in the LNP formulations may be increased or decreased and/or the carbon chain length of the PEG lipid may be modified from C14 to CI8 to alter the pharmacokinetics and/or biodistribution of the LNP formulations. As a non-limiting example, LNP formulations may contain 1-5% of the lipid molar ratio of PEG-c-DOMG as compared to the cationic lipid, DSPC and cholesterol. In another embodiment the PEG-c-DOMG may be replaced with a PEG lipid such as, but not limited to, PEG- DSG (1,2-Distearoyl-sn-glycerol, methoxypolyethylene glycol) or PEG-DPG (1,2-Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol). The

cationic lipid may be selected from any lipid known in the art such as, but not limited to, DLin-MC3 -DMA, DLin-DMA, C 12-200 and DLin-KC2-DMA.

[00507] In one embodiment, the cationic lipid may be selected from, but not limited to, a cationic lipid described in International Publication Nos. WO2012040184, WO201 1153120, WO201 1149733, WO201 1090965, WO201 1043913, WO201 1022460, WO2012061259, WO2012054365, WO2012044638, WO2010080724, WO201021865 and WO2008103276, US Patent Nos. 7,893,302 and 7,404,969 and US Patent Publication No. US20 1000361 15; each of which is herein incorporated by reference in their entirety. In another embodiment, the cationic lipid may be selected from, but not limited to, formula A described in International Publication Nos. WO2012040184, WO201 1153120, WO201 1149733, WO201 1090965, WO201 1043913, WO201 1022460, WO2012061259, WO2012054365 and WO2012044638; each of which is herein incorporated by reference in their entirety. In yet another embodiment, the cationic lipid may be selected from, but not limited to, formula CLI-CLXXIX of International Publication No. WO2008 103276, formula CLI-CLXXIX of US Patent No. 7,893,302, formula CLI-CLXXXXII of US Patent No. 7,404,969 and formula I-VI of US Patent Publication No. US201000361 15; each of which is herein incorporated by reference in their entirety. As a non-limiting example, the cationic lipid may be selected from (20Z,23Z)-N,N-dimethylnonacosa-20,23-dien-10-amine, (17Z,20Z)-N,N-dimemylhexacosa-17,20-dien-9-amine, (1 Z, 19Z)-N5N~dimethylpentacosa~l 6, 19-dien-8-amine, (13Z,16Z)-N,N-dimethyldocosa-13J16-dien-5-amine, (12Z, 15Z)-NJN-dimethylhenicosa- 12, 15-dien-4-amine, (14Z, 17Z)-N,N-dimethyltricosa- 14,1 7-dien-6-amine, (15Z, 18Z)-N,N-dimethyltetracosa- 15,1 8-dien-7-amine, (18Z,2 1Z)-N,N-dimethylheptacosa- 18,2 1-dien- 10-amine, (15Z, 18Z)-N,N-dimethyltetracosa- 15,1 8-dien-5-amine, (14Z, 17Z)-N,N-dimethyltricosa- 14,1 7-dien-4-amine, (19Z,22Z)-N,N-dimeihyloctacosa-19,22-dien-9-amine, (18Z,21 Z)-N ,N-dimethylheptacosa- 18 ,21 -dien-8 -amine, (17Z,20Z)-N,N-dimethylhexacosa- 17„20-dien-7-amine, (16Z; 19Z)-N,N-dimethylpentacosa- 16,1 9-dien-6-amine, (22Z,25Z)-N,N-dimethylhentriaconta-22,25-dien- 10-amine, (21 Z ,24Z)-N;N-dimethyltriaconta-21 ,24-dien-9-amine, (18Z)-N,N-dimetylheptacos- 18-en- 10-amine, (17Z)-N,N-dimethylhexacos-17-en-9-amine, (19Z,22Z)-NJN-dimethyloctacosa- 19,22-dien-7-amine, N,N-dimethylheptacosan- 10-amine, (20Z,23Z)-N-ethyl-N-methylnonacosa-20J23-dien-l 0-

amine, 1-[(1 lZ,14Z)-l-nonylicosa-l 1,14-dien-l-yl] pyrrolidine, (20Z)-N,N-dimethylheptacos-20-en-l 0-amine, (15Z)-N,N-dimethyl eptacos-15-en-l 0-amine, (14Z)-N,N-dimethylnonacos-14-en-l 0-amine, (17Z)-N,N-dimethylnonacos-17-en-l 0-amine, (24Z)-N,N-dimethyltritriacont-24-en-l 0-amine, (20Z)-N,N-dimethylnonacos-20-en-l 0-amine, (22Z)-N,N-dimethylhentriacont-22-en-l 0-amine, (16Z)-N,N-dimethylpentacos-16-en-8-amine, (12Z, 15Z)-N,N-dimethyl-2-nonylhenico sa- 12 , 15 -dien- 1 -amine, (13Z, 16Z)-N,N-dimethyl-3-nonyldocosa-l 3, 16-dien-l -amine, N,N-dimethyl-l-[(l S,2R)-2-octylcyclopropyl] eptadecan-8-amine, 1 -[(1 S,2R)-2-hexylcyclopropyl]-N,N-dimethylnonadecan- 10-amine, N,N-dimethyl- 1 - [(1 S ,2R)-2-octylcyclopropyl]nonadecan- 10-amine, N,N-dimethyl-21~[(lS,2R)-2-octylcyclopropyl]henicosan-l 0-amine, N,N-dimethyl- 1 -[(1 S ,2S)-2- { [(lR,2R)-2-pentylcycIopropyl]methyl} cyclopropyl]nonadecan- 10-amine, N ,N -dimethyl- 1 -[(1 S,2R)-2-octylcyclopropyl]hexadecan-8-amine, N ,N -dimethyH -[(lR,2S)-2-undecyIcyclopropyl]tetradecan-5-amine, N,N-dimethyl-3- {7-[(1 S,2R)-2-octylcyclopropyl]heptyl} dodecan- 1 -amine, 1 - [(1 R,2 S)-2-hepty lcyclopropy 1] - N,N-dimethyloctadecan-9 -amine, 1-[(1 S,2R)-2-decylcyclopropyl]-N,N-dimethylpentadecan-6-amine, N,N-dimethyl-l-[(lS,2R)-2-octylcyclopropyl]pentadecan-8-amine, R -N ,N -dimethyl- 1-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy] -3 -(octyloxy)propan-2-amine, S -N ,N -dimethyl- 1-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy] -3 -(octyloxy)propan-2-amine, 1- {2-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy]- 1-[(octyloxy) methyl]ethyl}pyrrolidine, (2S)-N,N-dimethyl- 1-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy]-3-[(5Z)-oct -5-en- 1-yloxy]propan-2-amine, 1- {2- [(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy] - 1-[(octyloxy) methyl]ethyl}azetidine, (2S)-l-(hexyloxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9, 12-dien- 1-yloxy]propan-2-amine, (2S)- 1-(heptyloxy)-N,N-dimethyl-3-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy]propan-2-amine, N ,N -dimethyl- 1-(nonyloxy)-3-[(9Z, 12Z)-octadeca-9, 12-dien- 1-yloxy]propan-2-amine, N ,N -dimethyl- 1-[(9Z)-octadec-9-en-l -yloxy] -3 -(octyloxy)propan-2-amine (Compound 9); (2S)-N,N-dimethyl-l-[(6Z,9Z, 12Z)-octadeca-6,9, 12-trien- 1-yloxy]-3-(octyloxy)propan-2-amine, (2S)- 1-[(1 lZ,14Z)-icosa-l l,14-dien-l-yloxy]-N,N-dimethyl-3-(pentyloxy)propan-2-amine, (2S)-l-(hexyloxy)-3-[(l lZ,14Z)-icosa-l l,14-dien-l-yloxy]-N,N-dimethylpropan-2-amine, 1-[(1 lZ,14Z)-icosa-l l,14-dien-l-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, 1-

[(13Z, 16Z)-docosa-13, 16-dien-l-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, (2S)-1-[(13Z, 16Z)-docosa-13,16-dien-1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, (2S)-1-[(13Z)-docos-13-en-1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, 1-[(13Z)-docos-13-en-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, 1-[(9Z)-hexadec-9-en-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, (2R)-N,N-dimethyl-H(1-metoylo ctyl)oxy]-3-[(9Z, 12Z)-octadeca-9, 12-dien-1-yloxy]propan-2-amine, (2R)-1-[(3,7-dimethyloctyl)oxy]-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-l-yloxy]propan-2-amine, N,N-dimethyl-1-(octyloxy)-3-({8-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl]methyl} cyclopropyl]octyl} oxy)propan-2-amine, N,N-dimethyl-1-{[8-(2-oclylcyclopropyl)octyl]oxy}-3-(octyloxy)propan-2-amine and (11E,20Z,23Z)-N;N-dimethylnonacosa-ll,20,2-trien-10-amine or a pharmaceutically acceptable salt or stereoisomer thereof.

[00508] In one embodiment, the cationic lipid may be synthesized by methods known in the art and/or as described in International Publication Nos. WO20 12040 184, WO201 1153120, WO201 1149733, WO201 1090965, WO201 1043913, WO201 1022460, WO2012061259, WO2012054365, WO2012044638, WO2010080724 and WO20 102 1865; each of which is herein incorporated by reference in their entirety.

[00509]

[00510] In one embodiment, the LNP formulations of the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may contain PEG-c-DOMG 3% lipid molar ratio. In another embodiment, the LNP formulations of the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may contain PEG-c-DOMG 1.5% lipid molar ratio.

[00511] In one embodiment, the pharmaceutical compositions of the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may include at least one of the PEGylated lipids described in International Publication No. 2012099755, herein incorporated by reference.

[00512] In one embodiment, the LNP formulation may contain PEG-DMG 2000 (1,2-dimyristoyl-sn-glycero-3-phophoethanolamine-N-[methoxy(poly ethylene glycol)-2000). In one embodiment, the LNP formulation may contain PEG-DMG 2000, a cationic lipid known in the art and at least one other component. In another embodiment, the LNP

formulation may contain PEG-DMG 2000, a cationic lipid known in the art, DSPC and cholesterol. As a non-limiting example, the LNP formulation may contain PEG-DMG 2000, DLin-DMA, DSPC and cholesterol. As another non-limiting example the LNP formulation may contain PEG-DMG 2000, DLin-DMA, DSPC and cholesterol in a molar ratio of 2:40:10:48 (see Geall et al., Nonviral delivery of self-amplifying RNA vaccines, PNAS 2012; PMID: 22908294).

[00513] In one embodiment, the LNP formulation may be formulated by the methods described in International Publication Nos. WO201 1127255 or WO2008103276, each of which is herein incorporated by reference in their entirety. As a non-limiting example, modified RNA described herein may be encapsulated in LNP formulations as described in WO201 1127255 and/or WO2008103276; each of which is herein incorporated by reference in their entirety.

[00514] In one embodiment, LNP formulations described herein may comprise a polycationic composition. As a non-limiting example, the polycationic composition may be selected from formula 1-60 of US Patent Publication No. US20050222064; herein incorporated by reference in its entirety. In another embodiment, the LNP formulations comprising a polycationic composition may be used for the delivery of the modified RNA described herein *in vivo* and/or *in vitro.*

[00515] In one embodiment, the LNP formulations described herein may additionally comprise a permeability enhancer molecule. Non-limiting permeability enhancer molecules are described in US Patent Publication No. US20050222064; herein incorporated by reference in its entirety.

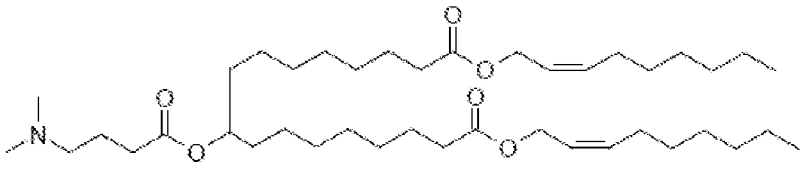
[00516]

[00517] In one embodiment, the pharmaceutical compositions may be formulated in liposomes such as, but not limited to, DiLa2 liposomes (Marina Biotech, Bothell, WA), SMARTICLES® (Marina Biotech, Bothell, WA), neutral DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) based liposomes (e.g., siRNA delivery for ovarian cancer (Landen et al. Cancer Biology & Therapy 2006 5(12)1708-1713)) and hyaluronan-coated liposomes (Quiet Therapeutics, Israel).

[00518] Lipid nanoparticle formulations may be improved by replacing the cationic lipid with a biodegradable cationic lipid which is known as a rapidly eliminated lipid

nanoparticle (reLNP). Ionizable cationic lipids, such as, but not limited to, DLinDMA, DLin-KC2-DMA, and DLin-MC3-DMA, have been shown to accumulate in plasma and tissues over time and may be a potential source of toxicity. The rapid metabolism of the rapidly eliminated lipids can improve the tolerability and therapeutic index of the lipid nanoparticles by an order of magnitude from a 1 mg/kg dose to a 10 mg/kg dose in rat. Inclusion of an enzymatically degraded ester linkage can improve the degradation and metabolism profile of the cationic component, while still maintaining the activity of the reLNP formulation. The ester linkage can be internally located within the lipid chain or it may be terminally located at the terminal end of the lipid chain. The internal ester linkage may replace any carbon in the lipid chain.

[00519] In one embodiment, the internal ester linkage may be located on either side of the saturated carbon. Non-limiting examples of reLNPs include,



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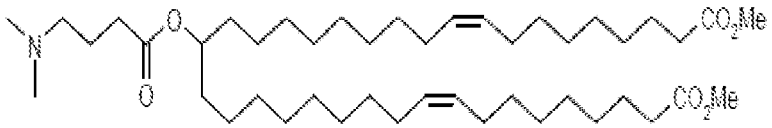

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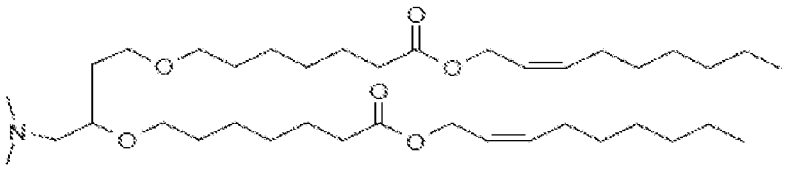

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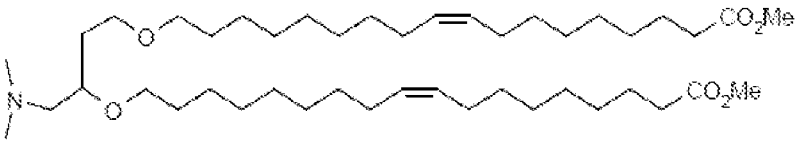

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[00520] In one embodiment, an immune response may be elicited by delivering a lipid nanoparticle which may include a nanospecies, a polymer and an immunogen. (U.S. Publication No. 20120189700 and International Publication No. WO2012099805; each of

which is herein incorporated by reference in their entirety). The polymer may encapsulate the nanospecies or partially encapsulate the nanospecies.

[00521] Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limted to, oral (e.g., the buccal and esophageal membranes and tonsil tissue), ophthalmic, gastrointestinal (e.g., stomach, small intestine, large intestine, colon, rectum), nasal, respiratory (e.g., nasal, pharyngeal, tracheal and bronchial membranes), genital (e.g., vaginal, cervical and urethral membranes). Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers. Mucus is continuously secreted, shed, discarded or digested and recycled so most of the trapped particles may be removed from the mucosla tissue within seconds or within a few hours. Large polymeric nanoparticles (200nm -500nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai et al. PNAS 2007 104(5): 1482-487; Lai et al. Adv Drug Deliv Rev. 2009 61(2): 158-171; each of which is herein incorporated by reference in their entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT).

[00522] The lipid nanoparticle engineered to penetrate mucus may comprise a polymeric material (i.e. a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material may include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyeneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. The polymeric material may be biodegradable and/or biocompatible. Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA),

poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacralate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), and trimethylene carbonate, polyvinylpyrrolidone. The lipid nanoparticle may be coated or associated with a co-polymer such as, but not limited to, a block co-polymer, and (poly(ethylene glycol))-(poly(propylene oxide))-(poly(ethylene glycol)) triblock copolymer (see US Publication 20120121718 and US Publication 20100003337; each of which is herein incorporated by reference in their entirety). The co-polymer may be a polymer that is generally regarded as safe (GRAS) and the formation of the lipid nanoparticle may be in such a way that no new chemical entities are created. For example, the lipid nanoparticle may comprise poloxamers coating PLGA nanoparticles without forming new chemical entities which are still able to rapidly penetrate human mucus (Yang et al. Angew. Chem. Int. Ed. 201 1 50:2597-2600; herein incorporated by reference in its entirety).

[00523] The vitamin of the polymer-vitamin conjugate may be vitamin E. The vitamin portion of the conjugate may be substituted with other suitable components such as, but not limited to, vitamin A, vitamin E, other vitamins, cholesterol, a hydrophobic moiety, or a hydrophobic component of other surfactants (e.g., sterol chains, fatty acids, hydrocarbon chains and alkylene oxide chains).

[00524] The lipid nanoparticle engineered to penetrate mucus may include surface altering agents such as, but not limited to, cell phenotype altering mmRNA, anionic protein (e.g., bovine serum albumin), surfactants (e.g., cationic surfactants such as for example dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (e.g., cyclodextrin), nucleic acids, polymers (e.g., heparin, polyethylene glycol and poloxamer), mucolytic agents (e.g., N-acetylcysteine, mugwort, bromelain, papain, clerodendrum, acetylcysteine, bromhexine, carbocisteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β4 dornase alfa, neltenexine, erdosteine) and various DNases including rhDNase. The surface altering agent may be embedded or enmeshed in the particle's surface or disposed (e.g., by coating, adsorption, covalent linkage, or other process) on the surface of the lipid nanoparticle. (see US Publication 20100215580 and US Publication 20080166414; each of which is herein incorporated by reference in their entirety).

[00525] The mucus penetrating lipid nanoparticles may comprise at least one cell phenotype altering mmRNA described herein. The mmRNA may be encapsulated in the lipid nanoparticle and/or disposed on the surface of the paricle. The mmRNA may be covalently coupled to the lipid nanoparticle. Formulations of mucus penetrating lipid nanoparticles may comprise a plurality of nanoparticles. Further, the formulations may contain particles which may interact with the mucus and alter the structural and/or adhesive properties of the surrounding mucus to decrease mucoadhesion which may increase the delivery of the mucus penetrating lipid nanoparticles to the mucosal tissue.

[00526] In one embodiment, the cell phenotype altering polynucleotide, primary construct, or mmRNA is formulated as a lipoplex, such as, without limitation, the ATUPLEX™ system, the DACC system, the DBTC system and other siRNA-lipoplex technology from Silence Therapeutics (London, United Kingdom), STEMFECT™ from STEMGENT® (Cambridge, MA), and polyethylenimine (PEI) or protamine-based

targeted and non-targeted delivery of nucleic acids acids (Aleku et al. Cancer Res. 2008 68:9788-9798; Strumberg et al. Int J Clin Pharmacol Ther 2012 50:76-78; Santel et al, Gene Ther 2006 13:1222-1234; Santel et al., Gene Ther 2006 13:1360-1370; Gutbier et al, Pulm Pharmacol. Ther. 2010 23:334-344; Kaufmann et al. Microvasc Res 2010 80:286-293Weide et al. J Immunother. 2009 32:498-507; Weide et al. J Immunother. 2008 31:180-188; Pascolo Expert Opin. Biol. Ther. 4:1285-1294; Fotin-Mleczek et al, 201 1 J. Immunother. 34:1-15; Song et al, Nature Biotechnol. 2005, 23:709-717; Peer et al, Proc Natl Acad Sci U S A. 2007 6;104:4095-4100; deFougerolles *Hum Gene Ther.* 2008 19:125-132; all of which are incorporated herein by reference in its entirety).

[00527] In one embodiment such formulations may also be constructed or compositions altered such that they passively or actively are directed to different cell types *in vivo,* including but not limited to hepatocytes, immune cells, tumor cells, endothelial cells, antigen presenting cells, and leukocytes (Akinc et al. Mol Ther. 2010 18:1357-1364; Song et al, Nat Biotechnol. 2005 23:709-717; Judge et al, J Clin Invest. 2009 119:661-673; Kaufmann et al, Microvasc Res 2010 80:286-293; Santel et al, Gene Ther 2006 13:1222-1234; Santel et al, Gene Ther 2006 13:1360-1370; Gutbier et al, Pulm Pharmacol. Ther. 2010 23:334-344; Basha et al, Mol. Ther. 201 1 19:2186-2200; Fenske and Cullis, Expert Opin Drug Deliv. 2008 5:25-44; Peer et al, Science. 2008 319:627-630; Peer and Lieberman, Gene Ther. 201 1 18:1 127-1 133; all of which are incorporated herein by reference in its entirety). One example of passive targeting of formulations to liver cells includes the DLin-DMA, DLin-KC2-DMA and MC3-based lipid nanoparticle formulations which have been shown to bind to apolipoprotein E and promote binding and uptake of these formulations into hepatocytes in vivo (Akinc et al. Mol Ther. 2010 18:1357-1364; herein incorporated by reference in its entirety). Formulations can also be selectively targeted through expression of different ligands on their surface as exemplified by, but not limited by, folate, transferrin, N-acetylgalactosamine (GalNAc), and antibody targeted approaches (Kolhatkar et al., Curr Drug Discov Technol. 201 1 8:197-206; Musacchio and Torchilin, *Front Biosci.* 201 1 16:1388-1412; Yu et al, Mol Membr Biol. 2010 27:286-298; Patil et al, Crit Rev Ther Drug Carrier Syst. 2008 25:1-61; Benoit et al., Biomacromolecules. 201 1 12:2708-2714Zhao et al., Expert Opin Drug Deliv. 2008 5:309-319; Akinc et al, Mol Ther. 2010 18:1357-1364; Srinivasan et al,

Methods Mol Biol. 2012 820:105-1 16; Ben-Arie et al, Methods Mol Biol. 2012 757:497-507; Peer 2010 J Control Release. 20:63-68; Peer et al, Proc Natl Acad Sci U S A. 2007 104:4095-4100; Kim et al, Methods Mol Biol. 201 1 721:339-353; Subramanya et al, Mol Ther. 2010 18:2028-2037; Song et al, Nat Biotechnol. 2005 23:709-717; Peer et al, Science. 2008 319:627-630; Peer and Lieberman, Gene Ther. 201 1 18:1 127-1 133; all of which are incorporated herein by reference in its entirety)..

[00528] In one embodiment, the cell phenotype altering polynucleotide, primary construct, or mmRNA is formulated as a solid lipid nanoparticle. A solid lipid nanoparticle (SLN) may be spherical with an average diameter between 10 to 1000 nm. SLN possess a solid lipid core matrix that can solubilize lipophilic molecules and may be stabilized with surfactants and/or emulsifiers. In a further embodiment, the lipid nanoparticle may be a self-assembly lipid-polymer nanoparticle (see Zhang et al., ACS Nano, 2008, 2 (8), pp 1696-1702; herein incorporated by reference in its entirety).

[00529] Liposomes, lipoplexes, or lipid nanoparticles may be used to improve the efficacy of polynucleotide, primary construct, or mmRNA directed protein production as these formulations may be able to increase cell transfection by the polynucleotide, primary construct, or mmRNA; and/or increase the translation of encoded protein. One such example involves the use of lipid encapsulation to enable the effective systemic delivery of polyplex plasmid DNA (Heyes et al, Mol Ther. 2007 15:713-720; herein incorporated by reference in its entirety). The liposomes, lipoplexes, or lipid nanoparticles may also be used to increase the stability of the cell phenotype altering polynucleotide, primary construct, or mmRNA.

[00530] In one embodiment, the the cell phenotype altering polynucleotides, primary constructs, and/or the mmRNA of the present invention can be formulated for controlled release and/or targeted delivery. As used herein, "controlled release" refers to a pharmaceutical composition or compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome. In one embodiment, the cell phenotype altering polynucleotides, primary constructs or the mmRNA may be encapsulated into a delivery agent described herein and/or known in the art for controlled release and/or targeted delivery. As used herein, the term "encapsulate" means to enclose, surround or encase. As it relates to the formulation of the compounds of the invention, encapsulation

may be substantial, complete or partial. The term "substantially encapsulated" means that at least greater than 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.9 or greater than 99.999% of the pharmaceutical composition or compound of the invention may be enclosed, surrounded or encased within the delivery agent. "Partially encapsulation" means that less than 10, 10, 20, 30, 40 50 or less of the pharmaceutical composition or compound of the invention may be enclosed, surrounded or encased within the delivery agent. Advantageously, encapsulation may be determined by measuring the escape or the activity of the pharmaceutical composition or compound of the invention using fluorescence and/or electron micrograph. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the pharmaceutical composition or compound of the invention are encapsulated in the delivery agent.

[00531] In another embodiment, the cell phenotype altering polynucleotides, primary constructs, or the mmRNA may be encapsulated into a lipid nanoparticle or a rapidly eliminating lipid nanoparticle and the lipid nanoparticles or a rapidly eliminating lipid nanoparticle may then be encapsulated into a polymer, hydrogel and/or surgical sealant described herein and/or known in the art. As a non-limiting example, the polymer, hydrogel or surgical sealant may be PLGA, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, FL), HYLENEX® (Halozyme Therapeutics, San Diego CA), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, GA), TISSELL® (Baxter International, Inc Deerfield, IL), PEG-based sealants, and COSEAL® (Baxter International, Inc Deerfield, IL).

[00532] In one embodiment, the lipid nanoparticle may be encapsulated into any polymer or hydrogel known in the art which may form a gel when injected into a subject. As another non-limiting example, the lipid nanoparticle may be encapsulated into a polymer matrix which may be biodegradable.

[00533] In one embodiment, the cell phenotype altering polynucleotide, primary construct, or mmRNA formulation for controlled release and/or targeted delivery may also include at least one controlled release coating. Controlled release coatings include, but are not limited to, OPADRY®, polyvinylpyrrolidone/vinyl acetate copolymer, polyvinylpyrrolidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose,

hydroxyethyl cellulose, EUDRAGIT RL®, EUDRAGIT RS® and cellulose derivatives such as ethylcellulose aqueous dispersions (AQUACOAT® and SURELEASE®).

[00534] In one embodiment, the controlled release and/or targeted delivery formulation may comprise at least one degradable polyester which may contain polycationic side chains. Degradeable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In another embodiment, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

[00535] In one embodiment, the cell phenotype altering polynucleotides, primary constructs, and/or the mmRNA of the present invention may be encapsulated in a therapeutic nanoparticle. Therapeutic nanoparticles may be formulated by methods described herein and known in the art such as, but not limited to, International Pub Nos. WO2010005740, WO2010030763, WO2010005721, WO2010005723, WO2012054923, US Pub. Nos. US20110262491, US20100104645, US20100087337, US20100068285, US201 10274759, US20100068286, and US Pat No. 8,206,747; each of which is herein incorporated by reference in their entirety. In another embodiment, therapeutic polymer nanoparticles may be identified by the methods described in US Pub No. US20120140790, herein incorporated by reference in its entirety.

[00536] In one embodiment, the therapeutic nanoparticle of may be formulated for sustained release. As used herein, "sustained release" refers to a pharmaceutical composition or compound that conforms to a release rate over a specific period of time. The period of time may include, but is not limited to, hours, days, weeks, months and years. As a non-limiting example, the sustained release nanoparticle may comprise a polymer and a therapeutic agent such as, but not limited to, the the polynucleotides, primary constructs, and mmRNA of the present invention (see International Pub No. 2010075072 and US Pub No. US20100216804 and US201 10217377, each of which is herein incorporated by reference in their entirety).

[00537] In one embodiment, the therapeutic nanoparticles may be formulated to be target specific. As a non-limiting example, the thereapeutic nanoparticles may include a corticosteroid (see International Pub. No. WO201 1084518). In one embodiment, the therapeutic nanoparticles may be formulated to be cancer specific. As a non-limiting

example, the therapeutic nanoparticles may be formulated in nanoparticles described in International Pub No. WO2008121949, WO2010005726, WO2010005725, WO201 1084521 and US Pub No. US20 100069426, US20120004293 and US20100104655, each of which is herein incorporated by reference in their entirety.

[00538] In one embodiment, the nanoparticles of the present invention may comprise a polymeric matrix. As a non-limiting example, the nanoparticle may comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester) or combinations thereof.

[00539] In one embodiment, the diblock copolymer may include PEG in combination with a polymer such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester) or combinations thereof.

[00540] In one embodiment, the therapeutic nanoparticle comprises a diblock copolymer. As a non-limiting example the therapeutic nanoparticle comprises a PLGA-PEG block copolymer (see US Pub. No. US20120004293 and US Pat No. 8,236,330, herein incorporated by reference in their entireties). In another non-limiting example, the therapeutic nanoparticle is a stealth nanoparticle comprising a diblock copolymer of PEG and PLA or PEG and PLGA (see US Pat No 8,246,968, each of which is herein incorporated by reference in its entirety).

[00541] In one embodiment, the therapeutic nanoparticle may comprise at least one acrylic polymer. Acrylic polymers include but are not limited to, acrylic acid, methacrylic acid, acrylic acid and methacrylic acid copolymers, methyl methacrylate

copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, amino alkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), polycyanoacrylates and combinations thereof.

[00542] In one embodiment, the therapeutic nanoparticles may comprise at least one cationic polymer described herein and/or known in the art.

[00543] In one embodiment, the therapeutic nanoparticles may comprise at least one amine-containing polymer such as, but not limited to polylysine, polyethylene imine, poly(amidoamine) dendrimers and combinations thereof.

[00544] In one embodiment, the therapeutic nanoparticles may comprise at least one degradable polyester which may contain polycationic side chains. Degradeable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In another embodiment, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

[00545] In another embodiment, the therapeutic nanoparticle may include a conjugation of at least one targeting ligand.

[00546] In one embodiment, the therapeutic nanoparticle may be formulated in an aqueous solution which may be used to target cancer (see International Pub No. WO201 1084513 and US Pub No. US201 10294717, each of which is herein incorporated by reference in their entirety).

[00547] In one embodiment, the cell phenotype altering polynucleotides, primary constructs, or mmRNA may be encapsulated in, linked to and/or associated with synthetic nanocarriers. The synthetic nanocarriers may be formulated using methods known in the art and/or described herein. As a non-limiting example, the synthetic nanocarriers may be formulated by the methods described in International Pub Nos. WO20 10005740, WO20 10030763 and US Pub. Nos. US20 110262491, US20 100 104645 and US20 100087337, each of which is herein incorporated by reference in their entirety. In another embodiment, the synthetic nanocarrier formulations may be lyophilized by methods described in International Pub. No. WO201 1072218 and US Pat No. 8,21 1,473; each of which is herein incorporated by reference in their entireties.

[00548] In one embodiment, the synthetic nanocarriers may contain reactive groups to release the cell phenotype altering polynucleotides, primary constructs and/or mmRNA

described herein (see International Pub. No. WO20120952552 and US Pub No. US20120171229, each of which is herein incorporated by reference in their entirety).

[00549] In one embodiment, the synthetic nanocarriers may contain an immunostimulatory agent to enhance the immune response from delivery of the synthetic nanocarrier. As a non-limiting example, the synthetic nanocarrier may comprise a Thl immunostimulatory agent which may enhance a Thl -based response of the immune system (see International Pub No. WO2010123569 and US Pub. No. US201 10223201, each of which is herein incorporated by reference in its entirety).

[00550] In one embodiment, the synthetic nanocarriers may be formulated for targeted release. In one embodiment, the synthetic nanocarrier is formulated to release the cell phenotype altering polynucleotides, primary constructs and/or mmRNA at a specified pH and/or after a desired time interval. As a non-limiting example, the synthetic nanoparticle may be formulated to release the cell phenotype altering polynucleotides, primary constructs and/or mmRNA after 24 hours and/or at a pH of 4.5 (see International Pub. Nos. WO2010138193 and WO2010138194 and US Pub Nos. US201 10020388 and US201 10027217, each of which is herein incorporated by reference in their entireties).

[00551] In one embodiment, the synthetic nanocarriers may be formulated for controlled and/or sustained release of the cell phenotype altering polynucleotides, primary constructs and/or mmRNA described herein. As a non-limiting example, the synthetic nanocarriers for sustained release may be formulated by methods known in the art, described herein and/or as described in International Pub No. WO2010138192 and US Pub No. 20100303850, each of which is herein incorporated by reference in their entireties.

Polymers, Biodegradable Nanoparticles, and Core-Shell Nanoparticles

[00552] The cell phenotype altering polynucleotide, primary construct, and mmRNA of the invention can be formulated using natural and/or synthetic polymers. Non-limiting examples of polymers which may be used for delivery include, but are not limited to, Dynamic POLYCONJUGATE™ formulations from MIRUS® Bio (Madison, WI) and Roche Madison (Madison, WI), PHASERX™ polymer formulations such as, without limitation, SMARTT POLYMER TECHNOLOGY™ (Seattle, WA), DMRI/DOPE, poloxamer, VAXFECTIN® adjuvant from Vical (San Diego, CA), chitosan, cyclodextrin

from Calando Pharmaceuticals (Pasadena, CA), dendrimers and poly(lactic-co-glycolic acid) (PLGA) polymers. RONDEL™ (RNAi/Oligonucleotide Nanoparticle Delivery) polymers (Arrowhead Research Corporation, Pasadena, CA) and pH responsive co-block polymers such as, but not limited to, PHASERX™ (Seattle, WA).

[00553] A non-limiting example of PLGA formulations include, but are not limited to, PLGA injectable depots (e.g., ELIGARD® which is formed by dissolving PLGA in 66% N-methyl-2-pyrrolidone (NMP) and the remainder being aqueous solvent and leuprolide. Once injected, the PLGA and leuprolide peptide precipitates into the subcutaneous space).

[00554] Many of these polymer approaches have demonstrated efficacy in delivering oligonucleotides *in vivo* into the cell cytoplasm (reviewed in deFougerolles *Hum Gene Ther*. 2008 19:125-132; herein incorporated by reference in its entirety). Two polymer approaches that have yielded robust *in vivo* delivery of nucleic acids, in this case with small interfering RNA (siRNA), are dynamic polyconjugates and cyclodextrin-based nanoparticles. The first of these delivery approaches uses dynamic polyconjugates and has been shown *in vivo* in mice to effectively deliver siRNA and silence endogenous target mRNA in hepatocytes (Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887). This particular approach is a multicomponent polymer system whose key features include a membrane-active polymer to which nucleic acid, in this case siRNA, is covalently coupled via a disulfide bond and where both PEG (for charge masking) and *N*-acetylgalactosamine (for hepatocyte targeting) groups are linked via pH-sensitive bonds (Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887). On binding to the hepatocyte and entry into the endosome, the polymer complex disassembles in the low-pH environment, with the polymer exposing its positive charge, leading to endosomal escape and cytoplasmic release of the siRNA from the polymer. Through replacement of the ^-acetylgalactosamine group with a mannose group, it was shown one could alter targeting from asialoglycoprotein receptor-expressing hepatocytes to sinusoidal endothelium and Kupffer cells. Another polymer approach involves using transferrin-targeted cyclodextrin-containing polycation nanoparticles. These nanoparticles have demonstrated targeted silencing of the *EWS-FLI1* gene product in transferrin receptor-expressing Ewing's sarcoma tumor cells (Hu-Lieskovan *et al.,* Cancer Res.2005 65:

8984-8982) and siRNA formulated in these nanoparticles was well tolerated in non-human primates (Heidel *et al,* Proc Natl Acad Sci USA 2007 104:5715-21). Both of these delivery strategies incorporate rational approaches using both targeted delivery and endosomal escape mechanisms.

[00555] The polymer formulation can permit the sustained or delayed release of the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., following intramuscular or subcutaneous injection). The altered release profile for the cell phenotype altering polynucleotide, primary construct, or mmRNA can result in, for example, translation of an encoded protein over an extended period of time. The polymer formulation may also be used to increase the stability of the cell phenotype altering polynucleotide, primary construct, or mmRNA. Biodegradable polymers have been previously used to protect nucleic acids other than mmRNA from degradation and been shown to result in sustained release of payloads in vivo (Rozema et al., Proc Natl Acad Sci U S A. 2007 104:12982-12887; Sullivan et al, Expert Opin Drug Deliv. 2010 7:1433-1446; Convertine et al., Biomacromolecules. 2010 Oct 1; Chu et al, Acc Chem Res. 2012 Jan 13; Manganiello et al, Biomaterials. 2012 33:2301-2309; Benoit et al., Biomacromolecules. 201 1 12:2708-2714; Singha et al, Nucleic Acid Ther. 201 1 2:133-147; deFougerolles Hum Gene Ther. 2008 19:125-132; Schaffert and Wagner, Gene Ther. 2008 16:1 131-1 138; Chaturvedi et al, Expert Opin Drug Deliv. 201 1 8:1455-1468; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 2010 464:1067-1070; each of which is herein incorporated by reference in its entirety).

[00556] In one embodiment, the pharmaceutical compositions may be sustained release formulations. In a further embodiment, the sustained release formulations may be for subcutaneous delivery. Sustained release formulations may include, but are not limited to, PLGA microspheres, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, FL), HYLENEX® (Halozyme Therapeutics, San Diego CA), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, GA), TISSELL® (Baxter International, Inc Deerfield, IL), PEG-based sealants, and COSEAL® (Baxter International, Inc Deerfield, IL).

[00557] As a non-limiting example modified mRNA may be formulated in PLGA microspheres by preparing the PLGA microspheres with tunable release rates (e.g., days

and weeks) and encapsulating the modified mRNA in the PLGA microspheres while maintaining the integrity of the modified mRNA during the encapsulation process. EVAc are non-biodegradeable, biocompatible polymers which are used extensively in pre-clinical sustained release implant applications (e.g., extended release products Ocusert a pilocarpine ophthalmic insert for glaucoma or progestasert a sustained release progesterone intrauterine deivce; transdermal delivery systems Testoderm, Duragesic and Selegiline; catheters). Poloxamer F-407 NF is a hydrophilic, non-ionic surfactant triblock copolymer of polyoxyethylene-polyoxypropylene-polyoxyethylene having a low viscosity at temperatures less than 5°C and forms a solid gel at temperatures greater than 15°C. PEG-based surgical sealants comprise two synthetic PEG components mixed in a delivery device which can be prepared in one minute, seals in 3 minutes and is reabsorbed within 30 days. GELSITE® and natural polymers are capable of in-situ gelation at the site of administration. They have been shown to interact with protein and peptide therapeutic candidates through ionic ineraction to provide a stabilizing effect.

[00558] Polymer formulations can also be selectively targeted through expression of different ligands as exemplified by, but not limited by, folate, transferrin, and N-acetylgalactosamine (GalNAc) (Benoit et al., Biomacromolecules. 201 1 12:2708-2714; Rozema et al, Proc Natl Acad Sci U S A. 2007 104:12982-12887; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 2010 464:1067-1070; each of which is herein incorporated by reference in its entirety).

[00559] The cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the invention may be formulated with or in a polymeric compound. The polymer may include at least one polymer such as, but not limited to, polyethenes, polyethylene glycol (PEG), poly(l-lysine)(PLL), PEG grafted to PLL, cationic lipopolymer, biodegradable cationic lipopolymer, polyethyleneimine (PEI), cross-linked branched poly(alkylene imines), a polyamine derivative, a modified poloxamer, a biodegradable polymer, biodegradable block copolymer, biodegradable random copolymer, biodegradable polyester copolymer, biodegradable polyester block copolymer, biodegradable polyester block random copolymer, linear biodegradable copolymer, poly[a-(4-aminobutyl)-L-glycolic acid) (PAGA), biodegradable cross-linked cationic multi-block copolymers, polycarbonates, polyanhydrides, polyhydroxyacids,

polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), acrylic polymers, amine-containing polymers or combinations thereof .

[00560] As a non-limiting example, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the invention may be formulated with the polymeric compound of PEG grafted with PLL as described in U.S. Pat. No. 6,177,274 herein incorporated by reference in its entirety. The formulation may be used for transfecting cells *in vitro* or for *in vivo* delivery of the cell phenotype altering polynucleotides, primary constructs and/or mmRNA. In another example, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may be suspended in a solution or medium with a cationic polymer, in a dry pharmaceutical composition or in a solution that is capable of being dried as described in U.S. Pub. Nos. 20090042829 and 20090042825 each of which are herein incorporated by reference in their entireties.

[00561] As another non-limiting example the cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention may be formulated with a PLGA-PEG block copolymer (see US Pub. No. US20120004293 and US Pat No. 8,236,330, each of which is herein incorporated by reference in their entireties). As a non-limiting example, the cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention may be formulated with a diblock copolymer of PEG and PLA or PEG and PLGA (see US Pat No 8,246,968, herein incorporated by reference in its entirety).

[00562] A polyamine derivative may be used to deliver nucleic acids or to treat and/or prevent a disease or to be included in an implantable or injectable device (U.S. Pub. No. 20100260817 herein incorporated by reference in its entirety). As a non-limiting example, a pharmaceutical composition may include the modified nucleic acids and mmRNA and the polyamine derivative described in U.S. Pub. No. 20100260817 (the contents of which are incorporated herein by reference in its entirety.

[00563] The cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention may be formulated with at least one acrylic polymer. Acrylic polymers include but are not limited to, acrylic acid, methacrylic acid, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, amino alkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), polycyanoacrylates and combinations thereof.

[00564] In one embodiment, the cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention may be formulated with at least one polymer described in International Publication Nos. WO201 1115862, WO2012082574 and WO20 12068 187, each of which is herein incorporated by reference in their entireties. In another embodiment the cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention may be formulated with a polymer of formula Z as described in WO201 1115862, herein incorporated by reference in its entirety. In yet another embodiment, the cell phenotype altering polynucleotides, primary constructs or mmRNA may be formulated with a polymer of formula Z, Z' or Z" as described in WO2012082574 or WO2012068187, each of which are herein incorporated by reference in their entireties. The polymers formulated with the modified RNA of the present invention may be synthesized by the methods described in WO2012082574 or WO2012068187, each of which is herein incorporated by reference in their entireties.

[00565] Formulations of cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention may include at least one amine-containing polymer such as, but not limited to polylysine, polyethylene imine, poly(amidoamine) dendrimers or combinations thereof.

[00566] For example, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the invention may be formulated in a pharmaceutical compound including a poly(alkylene imine), a biodegradable cationic lipopolymer, a biodegradable block copolymer, a biodegradable polymer, or a biodegradable random copolymer, a biodegradable polyester block copolymer, a biodegradable polyester polymer, a biodegradable polyester random copolymer, a linear biodegradable copolymer, PAGA, a biodegradable cross-linked cationic multi-block copolymer or combinations thereof. The biodegradable cationic lipopolymer may be made my methods known in the art and/or

described in U.S. Pat. No. 6,696,038, U.S. App. Nos. 20030073619 and 20040142474 each of which is herein incorporated by reference in their entireties. The poly(alkylene imine) may be made using methods known in the art and/or as described in U.S. Pub. No. 201000043 15, herein incorporated by reference in its entirety. The biodegradabale polymer, biodegradable block copolymer, the biodegradable random copolymer, biodegradable polyester block copolymer, biodegradable polyester polymer, or biodegradable polyester random copolymer may be made using methods known in the art and/or as described in U.S. Pat. Nos. 6,517,869 and 6,267,987, the contents of which are each incorporated herein by reference in its entirety. The linear biodegradable copolymer may be made using methods known in the art and/or as described in U.S. Pat. No. 6,652,886. The PAGA polymer may be made using methods known in the art and/or as described in U.S. Pat. Nos. 6,217,912 herein incorporated by reference in its entirety. The PAGA polymer may be copolymerized to form a copolymer or block copolymer with polymers such as but not limited to, poly-L-lysine, polyargine, polyornithine, histones, avidin, protamines, polylactides and poly(lactide-co-glycolides). The biodegradable cross-linked cationic multi-block copolymers may be made my methods known in the art and/or as described in U.S. Pat. No. 8,057,821 or U.S. Pub. No. 2012009145 each of which is herein incorporated by reference in their entireties. For example, the multi-block copolymers may be synthesized using linear polyethyleneimine (LPEI) blocks which have distinct patterns as compared to branched polyethyleneimines. Further, the composition or pharmaceutical composition may be made by the methods known in the art, described herein, or as described in U.S. Pub. No. 20100004315 or U.S. Pat. Nos. 6,267,987 and 6,217,912 each of which is herein incorporated by reference in their entireties.

[00567] The cell phenotype altering polynucleotides, primary constructs, and mmRNA of the invention may be formulated with at least one degradable polyester which may contain polycationic side chains. Degradeable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In another embodiment, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

[00568] In one embodiment, the polymers described herein may be conjugated to a lipid-terminating PEG. As a non-limiting example, PLGA may be conjugated to a lipid-terminating PEG forming PLGA-DSPE-PEG. As another non-limiting example, PEG conjugates for use with the present invention are described in International Publication No. WO2008 103276, herein incorporated by reference in its entirety.

[00569] In one embodiment, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA described herein may be conjugated with another compound. Non-limiting examples of conjugates are described in US Patent Nos. 7,964,578 and 7,833,992, each of which are herein incorporated by reference in their entireties. In another embodiment, , the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention may be conjugated with conjugates of formula 1-122 as described in US Patent Nos. 7,964,578 and 7,833,992, each of which are herein incorporated by reference in their entireties.

[00570] As described in U.S. Pub. No. 201000043 13, herein incorporated by reference in its entirety, a gene delivery composition may include a nucleotide sequence and a poloxamer. For example, the cell phenotype altering polynucleotide, primary construct and/or mmRNA of the present inveition may be used in a gene delivery composition with the poloxamer described in U.S. Pub. No. 20100004313.

[00571] In one embodiment, the polymer formulation of the present invention may be stabilized by contacting the polymer formulation, which may include a cationic carrier, with a cationic lipopolymer which may be covalently linked to cholesterol and polyethylene glycol groups. The polymer formulation may be contacted with a cationic lipopolymer using the methods described in U.S. Pub. No. 20090042829 herein incorporated by reference in its entirety. The cationic carrier may include, but is not limited to, polyethylenimine, poly(trimethylenimine), poly(tetramethylenimine), polypropylenimine, aminoglycoside-polyamine, dideoxy-diamino-b-cyclodextrin, spermine, spermidine, poly(2-dimethylamino)ethyl methacrylate, poly(lysine), poly(histidine), poly(arginine), cationized gelatin, dendrimers, chitosan, l,2-Dioleoyl-3-Trimethylammonium-Propane(DOTAP), N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), l-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), 2,3-dioleyloxy-N-

[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA), 3B-[N—(N',N'-Dimethylaminoethane)-carbamoyl]Cholesterol Hydrochloride (DC-Cholesterol HC1) diheptadecylamidoglycyl spermidine (DOGS), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(l,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), N,N-dioleyl-N,N-dimethylammonium chloride DODAC) and combinations thereof.

[00572] The cell phenotype altering polynucleotide, primary construct, and mmRNA of the invention can also be formulated as a nanoparticle using a combination of polymers, lipids, and/or other biodegradable agents, such as, but not limited to, calcium phosphate. Components may be combined in a core-shell, hybrid, and/or layer-by-layer architecture, to allow for fine-tuning of the nanoparticle so to delivery of the cell phenotype altering polynucleotide, primary construct and mmRNA may be enhanced (Wang et al., Nat Mater. 2006 5:791-796; Fuller et al, Biomaterials. 2008 29:1526-1532; DeKoker et al, Adv Drug Deliv Rev. 201 1 63:748-761; Endres et al, Biomaterials. 201 1 32:7721-7731; Su et al, Mol Pharm. 201 1 Jun 6;8(3):774-87; herein incorporated by reference in its entirety).

[00573] Biodegradable calcium phosphate nanoparticles in combination with lipids and/or polymers have been shown to deliver cell phenotype altering polynucleotides, primary constructs and mmRNA *in vivo.* In one embodiment, a lipid coated calcium phosphate nanoparticle, which may also contain a targeting ligand such as anisamide, may be used to deliver the cell phenotype altering polynucleotide, primary construct and mmRNA of the present invention. For example, to effectively deliver siRNA in a mouse metastatic lung model a lipid coated calcium phosphate nanoparticle was used (Li et al, J Contr Rel. 2010 142: 416-421; Li et al, J Contr Rel. 2012 158:108-1 14; Yang et al, Mol Ther. 2012 20:609-615). This delivery system combines both a targeted nanoparticle and a component to enhance the endosomal escape, calcium phosphate, in order to improve delivery of the siRNA.

[00574] In one embodiment, calcium phosphate with a PEG-polyanion block copolymer may be used to delivery cell phenotype altering polynucleotides, primary constructs and mmRNA (Kazikawa et al., J Contr Rel. 2004 97:345-356; Kazikawa et al., J Contr Rel. 2006 111:368-370).

[00575] In one embodiment, a PEG-charge-conversional polymer (Pitella et al., Biomaterials. 201 1 32:3 106-3 114) may be used to form a nanoparticle to deliver the cell phenotype altering polynucleotides, primary constructs and mmRNA of the present invention. The PEG-charge-conversional polymer may improve upon the PEG-polyanion block copolymers by being cleaved into a polycation at acidic pH, thus enhancing endosomal escape.

[00576] The use of core-shell nanoparticles has additionally focused on a high-throughput approach to synthesize cationic cross-linked nanogel cores and various shells (Siegwart et al, Proc Natl Acad Sci U S A. 201 1 108:12996-13001). The complexation, delivery, and internalization of the polymeric nanoparticles can be precisely controlled by altering the chemical composition in both the core and shell components of the nanoparticle. For example, the core-shell nanoparticles may efficiently deliver siRNA to mouse hepatocytes after they covalently attach cholesterol to the nanoparticle.

[00577] In one embodiment, a hollow lipid core comprising a middle PLGA layer and an outer neutral lipid layer containg PEG may be used to delivery of the cell phenotype altering polynucleotide, primary construct and mmRNA of the present invention. As a non-limiting example, in mice bearing a luciferease-expressing tumor, it was determined that the lipid-polymer-lipid hybrid nanoparticle significantly suppressed luciferase expression, as compared to a conventional lipoplex (Shi et al, Angew Chem Int Ed. 201 1 50:7027-7031).

Peptides and Proteins

[00578] The cell phenotype altering polynucleotide, primary construct, and mmRNA of the invention can be formulated with peptides and/or proteins in order to increase transfection of cells by the cell phenotype altering polynucleotide, primary construct, or mmRNA. In one embodiment, peptides such as, but not limited to, cell penetrating peptides and proteins and peptides that enable intracellular delivery may be used to deliver pharmaceutical formulations. A non-limiting example of a cell penetrating peptide which may be used with the pharmaceutical formulations of the present invention includes a cell-penetrating peptide sequence attached to polycations that facilitates delivery to the intracellular space, e.g., HIV-derived TAT peptide, penetratins, transportans, or hCT derived cell-penetrating peptides (see, e.g., Caron et al, Mol. Ther.

3(3):3 10-8 (2001); Langel, Cell-Penetrating Peptides: Processes and Applications (CRC Press, Boca Raton FL, 2002); El-Andaloussi et al, Curr. Pharm. Des. 11(28):3597-61 1 (2003); and Deshayes et al, Cell. Mol. Life Sci. 62(16): 1839-49 (2005), all of which are incorporated herein by reference). The compositions can also be formulated to include a cell penetrating agent, e.g., liposomes, which enhance delivery of the compositions to the intracellular space. Cell phenotype altering polynucleotides, primary constructs, and mmRNA of the invention may be complexed to peptides and/or proteins such as, but not limited to, peptides and/or proteins from Aileron Therapeutics (Cambridge, MA) and Permeon Biologies (Cambridge, MA) in order to enable intracellular delivery (Cronican et al, ACS Chem. Biol. 2010 5:747-752; McNaughton et al, Proc. Natl. Acad. Sci. USA 2009 106:61 11-61 16; Sawyer, Chem Biol Drug Des. 2009 73:3-6; Verdine and Hilinski, Methods Enzymol. 2012;503:3-33; all of which are herein incorporated by reference in its entirety).

[00579] In one embodiment, the cell-penetrating polypeptide may comprise a first domain and a second domain. The first domain may comprise a supercharged polypeptide. The second domain may comprise a protein-binding partner. As used herein, "protein-binding partner" includes, but are not limited to, antibodies and functional fragments thereof, scaffold proteins, or peptides. The cell-penetrating polypeptide may further comprise an intracellular binding partner for the protein-binding partner. The cell-penetrating polypeptide may be capable of being secreted from a cell where the cell phenotype altering polynucleotide, primary construct, or mmRNA may be introduced.

[00580] Formulations of the including peptides or proteins may be used to increase cell transfection by the cell phenotype altering polynucleotide, primary construct, or mmRNA, alter the biodistribution of the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., by targeting specific tissues or cell types), and/or increase the translation of encoded protein.

Cells

[00581] The cell phenotype altering polynucleotide, primary construct, and mmRNA of the invention can be transfected *ex vivo* into cells, which are subsequently transplanted into a subject. As non-limiting examples, the pharmaceutical compositions may include red blood cells to deliver modified cell phenotype altering RNA to liver and myeloid

cells, virosomes to deliver modified RNA in virus-like particles (VLPs), and electroporated cells such as, but not limited to, from MAXCYTE® (Gaithersburg, MD) and from ERYTECH® (Lyon, France) to deliver modified RNA. Examples of use of red blood cells, viral particles and electroporated cells to deliver payloads other than mmRNA have been documented (Godfrin et al., Expert Opin Biol Ther. 2012 12:127-133; Fang et al, Expert Opin Biol Ther. 2012 12:385-389; Hu et al, Proc Natl Acad Sci U S A. 201 1 108:10980-10985; Lund et al, Pharm Res. 2010 27:400-420; Huckriede et al, J Liposome Res. 2007;17:39-47; Cusi, Hum Vaccin. 2006 2:1-7; de Jonge et al, Gene Ther. 2006 13:400-41 1; all of which are herein incorporated by reference in its entirety).

[00582] The cell phenotype altering polynucleotides, primary constructs and mmRNA may be delivered in synthetic VLPs synthesized by the methods described in International Pub No. WO201 1085231 and US Pub No. 201 10171248, each of which is herein incorporated by reference in their entireties.

[00583] Cell-based formulations of the cell phenotype altering polynucleotide, primary construct, and mmRNA of the invention may be used to ensure cell transfection (e.g., in the cellular carrier), alter the biodistribution of the cell phenotype altering polynucleotide, primary construct, or mmRNA (e.g., by targeting the cell carrier to specific tissues or cell types), and/or increase the translation of encoded protein.

[00584] A variety of methods are known in the art and suitable for introduction of nucleic acid into a cell, including viral and non-viral mediated techniques. Examples of typical non-viral mediated techniques include, but are not limited to, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock, magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG) and the like) or cell fusion.

[00585] The technique of sonoporation, or cellular sonication, is the use of sound (e.g., ultrasonic frequencies) for modifying the permeability of the cell plasma membrane. Sonoporation methods are known to those in the art and are used to deliver nucleic acids *in vivo* (Yoon and Park, Expert Opin Drug Deliv. 2010 7:321-330; Postema and Gilja, Curr Pharm Biotechnol. 2007 8:355-361; Newman and Bettinger, Gene Ther. 2007 14:465-475; all herein incorporated by reference in their entirety). Sonoporation methods

are known in the art and are also taught for example as it relates to bacteria in US Patent Publication 20100196983 and as it relates to other cell types in, for example, US Patent Publication 20100009424, each of which are incorporated herein by reference in their entirety.

[00586] Electroporation techniques are also well known in the art and are used to deliver nucleic acids *in vivo* and clinically (Andre et al., Curr Gene Ther. 2010 10:267-280; Chiarella et al, Curr Gene Ther. 2010 10:281-286; Hojman, Curr Gene Ther. 2010 10:128-138; all herein incorporated by reference in their entirety). In one embodiment, cell phenotype altering polynucleotides, primary constructs or mmRNA may be delivered by electroporation as described in Example 26.

Hyaluronidase

[00587] The intramuscular or subcutaneous localized injection of cell phenotype altering polynucleotide, primary construct, or mmRNA of the invention can include hyaluronidase, which catalyzes the hydrolysis of hyaluronan. By catalyzing the hydrolysis of hyaluronan, a constituent of the interstitial barrier, hyaluronidase lowers the viscosity of hyaluronan, thereby increasing tissue permeability (Frost, Expert Opin. Drug Deliv. (2007) 4:427-440; herein incorporated by reference in its entirety). It is useful to speed their dispersion and systemic distribution of encoded proteins produced by transfected cells. Alternatively, the hyaluronidase can be used to increase the number of cells exposed to a cell phenotype altering polynucleotide, primary construct, or mmRNA of the invention administered intramuscularly or subcutaneously.

Nanoparticle Mimics

[00588] The cell phenotype altering polynucleotide, primary construct or mmRNA of the invention may be encapsulated within and/or absorbed to a nanoparticle mimic. A nanoparticle mimic can mimic the delivery function organisms or particles such as, but not limited to, pathogens, viruses, bacteria, fungus, parasites, prions and cells. As a non-limiting example the cell phenotype altering polynucleotide, primary construct or mmRNA of the invention may be encapsulated in a non-viron particle which can mimic the delivery function of a virus (see International Pub. No. WO20 12006376 herein incorporated by reference in its entirety).

Nanotubes

[00589] The cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention can be attached or otherwise bound to at least one nanotube such as, but not limited to, rosette nanotubes, rosette nanotubes having twin bases with a linker, carbon nanotubes and/or single-walled carbon nanotubes, The cell phenotype altering polynucleotides, primary constructs or mmRNA may be bound to the nanotubes through forces such as, but not limited to, steric, ionic, covalent and/or other forces.

[00590] In one embodiment, the nanotube can release one or more cell phenotype altering polynucleotides, primary constructs or mmRNA into cells. The size and/or the surface structure of at least one nanotube may be altered so as to govern the interaction of the nanotubes within the body and/or to attach or bind to the cell phenotype altering polynucleotides, primary constructs or mmRNA disclosed herein. In one embodiment, the building block and/or the functional groups attached to the building block of the at least one nanotube may be altered to adjust the dimensions and/or properties of the nanotube. As a non-limiting example, the length of the nanotubes may be altered to hinder the nanotubes from passing through the holes in the walls of normal blood vessels but still small enough to pass through the larger holes in the blood vessels of tumor tissue.

[00591] In one embodiment, at least one nanotube may also be coated with delivery enhancing compounds including polymers, such as, but not limited to, polyethylene glycol. In another embodiment, at least one nanotube and/or the cell phenotype altering polynucleotides, primary constructs or mmRNA may be mixed with pharmaceutically acceptable excipients and/or delivery vehicles.

[00592] In one embodiment, the cell phenotype altering polynucleotides, primary constructs or mmRNA are attached and/or otherwise bound to at least one rosette nanotube. The rosette nanotubes may be formed by a process known in the art and/or by the process described in International Publication No. WO2012094304, herein incorporated by reference in its entirety. At least one cell phenotype altering polynucleotide, primary construct and/or mmRNA may be attached and/or otherwise bound to at least one rosette nanotube by a process as described in International Publication No. WO2012094304, herein incorporated by reference in its entirety, where rosette nanotubes or modules forming rosette nanotubes are mixed in aqueous media with

at least one cell phenotype altering polynucleotide, primary construct and/or mmRNA under conditions which may cause at least one cell phenotype altering polynucleotide, primary construct or mmRNA to attach or otherwise bind to the rosette nanotubes.

Conjugates

[00593] The cell phenotype altering polynucleotides, primary constructs, and mmRNA of the invention include conjugates, such as a cell phenotype altering polynucleotide, primary construct, or mmRNA covalently linked to a carrier or targeting group, or including two encoding regions that together produce a fusion protein (e.g., bearing a targeting group and therapeutic protein or peptide).

[00594] The conjugates of the invention include a naturally occurring substance, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), high-density lipoprotein (HDL), or globulin); an carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid, an oligonucleotide (e.g. an aptamer). Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

[00595] Representative U.S. patents that teach the preparation of polynucleotide conjugates, particularly to RNA, include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,1 18,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,1 12,963; 5,214,136; 5,082,830; 5,1 12,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098;

5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; each of which is herein incorporated by reference in their entireties.

[00596] In one embodiment, the conjugate of the present invention may function as a carrier for the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention. The conjugate may comprise a cationic polymer such as, but not limited to, polyamine, polylysine, polyalkylenimine, and polyethylenimine which may be grafted to with poly(ethylene glycol). As a non-limiting example, the conjugate may be similar to the polymeric conjugate and the method of synthesizing the polymeric conjugate described in U.S. Pat. No. 6,586,524 herein incorporated by reference in its entirety.

[00597] The conjugates can also include targeting groups, e.g., a cell or tissue targeting agent, e.g., a lectin, glycoprotein, lipid or protein, e.g., an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, an RGD peptide, an RGD peptide mimetic or an aptamer.

[00598] Targeting groups can be proteins, e.g., glycoproteins, or peptides, e.g., molecules having a specific affinity for a co-ligand, or antibodies e.g., an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell. Targeting groups may also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, or aptamers. The ligand can be, for example, a lipopolysaccharide, or an activator of p38 MAP kinase.

[00599] The targeting group can be any ligand that is capable of targeting a specific receptor. Examples include, without limitation, folate, GalNAc, galactose, mannose,

mannose-6P, apatamers, integrin receptor ligands, chemokine receptor ligands, transferrin, biotin, serotonin receptor ligands, PSMA, endothelin, GCPII, somatostatin, LDL, and HDL ligands. In particular embodiments, the targeting group is an aptamer. The aptamer can be unmodified or have any combination of modifications disclosed herein.

[00600] In one embodiment, pharmaceutical compositions of the present invention may include chemical modifications such as, but not limited to, modifications similar to locked nucleic acids.

[00601] Representative U.S. Patents that teach the preparation of locked nucleic acid (LNA) such as those from Santaris, include, but are not limited to, the following: U.S. Pat. Nos. 6,268,490; 6,670,461; 6,794,499; 6,998,484; 7,053,207; 7,084,125; and 7,399,845, each of which is herein incorporated by reference in its entirety.

[00602] Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found, for example, in Nielsen *et al.,* Science, 1991, 254, 1497-1500.

[00603] Some embodiments featured in the invention include cell phenotype altering polynucleotides, primary constructs or mmRNA with phosphorothioate backbones and oligonucleosides with other modified backbones, and in particular ~CH_2~NH~CH_2~, —CH_2—N(CH_3)—0—CH_2—[known as a methylene (methylimino) or MMI backbone], —CH_2--0-N(CH_3)~CH_2- , --CH_2-N(CH_3)~N(CH_3)--CH_2- and -N(CH_3)-CH_2-CH_2- [wherein the native phosphodiester backbone is represented as —O—P(0)$_2$—O—CH_2—] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. In some embodiments, the polynucletotides featured herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

[00604] Modifications at the 2' position may also aid in delivery. Preferably, modifications at the 2' position are not located in a polypeptide-coding sequence, i.e., not in a translatable region. Modifications at the *2'* position may be located in a 5'UTR, a 3'UTR and/or a tailing region. Modifications at the *2'* position can include one of the following at the 2' position: H (i.e., 2'-deoxy); F; 0-, S-, or N-alkyl; 0-, S-, or N-alkenyl;

O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted Ci to Cio alkyl or C2 to C10 alkenyl and alkynyl. Exemplary suitable modifications include 0[(CH 2)nO] mCHs, 0(CH 2).nOCH 3, 0(CH 2)nNH 2, 0(CH 2) nCH 3, 0(CH 2)nONH 2, and 0(CH 2)nON[(CH 2)nCH 3)]2, where n and m are from 1 to about 10. In other embodiments, the cell phenotype altering polynucleotides, primary constructs or mmRNA include one of the following at the 2' position: Ci to Cio lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, CI, Br, CN, CF_3, OCF_3, $SOCH_3$, SO2CFJ3, ONO2, NO2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties, or a group for improving the pharmacodynamic properties, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-0—CH2CH 2OCH_3, also known as 2'-0-(2-methoxyethyl) or 2'-MOE) (Martin *et al, Helv. Chim. Acta,* 1995, 78:486-504) *i.e.,* an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.,* a 0(CH 2)2ON(CFI3)2 group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-0-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.,* 2'-0~CH2~0~CH2~N(CH 2)2, also described in examples herein below. Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. Cell phenotype altering polynucleotides of the invention may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,1 18,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,81 1; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920 and each of which is herein incorporated by reference.

[00605] In still other embodiments, the cell phenotype altering polynucleotide, primary construct, or mmRNA is covalently conjugated to a cell penetrating polypeptide. The

cell-penetrating peptide may also include a signal sequence. The conjugates of the invention can be designed to have increased stability; increased cell transfection; and/or altered the biodistribution (e.g., targeted to specific tissues or cell types).

Self-Assembled Nucleic Acid Nanoparticles

[00606] Self-assembled nanoparticles have a well-defined size which may be precisely controlled as the nucleic acid strands may be easily reprogrammable. For example, the optimal particle size for a cancer-targeting nanodelivery carrier is 20-100 nm as a diameter greater than 20 nm avoids renal clearance and enhances delivery to certain tumors through enhanced permeability and retention effect. Using self-assembled nucleic acid nanoparticles a single uniform population in size and shape having a precisely controlled spatial orientation and density of cancer-targeting ligands for enhanced delivery. As a non-limiting example, oligonucleotide nanoparticles are prepared using programmable self-assembly of short DNA fragments and therapeutic siRNAs. These nanoparticles are molecularly identical with controllable particle size and target ligand location and density. The DNA fragments and siRNAs self-assembled into a one-step reaction to generate DNA/siRNA tetrahedral nanoparticles for targeted *in vivo* delivery. (Lee et al, Nature Nanotechnology 2012 7:389-393).

Excipients

[00607] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's *The Science and Practice of Pharmacy*, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

[00608] In some embodiments, a pharmaceutically acceptable excipient is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use in humans and for veterinary use. In some embodiments, an excipient is approved by United States Food and Drug Administration. In some embodiments, an excipient is pharmaceutical grade. In some embodiments, an excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[00609] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in pharmaceutical compositions.

[00610] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, *etc.*, and/or combinations thereof.

[00611] Exemplary granulating and/or dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (VEEGUM®), sodium lauryl sulfate, quaternary ammonium compounds, *etc.*, and/or combinations thereof.

[00612] Exemplary surface active agents and/or emulsifiers include, but are not limited to, natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and VEEGUM®

[magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g. stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (e.g. carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate [TWEEN®20], polyoxy ethylene sorbitan [TWEENn®60], polyoxyethylene sorbitan monooleate [TWEEN®80], sorbitan monopalmitate [SPAN®40], sorbitan monostearate [Span®60], sorbitan tristearate [Span®65], glyceryl monooleate, sorbitan monooleate [SPAN®80]), polyoxyethylene esters (e.g. polyoxyethylene monostearate [MYRJ®45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and SOLUTOL®), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. CREMOPHOR®), polyoxyethylene ethers, (e.g. polyoxyethylene lauryl ether [BRIJ®30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, PLUORINC®F 68, POLOXAMER®188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof.

[00613] Exemplary binding agents include, but are not limited to, starch *(e.g.* cornstarch and starch paste); gelatin; sugars *(e.g.* sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol,); natural and synthetic gums *(e.g.* acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (Veegum®), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; *etc.;* and combinations thereof.

[00614] Exemplary preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol

preservatives, acidic preservatives, and/or other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and/or trisodium edetate. Exemplary antimicrobial preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and/or thimerosal. Exemplary antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Exemplary alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Exemplary acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluened (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, GLYDANT PLUS®, PHENONIP®, methylparaben, GERMALL®115, GERMABEN®II, NEOLONE™, KATHON™, and/or EUXYL®.

[00615] Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate,

phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, *etc.*, and/or combinations thereof.

[00616] Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behanate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, *etc.*, and combinations thereof.

[00617] Exemplary oils include, but are not limited to, almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and/or combinations thereof.

[00618] Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

Delivery

[00619] The present disclosure encompasses the delivery of cell phenotype altering polynucleotides, primary constructs or mmRNA for any of therapeutic, pharmaceutical,

diagnostic or imaging by any appropriate route taking into consideration likely advances in the sciences of drug delivery. Delivery may be naked or formulated.

Naked Delivery

[00620] The cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention may be delivered to a cell naked. As used herein in, "naked" refers to delivering cell phenotype altering polynucleotides, primary constructs or mmRNA free from agents which promote transfection. For example, the cell phenotype altering polynucleotides, primary constructs or mmRNA delivered to the cell may contain no modifications. The naked cell phenotype altering polynucleotides, primary constructs or mmRNA may be delivered to the cell using routes of administration known in the art and described herein.

Formulated Delivery

[00621] The cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention may be formulated, using the methods described herein. The formulations may contain cell phenotype altering polynucleotides, primary constructs or mmRNA which may be modified and/or unmodified. The formulations may further include, but are not limited to, cell penetration agents, a pharmaceutically acceptable carrier, a delivery agent, a bioerodible or biocompatible polymer, a solvent, and a sustained-release delivery depot. The formulated cell phenotype altering polynucleotides, primary constructs or mmRNA may be delivered to the cell using routes of administration known in the art and described herein.

[00622] The compositions may also be formulated for direct delivery to an organ or tissue in any of several ways in the art including, but not limited to, direct soaking or bathing, via a catheter, by gels, powder, ointments, creams, gels, lotions, and/or drops, by using substrates such as fabric or biodegradable materials coated or impregnated with the compositions, and the like.

Administration

[00623] The cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited to enteral, gastroenteral, epidural, oral, transdermal, epidural (peridural), intracerebral (into the cerebrum),

intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection, (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), insufflation (snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), or in ear drops. In specific embodiments, compositions may be administered in a way which allows them cross the blood-brain barrier, vascular barrier, or other epithelial barrier.Non-limiting routes of administration for the cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention are described below.

Parenteral and Injectible Administration

[00624] Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents. In certain embodiments for parenteral administration, compositions are mixed with solubilizing agents such as CREMOPHOR®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

[00625] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing

agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00626] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00627] In order to prolong the effect of an active ingredient, it is often desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Rectal and VaginalAdministration

[00628] Compositions for rectal or vaginal administration are typically suppositories which can be prepared by mixing compositions with suitable non-irritating excipients such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active ingredient.

Oral Administration

[00629] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, an active ingredient is mixed with at least one inert, pharmaceutically acceptable excipient such as sodium citrate or dicalcium phosphate and/or fillers or extenders *{e.g.* starches, lactose, sucrose, glucose, mannitol, and silicic acid), binders *{e.g.* carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia), humectants *{e.g.* glycerol), disintegrating agents *{e.g.* agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate), solution retarding agents *{e.g.* paraffin), absorption accelerators *{e.g.* quaternary ammonium compounds), wetting agents *{e.g.* cetyl alcohol and glycerol monostearate), absorbents *{e.g.* kaolin and bentonite clay), and lubricants *{e.g.* talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

Topical or Transdermal Administration

[00630] As described herein, compositions containing the cell phenotype altering polynucleotides, primary constructs or mmRNA of the invention may be formulated for administration topically. The skin may be an ideal target site for delivery as it is readily accessible. Gene expression may be restricted not only to the skin, potentially avoiding nonspecific toxicity, but also to specific layers and cell types within the skin.

[00631] The site of cutaneous expression of the delivered compositions will depend on the route of nucleic acid delivery. Three routes are commonly considered to deliver cell phenotype altering polynucleotides, primary constructs or mmRNA to the skin: (i) topical application (e.g. for local/regional treatment and/or cosmetic applications); (ii) intradermal injection (e.g. for local/regional treatment and/or cosmetic applications); and (iii) systemic delivery (e.g. for treatment of dermatologic diseases that affect both cutaneous and extracutaneous regions). Cell phenotype altering polynucleotides, primary constructs or mmRNA can be delivered to the skin by several different approaches known in the art. Most topical delivery approaches have been shown to work for delivery of DNA, such as but not limited to, topical application of non-cationic liposome-DNA complex, cationic liposome-DNA complex, particle-mediated (gene

gun), puncture-mediated gene transfections, and viral delivery approaches. After delivery of the nucleic acid, gene products have been detected in a number of different skin cell types, including, but not limited to, basal keratinocytes, sebaceous gland cells, dermal fibroblasts and dermal macrophages.

[00632] In one embodiment, the invention provides for a variety of dressings (e.g., wound dressings) or bandages (e.g., adhesive bandages) for conveniently and/or effectively carrying out methods of the present invention. Typically dressing or bandages may comprise sufficient amounts of pharmaceutical compositions and/or cell phenotype altering polynucleotides, primary constructs or mmRNA described herein to allow a user to perform multiple treatments of a subject(s).

[00633] In one embodiment, the invention provides for the cell phenotype altering polynucleotides, primary constructs or mmRNA compositions to be delivered in more than one injection.

[00634] In one embodiment, before topical and/or transdermal administration at least one area of tissue, such as skin, may be subjected to a device and/or solution which may increase permeability. In one embodiment, the tissue may be subjected to an abrasion device to increase the permeability of the skin (see U.S. Patent Publication No. 20080275468, herein incorporated by reference in its entirety). In another embodiment, the tissue may be subjected to an ultrasound enhancement device. An ultrasound enhancement device may include, but is not limited to, the devices described in U.S. Publication No. 20040236268 and U.S. Patent Nos. 6,491,657 and 6,234,990; each of which is herein incorporated by reference in their entireties. Methods of enhancing the permeability of tissue are described in U.S. Publication Nos. 20040171980 and 20040236268 and U.S. Pat. No. 6,190,315; each of whish are herein incorporated by reference in their entireties.

[00635] In one embodiment, a device may be used to increase permeability of tissue before delivering formulations of the cell phenotype altering polynucleotides, primary constructs and mmRNA described herein. The permeability of skin may be measured by methods known in the art and/or described in U.S. Patent No. 6,190,315, herein incorporated by reference in its entirety. As a non-limiting example, a modified cell

phenotype altering mRNA formulation may be delivered by the drug delivery methods described in U.S. Patent No. 6,190,315, herein incorporated by reference in its entirety.

[00636] In another non-limiting example tissue may be treated with a eutectic mixture of local anesthetics (EMLA) cream before, during and/or after the tissue may be subjected to a device which may increase permeability. Katz et al. (Anesth Analg (2004); 98:371-76; herein incorporated by reference in its entirety) showed that using the EMLA cream in combination with a low energy, an onset of superficial cutaneous analgesia was seen as fast as 5 minutes after a pretreatment with a low energy ultrasound.

[00637] In one embodiment, enhancers may be applied to the tissue before, during, and/or after the tissue has been treated to increase permeability. Enhancers include, but are not limited to, transport enhancers, physical enhancers, and cavitation enhancers. Non-limiting examples of enhancers are described in U.S. Patent No. 6,190,315, herein incorporated by reference in its entirety.

[00638] In one embodiment, a device may be used to increase permeability of tissue before delivering formulations of cell phenotype altering polynucleotides, primary constructs and/or mmRNA described herein, which may further contain a substance that invokes an immune response. In another non-limiting example, a formulation containing a substance to invoke an immune response may be delivered by the methods described in U.S. Publication Nos. 20040171980 and 20040236268; each of which is herein incorporated by reference in their entirety.

[00639] Dosage forms for topical and/or transdermal administration of a composition may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants and/or patches. Generally, an active ingredient is admixed under sterile conditions with a pharmaceutically acceptable excipient and/or any needed preservatives and/or buffers as may be required.

[00640] Additionally, the present invention contemplates the use of transdermal patches, which often have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms may be prepared, for example, by dissolving and/or dispensing the compound in the proper medium. Alternatively or additionally, rate may be controlled by either providing a rate controlling membrane and/or by dispersing the compound in a polymer matrix and/or gel.

[00641] Formulations suitable for topical administration include, but are not limited to, liquid and/or semi liquid preparations such as liniments, lotions, oil in water and/or water in oil emulsions such as creams, ointments and/or pastes, and/or solutions and/or suspensions. Topically-administrable formulations may, for example, comprise from about 0.1% to about 10% (w/w) active ingredient, although the concentration of active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

Depot Administration

[00642] As described herein, in some embodiments, the composition is formulated in depots for extended release. Generally, a specific organ or tissue (a "target tissue") is targeted for administration.

[00643] In some aspects of the invention, the cell phenotype altering polynucleotides, primary constructs or mmRNA are spatially retained within or proximal to a target tissue. Provided are method of providing a composition to a target tissue of a mammalian subject by contacting the target tissue (which contains one or more target cells) with the composition under conditions such that the composition, in particular the nucleic acid component(s) of the composition, is substantially retained in the target tissue, meaning that at least 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the composition is retained in the target tissue. Advantageously, retention is determined by measuring the amount of the nucleic acid present in the composition that enters one or more target cells. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the nucleic acids administered to the subject are present intracellularly at a period of time following administration. For example, intramuscular injection to a mammalian subject is performed using an aqueous composition containing a ribonucleic acid and a transfection reagent, and retention of the composition is determined by measuring the amount of the ribonucleic acid present in the muscle cells.

[00644] Aspects of the invention are directed to methods of providing a composition to a target tissue of a mammalian subject, by contacting the target tissue (containing one or more target cells) with the composition under conditions such that the composition is

substantially retained in the target tissue. The composition contains an effective amount of a cell phenotype altering polynucleotide, primary construct or mmRNA such that the cell phenotype altering polypeptide of interest is produced in at least one target cell. The compositions generally contain a cell penetration agent, although "naked" nucleic acid (such as nucleic acids without a cell penetration agent or other agent) is also contemplated, and a pharmaceutically acceptable carrier.

[00645] In some circumstances, the amount of a protein produced by cells in a tissue is desirably increased. Preferably, this increase in protein production is spatially restricted to cells within the target tissue. Thus, provided are methods of increasing production of a protein of interest in a tissue of a mammalian subject. A composition is provided that contains cell phenotype altering polynucleotides, primary constructs or mmRNA characterized in that a unit quantity of composition has been determined to produce the cell phenotype altering polypeptide of interest in a substantial percentage of cells contained within a predetermined volume of the target tissue.

[00646] In some embodiments, the composition includes a plurality of different cell phenotype altering polynucleotides, primary constructs or mmRNA, where one or more than one of the cell phenotype altering polynucleotides, primary constructs or mmRNA encodes a polypeptide of interest. Optionally, the composition also contains a cell penetration agent to assist in the intracellular delivery of the composition. A determination is made of the dose of the composition required to produce the polypeptide of interest in a substantial percentage of cells contained within the predetermined volume of the target tissue (generally, without inducing significant production of the cell phenotype altering polypeptide of interest in tissue adjacent to the predetermined volume, or distally to the target tissue). Subsequent to this determination, the determined dose is introduced directly into the tissue of the mammalian subject.

[00647] In one embodiment, the invention provides for the cell phenotype altering polynucleotides, primary constructs or mmRNA to be delivered in more than one injection or by split dose injections.

[00648] In one embodiment, the invention may be retained near target tissue using a small disposable drug reservoir or patch pump. Non-limiting examples of patch pumps include those manufactured and/or sold by BD® (Franklin Lakes, NJ), Insulet

Corporation (Bedford, MA), SteadyMed Therapeutics (San Francisco, CA), Medtronic (Minneapolis, MN), UniLife (York, PA), Valeritas (Bridgewater, NJ), and SpringLeaf Therapeutics (Boston, MA).

Pulmonary Administration

[00649] A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 nm to about 7 nm or from about 1 nm to about 6 nm. Such compositions are suitably in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder and/or using a self propelling solvent/powder dispensing container such as a device comprising the active ingredient dissolved and/or suspended in a low-boiling propellant in a sealed container. Such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nm and at least 95% of the particles by number have a diameter less than 7 nm. Alternatively, at least 95% of the particles by weight have a diameter greater than 1 nm and at least 90% of the particles by number have a diameter less than 6 nm. Dry powder compositions may include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

[00650] Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally the propellant may constitute 50% to 99.9% (w/w) of the composition, and active ingredient may constitute 0.1% to 20% (w/w) of the composition. A propellant may further comprise additional ingredients such as a liquid non-ionic and/or solid anionic surfactant and/or a solid diluent (which may have a particle size of the same order as particles comprising the active ingredient).

[00651] Pharmaceutical compositions formulated for pulmonary delivery may provide an active ingredient in the form of droplets of a solution and/or suspension. Such formulations may be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic solutions and/or suspensions, optionally sterile, comprising active ingredient, and may conveniently be administered using any nebulization and/or atomization device. Such formulations may further comprise one or more additional ingredients including, but not

limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, and/or a preservative such as methylhydroxybenzoate. Droplets provided by this route of administration may have an average diameter in the range from about 0.1 nm to about 200 nm.

Intranasal, nasal and buccal Administration

[00652] Formulations described herein as being useful for pulmonary delivery are useful for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 μιη to 500 μιη. Such a formulation is administered in the manner in which snuff is taken, *i.e.* by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00653] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, 0.1% to 20% (w/w) active ingredient, the balance comprising an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

Ophthalmic Administration

[00654] A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1/1.0% (w/w) solution and/or suspension of the active ingredient in an aqueous or oily liquid excipient. Such drops

may further comprise buffering agents, salts, and/or one or more other of any additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form and/or in a liposomal preparation. Ear drops and/or eye drops are contemplated as being within the scope of this invention.

Payload Administration: Detectable Agents and Therapeutic Agents

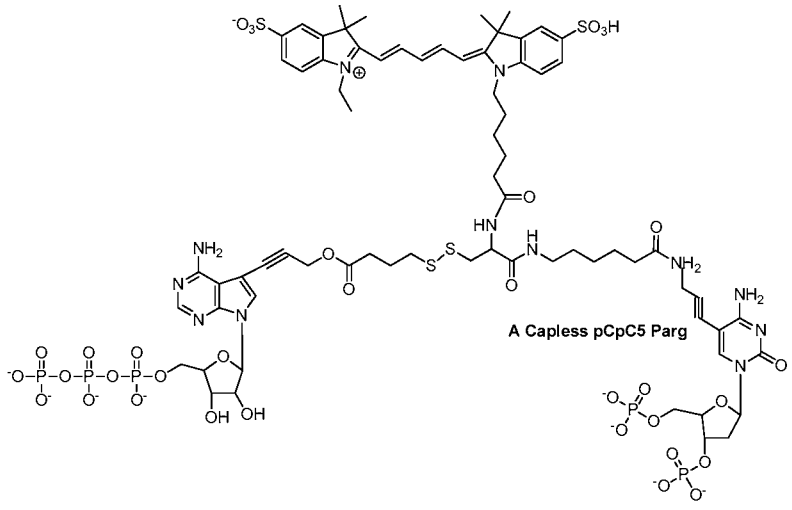
[00655] The cell phenotype altering polynucleotides, primary constructs or mmRNA described herein can be used in a number of different scenarios in which delivery of a substance (the "payload") to a biological target is desired, for example delivery of detectable substances for detection of the target, or delivery of a therapeutic agent. Detection methods can include, but are not limited to, both imaging *in vitro* and *in vivo* imaging methods, *e.g.*, immunohistochemistry, bioluminescence imaging (BLI), Magnetic Resonance Imaging (MRI), positron emission tomography (PET), electron microscopy, X-ray computed tomography, Raman imaging, optical coherence tomography, absorption imaging, thermal imaging, fluorescence reflectance imaging, fluorescence microscopy, fluorescence molecular tomographic imaging, nuclear magnetic resonance imaging, X-ray imaging, ultrasound imaging, photoacoustic imaging, lab assays, or in any situation where tagging/staining/imaging is required.

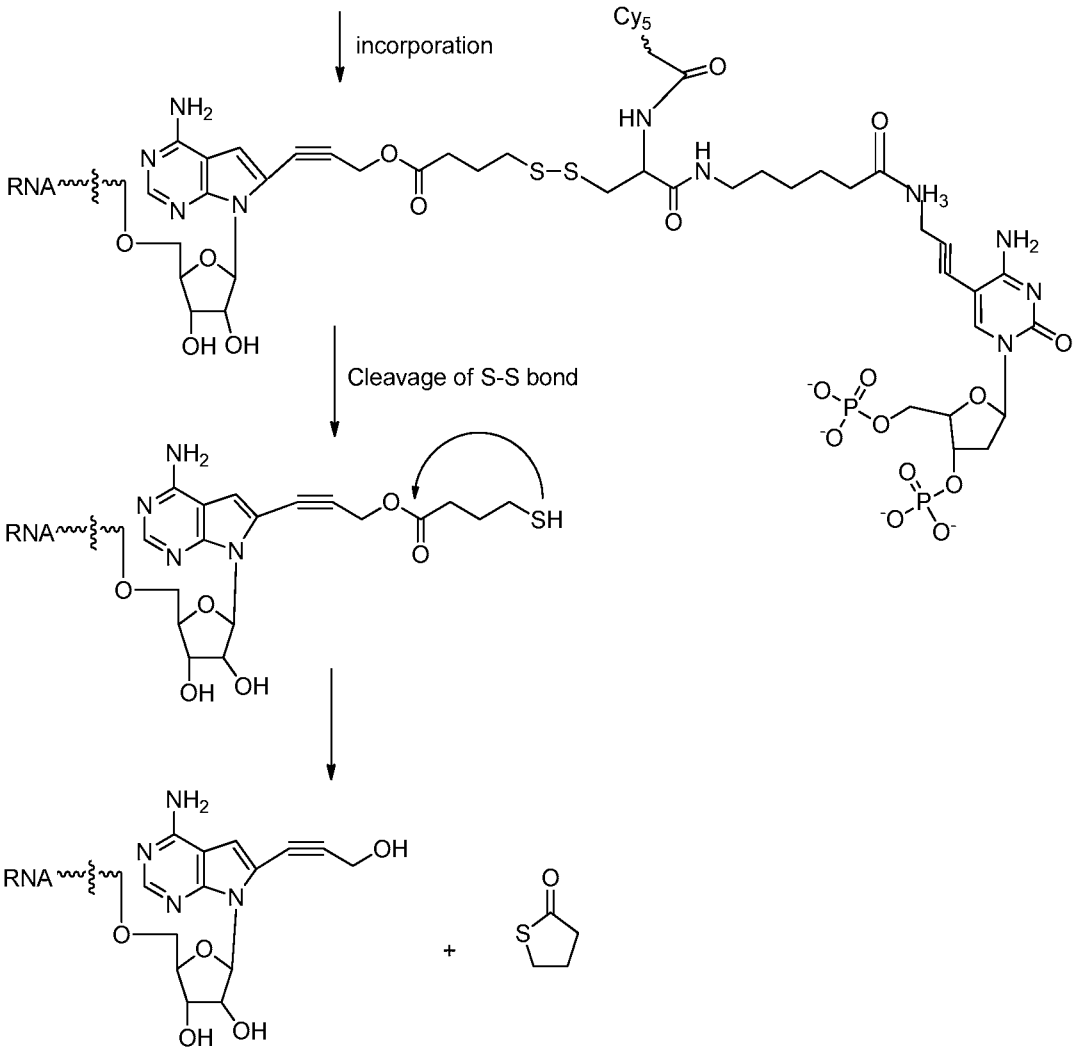
[00656] The cell phenotype altering polynucleotides, primary constructs or mmRNA can be designed to include both a linker and a payload in any useful orientation. For example, a linker having two ends is used to attach one end to the payload and the other end to the nucleobase, such as at the C-7 or C-8 positions of the deaza-adenosine or deaza-guanosine or to the N-3 or C-5 positions of cytosine or uracil. The cell phenotype altering polynucleotide of the invention can include more than one payload (e.g., a label and a transcription inhibitor), as well as a cleavable linker. In one embodiment, the modified nucleotide is a modified 7-deaza-adenosine triphosphate, where one end of a cleavable linker is attached to the C7 position of 7-deaza-adenine, the other end of the linker is attached to an inhibitor (e.g., to the C5 position of the nucleobase on a cytidine), and a label (e.g., Cy5) is attached to the center of the linker (see, e.g., compound 1 of A*pCp C5 Parg Capless in Fig. 5 and columns 9 and 10 of U.S. Pat. No. 7,994,304, incorporated herein by reference). Upon incorporation of the modified 7-deaza-

adenosine triphosphate to an encoding region, the resulting cell phenotype altering polynucleotide will have a cleavable linker attached to a label and an inhibitor (e.g., a polymerase inhibitor). Upon cleavage of the linker (e.g., with reductive conditions to reduce a linker having a cleavable disulfide moiety), the label and inhibitor are released. Additional linkers and payloads (e.g., therapeutic agents, detectable labels, and cell penetrating payloads) are described herein.

[00657] Scheme 12 below depicts an exemplary modified nucleotide wherein the nucleobase, adenine, is attached to a linker at the C-7 carbon of 7-deaza adenine. In addition, Scheme 12 depicts the modified nucleotide with the linker and payload, e.g., a detectable agent, incorporated onto the 3' end of the mRNA. Disulfide cleavage and 1,2-addition of the thiol group onto the propargyl ester releases the detectable agent. The remaining structure (depicted, for example, as pApC5Parg in Scheme 12) is the inhibitor. The rationale for the structure of the modified nucleotides is that the tethered inhibitor sterically interferes with the ability of the polymerase to incorporate a second base. Thus, it is critical that the tether be long enough to affect this function and that the inhibiter be in a stereochemical orientation that inhibits or prohibits second and follow on nucleotides into the growing polynucleotide strand.

Scheme 12





[00658] For example, the cell phenotype altering polynucleotides, primary constructs or mmRNA described herein can be used in cell phenotype altering induced pluripotent stem cells (iPS cells), which can directly track cells that are transfected compared to total cells in the cluster. In another example, a drug that may be attached to the cell phenotype altering polynucleotides, primary constructs or mmRNA via a linker and may be fluorescently labeled can be used to track the drug *in vivo, e.g.* intracellularly. Other examples include, but are not limited to, the use of a cell phenotype altering polynucleotide, primary construct or mmRNA in reversible drug delivery into cells.

[00659] The cell phenotype altering polynucleotides, primary constructs or mmRNA described herein can be used in intracellular targeting of a payload, *e.g.*, detectable or therapeutic agent, to specific organelle. Exemplary intracellular targets can include, but

are not limited to, the nuclear localization for advanced mRNA processing, or a nuclear localization sequence (NLS) linked to the mRNA containing an inhibitor.

[00660] In addition, the cell phenotype altering polynucleotides, primary constructs or mmRNA described herein can be used to deliver therapeutic agents to cells or tissues, *e.g.*, in living animals. For example, the cell phenotype altering polynucleotides, primary constructs or mmRNA attached to the therapeutic agent through a linker can facilitate member permeation allowing the therapeutic agent to travel into a cell to reach an intracellular target.

[00661] In some embodiments, the payload may be a therapeutic agent such as a cytotoxin, radioactive ion, chemotherapeutic, or other therapeutic agent. A cytotoxin or cytotoxic agent includes any agent that may be detrimental to cells. Examples include, but are not limited to, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxyanthracmedione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, *e.g.*, maytansinol (see U.S. Pat. No. 5,208,020 incorporated herein in its entirety), rachelmycin (CC-1065, see U.S. Pat. Nos. 5,475,092, 5,585,499, and 5,846,545, all of which are incorporated herein by reference), and analogs or homologs thereof. Radioactive ions include, but are not limited to iodine *(e.g.*, iodine 125 or iodine 131), strontium 89, phosphorous, palladium, cesium, iridium, phosphate, cobalt, yttrium 90, samarium 153, and praseodymium. Other therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thiotepa chlorambucil, rachelmycin (CC-1065), melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine, vinblastine, taxol and maytansinoids).

[00662] In some embodiments, the payload may be a detectable agent, such as various organic small molecules, inorganic compounds, nanoparticles, enzymes or enzyme

substrates, fluorescent materials, luminescent materials (e.g., luminol), bioluminescent materials (e.g., luciferase, luciferin, and aequorin), chemiluminescent materials, radioactive materials (e.g., ^{18}F, ^{67}Ga, ^{81m}Kr, ^{82}Rb, ^{111}In, ^{123}I, ^{133}Xe, $^{201}T1$, $^{125}1$, ^{35}S, ^{14}C, ^{3}H, or ^{99m}Tc (e.g., as pertechnetate (technetate(VII), $TCC\prime_4^-$)), and contrast agents (e.g., gold *(e.g.,* gold nanoparticles), gadolinium (e.g., chelated Gd), iron oxides (e.g., superparamagnetic iron oxide (SPIO), monocrystalline iron oxide nanoparticles (MIONs), and ultrasmall superparamagnetic iron oxide (USPIO)), manganese chelates (e.g., Mn-DPDP), barium sulfate, iodinated contrast media (iohexol), microbubbles, or perfluorocarbons). Such optically-detectable labels include for example, without limitation, 4-acetamido-4'-isothiocyanatostilbene-2,2'disulfonic acid; acridine and derivatives (e.g., acridine and acridine isothiocyanate); 5-(2'-aminoethyl)aminonaphthalene-l -sulfonic acid (EDANS); 4-amino-N-[3-vinylsulfonyl)phenyl]naphthalimide-3 ,5 disulfonate; N-(4-anilino-l-naphthyl)maleimide; anthranilamide; BODIPY; Brilliant Yellow; coumarin and derivatives (e.g., coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), and 7-amino-4-trifluoromethylcoumarin (Coumarin 151)); cyanine dyes; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5' 5''-dibromopyrogallol-sulfonaphthalein (Bromopyrogallol Red); 7-diethylamino-3 -(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; 5-[dimethylamino]-naphthalene-l-sulfonyl chloride (DNS, dansylchloride); 4-dimethylaminophenylazophenyl-4' -isothiocyanate (DABITC); eosin and derivatives (e.g., eosin and eosin isothiocyanate); erythrosin and derivatives (e.g., erythrosin B and erythrosin isothiocyanate); ethidium; fluorescein and derivatives (e.g., 5-carboxyfiuorescein (FAM), 5-(4,6-dichlorotriazin-2-yl)amino fluorescein (DTAF), 2',7'-dimethoxy-4 '5'-dichloro-6-carboxyfiuorescein, fluorescein, fluorescein isothiocyanate, X-rhodamine-5-(and-6)-isothiocyanate (QFITC or XRITC), and fluorescamine); 2-[2-[3-[[l,3-dihydro-l,l-dimethyl-3-(3-sulfopropyl)-2H-benz[e]indol-2-ylidene]ethylidene]-2-[4-(ethoxycarbonyl)-l-piperazinyl]-l-cyclopenten-1 -yljethenyl]- 1,1-dimethyl-3-(3-sulforpropyl)- lH-benz[e]indolium hydroxide, inner salt, compound with n,n-diethylethanamine(l:l) (IR144); 5-chloro-2-[2-[3-[(5-chloro-3-ethyl-2(3H)-benzothiazol- ylidene)ethylidene]-2-(diphenylamino)-l-

cyclopenten-l-yl]ethenyl]-3-ethyl benzothiazolium perchlorate (IR140); Malachite Green isothiocyanate; 4-methylumbelliferone orthocresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthaldialdehyde; pyrene and derivatives(e.g., pyrene, pyrene butyrate, and succinimidyl 1-pyrene); butyrate quantum dots; Reactive Red 4 (CIBACRON™ Brilliant Red 3B-A); rhodamine and derivatives (e.g., 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red), N,N,N ',N 'tetramethyl-6-carboxyrhodamine (TAMRA) tetramethyl rhodamine, and tetramethyl rhodamine isothiocyanate (TRITC)); riboflavin; rosolic acid; terbium chelate derivatives; Cyanine-3 (Cy3); Cyanine-5 (Cy5); cyanine-5.5 (Cy5.5), Cyanine-7 (Cy7); IRD 700; IRD 800; Alexa 647; La Jolta Blue; phthalo cyanine; and naphthalo cyanine.

[00663] In some embodiments, the detectable agent may be a non-detectable pre-cursor that becomes detectable upon activation (e.g., fluorogenic tetrazine-fluorophore constructs (e.g., tetrazine-BODIPY FL, tetrazine-Oregon Green 488, or tetrazine-BODIPY TMR-X) or enzyme activatable fluorogenic agents (e.g., PROSENSE® (VisEn Medical))). *In vitro* assays in which the enzyme labeled compositions can be used include, but are not limited to, enzyme linked immunosorbent assays (ELISAs), immunoprecipitation assays, immunofluorescence, enzyme immunoassays (EIA), radioimmunoassays (RIA), and Western blot analysis.

Combinations

[00664] The cell phenotype altering polynucleotides, primary constructs or mmRNA may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. By "in combination with," it is not intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of pharmaceutical, prophylactic, diagnostic,

or imaging compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body. As a non-limiting example, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may be used in combination with a pharmaceutical agent for the treatment of cancer or to control hyperproliferative cells. In U.S. Pat. No. 7,964,571, herein incorporated by reference in its entirety, a combination therapy for the treatment of solid primary or metastasized tumor is described using a pharmaceutical composition including a DNA plasmid encoding for interleukin-12 with a lipopolymer and also administering at least one anticancer agent or chemotherapeutic. Further, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention that encodes anti-proliferative molecules may be in a pharmaceutical composition with a lipopolymer (see e.g., U.S. Pub. No. 201 10218231, herein incorporated by reference in its entirety, claiming a pharmaceutical composition comprising a DNA plasmid encoding an anti-proliferative molecule and a lipopolymer) which may be administered with at least one chemotherapeutic or anticancer agent.

Dosing

[00665] The present invention provides methods comprising administering cell phenotype altering polynucleotides, primary constructs and/or mmRNA and their encoded cell phenotype altering proteins or complexes in accordance with the invention to a subject in need thereof. Cell phenotype altering nucleic acids, cell phenotype altering proteins or complexes, or pharmaceutical, imaging, diagnostic, or prophylactic compositions thereof, may be administered to a subject using any amount and any route of administration effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition *(e.g., a* disease, disorder, and/or condition relating to working memory deficits). The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. Compositions in accordance with the invention are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present

invention may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound 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 rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[00666] In certain embodiments, compositions in accordance with the present invention may be administered at dosage levels sufficient to deliver from about 0.0001 mg/kg to about 100 mg/kg, from about 0.001 mg/kg to about 0.05 mg/kg, from about 0.005 mg/kg to about 0.05 mg/kg, from about 0.001 mg/kg to about 0.005 mg/kg, from about 0.05 mg/kg to about 0.5 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect. The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage may be delivered using multiple administrations *(e.g.,* two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations).

[00667] According to the present invention, it has been discovered that administration of cell phenotype altering mmRNA in split-dose regimens produce higher levels of cell phenotype altering proteins in mammalian subjects. As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses, e.g, two or more administrations of the single unit dose. As used herein, a "single unit dose" is a dose of any therapeutic administed in one dose/at one time/single route/single point of contact, i.e., single administration event. As used herein, a "total daily dose" is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose. In one

embodiment, the mmRNA of the present invention are administed to a subject in split doses. The mmRNA may be formulated in buffer only or in a formulation described herein.

Dosage Forms

[00668] A pharmaceutical composition described herein can be formulated into a dosage form described herein, such as a topical, intranasal, intratracheal, or injectable (e.g., intravenous, intraocular, intravitreal, intramuscular, intracardiac, intraperitoneal, subcutaneous).

Liquid dosageforms

[00669] Liquid dosage forms for parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art including, but not limited to, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. In certain embodiments for parenteral administration, compositions may be mixed with solubilizing agents such as CREMOPHOR®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

Injectable

[00670] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art and may include suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed include, but are not limited to, are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono-

or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00671] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00672] In order to prolong the effect of an active ingredient, it may be desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the cell phenotype altering polynucleotide, primary construct or mmRNA then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered cell phenotype altering polynucleotide, primary construct or mmRNA may be accomplished by dissolving or suspending the cell phenotype altering polynucleotide, primary construct or mmRNA in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the cell phenotype altering polynucleotide, primary construct or mmRNA in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of cell phenotype altering polynucleotide, primary construct or mmRNA to polymer and the nature of the particular polymer employed, the rate of cell phenotype altering polynucleotide, primary construct or mmRNA release can be controlled. Examples of other biodegradable polymers include, but are not limited to, poly(orthoesters) and poly(anhydrides). Depot injectable formulations may be prepared by entrapping the cell phenotype altering polynucleotide, primary construct or mmRNA in liposomes or microemulsions which are compatible with body tissues.

Pulmonary

[00673] Formulations described herein as being useful for pulmonary delivery may also be use for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration may be a coarse powder comprising the active ingredient and having an average particle from about 0.2 μιη to 500 μιη. Such a

formulation may be administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00674] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, contain about 0.1% to 20% (w/w) active ingredient, where the balance may comprise an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

[00675] General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

Coatings or Shells

[00676] Solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

Properties of Pharmaceutical Compositions

[00677] The pharmaceutical compositions described herein can be characterized by one or more of bioavailability, therapeutic window and/or volume of distribution.

Bioavailability

[00678] The cell phenotype altering polynucleotides, primary constructs or mmRNA, when formulated into a composition with a delivery agent as described herein, can exhibit an increase in bioavailability as compared to a composition lacking a delivery agent as described herein. As used herein, the term "bioavailability" refers to the systemic availability of a given amount of cell phenotype altering polynucleotides, primary constructs or mmRNA administered to a mammal. Bioavailability can be assessed by measuring the area under the curve (AUC) or the maximum serum or plasma concentration **(Cmax)** of the unchanged form of a compound following administration of the compound to a mammal. AUC is a determination of the area under the curve plotting the serum or plasma concentration of a compound along the ordinate (Y-axis) against time along the abscissa (X-axis). Generally, the AUC for a particular compound can be calculated using methods known to those of ordinary skill in the art and as described in G. S. Banker, Modern Pharmaceutics, Drugs and the Pharmaceutical Sciences, v. 72, Marcel Dekker, New York, Inc., 1996, herein incorporated by reference.

[00679] The **Cmax** value is the maximum concentration of the compound achieved in the serum or plasma of a mammal following administration of the compound to the mammal. The **Cmax** value of a particular compound can be measured using methods known to those of ordinary skill in the art. The phrases "increasing bioavailability" or "improving the pharmacokinetics," as used herein mean that the systemic availability of a first cell phenotype altering polynucleotide, primary construct or mmRNA, measured as AUC, **C̃max,** or **C̃min** in a mammal is greater, when co-administered with a delivery agent as described herein, than when such co-administration does not take place. In some embodiments, the bioavailability of the cell phenotype altering polynucleotide, primary construct or mmRNA can increase by at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%>, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%>, at least about 65%, at least about 70%>, at least about 75%, at

least about 80%, at least about 85%, at least about 90%>, at least about 95%, or about 100%.

Therapeutic Window

[00680] The cell phenotype altering polynucleotides, primary constructs or mmRNA, when formulated into a composition with a delivery agent as described herein, can exhibit an increase in the therapeutic window of the administered cell phenotype altering polynucleotide, primary construct or mmRNA composition as compared to the therapeutic window of the administered cell phenotype altering polynucleotide, primary construct or mmRNA composition lacking a delivery agent as described herein. As used herein "therapeutic window" refers to the range of plasma concentrations, or the range of levels of therapeutically active substance at the site of action, with a high probability of eliciting a therapeutic effect. In some embodiments, the therapeutic window of the cell phenotype altering polynucleotide, primary construct or mmRNA when co-administered with a delivery agent as described herein can increase by at least about 2%, at least about 5%, at least about 10%>, at least about 15%, at least about 20%>, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%.

Volume of Distribution

[00681] The cell phenotype altering polynucleotides, primary constructs or mmRNA, when formulated into a composition with a delivery agent as described herein, can exhibit an improved volume of distribution (Vdist), e.g., reduced or targeted, relative to a composition lacking a delivery agent as described herein. The volume of distribution (Vdist) relates the amount of the drug in the body to the concentration of the drug in the blood or plasma. As used herein, the term "volume of distribution" refers to the fluid volume that would be required to contain the total amount of the drug in the body at the same concentration as in the blood or plasma: Vdist equals the amount of drug in the body/concentration of drug in blood or plasma. For example, for a 10 mg dose and a plasma concentration of 10 mg/L, the volume of distribution would be 1 liter. The volume of distribution reflects the extent to which the drug is present in the extravascular

tissue. A large volume of distribution reflects the tendency of a compound to bind to the tissue components compared with plasma protein binding. In a clinical setting, **Vdist** can be used to determine a loading dose to achieve a steady state concentration. In some embodiments, the volume of distribution of the cell phenotype altering polynucleotide, primary construct or mmRNA when co-administered with a delivery agent as described herein can decrease at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%.

Biological Effect

[00682] In one embodiment, the biological effect of the modified cell phenotype altering mRNA delivered to the animals may be categorized by analyzing the protein expression in the animals. The reprogrammed protein expression may be determined from analyzing a biological sample collected from a mammal administered the modified cell phenotype altering mRNA of the present invention. In one embodiment, the expression protein encoded by the modified cell phenotype altering mRNA administered to the mammal of at least 50 pg/ml may be preferred. For example, a protein expression of 50-200 pg/ml for the protein encoded by the modified cell phenotype altering mRNA delivered to the mammal may be seen as a therapeutically effective amount of protein in the mammal.

Detection of Modified Nucleic Acids by Mass Spectrometry

[00683] Mass spectrometry (MS) is an analytical technique that can provide structural and molecular mass/concentration information on molecules after their conversion to ions. The molecules are first ionized to acquire positive or negative charges and then they travel through the mass analyzer to arrive at different areas of the detector according to their mass/charge (m/z) ratio.

[00684] Mass spectrometry is performed using a mass spectrometer which includes an ion source for ionizing the fractionated sample and creating charged molecules for further analysis. For example ionization of the sample may be performed by electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), photoionization, electron ionization, fast atom bombardment (FAB)/liquid secondary ionization (LSIMS),

matrix assisted laser desorption/ionization (MALDI), field ionization, field desorption, thermospray/plasmaspray ionization, and particle beam ionization. The skilled artisan will understand that the choice of ionization method can be determined based on the analyte to be measured, type of sample, the type of detector, the choice of positive versus negative mode, etc.

[00685] After the sample has been ionized, the positively charged or negatively charged ions thereby created may be analyzed to determine a mass-to-charge ratio (i.e., m/z). Suitable analyzers for determining mass-to-charge ratios include quadropole analyzers, ion traps analyzers, and time-of-flight analyzers. The ions may be detected using several detection modes. For example, selected ions may be detected (i.e., using a selective ion monitoring mode (SIM)), or alternatively, ions may be detected using a scanning mode, e.g., multiple reaction monitoring (MRM) or selected reaction monitoring (SRM).

[00686] Liquid chromatography-multiple reaction monitoring (LC-MS/MRM) coupled with stable isotope labeled dilution of peptide standards has been shown to be an effective method for protein verification (e.g., Keshishian et al., Mol Cell Proteomics 2009 8: 2339-2349; Kuhn et al, Clin Chem 2009 55:1 108-1 117; Lopez et al, Clin Chem 2010 56:281-290). Unlike untargeted mass spectrometry frequently used in biomarker discovery studies, targeted MS methods are peptide sequence-based modes of MS that focus the full analytical capacity of the instrument on tens to hundreds of selected peptides in a complex mixture. By restricting detection and fragmentation to only those peptides derived from proteins of interest, sensitivity and reproducibility are improved dramatically compared to discovery-mode MS methods. This method of mass spectrometry-based multiple reaction monitoring (MRM) quantitation of proteins can dramatically impact the discovery and quantitation of biomarkers via rapid, targeted, multiplexed protein expression profiling of clinical samples.

[00687] In one embodiment, a biological sample which may contain at least one protein encoded by at least one modified cell phenotype altering mRNA of the present invention may be analyzed by the method of MRM-MS. The quantification of the biological sample may further include, but is not limited to, isotopically labeled peptides or proteins as internal standards.

[00688] According to the present invention, the biological sample, once obtained from the subject, may be subjected to enzyme digestion. As used herein, the term "digest" means to break apart into shorter peptides. As used herein, the phrase "treating a sample to digest proteins" means manipulating a sample in such a way as to break down proteins in a sample. These enzymes include, but are not limited to, trypsin, endoproteinase Glu-C and chymotrypsin. In one embodiment, a biological sample which may contain at least one protein encoded by at least one modified cell phenotype altering mRNA of the present invention may be digested using enzymes.

[00689] In one embodiment, a biological sample which may contain protein encoded by modified cell phenotype altering mRNA of the present invention may be analyzed for protein using electrospray ionization. Electrospray ionization (ESI) mass spectrometry (ESIMS) uses electrical energy to aid in the transfer of ions from the solution to the gaseous phase before they are analyzed by mass spectrometry. Samples may be analyzed using methods known in the art (e.g., Ho et al., Clin Biochem Rev. 2003 24(1):3-12). The ionic species contained in solution may be transferred into the gas phase by dispersing a fine spray of charge droplets, evaporating the solvent and ejecting the ions from the charged droplets to generate a mist of highly charged droplets. The mist of highly charged droplets may be analyzed using at least 1, at least 2, at least 3 or at least 4 mass analyzers such as, but not limited to, a quadropole mass analyzer. Further, the mass spectrometry method may include a purification step. As a non-limiting example, the first quadrapole may be set to select a single m/z ratio so it may filter out other molecular ions having a different m/z ratio which may eliminate complicated and time-consuming sample purification procedures prior to MS analysis.

[00690] In one embodiment, a biological sample which may contain protein encoded by modified cell phenotype altering mRNA of the present invention may be analyzed for protein in a tandem ESIMS system (e.g., MS/MS). As non-limiting examples, the droplets may be analyzed using a product scan (or daughter scan) a precursor scan (parent scan) a neutral loss or a multiple reaction monitoring.

[00691] In one embodiment, a biological sample which may contain protein encoded by modified cell phenotype altering mRNA of the present invention may be analyzed using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MALDIMS).

MALDI provides for the nondestructive vaporization and ionization of both large and small molecules, such as proteins. In MALDI analysis, the analyte is first co-crystallized with a large molar excess of a matrix compound, which may also include, but is not limited to, an ultraviolet absorbing weak organic acid. Non-limiting examples of matrices used in MALDI are a-cyano-4-hydroxycinnamic acid, 3,5-dimethoxy-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid. Laser radiation of the analyte-matrix mixture may result in the vaporization of the matrix and the analyte. The laser induced desorption provides high ion yields of the intact analyte and allows for measurement of compounds with high accuracy. Samples may be analyzed using methods known in the art (e.g., Lewis, Wei and Siuzdak, Encyclopedia of Analytical Chemistry 2000:5880-5894). As non-limiting examples, mass analyzers used in the MALDI analysis may include a linear time-of-flight (TOF), a TOF reflectron or a Fourier transform mass analyzer.

[00692] In one embodiment, the analyte-matrix mixture may be formed using the dried-droplet method. A biologic sample is mixed with a matrix to create a saturated matrix solution where the matrix-to-sample ratio is approximately 5000: 1. An aliquot (approximately 0.5-2.0 uL) of the saturated matrix solution is then allowed to dry to form the analyte-matrix mixture.

[00693] In one embodiment, the analyte-matrix mixture may be formed using the thin-layer method. A matrix homogeneous film is first formed and then the sample is then applied and may be absorbed by the matrix to form the analyte-matrix mixture.

[00694] In one embodiment, the analyte-matrix mixture may be formed using the thick-layer method. A matrix homogeneous film is formed with a nitro-cellulose matrix additive. Once the uniform nitro-cellulose matrix layer is obtained the sample is applied and absorbed into the matrix to form the analyte-matrix mixture.

[00695] In one embodiment, the analyte-matrix mixture may be formed using the sandwich method. A thin layer of matrix crystals is prepared as in the thin-layer method followed by the addition of droplets of aqueous trifluoroacetic acid, the sample and matrix. The sample is then absorbed into the matrix to form the analyte-matrix mixture.

V. Uses of Polynucleotides, primary constructs and mmRNA of the Invention

[00696] The polynucleotides, primary constructs and mmRNA of the present invention may be used to alter the phenotype of cells. The polynucleotides, primary constructs and mmRNA of the invention may encode peptides, polypeptides or multiple proteins to produce cell phenotype altering polypeptides of interest. The cell phenotype altering polypeptides of interest may be used in therapeutics and/or clinical and research settings. As a non-limiting example, the cell phenotype altering polypeptides of interest may include reprogramming factors, differentiation factors and de-differentiation factors.

Therapeutics

Therapeutic Agents

[00697] The cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention, such as modified cell phenotype altering nucleic acids and modified cell phenotype altering RNAs, and the cell phenotype altering proteins translated from them described herein can be used as therapeutic or prophylactic agents. They are provided for use in medicine, therapy and preventative treatments. For example, a cell phenotype altering polynucleotide, primary construct or mmRNA described herein can be administered to a subject, wherein the cell phenotype altering polynucleotide, primary construct or mmRNA is translated *in vivo* to produce a therapeutic or prophylactic polypeptide in the subject. Provided are compositions, methods, kits, and reagents for diagnosis, treatment or prevention of a disease or condition in humans and other mammals. The active therapeutic agents of the invention include cell phenotype altering polynucleotides, primary constructs or mmRNA, cells containing the cell phenotype altering polynucleotides, primary constructs or mmRNA or cell phenotype altering polypeptides translated from the cell phenotype altering polynucleotides, primary constructs or mmRNA.

[00698] In certain embodiments, provided herein are combination therapeutics containing one or more cell phenotype altering polynucleotide, primary construct or mmRNA containing translatable regions that encode for a cell phenotype altering protein or proteins such as a reprogramming, differentiation or de-differentiation protein..

[00699] Provided herein are methods of inducing translation of a recombinant cell phenotype altering polypeptide in a cell population using the cell phenotype altering polynucleotide, primary construct or mmRNA described herein. Such translation can be

in vivo, ex vivo, in culture, or *in vitro.* The cell population is contacted with an effective amount of a composition containing a cell phenotype altering nucleic acid that has at least one nucleoside modification, and a translatable region encoding the recombinant polypeptide. The population is contacted under conditions such that the nucleic acid is localized into one or more cells of the cell population and the recombinant cell phenotype altering polypeptide is translated in the cell from the cell phenotype altering nucleic acid.

[00700] An "effective amount" of the composition is provided based, at least in part, on the target tissue, target cell type, means of administration, physical characteristics of the nucleic acid (e.g., size, and extent of modified nucleosides), and other determinants. In general, an effective amount of the composition provides efficient protein production in the cell, preferably more efficient than a composition containing a corresponding unmodified nucleic acid. Increased efficiency may be demonstrated by increased cell transfection (i.e., the percentage of cells transfected with the nucleic acid), increased protein translation from the nucleic acid, decreased nucleic acid degradation (as demonstrated, e.g., by increased duration of protein translation from a modified nucleic acid), or reduced innate immune response of the host cell.

[00701] Aspects of the invention are directed to methods of inducing *in vivo* translation of a recombinant cell phenotype altering polypeptide in a mammalian subject in need thereof. Therein, an effective amount of a composition containing a nucleic acid that has at least one structural or chemical modification and a translatable region encoding the recombinant cell phenotype altering polypeptide is administered to the subject using the delivery methods described herein. The cell phenotype altering nucleic acid is provided in an amount and under other conditions such that the nucleic acid is localized into a cell of the subject and the recombinant cell phenotype altering polypeptide is translated in the cell from the cell phenotype altering nucleic acid. The cell in which the cell phenotype altering nucleic acid is localized, or the tissue in which the cell is present, may be targeted with one or more than one rounds of cell phenotype altering nucleic acid administration.

[00702] In certain embodiments, the administered cell phenotype altering polynucleotide, primary construct or mmRNA directs production of one or more recombinant cell phenotype altering polypeptides that provide a functional activity which

is substantially absent in the cell, tissue or organism in which the recombinant polypeptide is translated. For example, the missing functional activity may be enzymatic, structural, or gene regulatory in nature. In related embodiments, the administered cell phenotype altering polynucleotide, primary construct or mmRNA directs production of one or more recombinant cell phenotype altering polypeptides that increases (e.g., synergistically) a functional activity which is present but substantially deficient in the cell in which the recombinant cell phenotype altering polypeptide is translated.

[00703] In other embodiments, the administered cell phenotype altering polynucleotide, primary construct or mmRNA directs production of one or more recombinant polypeptides that replace a polypeptide (or multiple polypeptides) that is substantially absent in the cell in which the recombinant cell phenotype altering polypeptide is translated. Such absence may be due to genetic mutation of the encoding cell phenotype altering gene or regulatory pathway thereof. In some embodiments, the recombinant cell phenotype altering polypeptide increases the level of an endogenous cell phenotype altering protein in the cell to a desirable level; such an increase may bring the level of the endogenous cell phenotype altering protein from a subnormal level to a normal level or from a normal level to a super-normal level.

[00704] Alternatively, the recombinant cell phenotype altering polypeptide functions to antagonize the activity of an endogenous protein present in, on the surface of, or secreted from the cell. Usually, the activity of the endogenous cell phenotype altering protein is deleterious to the subject; for example, due to mutation of the endogenous protein resulting in altered activity or localization. Additionally, the recombinant cell phenotype altering polypeptide antagonizes, directly or indirectly, the activity of a biological moiety present in, on the surface of, or secreted from the cell. Examples of antagonized biological moieties include lipids (e.g., cholesterol), a lipoprotein (e.g., low density lipoprotein), a nucleic acid, a carbohydrate, a protein toxin such as shiga and tetanus toxins, or a small molecule toxin such as botulinum, cholera, and diphtheria toxins. Additionally, the antagonized biological molecule may be an endogenous protein that exhibits an undesirable activity, such as a cytotoxic or cytostatic activity.

[00705] The recombinant cell phenotype altering proteins described herein may be engineered for localization within the cell, potentially within a specific compartment such

as the nucleus, or are engineered for secretion from the cell or translocation to the plasma membrane of the cell.

[00706] In some embodiments, modified cell phenotype altering mRNAs and their encoded cell phenotype altering polypeptides in accordance with the present invention may be used for treatment of any of a variety of diseases, disorders, and/or conditions, including but not limited to one or more of the following: autoimmune disorders *(e.g.* diabetes, lupus, multiple sclerosis, psoriasis, rheumatoid arthritis); inflammatory disorders *(e.g.* arthritis, pelvic inflammatory disease); infectious diseases *(e.g.* viral infections *(e.g.,* HIV, HCV, RSV), bacterial infections, fungal infections, sepsis); neurological disorders *(e.g.* Alzheimer's disease, Huntington's disease; autism; Duchenne muscular dystrophy); cardiovascular disorders *(e.g.* atherosclerosis, hypercholesterolemia, thrombosis, clotting disorders, angiogenic disorders such as macular degeneration); proliferative disorders *(e.g.* cancer, benign neoplasms); respiratory disorders *(e.g.* chronic obstructive pulmonary disease); digestive disorders *(e.g.* inflammatory bowel disease, ulcers); musculoskeletal disorders *(e.g.* fibromyalgia, arthritis); endocrine, metabolic, and nutritional disorders *(e.g.* diabetes, osteoporosis); urological disorders *(e.g.* renal disease); psychological disorders *(e.g.* depression, schizophrenia); skin disorders *(e.g.* wounds, eczema); blood and lymphatic disorders *(e.g.* anemia, hemophilia); *etc.*

[00707] Diseases characterized by dysfunctional or aberrant protein activity include cystic fibrosis, sickle cell anemia, epidermolysis bullosa, amyotrophic lateral sclerosis, and glucose-6-phosphate dehydrogenase deficiency. The present invention provides a method for treating such conditions or diseases in a subject by introducing nucleic acid or cell-based therapeutics containing the cell phenotype altering polynucleotide, primary construct or mmRNA provided herein, wherein the cell phenotype altering polynucleotide, primary construct or mmRNA encode for a protein that antagonizes or otherwise overcomes the aberrant protein activity present in the cell of the subject. Specific examples of a dysfunctional protein are the missense mutation variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which produce a dysfunctional protein variant of CFTR protein, which causes cystic fibrosis.

[00708] Diseases characterized by missing (or substantially diminished such that proper (normal or physiological protein function does not occur) protein activity include cystic fibrosis, Niemann-Pick type C, β thalassemia major, Duchenne muscular dystrophy, Hurler Syndrome, Hunter Syndrome, and Hemophilia A. Such proteins may not be present, or are essentially non-functional. The present invention provides a method for treating such conditions or diseases in a subject by introducing nucleic acid or cell-based therapeutics containing the cell phenotype altering polynucleotide, primary construct or mmRNA provided herein, wherein the cell phenotype altering polynucleotide, primary construct or mmRNA encode for a protein that replaces the protein activity missing from the target cells of the subject. Specific examples of a dysfunctional protein are the nonsense mutation variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which produce a nonfunctional protein variant of CFTR protein, which causes cystic fibrosis.

[00709] Thus, provided are methods of treating cystic fibrosis in a mammalian subject by contacting a cell of the subject with a cell phenotype altering polynucleotide, primary construct or mmRNA having a translatable region that encodes a functional CFTR polypeptide, under conditions such that an effective amount of the CTFR polypeptide is present in the cell. Preferred target cells are epithelial, endothelial and mesothelial cells, such as the lung, and methods of administration are determined in view of the target tissue; i.e., for lung delivery, the RNA molecules are formulated for administration by inhalation.

[00710] Other aspects of the present disclosure relate to transplantation of cells containing cell phenotype altering polynucleotide, primary construct, or mmRNA to a mammalian subject. Administration of cells to mammalian subjects is known to those of ordinary skill in the art, and include, but is not limited to, local implantation (e.g., topical or subcutaneous administration), organ delivery or systemic injection (e.g., intravenous injection or inhalation), and the formulation of cells in pharmaceutically acceptable carrier. Such compositions containing polynucleotide, primary construct, or mmRNA can be formulated for administration intramuscularly, transarterially, intraperitoneally, intravenously, intranasally, subcutaneously, endoscopically, transdermally, or

intrathecally. In some embodiments, the composition may be formulated for extended release.

The subject to whom the therapeutic agent may be administered suffers from or may be at risk of developing a disease, disorder, or deleterious condition. Provided are methods of identifying, diagnosing, and classifying subjects on these bases, which may include clinical diagnosis, biomarker levels, genome-wide association studies (GWAS), and other methods known in the art.

Reprogramming of Cells

[00711] In one embodiment, the cell phenotype altering polynucleotides, primary constructs and/or mmRNA may be used to reprogram a cell. The reprogramming of a cell may be accomplished by a single or repeated transfection of a cell, such as, but not limited to, a somatic cell, with a cell phenotype altering polynucleotide, primary construct and/or mmRNA of the present invention encoding a reprogramming factor. As a non-limiting example, the reprogramming factor may include, OCT4, SOX1, SOX2, SOX3, SOX15, SOX18, NANOG, KLF1, KLF2, KLF4, KL5, NR5A2, c-MYC, 1-MYC, n-MYC, REM2, TERT and LIN28.

[00712] In another embodiment, a cell may be reprogrammed by contacting the cell at least once with a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding OCT4. Further, the cell may be contacted at least once with a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the SOX family. Additionally the cell may be contacted at least once with a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the KLF family or a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the MYC family.

[00713] In one embodiment, a cell may be reprogrammed by contacting the cell to be reprogrammed at least once with a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding OCT4 and a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the SOX family. In a further embodiment, the cell may additionally be contacted at least once with a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the KLF family or LIN28. In yet another embodiment, the cell may be contacted at least

once with a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the MYC family or NANOG.

[00714] In one embodiment, a cell is repeatedly contacted with a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding OCT4, a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the SOX family, a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the KLF family and a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the MYC family. In a further embodiment, the cell is a somatic cell.

[00715] In one embodiment, a cell is repeatedly contacted with a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding OCT4, a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the SOX family, a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the LIN28 and a cell phenotype altering polynucleotide, primary construct and/or mmRNA encoding a member of the NANOG. In a further embodiment, the cell is a somatic cell.

[00716] Cells may be transfected with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding reprogramming factors once or multiple times in order to permit sufficient expression of the reprogramming factors in cells being contacted. Repeated transfection can include, but is not limited to, at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least fifteen, at least twenty, at least twenty five, at least thirty, at least thirty five or more transfections. The transfection of cells with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding reprogramming factors may be repeated as many times as necessary to achieve the desired phenotype of the cell or population of cells contacted.

[00717] In one embodiment, transfection of cells with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding reprogramming factors may produce pluripotent cells from a starting population of cells in less than 30 days, less than 29 days, less than 28 days, less than 27 days, less than 26

days, less than 25 days, less than 24 days, less than 23 days, less than 22 days, less than 21 days, less than 20 days, less than 19 days, less than 18 days, less than 17 days, less than 16 days, less than 15 days, less than 14 days, less than 13 days, less than 12 days, less than 11 days, less than 10 days, less than 9 days, less than 8 days, less than 7 days. In another embodiment, transfection of cells with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding reprogramming factors may produce pluripotent cells from a starting population of cells in greater than 7 days.

[00718] In order to enhance the efficiency of reprogramming cells with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding a reprogramming factor, at least one small molecule enhancing the efficiency of reprogramming may be added to the process. These small molecules include, but are not limited to, the small molecules shown by Shi et al. *{Cell-Stem Cell,* 2008, 2:525-528; herein incorporated by reference in its entirety), Huangfu et al. *{Nature Biotechnology,* 2008, 26(7):795-797; herein incorporated by reference in its entirety) and Marson et al. *{Cell-Stem Cell,* 2008, 3:132-135; herein incorporated by reference in its entirety). As a non-limiting example, small molecules that may enhance the efficiency of reprogramming include trichostatin (TSA), soluble Wnt, Wnt conditioned media, hydroxamic acid (SAHA), BIX-01294 (a G9a histone methyltransferase), suberoylanide, dexamethasone, 5'-azacytidine, MEK inhibitor PD0325901, valproic acid, DNA mehtyltransferase inhibitors, and histone deacetylase (HDAC) inhibitors.

[00719] The efficiency of reprogramming cells with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding reprogramming factors to a pluripotent cell from a starting population of cells can be at least l%,at least 1.2%, at least 1.4%, at least 1.6%, at least 1.8%, at least 2.1 %, at least 2.2%, at least 2.3%, at least 2.4%, at least 2.5%, at least 2.6%, at least 2.7%, at least 2.8%, at least 2.9%, at least 3.0%, at least 3.1 %, at least 3.2%, at least 3.3%, at least 3.4%, at least 3.5%, at least 3.6%, at least 3.7%, at least 3.8%, at least 3.9%, at least 4.0%, at least 4.1 %, at least 4.2%, at least 4.3%, at least 4.4%, at least 4.5%, at least 4.6%, at least 4.7%, at least 4.8%, at least 4.9%, at least 5.0%, 5.1 %, at least 5.2%, at least 5.3%, at least 5.4%, at least 5.5%, at least 5.6%, at least 5.7%, at least 5.8%, at least

5.9%, at least 6.0%, 6.1 %, at least 6.2%, at least 6.3%, at least 6.4%, at least 6.5%, at least 6.6%, at least 6.7%, at least 6.8%, at least 6.9%, at least 7.0%, 7.1 %, at least 8.2%, at least 8.3%, at least 8.4%, at least 8.5%, at least 8.6%, at least 8.7%, at least 8.8%, at least 8.9%, at least 9.0%, 9.1 %, at least 9.2%, at least 9.3%, at least 9.4%, at least 9.5%, at least 1.6%, at least 9.7%, at least 9.8%, at least 9.9%, at least 10.0%, at least 15%, at least 20% or more.

[00720] In one embodiment, the reprogramming of a somatic cell, a precursor somatic cell, partially reprogrammed somatic cell, pluripotent cell, multipotent cell, differentiated cell or an embryonic cell into a pluripotent stem cell or its immediate precursor cell may cause an induction of at least one stem cell genes such as, but not limited to, DNMT3B, NANOG, OCT4, SSEA3, SSEA4, SOX2, REX1, TRA-1-60 and TRA-1-81.

[00721] In one embodiment, to reduce the stress response during the reprogramming of cells with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding reprogramming factors a p53 inhibitor may be used to direct the cell toward reprogramming instead of apoptotic stimulus. Non-limiting examples of methods to enhance efficiency of translation are described in International Publication No. WO201 1130624; herein incorporated by reference in its entirety.

[00722] In order to determine if the cell-altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding reprogramming factors were able to induce pluripotent stem cells, testing of isolated clones can be done to determine the expression of an endogenous stem cell marker to identify the cell as an induced pluripotent stem cell. Stem cell markers include, but are not limited to, NAT1, SSEA1, UTF1, CD9, REX1, NANOG, SLC2A3, FBX15, ZPF296, ECAT1, ESG1, DAX1, CRIPTO, ERAS, GDF2 and FGF4. Methods for the detection of stem-cell markers are known in the art and may include reduction in or loss of lamin A/C protein expression detected by measuring an increase in acetylation or decrease in methylation, detection of chromatin remodeling to lead to the activation of an embryonic stem cell marker and the detection of the expression of stem cell markers by reverse transcription polymerase chain reaction (RT-PCR) and other immunological detection methods.

[00723] Additional information related to reprogramming of cells using RNA is described in International Publication No. WO201 1130624; the contents of which is herein incorporated by reference in its entirety.

[00724] Cells transfected with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding reprogramming factors may further be cultured with in the presence of cell specific growth factors such as, but not limited to, angiogenin, bone morphogenic protein-I, bone morphogenic protein-2, bone morphogenic protein-3, bone morphogenic protein-4, bone morphogenic protein-5, bone morphogenic protein-6, bone morphogenic protein-7, bone morphogenic protein-8, bone morphogenic protein-9, bone morphogenic protein-10, bone morphogenic protein-11, bone morphogenic protein-12, bone morphogenic protein-13, bone morphogenic protein-14, bone morphogenic protein-15, bone morphogenic protein receptor 1A, bone morphogenic protein receptor IB, brain derived neurotrophic factor, ciliary neutrophic factor, ciliary neutrophic factor receptor-alpha, cytokine-induced neutrophil chemotactic factor 1, cytokine-induced neutrophil, chemotactic factor 2-alpha, cytokine-induced neutrophil chemotactic factor 2-beta, betaendothelial cell growth factor, endothelia 1, epidermal growth factor, epithelial-derived neutrophil attractant, fibroblast growth factor 4, fibroblast growth factor 5, fibroblast growth factor 6 fibroblast growth factor 7, fibroblast growth factor 8, fibroblast growth factor b, fibroblast growth factor c, fibroblast growth factor 9, fibroblast growth factor 10, fibroblast growth factor acidic, fibroblast growth factor basic, glial cell line-derived neutrophil factor receptor-alpha-1, glial cell line-derived neutrophil factor receptor-alpha-2, growth related protein, growth related protein-alpha, growth related protein-beta, growth related protein-gamma, heparin binding epidermal growth factor, hepatocyte growth factor, hepatocyte growth factor receptor, insulin-like growth factor I, insulin-like growth factor receptor, insulin-like growth factor II, insulin-like growth factor binding protein, keratinocyte growth factor, leukemia inhibitory factor, leukemia inhibitory factor receptor-alpha, nerve growth factor, nerve growth factor receptor, neurotrophin-3, neurotrophin-4, placenta growth factor, placenta growth factor 2, platelet-derived endothelial cell growth factor, platelet derived growth factor, platelet derived growth factor A chain, platelet derived growth factor AA, platelet derived growth factor AB, platelet derived growth factor B chain,

platelet derived growth factor BB, platelet derived growth factor receptor-alpha, platelet derived growth factor receptor-beta, pre-B cell growth stimulating factor, stem cell factor, stem cell factor receptor, transforming growth factor-alpha, transforming growth factor-beta, transforming growth factor-beta- 1, transforming growth factor-beta- 1-2, transforming growth factor-beta-2, transforming growth factor-beta-3, transforming growth factor-beta-5, latent transforming growth factor-beta- 1, transforming growth factor-beta-binding protein I, transforming growth factor-beta-binding protein II, transforming growth factor-beta-binding protein III, tumor necrosis factor receptor type I, tumor necrosis factor receptor type II, urokinase-type plasminogen activator receptor, vascular endothelial growth factor, and chimeric proteins and biologically or immunologically active fragments thereof.

[00725] Cell may be transfected with ribonuclease inhibitors, such as, but not limited to B18R, TLR signaling inhibitors and PKR inhibitors, to reduce the innate immune response by inhibiting the activity and cellular binding.

Differentiation and De-Differentiation of Cells

[00726] In one embodiment, the directing of the differentiation of cells may be accomplished by a single or repeated transfection of a cell with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention. The cell phenotype altering polynucleotides, primary constructs and/or mmRNA may encode differentiation or de-differentiation factors.

[00727] Cells may be transfected once or multiple times with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding differentiation or de-differentiation factors in order to permit the desired expression of the differentiation or de-differentiation factors in cells being contacted. Repeated transfection can include, but is not limited to, at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least fifteen, at least twenty, at least twenty five, at least thirty, at least thirty five or more transfections. The transfection of cells with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding differentiation or de-differentiation factors may be repeated as many times as necessary to achieve the desired phenotype of the cell or population of cells contacted.

[00728] In one embodiment, transfection of cells with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding differentiation or de-differentiation factors may produce pluripotent cells from a starting population of cells in less than 30 days, less than 29 days, less than 28 days, less than 27 days, less than 26 days, less than 25 days, less than 24 days, less than 23 days, less than 22 days, less than 2 1 days, less than 20 days, less than 19 days, less than 18 days, less than 17 days, less than 16 days, less than 15 days, less than 14 days, less than 13 days, less than 12 days, less than 11 days, less than 10 days, less than 9 days, less than 8 days, less than 7 days. In another embodiment, transfection of cells with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding differentiation or de-differentiation factors may produce pluripotent cells from a starting population of cells in greater than 7 days.

[00729] In one embodiment, the gene expression of cell type specific markers can be measured by methods known in the art such as, but not limited to, Western blotting and cell function assays to determine if the transfection of the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding differentiation or de-differentiation factors has created the desired phenotype.

[00730] The efficiency of differentiation or de-differentiation of cells using the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding differentiation or de-differentiation factors to a pluripotent cell from a starting population of cells can be at least l%,at least 1.2%, at least 1.4%, at least 1.6%, at least 1.8%, at least 2.1 %, at least 2.2%, at least 2.3%, at least 2.4%, at least 2.5%, at least 2.6%, at least 2.7%, at least 2.8%, at least 2.9%, at least 3.0%, at least 3.1 %, at least 3.2%, at least 3.3%, at least 3.4%, at least 3.5%, at least 3.6%, at least 3.7%, at least 3.8%, at least 3.9%, at least 4.0%, at least 4.1 %, at least 4.2%, at least 4.3%, at least 4.4%, at least 4.5%, at least 4.6%, at least 4.7%, at least 4.8%, at least 4.9%, at least 5.0%, 5.1 %, at least 5.2%, at least 5.3%, at least 5.4%, at least 5.5%, at least 5.6%, at least 5.7%, at least 5.8%, at least 5.9%, at least 6.0%, 6.1 %, at least 6.2%, at least 6.3%, at least 6.4%, at least 6.5%, at least 6.6%, at least 6.7%, at least 6.8%, at least 6.9%, at least 7.0%, 7.1 %, at least 8.2%, at least 8.3%, at least 8.4%, at least 8.5%, at least 8.6%, at least 8.7%, at least 8.8%, at least 8.9%, at least 9.0%, 9.1 %, at least 9.2%, at least

9.3%, at least 9.4%, at least 9.5%, at least 1.6%, at least 9.7%, at least 9.8%, at least 9.9%, at least 10.0%, at least 15%, at least 20% or more.

[00731] As a non-limiting example, cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding a neuronal differentiation factor such as ASCI 1, BRN2, MYT1 may be used to differentiate a cell into a neuronal cell phenotype. Other factors to promote differentiation and other information related to differentiation and de-differentiation of cells using RNA is described in International Publication No. WO201 1130624; herein incorporated by reference in its entirety.

[00732] Cells transfected with the cell phenotype altering polynucleotides, primary constructs and/or mmRNA of the present invention encoding differentiation or de-differentation factors may further be cultured with in the presence of cell specific growth factors such as, but not limited to, angiogenin, bone morphogenic protein-I, bone morphogenic protein-2, bone morphogenic protein-3, bone morphogenic protein-4, bone morphogenic protein-5, bone morphogenic protein-6, bone morphogenic protein-7, bone morphogenic protein-8, bone morphogenic protein-9, bone morphogenic protein- 10, bone morphogenic protein- 11, bone morphogenic protein- 12, bone morphogenic protein- 13, bone morphogenic protein- 14, bone morphogenic protein-15, bone morphogenic protein receptor 1A, bone morphogenic protein receptor IB, brain derived neurotrophic factor, ciliary neutrophic factor, ciliary neutrophic factor receptor-alpha, cytokine-induced neutrophil chemotactic factor 1, cytokine-induced neutrophil, chemotactic factor 2-alpha, cytokine-induced neutrophil chemotactic factor 2-beta, betaendothelial cell growth factor, endothelia 1, epidermal growth factor, epithelial-derived neutrophil attractant, fibroblast growth factor 4, fibroblast growth factor 5, fibroblast growth factor 6 fibroblast growth factor 7, fibroblast growth factor 8, fibroblast growth factor b, fibroblast growth factor c, fibroblast growth factor 9, fibroblast growth factor 10, fibroblast growth factor acidic, fibroblast growth factor basic, glial cell line-derived neutrophil factor receptor-alpha- 1, glial cell line-derived neutrophil factor receptor-alpha-2, growth related protein, growth related protein-alpha, growth related protein-beta, growth related protein-gamma, heparin binding epidermal growth factor, hepatocyte growth factor, hepatocyte growth factor receptor, insulin-like growth factor I, insulin-like growth factor receptor, insulin-like growth factor II, insulin-like growth factor binding protein, keratinocyte growth factor,

leukemia inhibitory factor, leukemia inhibitory factor receptor-alpha, nerve growth factor, nerve growth factor receptor, neurotrophin-3, neurotrophin-4, placenta growth factor, placenta growth factor 2, platelet-derived endothelial cell growth factor, platelet derived growth factor, platelet derived growth factor A chain, platelet derived growth factor AA, platelet derived growth factor AB, platelet derived growth factor B chain, platelet derived growth factor BB, platelet derived growth factor receptor-alpha, platelet derived growth factor receptor-beta, pre-B cell growth stimulating factor, stem cell factor, stem cell factor receptor, transforming growth factor-alpha, transforming growth factor-beta, transforming growth factor-beta- 1, transforming growth factor-beta- 1-2, transforming growth factor-beta-2, transforming growth factor-beta-3, transforming growth factor-beta-5, latent transforming growth factor-beta- 1, transforming growth factor-beta-binding protein I, transforming growth factor-beta-binding protein II, transforming growth factor-beta-binding protein III, tumor necrosis factor receptor type I, tumor necrosis factor receptor type II, urokinase-type plasminogen activator receptor, vascular endothelial growth factor, and chimeric proteins and biologically or immunologically active fragments thereof.

Transdifferentiation of cells

[00733] The cell phenotype altering polynucleotides, primary constructs or mmRNA disclosed herein, may encode one or more differentiation factors which can be used in transdifferentiation. As used herein, a "transdifferentiation" refers the process of differentiated cells of one type to lose their identifying characteristics and change their phenotype to that of other fully differentiated cells. The cells may have been reprogramming before they are differentiated into another cell type but it is not required.

[00734] In one embodiment, the cell phenotype altering polynucleotides, primary constructs or mmRNA disclosed herein, may encode one or more differentiation factors to inhibit the expression of a cell type specific polypeptide of the starting cell which may be used to turn off the original phenotype of the cell. In another embodiment, the cell phenotype altering polynucleotides, primary constructs or mmRNA disclosed herein, may encode one or more differentiation factors of the desired cell to turn on the expression of a cell type specific polypeptide of the desired cell which is not part the original phenotype of the starting cell. In a further embodiment, the cell phenotype altering

polynucleotides, primary constructs or mmRNA turning off the cell type specific polypeptide may be contacted at the same time, before and/or after the cell is contacted with a cell phenotype altering polynucleotides, primary constructs or mmRNA turning on the desired expression of a cell type specific polypeptide.

[00735] Other information relating to transdifferentiation with RNA is described in International Application No. WO201 1130624; herein incorporated by reference in its entirety.

Major Groove Interacting Partners

[00736] As described herein, the phrase "major groove interacting partner" refers to RNA recognition receptors that detect and respond to RNA ligands through interactions, *e.g.* binding, with the major groove face of a nucleotide or nucleic acid. As such, RNA ligands comprising modified nucleotides or nucleic acids such as the cell phenotype altering polynucleotide, primary construct or mmRNA as described herein decrease interactions with major groove binding partners, and therefore decrease an innate immune response.

[00737] Example major groove interacting, *e.g.* binding, partners include, but are not limited to the following nucleases and helicases. Within membranes, TLRs (Toll-like Receptors) 3, 7, and 8 can respond to single- and double-stranded RNAs. Within the cytoplasm, members of the superfamily 2 class of DEX(D/H) helicases and ATPases can sense RNAs to initiate antiviral responses. These helicases include the RIG-I (retinoic acid-inducible gene I) and MDA5 (melanoma differentiation-associated gene 5). Other examples include laboratory of genetics and physiology 2 (LGP2), HΓN-200 domain containing proteins, or Helicase-domain containing proteins.

Targeting of pathogenic organisms or diseased cells

[00738] Provided herein are methods for targeting pathogenic microorganisms, such as bacteria, yeast, protozoa, helminthes and the like, or diseased cells such as cancer cells using cell phenotype altering polynucleotides, primary constructs or mmRNA that encode cytostatic or cytotoxic polypeptides. Preferably the mRNA introduced contains modified nucleosides or other nucleic acid sequence modifications that are translated exclusively, or preferentially, in the target pathogenic organism, to reduce possible off-target effects of the therapeutic. Such methods are useful for removing pathogenic organisms or killing

diseased cells found in any biological material, including blood, semen, eggs, and transplant materials including embryos, tissues, and organs.

Bioprocessing

[00739] The methods provided herein may be useful for enhancing protein product yield in a cell culture process. In a cell culture containing a plurality of host cells, introduction of a cell phenotype altering polynucleotide, primary construct or mmRNA described herein results in increased protein production efficiency relative to a corresponding unmodified nucleic acid. Such increased protein production efficiency can be demonstrated, e.g., by showing increased cell transfection, increased cell phenotype altering protein translation from the cell phenotype altering polynucleotide, primary construct or mmRNA, decreased nucleic acid degradation, and/or reduced innate immune response of the host cell. Protein production can be measured by enzyme-linked immunosorbent assay (ELISA), and protein activity can be measured by various functional assays known in the art. The protein production may be generated in a continuous or a batch-fed mammalian process.

[00740] Additionally, it is useful to optimize the expression of a specific cell phenotype altering polypeptide in a cell line or collection of cell lines of potential interest, particularly a cell phenotype altering polypeptide of interest such as a protein variant of a reference cell phenotype altering protein having a known activity. In one embodiment, provided is a method of optimizing expression of a cell phenotype altering polypeptide of interest in a target cell, by providing a plurality of target cell types, and independently contacting with each of the plurality of target cell types a cell phenotype altering polynucleotide, primary construct or mmRNA encoding a cell phenotype altering polypeptide of interest. The cells may be transfected with two or more cell phenotype altering polynucleotide, primary construct or mmRNA simultaneously or sequentially.

[00741] In certain embodiments, multiple rounds of the methods described herein may be used to obtain cells with increased expression of one or more cell phenotype altering nucleic acids or cell phenotype altering proteins of interest. For example, cells may be transfected with one or more cell phenotype altering polynucleotide, primary construct or mmRNA that encode a cell phenotype altering nucleic acid or cell phenotype altering protein of interest. The cells may be isolated according to methods described herein

before being subjected to further rounds of transfections with one or more other nucleic acids which encode a cell phenotype altering nucleic acid or cell phenotype altering protein of interest before being isolated again. This method may be useful for generating cells with increased expression of a complex of cell phenotype altering proteins, cell phenotype altering nucleic acids or cell phenotype altering proteins in the same or related biological pathway, cell phenotype altering nucleic acids or cell phenotype altering proteins that act upstream or downstream of each other, cell phenotype altering nucleic acids or cell phenotype altering proteins that have a modulating, activating or repressing function to each other, cell phenotype altering nucleic acids or cell phenotype altering proteins that are dependent on each other for function or activity, or cell phenotype altering nucleic acids or cell phenotype altering proteins that share homology.

[00742] Additionally, culture conditions may be altered to increase cell phenotype altering protein production efficiency. Subsequently, the presence and/or level of the cell phenotype altering polypeptide of interest in the plurality of target cell types is detected and/or quantitated, allowing for the optimization of a cell phenotype altering polypeptide's expression by selection of an efficient target cell and cell culture conditions relating thereto. Such methods are particularly useful when the cell phenotype altering polypeptide contains one or more post-translational modifications or has substantial tertiary structure, situations which often complicate efficient protein production.

[00743] In one embodiment, the cells used in the methods of the present invention may be cultured. The cells may be cultured in suspension or as adherent cultures. The cells may be cultured in a varied of vessels including, but not limited to, bioreactors, cell bags, wave bags, culture plates, flasks and other vessels well known to those of ordinary skill in the art. Cells may be cultured in IMDM (Invitrogen, Catalog number 12440-53) or any other suitable media including, but not limited to, chemically defined media formulations. The ambient conditions which may be suitable for cell culture, such as temperature and atmospheric composition, are well known to those skilled in the art. The methods of the invention may be used with any cell that is suitable for use in cell phenotype altering protein production.

[00744] The invention provides for the repeated introduction (e.g., transfection) of modified cell phenotype altering nucleic acids into a target cell population, e.g., *in vitro*,

ex vivo, *in situ*, or *in vivo*. For example, contacting the same cell population may be repeated one or more times (such as two, three, four, five or more than five times). In some embodiments, the step of contacting the cell population with the cell phenotype altering polynucleotides, primary constructs or mmRNA is repeated a number of times sufficient such that a predetermined efficiency of cell phenotype altering protein translation in the cell population is achieved. Given the often reduced cytotoxicity of the target cell population provided by the nucleic acid modifications, repeated transfections are achievable in a diverse array of cell types and within a variety of tissues, as provided herein.

[00745] In some embodiments, the contacting step is repeated multiple times at a frequency selected from the group consisting of: 6 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 84 hr, 96 hr, and 108 hr and at concentrations of less than 20 nM, less than 50 nM, less than 80 nM or less than 100 nM. Compositions may also be administered at less than lmM, less than 5mM, less than lOmM, less than lOOmM or less than 500 mM.

[00746] In some embodiments, the cell phenotype altering polynucleotides, primary constructs or mmRNA are added at an amount of 50 molecules per cell, 100 molecules/cell, 200 molecules/cell, 300 molecules/cell, 400 molecules/cell, 500 molecules/ cell, 600 molecules/cell, 700 molecules/ cell, 800 molecules/cell, 900 molecules/cell, 1000 molecules/cell, 2000 molecules/cell, or 5000 molecules/cell.

[00747] In other embodiments, the cell phenotype altering polynucleotides, primary constructs or mmRNA are added at a concentration selected from the group consisting of: 0.01 fmol/106 cells, 0.1 fmol/106 cells, 0.5 fmol/106 cells, 0.75 fmol/106 cells, 1 fmol/106 cells, 2 fmol/106 cells, 5 fmol/106 cells, 10 fmol/106 cells, 20 fmol/106 cells, 30 fmol/106 cells, 40 fmol/106 cells, 50 fmol/106 cells, 60 fmol/106 cells, 100 fmol/106 cells, 200 fmol/106 cells, 300 fmol/106 cells, 400 fmol/106 cells, 500 fmol/106 cells, 700 fmol/106 cells, 800 fmol/106 cells, 900 fmol/106 cells, and 1 pmol/106 cells.

[00748] In some embodiments, the production of a biological product upon is detected by monitoring one or more measurable bioprocess parameters, such as a parameter selected from the group consisting of: cell density, pH, oxygen levels, glucose levels, lactic acid levels, temperature, and protein production. Protein production can be measured as specific productivity (SP) (the concentration of a product, such as a

heterologously expressed polypeptide, in solution) and can be expressed as mg/L or g/L; in the alternative, specific productivity can be expressed as pg/cell/day. An increase in SP can refer to an absolute or relative increase in the concentration of a product produced under two defined set of conditions (e.g., when compared with controls not treated with modified cell phenotype altering mRNA(s)).

Cells

[00749] In one embodiment, the cells are selected from the group consisting of mammalian cells, bacterial cells, plant, microbial, algal and fungal cells. In some embodiments, the cells are mammalian cells, such as, but not limited to, human, mouse, rat, goat, horse, rabbit, hamster or cow cells. In a further embodiment, the cells may be from an established cell line, including, but not limited to, HeLa, NSO, SP2/0, KEK 293T, Vero, Caco, Caco-2, MDCK, COS-1, COS-7, K562, Jurkat, CHO-K1, DG44, CHOKISV, CHO-S, Huvec, CV-1, Huh-7, NIH3T3, HEK293, 293, A549, HepG2, IMR-90, MCF-7, U-20S, Per.C6, SF9, SF21 or Chinese Hamster Ovary (CHO) cells.

[00750] In certain embodiments, the cells are fungal cells, such as, but not limited to, Chrysosporium cells, Aspergillus cells, Trichoderma cells, Dictyostelium cells, Candida cells, Saccharomyces cells, Schizosaccharomyces cells, and Penicillium cells.

[00751] In certain embodiments, the cells are bacterial cells such as, but not limited to, E. coli, B. subtilis, or BL21 cells. Primary and secondary cells to be transfected by the methods of the invention can be obtained from a variety of tissues and include, but are not limited to, all cell types which can be maintained in culture. For examples, primary and secondary cells which can be transfected by the methods of the invention include, but are not limited to, fibroblasts, keratinocytes, epithelial cells (e.g., mammary epithelial cells, intestinal epithelial cells), endothelial cells, glial cells, neural cells, formed elements of the blood (e.g., lymphocytes, bone marrow cells), muscle cells and precursors of these somatic cell types. Primary cells may also be obtained from a donor of the same species or from another species (e.g., mouse, rat, rabbit, cat, dog, pig, cow, bird, sheep, goat, horse).

[00752] In one embodiment, the cells used in the present invention are somatic cells. Almost any primary somatic cell type can be used to prepare cells with an altered phenotype or altered developmental potential. A primary somatic cell may include, but is

not limited to, fibroblast, epithelial, endothelial, neuronal, adipose, cardiac, skeletal muscle, immune cells, hepatic, splenic, lung, circulating blood cells, gastrointestinal, renal, bone marrow and pancreatic cells. Further, a primary somatic cell may be isolated from somatic tissue such as, but not limited to, brain, liver, lung, gut, stomach, intestine, fat, muscle, uterus, skin, spleen, endocrine organ, and bone.

[00753] In one embodiment, the cells of the present invention are stem cells. Stem cells are classified by their development potention to be totipotent, pluripotent, multipotent, oligopotent and unipotent. The stem cells may be adult stem cells or derived from embryonic sources. Cell phenotype altering polynucleotides, primary constructs and mmRNA may be used to generate a stem cell from a differentiated cell. Further, the cell phenotype altering polynucleotides, primary constructs and mmRNA of the present invention may be used to direct the differentiation of the stem cell to one or more desired cell types. Stem cells are described in International Publication No. WO201 1130624, US Patent Nos 5,750,376, 5,851,832, 5,753,506, 5,589,379, 5,824,489, 5,654,183, 5,693,482, 5,672,499 and 5,849,553; each of which is incorporated by reference in its entirety.

[00754] In one embodiment, the cells are cancer stem cells. The cell phenotype altering polynucleotides, primary constructs and mmRNA of the present invention may be used to alter the phenotype of a cancer stem cell to a non-tumorigenic state. Non-limiting examples of tumors form which samples containing cancer stem cells can be isolated from or enriched for use with the present invention include sarcomas and carcinomas such as, but not limited to, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, mesothelioma, Ewing's tumor, lymphangioendotheliosarcoma, synovioma, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, astrocytic tumors (e.g., diffuse, infiltrating gliomas, anaplastic astrocytoma,

glioblastoma, gliosarcoma, pilocytic astrocytoma, pleomorphic xanthoastrocytoma), oligodendroglia! tumors and mixed gliomas (e.g., oligodendroglioma, anaplastic oligodendroglioma, oligoastrocytoma, anaplastic oligoastrocytoma), ependymal tumors (e.g., ependymoma, anaplastic ependymoma, myxopapillary ependymoma, subependymoma), choroid plexus tumors, neuroepithelial tumors of uncertain origin (astroblastoma, chordoid glioma, gliomatosis cerebri), neuronal and mixed-neuronal-glial tumors (e.g., ganglioglioma and gangliocytoma, desmoplastic infantile astrocytoma and ganglioglioma, dysembryoplastic neuroepithelial tumor, central neurocytoma, cerebellar liponeurocytoma, paraganglioglioma), pineal parenchymal tumors, embryonal tumors (medulloepithelioma, ependymoblastoma, medulloblastoma, primitive neuroectodemmal tumor, atypical teratoid/rhabdoid tumor), peripheral neuroblastic tumors, tumors of cranial and peripheral nerves (e.g., schwannoma, neurinofibroma, perineurioma, malignant peripheral nerve sheath tumor), meningeal tumors (e.g., meningeomas, mesenchymal, nonmeningothelial tumors, haemangiopericytomas, melanocytic lesions), germ cell tumors, tumors of the sellar region (e.g., craniopharyngioma, granular cell tumor of the neurohypophysis), hemangioblastoma, melanoma, and retinoblastoma.

Purification and Isolation

[00755] Those of ordinary skill in the art should be able to make a determination of the methods to use to purify or isolate of a cell phenotype altering protein of interest from cultured cells. Generally, this is done through a capture method using affinity binding or non-affinity purification. If the cell phenotype altering protein of interest is not secreted by the cultured cells, then a lysis of the cultured cells should be performed prior to purification or isolation. One may use unclarified cell culture fluid containing the cell phenotype altering protein of interest along with cell culture media components as well as cell culture additives, such as anti-foam compounds and other nutrients and supplements, cells, cellular debris, host cell proteins, DNA, viruses and the like in the present invention. The process may be conducted in the bioreactor itself. The fluid may either be preconditioned to a desired stimulus such as pH, temperature or other stimulus characteristic or the fluid can be conditioned upon the addition of polymer(s) or the polymer(s) can be added to a carrier liquid that is properly conditioned to the required parameter for the stimulus condition required for that polymer to be solubilized in the

fluid. The polymer may be allowed to circulate thoroughly with the fluid and then the stimulus may be applied (change in pH, temperature, salt concentration, etc) and the desired cell phenotype altering protein and polymer(s) precipitate can out of the solution. The polymer and the desired cell phenotype altering protein(s) can be separated from the rest of the fluid and optionally washed one or more times to remove any trapped or loosely bound contaminants. The desired cell phenotype altering protein may then be recovered from the polymer(s) by, for example, elution and the like. Preferably, the elution may be done under a set of conditions such that the polymer remains in its precipitated form and retains any impurities to it during the selected elution of the desired cell phenotype altering protein. The polymer and cell phenotype altering protein as well as any impurities may be solubilized in a new fluid such as water or a buffered solution and the cell phenotype altering protein may be recovered by a means such as affinity, ion exchanged, hydrophobic, or some other type of chromatography that has a preference and selectivity for the protein over that of the polymer or impurities. The eluted protein may then be recovered and may be subjected to additional processing steps, either batch like steps or continuous flow through steps if appropriate.

[00756] In another embodiment, it may be useful to optimize the expression of a specific cell phenotype altering polypeptide in a cell line or collection of cell lines of potential interest, particularly a cell phenotype altering polypeptide of interest such as a protein variant of a reference cell phenotype altering protein having a known activity. In one embodiment, provided is a method of optimizing expression of a cell phenotype altering polypeptide of interest in a target cell, by providing a plurality of target cell types, and independently contacting with each of the plurality of target cell types a modified cell phenotype altering mRNA encoding a polypeptide. Additionally, culture conditions may be altered to increase protein production efficiency. Subsequently, the presence and/or level of the cell phenotype altering polypeptide of interest in the plurality of target cell types is detected and/or quantitated, allowing for the optimization of a cell phenotype altering polypeptide of interest's expression by selection of an efficient target cell and cell culture conditions relating thereto. Such methods may be useful when the cell phenotype altering polypeptide of interest contains one or more post-translational

modifications or has substantial tertiary structure, which often complicate efficient protein production.

Protein recovery

[00757] The cell phenotype altering protein of interest may be preferably recovered from the culture medium as a secreted polypeptide, or it can be recovered from host cell lysates if expressed without a secretory signal. It may be necessary to purify the cell phenotype altering protein of interest from other recombinant proteins and host cell proteins in a way that substantially homogenous preparations of the cell phenotype altering protein of interest are obtained. The cells and/or particulate cell debris may be removed from the culture medium or lysate. The cell phenotype altering product of interest may then be purified from contaminant soluble repgroamming proteins, polypeptides and nucleic acids by, for example, fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC (RP-HPLC), SEPHADEX® chromatography, chromatography on silica or on a cation exchange resin such as DEAE. Methods of purifying a protein heterologous expressed by a host cell are well known in the art.

[00758] Methods and compositions described herein may be used to produce cell phenotype altering proteins which are capable of attenuating or blocking the endogenous agonist biological response and/or antagonizing a receptor or signaling molecule in a mammalian subject. For example, IL-12 and IL-23 receptor signaling may be enhanced in chronic autoimmune disorders such as multiple sclerosis and inflammatory diseases such as rheumatoid arthritis, psoriasis, lupus erythematosus, ankylosing spondylitis and Chron's disease (Kikly K, Liu L, Na S, Sedgwich JD (2006) Cur. Opin. Immunol. 18(6): 670-5). In another embodiment, a cell phenotype altering nucleic acid encodes an antagonist for chemokine receptors. Chemokine receptors CXCR-4 and CCR-5 are required for HIV enry into host cells (Arenzana-Seisdedos F et al, (1996) Nature. Oct 3; 383 (6599):400).

Gene Silencing

[00759] The cell phenotype altering polynucleotides, primary constructs and mmRNA described herein are useful to silence (i.e., prevent or substantially reduce) expression of one or more target genes in a cell population. A cell phenotype altering polynucleotide,

primary construct or mmRNA encoding a cell phenotype altering polypeptide of interest capable of directing sequence-specific histone H3 methylation is introduced into the cells in the population under conditions such that the polypeptide is translated and reduces gene transcription of a target gene via histone H3 methylation and subsequent heterochromatin formation. In some embodiments, the silencing mechanism is performed on a cell population present in a mammalian subject. By way of non-limiting example, a useful target gene is a mutated Janus Kinase-2 family member, wherein the mammalian subject expresses the mutant target gene suffers from a myeloproliferative disease resulting from aberrant kinase activity.

[00760] Co-administration of cell phenotype altering polynucleotides, primary constructs and mmRNA and RNAi agents are also provided herein.

Modulation of Biological Pathways

[00761] The rapid translation cell phenotype altering polynucleotides, primary constructs and mmRNA introduced into cells provides a desirable mechanism of modulating target biological pathways. Such modulation includes antagonism or agonism of a given pathway. In one embodiment, a method is provided for antagonizing a biological pathway in a cell by contacting the cell with an effective amount of a composition comprising a cell phenotype altering polynucleotide, primary construct or mmRNA encoding a polypeptide of interest, under conditions such that the cell phenotype altering polynucleotides, primary constructs and mmRNA is localized into the cell and the polypeptide is capable of being translated in the cell from the cell phenotype altering polynucleotides, primary constructs and mmRNA, wherein the cell phenotype altering polypeptide inhibits the activity of a polypeptide functional in the biological pathway. Exemplary biological pathways are those defective in an autoimmune or inflammatory disorder such as multiple sclerosis, rheumatoid arthritis, psoriasis, lupus erythematosus, ankylosing spondylitis colitis, or Crohn's disease; in particular, antagonism of the IL-12 and IL-23 signaling pathways are of particular utility. (See Kikly K, Liu L, Na S, Sedgwick JD (2006) Curr. Opin. Immunol. 18 (6): 670-5).

[00762] Further, provided are cell phenotype altering polynucleotide, primary construct or mmRNA encoding an antagonist for chemokine receptors; chemokine receptors

CXCR-4 and CCR-5 are required for, e.g., HIV entry into host cells (Arenzana-Seisdedos F et al, (1996) Nature, Oct 3;383(6599):400).

[00763] Alternatively, provided are methods of agonizing a biological pathway in a cell by contacting the cell with an effective amount of a cell phenotype altering polynucleotide, primary construct or mmRNA encoding a recombinant polypeptide under conditions such that the nucleic acid is localized into the cell and the recombinant polypeptide is capable of being translated in the cell from the nucleic acid, and the recombinant polypeptide induces the activity of a cell phenotype altering polypeptide functional in the biological pathway. Exemplary agonized biological pathways include pathways that modulate cell fate determination. Such agonization is reversible or, alternatively, irreversible.

Expression of Ligand or Receptor on Cell Surface

[00764] In some aspects and embodiments of the aspects described herein, the cell phenotype altering polynucleotides, primary constructs or mmRNA described herein can be used to express a ligand or ligand receptor on the surface of a cell *(e.g.,* a homing moiety). A ligand or ligand receptor moiety attached to a cell surface can permit the cell to have a desired biological interaction with a tissue or an agent *in vivo.* A ligand can be an antibody, an antibody fragment, an aptamer, a peptide, a vitamin, a carbohydrate, a protein or polypeptide, a receptor, *e.g.,* cell-surface receptor, an adhesion molecule, a glycoprotein, a sugar residue, a therapeutic agent, a drug, a glycosaminoglycan, or any combination thereof. For example, a ligand can be an antibody that recognizes a cancer-cell specific antigen, rendering the cell capable of preferentially interacting with tumor cells to permit tumor-specific localization of a modified cell. A ligand can confer the ability of a cell composition to accumulate in a tissue to be treated, since a preferred ligand may be capable of interacting with a target molecule on the external face of a tissue to be treated. Ligands having limited cross-reactivity to other tissues are generally preferred.

[00765] In some cases, a ligand can act as a homing moiety which permits the cell to target to a specific tissue or interact with a specific ligand. Such homing moieties can include, but are not limited to, any member of a specific binding pair, antibodies, monoclonal antibodies, or derivatives or analogs thereof, including without limitation: Fv

fragments, single chain Fv (scFv) fragments, Fab' fragments, F(ab')2 fragments, single domain antibodies, camelized antibodies and antibody fragments, humanized antibodies and antibody fragments, and multivalent versions of the foregoing; multivalent binding reagents including without limitation: monospecific or bispecific antibodies, such as disulfide stabilized Fv fragments, scFv tandems ((SCFV)2 fragments), diabodies, tribodies or tetrabodies, which typically are covalently linked or otherwise stabilized *(i.e.,* leucine zipper or helix stabilized) scFv fragments; and other homing moieties include for example, aptamers, receptors, and fusion proteins.

[00766] In some embodiments, the homing moiety may be a surface-bound antibody, which can permit tuning of cell targeting specificity. This is especially useful since highly specific antibodies can be raised against an epitope of interest for the desired targeting site. In one embodiment, multiple antibodies are expressed on the surface of a cell, and each antibody can have a different specificity for a desired target. Such approaches can increase the avidity and specificity of homing interactions.

[00767] A skilled artisan can select any homing moiety based on the desired localization or function of the cell, for example an estrogen receptor ligand, such as tamoxifen, can target cells to estrogen-dependent breast cancer cells that have an increased number of estrogen receptors on the cell surface. Other non-limiting examples of ligand/receptor interactions include CCRI *(e.g.,* for treatment of inflamed joint tissues or brain in rheumatoid arthritis, and/or multiple sclerosis), CCR7, CCR8 *(e.g.,* targeting to lymph node tissue), CCR6, CCR9,CCR10 *(e.g.,* to target to intestinal tissue), CCR4, CCR10 *(e.g.,* for targeting to skin), CXCR4 *(e.g.,* for general enhanced transmigration), HCELL *(e.g.,* for treatment of inflammation and inflammatory disorders, bone marrow), Alpha4beta7 *(e.g.,* for intestinal mucosa targeting), VLA-4/VCAM-1 *(e.g.,* targeting to endothelium). In general, any receptor involved in targeting *(e.g.,* cancer metastasis) can be harnessed for use in the methods and compositions described herein.

Modulation of Cell Lineage

[00768] Provided are methods of inducing an alteration in cell fate in a target mammalian cell. The target mammalian cell may be a precursor cell and the alteration may involve driving differentiation into a lineage, or blocking such differentiation. Alternatively, the target mammalian cell may be a differentiated cell, and the cell fate

alteration includes driving de-differentiation into a pluripotent precursor cell, or blocking such de-differentiation, such as the dedifferentiation of cancer cells into cancer stem cells. In situations where a change in cell fate is desired, effective amounts of cell phenotype altering mRNAs encoding a cell fate inductive polypeptide is introduced into a target cell under conditions such that an alteration in cell fate is induced. In some embodiments, the modified cell phenotype altering mRNAs are useful to reprogram a subpopulation of cells from a first phenotype to a second phenotype. Such a cell phenotype altering may be temporary or permanent. Optionally, the cell phenotype altering induces a target cell to adopt an intermediate phenotype.

[00769] In one embodiment, the methods and compositions of the present invention are particularly useful to generate induced pluripotent stem cells (iPS cells). The cell phenotype altering polynucleotides, primary constructs and/or mmRNA can have a high efficiency of transfection, the ability to re-transfect cells, and the tenability of the amount of recombinant polypeptides produced in the target cells. Further, the use of iPS cells generated using the cell phenotype altering polynucleotides, primary constructs and/or mmRNA and methods described herein is expected to have a reduced incidence of teratoma formation.

[00770] Also provided herein are methods to reduce cellular differentiation in a target cell population using cell phenotype altering polynucleotides, primary constructs and/or mmRNA which may encode differentiation or de-differentiation factors. For example, a target cell population containing one or more precursor cell types may be contacted with a composition of the present invention having an effective amount of a cell phenotype altering polynucleotides, primary constructs and mmRNA encoding a cell phenotype altering polypeptide, under conditions such that the cell phenotype altering polypeptide is translated and reduces the differentiation of the precursor cell. In non-limiting embodiments, the target cell population contains injured tissue in a mammalian subject or tissue affected by a surgical procedure. The precursor cell is, e.g., a stromal precursor cell, a neural precursor cell, or a mesenchymal precursor cell.

[00771] In a specific embodiment, provided are cell phenotype altering polynucleotide, primary construct or mmRNA that encode one or more differentiation factors GATA4, MEF2C and TBX4. These mRNA-generated factors can be introduced into fibroblasts

and drive the reprogramming into cardiomyocytes. Such a reprogramming can be performed *in vivo*, by contacting an mRNA-containing patch or other material to damaged cardiac tissue to facilitate cardiac regeneration. Such a process promotes cardiomyocyte genesis as opposed to fibrosis.

Mediation of cell death

[00772] In one embodiment, cell phenotype altering polynucleotides, primary constructs or mmRNA compositions can be used to induce apoptosis in a cell *(e.g.,* a cancer cell) by increasing the expression of a death receptor, a death receptor ligand or a combination thereof. This method can be used to induce cell death in any desired cell and has particular usefulness in the treatment of cancer where cells escape natural apoptotic signals.

[00773] Apoptosis can be induced by multiple independent signaling pathways that converge upon a final effector mechanism consisting of multiple interactions between several "death receptors" and their ligands, which belong to the tumor necrosis factor (TNF) receptor/ligand superfamily. The best-characterized death receptors are CD95 ("Fas"), TNFRI (p55), death receptor 3 (DR3 or Apo3/TRAMO), DR4 and DR5 (apo2-TRAIL-R2). The final effector mechanism of apoptosis may be the activation of a series of proteinases designated as caspases. The activation of these caspases results in the cleavage of a series of vital cellular proteins and cell death. The molecular mechanism of death receptors/ligands-induced apoptosis is well known in the art. For example, Fas/FasL-mediated apoptosis is induced by binding of three FasL molecules which induces trimerization of Fas receptor via C-terminus death domains (DDs), which in turn recruits an adapter protein FADD (Fas-associated protein with death domain) and Caspase-8. The oligomerization of this trimolecular complex, Fas/FAIDD/caspase-8, results in proteolytic cleavage of proenzyme caspase-8 into active caspase-8 that, in turn, initiates the apoptosis process by activating other downstream caspases through proteolysis, including caspase-3. Death ligands in general are apoptotic when formed into trimers or higher order of structures. As monomers, they may serve as antiapoptotic agents by competing with the trimers for binding to the death receptors.

[00774] In one embodiment, the cell phenotype altering polynucleotides, primary constructs or mmRNA composition encodes for a death receptor *(e.g.,* Fas, TRAIL,

TRAMO, TNFR, TLR etc). Cells made to express a death receptor by transfection of cell phenotype altering polynucleotides, primary constructs and mmRNA become susceptible to death induced by the ligand that activates that receptor. Similarly, cells made to express a death ligand, *e.g.,* on their surface, will induce death of cells with the receptor when the transfected cell contacts the target cell. In another embodiment, the polynucleotides, primary constructs and mmRNA composition encodes for a death receptor ligand *(e.g.,* FasL, TNF, etc). In another embodiment, the polynucleotides, primary constructs and mmRNA composition encodes a caspase *(e.g.,* caspase 3, caspase 8, caspase 9 etc). Where cancer cells often exhibit a failure to properly differentiate to a non-proliferative or controlled proliferative form, in another embodiment, the synthetic, cell phenotype altering polynucleotides, primary constructs and mmRNA composition encodes for both a death receptor and its appropriate activating ligand. In another embodiment, the synthetic, cell phenotype altering polynucleotides, primary constructs and mmRNA composition encodes for a differentiation factor that when expressed in the cancer cell, such as a cancer stem cell, will induce the cell to differentiate to a non-pathogenic or nonself-renewing phenotype *(e.g.,* reduced cell growth rate, reduced cell division etc) or to induce the cell to enter a dormant cell phase *(e.g.,* **Go** resting phase).

[00775] One of skill in the art will appreciate that the use of apoptosis-inducing techniques may require that the cell phenotype altering polynucleotides, primary constructs or mmRNA are appropriately targeted to *e.g.,* tumor cells to prevent unwanted wide-spread cell death. Thus, one can use a delivery mechanism *(e.g.,* attached ligand or antibody, targeted liposome etc) that recognizes a cancer antigen such that the cell phenotype altering polynucleotides, primary constructs or mmRNA are expressed only in cancer cells.

VI. Kits and Devices

Kits

[00776] The invention provides a variety of kits for conveniently and/or effectively carrying out methods of the present invention. Typically kits will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments, and contact cells and/or a population of cells at least once.

[00777] In one aspect, the present invention provides kits comprising the molecules (cell phenotype altering polynucleotides, primary constructs or mmRNA) of the invention. In one embodiment, the kit comprises one or more functional antibodies or function fragments thereof.

[00778] Said kits can be for cell phenotype altering protein production, comprising a first cell phenotype altering polynucleotide, primary construct or mmRNA comprising a translatable region. The kit may further comprise packaging and instructions and/or a delivery agent to form a formulation composition. The delivery agent may comprise a saline, a buffered solution, a lipidoid or any delivery agent disclosed herein.

[00779] In one embodiment, the buffer solution may include sodium chloride, calcium chloride, phosphate and/or EDTA. In another embodiment, the buffer solution may include, but is not limited to, saline, saline with 2mM calcium, 5% sucrose, 5% sucrose with 2mM calcium, 5% Mannitol, 5% Mannitol with 2mM calcium, Ringer's lactate, sodium chloride, sodium chloride with 2mM calcium. In a futher embodiment, the buffer solutions may be precipitated or it may be lyophilized. The amount of each component may be varied to enable consistent, reproducible higher concentration saline or simple buffer formulations. The components may also be varied in order to increase the stability of modified cell phenotype altering RNA in the buffer solution over a period of time and/or under a variety of conditions. In one aspect, the present invention provides kits for protein production, comprising: a cell phenotype altering polynucleotide, primary construct or mmRNA comprising a translatable region, provided in an amount effective to produce a desired amount of a cell phenotype altering protein encoded by the translatable region when introduced into a target cell; a second polynucleotide comprising an inhibitory nucleic acid, provided in an amount effective to substantially inhibit the innate immune response of the cell; and packaging and instructions.

[00780] In one aspect, the present invention provides kits for cell phenotype altering protein production, comprising a cell phenotype altering polynucleotide, primary construct or mmRNA comprising a translatable region, wherein the polynucleotide exhibits reduced degradation by a cellular nuclease, and packaging and instructions.

[00781] In one aspect, the present invention provides kits for protein production, comprising a cell phenotype altering polynucleotide, primary construct or mmRNA

comprising a translatable region, wherein the polynucleotide exhibits reduced degradation by a cellular nuclease, and a mammalian cell suitable for translation of the translatable region of the first nucleic acid.

Devices

[00782] The present invention provides for devices which may incorporate cell phenotype altering polynucleotides, primary constructs or mmRNA that encode cell phenotype altering polypeptides of interest. These devices contain in a stable formulation the reagents to synthesize a cell phenotype altering polynucleotide in a formulation available to be immediately delivered to a subject in need thereof, such as a human patient. Non-limiting examples of such a cell phenotype altering polypeptide of interest include reprogramming factors, differentiation factors and de-differentiation factors. .

[00783] In some embodiments the device is self-contained, and is optionally capable of wireless remote access to obtain instructions for synthesis and/or analysis of the generated cell phenotype altering polynucleotide, primary construct or mmRNA. The device is capable of mobile synthesis of at least one cell phenotype altering polynucleotide, primary construct or mmRNA and preferably an unlimited number of different cell phenotype altering polynucleotides, primary constructs or mmRNA. In certain embodiments, the device is capable of being transported by one or a small number of individuals. In other embodiments, the device is scaled to fit on a benchtop or desk. In other embodiments, the device is scaled to fit into a suitcase, backpack or similarly sized object. In another embodiment, the device may be a point of care or handheld device. In further embodiments, the device is scaled to fit into a vehicle, such as a car, truck or ambulance, or a military vehicle such as a tank or personnel carrier. The information necessary to generate a modified cell phenotype altering mRNA encoding a cell phenotype altering polypeptide of interest is present within a computer readable medium present in the device.

[00784] In one embodiment, a device may be used to assess levels of a protein which has been administered in the form of a cell phenotype altering polynucleotide, primary construct or mmRNA. The device may comprise a blood, urine or other biofluidic test.

[00785] In some embodiments, the device is capable of communication (e.g., wireless communication) with a database of nucleic acid and polypeptide sequences. The device

contains at least one sample block for insertion of one or more sample vessels. Such sample vessels are capable of accepting in liquid or other form any number of materials such as template DNA, nucleotides, enzymes, buffers, and other reagents. The sample vessels are also capable of being heated and cooled by contact with the sample block. The sample block is generally in communication with a device base with one or more electronic control units for the at least one sample block. The sample block preferably contains a heating module, such heating molecule capable of heating and/or cooling the sample vessels and contents thereof to temperatures between about -20C and above +100C. The device base is in communication with a voltage supply such as a battery or external voltage supply. The device also contains means for storing and distributing the materials for RNA synthesis.

[00786] Optionally, the sample block contains a module for separating the synthesized cell phenotype altering nucleic acids. Alternatively, the device contains a separation module operably linked to the sample block. Preferably the device contains a means for analysis of the synthesized cell phenotype altering nucleic acid. Such analysis includes sequence identity (demonstrated such as by hybridization), absence of non-desired sequences, measurement of integrity of synthesized cell phenotype altering mRNA (such has by microfluidic viscometry combined with spectrophotometry), and concentration and/or potency of modified cell phenotype altering RNA (such as by spectrophotometry).

[00787] In certain embodiments, the device is combined with a means for detection of pathogens present in a biological material obtained from a subject, e.g., the IBIS PLEX-ID system (Abbott, Abbott Park, IL) for microbial identification.

[00788] Suitable devices for use in delivering intradermal pharmaceutical compositions described herein include short needle devices such as those described in U.S. Patents 4,886,499; 5,190,521; 5,328,483; 5,527,288; 4,270,537; 5,015,235; 5,141,496; and 5,417,662. Intradermal compositions may be administered by devices which limit the effective penetration length of a needle into the skin, such as those described in PCT publication WO 99/34850 and functional equivalents thereof. Jet injection devices which deliver liquid compositions to the dermis via a liquid jet injector and/or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis are suitable. Jet injection devices are described, for example, in U.S. Patents 5,480,381;

5,599,302; 5,334,144; 5,993,412; 5,649,912; 5,569,189; 5,704,91 1; 5,383,851; 5,893,397; 5,466,220; 5,339,163; 5,312,335; 5,503,627; 5,064,413; 5,520,639; 4,596,556; 4,790,824; 4,941,880; 4,940,460; and PCT publications WO 97/37705 and WO 97/13537. Ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis are suitable. Alternatively or additionally, conventional syringes may be used in the classical mantoux method of intradermal administration.

[00789] In some embodiments, the device may be a pump or comprise a catheter for administration of compounds or compositions of the invention across the blood brain barrier. Such devices include but are not limited to a pressurized olfactory delivery device, iontophoresis devices, multi-layered microfluidic devices, and the like. Such devices may be portable or stationary. They may be implantable or externally tethered to the body or combinations thereof.

[00790] Devices for administration may be employed to deliver the cell phenotype altering polynucleotides, primary constructs or mmRNA of the present invention according to single, multi- or split-dosing regimens taught herein. Such devices are described below.

[00791] Method and devices known in the art for multi-administration to cells, organs and tissues are contemplated for use in conjunction with the methods and compositions disclosed herein as embodiments of the present invention. These include, for example, those methods and devices having multiple needles, hybrid devices employing for example lumens or catheters as well as devices utilizing heat, electric current or radiation driven mechanisms.

[00792] According to the present invention, these multi-administration devices may be utilized to deliver the single, multi- or split doses contemplated herein.

[00793] A method for delivering therapeutic agents to a solid tissue has been described by Bahrami et al. and is taught for example in US Patent Publication 201 10230839, the contents of which are incorporated herein by reference in their entirety. According to Bahrami, an array of needles is incorporated into a device which delivers a substantially equal amount of fluid at any location in said solid tissue along each needle's length.

[00794] A device for delivery of biological material across the biological tissue has been described by Kodgule et al. and is taught for example in US Patent Publication 201 10172610, the contents of which are incorporated herein by reference in their entirety. According to Kodgule, multiple hollow micro-needles made of one or more metals and having outer diameters from about 200 microns to about 350 microns and lengths of at least 100 microns are incorporated into the device which delivers peptides, proteins, carbohydrates, nucleic acid molecules, lipids and other pharmaceutically active ingredients or combinations thereof.

[00795] A delivery probe for delivering a therapeutic agent to a tissue has been described by Gunday et al. and is taught for example in US Patent Publication 201 10270184, the contents of which are incorporated herein by reference in their entirety. According to Gunday, multiple needles are incorporated into the device which moves the attached capsules between an activated position and an inactivated position to force the agent out of the capsules through the needles.

[00796] A multiple-injection medical apparatus has been described by Assaf and is taught for example in US Patent Publication 201 10218497, the contents of which are incorporated herein by reference in their entirety. According to Assaf, multiple needles are incorporated into the device which has a chamber connected to one or more of said needles and a means for continuously refilling the chamber with the medical fluid after each injection.

[00797] In one embodiment, the cell phenotype altering polynucleotide, primary construct, or mmRNA is administered subcutaneously or intramuscularly via at least 3 needles to three different, optionally adjacent, sites simultaneously, or within a 60 minutes period (e.g., administration to 4 ,5, 6, 7, 8, 9, or 10 sites simultaneously or within a 60 minute period). The split doses can be administered simultaneously to adjacent tissue using the devices described in U.S. Patent Publication Nos. 201 10230839 and 201 10218497, each of which is incorporated herein by reference.

[00798] An at least partially implantable system for injecting a substance into a patient's body, in particular a penis erection stimulation system has been described by Forsell and is taught for example in US Patent Publication 201 10196198, the contents of which are incorporated herein by reference in their entirety. According to Forsell,

multiple needles are incorporated into the device which is implanted along with one or more housings adjacent the patient's left and right corpora cavernosa. A reservoir and a pump are also implanted to supply drugs through the needles.

[00799] A method for the transdermal delivery of a therapeutic effective amount of iron has been described by Berenson and is taught for example in US Patent Publication 20100130910, the contents of which are incorporated herein by reference in their entirety. According to Berenson, multiple needles may be used to create multiple micro channels in stratum corneum to enhance transdermal delivery of the ionic iron on an iontophoretic patch.

[00800] A method for delivery of biological material across the biological tissue has been described by Kodgule et al and is taught for example in US Patent Publication 201 10196308, the contents of which are incorporated herein by reference in their entirety. According to Kodgule, multiple biodegradable microneedles containing a therapeutic active ingredient are incorporated in a device which delivers proteins, carbohydrates, nucleic acid molecules, lipids and other pharmaceutically active ingredients or combinations thereof.

[00801] A transdermal patch comprising a botulinum toxin composition has been described by Donovan and is taught for example in US Patent Publication 20080220020, the contents of which are incorporated herein by reference in their entirety. According to Donovan, multiple needles are incorporated into the patch which delivers botulinum toxin under stratum corneum through said needles which project through the stratum corneum of the skin without rupturing a blood vessel.

[00802] A small, disposable drug reservoir, or patch pump, which can hold approximately 0.2 to 15 mL of liquid formulations can be placed on the skin and deliver the formulation continuously subcutaneously using a small bore needed (e.g., 26 to 34 gauge). As non-limiting examples, the patch pump may be 50 mm by 76 mm by 20 mm spring loaded having a 30 to 34 gauge needle (BD™ Microinfuser, Franklin Lakes NJ), 4 1 mm by 62 mm by 17 mm with a 2 mL reservoir used for drug delivery such as insulin (OMNIPOD®, Insulet Corporation Bedford, MA), or 43-60 mm diameter, 10 mm thick with a 0.5 to 10 mL reservoir (PATCHPUMP®, SteadyMed Therapeutics, San Francisco, CA). Further, the patch pump may be battery powered and/or rechargeable.

[00803] A cryoprobe for administration of an active agent to a location of cryogenic treatment has been described by Toubia and is taught for example in US Patent Publication 20080140061, the contents of which are incorporated herein by reference in their entirety. According to Toubia, multiple needles are incorporated into the probe which receives the active agent into a chamber and administers the agent to the tissue.

[00804] A method for treating or preventing inflammation or promoting healthy joints has been described by Stock et al and is taught for example in US Patent Publication 20090155186, the contents of which are incorporated herein by reference in their entirety. According to Stock, multiple needles are incorporated in a device which administers compositions containing signal transduction modulator compounds.

[00805] A multi-site injection system has been described by Kimmell et al. and is taught for example in US Patent Publication 20100256594, the contents of which are incorporated herein by reference in their entirety. According to Kimmell, multiple needles are incorporated into a device which delivers a medication into a stratum corneum through the needles.

[00806] A method for delivering interferons to the intradermal compartment has been described by Dekker et al. and is taught for example in US Patent Publication 20050181033, the contents of which are incorporated herein by reference in their entirety. According to Dekker, multiple needles having an outlet with an exposed height between 0 and 1 mm are incorporated into a device which improves pharmacokinetics and bioavailability by delivering the substance at a depth between 0.3 mm and 2 mm.

[00807] A method for delivering genes, enzymes and biological agents to tissue cells has described by Desai and is taught for example in US Patent Publication 20030073908, the contents of which are incorporated herein by reference in their entirety. According to Desai, multiple needles are incorporated into a device which is inserted into a body and delivers a medication fluid through said needles.

[00808] A method for treating cardiac arrhythmias with fibroblast cells has been described by Lee et al and is taught for example in US Patent Publication 20040005295, the contents of which are incorporated herein by reference in their entirety. According to Lee, multiple needles are incorporated into the device which delivers fibroblast cells into the local region of the tissue.

[00809] A method using a magnetically controlled pump for treating a brain tumor has been described by Shachar et al. and is taught for example in US Patent 7799012 (method) and 7799016 (device), the contents of which are incorporated herein by reference in their entirety. According Shachar, multiple needles were incorporated into the pump which pushes a medicating agent through the needles at a controlled rate.

[00810] Methods of treating functional disorders of the bladder in mammalian females have been described by Versi et al. and are taught for example in US Patent 8,029,496, the contents of which are incorporated herein by reference in their entirety. According to Versi, an array of micro-needles is incorporated into a device which delivers a therapeutic agent through the needles directly into the trigone of the bladder.

[00811] A micro-needle transdermal transport device has been described by Angel et al and is taught for example in US Patent 7,364,568, the contents of which are incorporated herein by reference in their entirety. According to Angel, multiple needles are incorporated into the device which transports a substance into a body surface through the needles which are inserted into the surface from different directions. The micro-needle transdermal transport device may be a solid micro-needle system or a hollow micro-needle system. As a non-limiting example, the solid micro-needle system may have up to a 0.5 mg capacity, with 300-1500 solid micro-needles per cm^2 about 150-700 μιη tall coated with a drug. The micro-needles penetrate the stratum corneum and remain in the skin for short duration (e.g., 20 seconds to 15 minutes). In another example, the hollow micro-needle system has up to a 3 mL capacity to deliver liquid formulations using 15-20 microneedles per cm2 being approximately 950 μιη tall. The micro-needles penetrate the skin to allow the liquid formulations to flow from the device into the skin. The hollow micro-needle system may be worn from 1 to 30 minutes depending on the formulation volume and viscocity.

[00812] A device for subcutaneous infusion has been described by Dalton et al and is taught for example in US Patent 7,150,726, the contents of which are incorporated herein by reference in their entirety. According to Dalton, multiple needles are incorporated into the device which delivers fluid through the needles into a subcutaneous tissue.

[00813] A device and a method for intradermal delivery of vaccines and gene therapeutic agents through microcannula have been described by Mikszta et al. and are

taught for example in US Patent 7,473,247, the contents of which are incorporated herein by reference in their entirety. According to Mitszta, at least one hollow micro-needle is incorporated into the device which delivers the vaccines to the subject's skin to a depth of between 0.025 mm and 2 mm.

[00814] A method of delivering insulin has been described by Pettis et al and is taught for example in US Patent 7,722,595, the contents of which are incorporated herein by reference in their entirety. According to Pettis, two needles are incorporated into a device wherein both needles insert essentially simultaneously into the skin with the first at a depth of less than 2.5 mm to deliver insulin to intradermal compartment and the second at a depth of greater than 2.5 mm and less than 5.0 mm to deliver insulin to subcutaneous compartment.

[00815] Cutaneous injection delivery under suction has been described by Kochamba et al. and is taught for example in US Patent 6,896,666, the contents of which are incorporated herein by reference in their entirety. According to Kochamba, multiple needles in relative adjacency with each other are incorporated into a device which injects a fluid below the cutaneous layer.

[00816] A device for withdrawing or delivering a substance through the skin has been described by Down et al and is taught for example in US Patent 6,607,513, the contents of which are incorporated herein by reference in their entirety. According to Down, multiple skin penetrating members which are incorporated into the device have lengths of about 100 microns to about 2000 microns and are about 30 to 50 gauge.

[00817] A device for delivering a substance to the skin has been described by Palmer et al and is taught for example in US Patent 6,537,242, the contents of which are incorporated herein by reference in their entirety. According to Palmer, an array of micro-needles is incorporated into the device which uses a stretching assembly to enhance the contact of the needles with the skin and provides a more uniform delivery of the substance.

[00818] A perfusion device for localized drug delivery has been described by Zamoyski and is taught for example in US Patent 6,468,247, the contents of which are incorporated herein by reference in their entirety. According to Zamoyski, multiple hypodermic

needles are incorporated into the device which injects the contents of the hypodermics into a tissue as said hypodermics are being retracted.

[00819] A method for enhanced transport of drugs and biological molecules across tissue by improving the interaction between micro-needles and human skin has been described by Prausnitz et al. and is taught for example in US Patent 6,743,21 1, the contents of which are incorporated herein by reference in their entirety. According to Prausnitz, multiple micro-needles are incorporated into a device which is able to present a more rigid and less deformable surface to which the micro-needles are applied.

[00820] A device for intraorgan administration of medicinal agents has been described by Ting et al and is taught for example in US Patent 6,077,251, the contents of which are incorporated herein by reference in their entirety. According to Ting, multiple needles having side openings for enhanced administration are incorporated into a device which by extending and retracting said needles from and into the needle chamber forces a medicinal agent from a reservoir into said needles and injects said medicinal agent into a target organ.

[00821] A multiple needle holder and a subcutaneous multiple channel infusion port has been described by Brown and is taught for example in US Patent 4,695,273, the contents of which are incorporated herein by reference in their entirety. According to Brown, multiple needles on the needle holder are inserted through the septum of the infusion port and communicate with isolated chambers in said infusion port.

[00822] A dual hypodermic syringe has been described by Horn and is taught for example in US Patent 3,552,394, the contents of which are incorporated herein by reference in their entirety. According to Horn, two needles incorporated into the device are spaced apart less than 68 mm and may be of different styles and lengths, thus enabling injections to be made to different depths.

[00823] A syringe with multiple needles and multiple fluid compartments has been described by Hershberg and is taught for example in US Patent 3,572,336, the contents of which are incorporated herein by reference in their entirety. According to Hershberg, multiple needles are incorporated into the syringe which has multiple fluid compartments and is capable of simultaneously administering incompatible drugs which are not able to be mixed for one injection.

[00824] A surgical instrument for intradermal injection of fluids has been described by Eliscu et al. and is taught for example in US Patent 2,588,623, the contents of which are incorporated herein by reference in their entirety. According to Eliscu, multiple needles are incorporated into the instrument which injects fluids intradermally with a wider disperse.

[00825] An apparatus for simultaneous delivery of a substance to multiple breast milk ducts has been described by Hung and is taught for example in EP 1818017, the contents of which are incorporated herein by reference in their entirety. According to Hung, multiple lumens are incorporated into the device which inserts though the orifices of the ductal networks and delivers a fluid to the ductal networks.

[00826] A catheter for introduction of medications to the tissue of a heart or other organs has been described by Tkebuchava and is taught for example in WO2006138109, the contents of which are incorporated herein by reference in their entirety. According to Tkebuchava, two curved needles are incorporated which enter the organ wall in a flattened trajectory.

[00827] Devices for delivering medical agents have been described by Mckay et al. and are taught for example in WO20061 18804, the content of which are incorporated herein by reference in their entirety. According to Mckay, multiple needles with multiple orifices on each needle are incorporated into the devices to facilitate regional delivery to a tissue, such as the interior disc space of a spinal disc.

[00828] A method for directly delivering an immunomodulatory substance into an intradermal space within a mammalian skin has been described by Pettis and is taught for example in WO2004020014, the contents of which are incorporated herein by reference in their entirety. According to Pettis, multiple needles are incorporated into a device which delivers the substance through the needles to a depth between 0.3 mm and 2 mm.

[00829] Methods and devices for administration of substances into at least two compartments in skin for systemic absorption and improved pharmacokinetics have been described by Pettis et al. and are taught for example in WO2003094995, the contents of which are incorporated herein by reference in their entirety. According to Pettis, multiple needles having lengths between about 300 μιη and about 5 mm are incorporated into a

device which delivers to intradermal and subcutaneous tissue compartments simultaneously.

[00830] A drug delivery device with needles and a roller has been described by Zimmerman et al. and is taught for example in WO2012006259, the contents of which are incorporated herein by reference in their entirety. According to Zimmerman, multiple hollow needles positioned in a roller are incorporated into the device which delivers the content in a reservoir through the needles as the roller rotates.

Methods and Devices utilizing catheters and/or lumens

[00831] Methods and devices using catheters and lumens may be employed to administer the cell phenotype altering mmRNA of the present invention on a single, multi- or split dosing schedule. Such methods and devices are described below.

[00832] A catheter-based delivery of skeletal myoblasts to the myocardium of damaged hearts has been described by Jacoby et al and is taught for example in US Patent Publication 20060263338, the contents of which are incorporated herein by reference in their entirety. According to Jacoby, multiple needles are incorporated into the device at least part of which is inserted into a blood vessel and delivers the cell composition through the needles into the localized region of the subject's heart.

[00833] An apparatus for treating asthma using neurotoxin has been described by Deem et al and is taught for example in US Patent Publication 20060225742, the contents of which are incorporated herein by reference in their entirety. According to Deem, multiple needles are incorporated into the device which delivers neurotoxin through the needles into the bronchial tissue.

[00834] A method for administering multiple-component therapies has been described by Nayak and is taught for example in US Patent 7,699,803, the contents of which are incorporated herein by reference in their entirety. According to Nayak, multiple injection cannulas may be incorporated into a device wherein depth slots may be included for controlling the depth at which the therapeutic substance is delivered within the tissue.

[00835] A surgical device for ablating a channel and delivering at least one therapeutic agent into a desired region of the tissue has been described by Mclntyre et al and is taught for example in US Patent 8,012,096, the contents of which are incorporated herein by reference in their entirety. According to Mclntyre, multiple needles are incorporated

into the device which dispenses a therapeutic agent into a region of tissue surrounding the channel and is particularly well suited for transmyocardial revascularization operations.

[00836] Methods of treating functional disorders of the bladder in mammalian females have been described by Versi et al and are taught for example in US Patent 8,029,496, the contents of which are incorporated herein by reference in their entirety. According to Versi, an array of micro-needles is incorporated into a device which delivers a therapeutic agent through the needles directly into the trigone of the bladder.

[00837] A device and a method for delivering fluid into a flexible biological barrier have been described by Yeshurun et al. and are taught for example in US Patent 7,998,119 (device) and 8,007,466 (method), the contents of which are incorporated herein by reference in their entirety. According to Yeshurun, the micro-needles on the device penetrate and extend into the flexible biological barrier and fluid is injected through the bore of the hollow micro-needles.

[00838] A method for epicardially injecting a substance into an area of tissue of a heart having an epicardial surface and disposed within a torso has been described by Bonner et al and is taught for example in US Patent 7,628,780, the contents of which are incorporated herein by reference in their entirety. According to Bonner, the devices have elongate shafts and distal injection heads for driving needles into tissue and injecting medical agents into the tissue through the needles.

[00839] A device for sealing a puncture has been described by Nielsen et al and is taught for example in US Patent 7,972,358, the contents of which are incorporated herein by reference in their entirety. According to Nielsen, multiple needles are incorporated into the device which delivers a closure agent into the tissue surrounding the puncture tract.

[00840] A method for myogenesis and angiogenesis has been described by Chiu et al. and is taught for example in US Patent 6,551,338, the contents of which are incorporated herein by reference in their entirety. According to Chiu, 5 to 15 needles having a maximum diameter of at least 1.25 mm and a length effective to provide a puncture depth of 6 to 20 mm are incorporated into a device which inserts into proximity with a myocardium and supplies an exogeneous angiogenic or myogenic factor to said myocardium through the conduits which are in at least some of said needles.

[00841] A method for the treatment of prostate tissue has been described by Bolmsj et al. and is taught for example in US Patent 6,524,270, the contents of which are incorporated herein by reference in their entirety. According to Bolmsj, a device comprising a catheter which is inserted through the urethra has at least one hollow tip extendible into the surrounding prostate tissue. An astringent and analgesic medicine is administered through said tip into said prostate tissue.

[00842] A method for infusing fluids to an intraosseous site has been described by Findlay et al. and is taught for example in US Patent 6,761,726, the contents of which are incorporated herein by reference in their entirety. According to Findlay, multiple needles are incorporated into a device which is capable of penetrating a hard shell of material covered by a layer of soft material and delivers a fluid at a predetermined distance below said hard shell of material.

[00843] A device for injecting medications into a vessel wall has been described by Vigil et al. and is taught for example in US Patent 5,713,863, the contents of which are incorporated herein by reference in their entirety. According to Vigil, multiple injectors are mounted on each of the flexible tubes in the device which introduces a medication fluid through a multi-lumen catheter, into said flexible tubes and out of said injectors for infusion into the vessel wall.

[00844] A catheter for delivering therapeutic and/or diagnostic agents to the tissue surrounding a bodily passageway has been described by Faxon et al. and is taught for example in US Patent 5,464,395, the contents of which are incorporated herein by reference in their entirety. According to Faxon, at least one needle cannula is incorporated into the catheter which delivers the desired agents to the tissue through said needles which project outboard of the catheter.

[00845] Balloon catheters for delivering therapeutic agents have been described by Orr and are taught for example in WO2010024871, the contents of which are incorporated herein by reference in their entirety. According to Orr, multiple needles are incorporated into the devices which deliver the therapeutic agents to different depths within the tissue.

Methods and Devices utilizing electrical current

[00846] Methods and devices utilizing electric current may be employed to deliver the cell phenotype altering mmRNA of the present invention according to the single, multi- or split dosing regimens taught herein. Such methods and devices are described below.

[00847] An electro collagen induction therapy device has been described by Marquez and is taught for example in US Patent Publication 20090137945, the contents of which are incorporated herein by reference in their entirety. According to Marquez, multiple needles are incorporated into the device which repeatedly pierce the skin and draw in the skin a portion of the substance which is applied to the skin first.

[00848] An electrokinetic system has been described by Etheredge et al. and is taught for example in US Patent Publication 20070185432, the contents of which are incorporated herein by reference in their entirety. According to Etheredge, micro-needles are incorporated into a device which drives by an electrical current the medication through the needles into the targeted treatment site.

[00849] An iontophoresis device has been described by Matsumura et al. and is taught for example in US Patent 7,437,189, the contents of which are incorporated herein by reference in their entirety. According to Matsumura, multiple needles are incorporated into the device which is capable of delivering ionizable drug into a living body at higher speed or with higher efficiency.

[00850] Intradermal delivery of biologically active agents by needle-free injection and electroporation has been described by Hoffmann et al and is taught for example in US Patent 7,171,264, the contents of which are incorporated herein by reference in their entirety. According to Hoffmann, one or more needle-free injectors are incorporated into an electroporation device and the combination of needle-free injection and electroporation is sufficient to introduce the agent into cells in skin, muscle or mucosa.

[00851] A method for electropermeabilization-mediated intracellular delivery has been described by Lundkvist et al. and is taught for example in US Patent 6,625,486, the contents of which are incorporated herein by reference in their entirety. According to Lundkvist, a pair of needle electrodes is incorporated into a catheter. Said catheter is positioned into a body lumen followed by extending said needle electrodes to penetrate into the tissue surrounding said lumen. Then the device introduces an agent through at least one of said needle electrodes and applies electric field by said pair of needle

electrodes to allow said agent pass through the cell membranes into the cells at the treatment site.

[00852] A delivery system for transdermal immunization has been described by Levin et al. and is taught for example in WO2006003659, the contents of which are incorporated herein by reference in their entirety. According to Levin, multiple electrodes are incorporated into the device which applies electrical energy between the electrodes to generate micro channels in the skin to facilitate transdermal delivery.

[00853] A method for delivering RF energy into skin has been described by Schomacker and is taught for example in WO201 1163264, the contents of which are incorporated herein by reference in their entirety. According to Schomacker, multiple needles are incorporated into a device which applies vacuum to draw skin into contact with a plate so that needles insert into skin through the holes on the plate and deliver RF energy.

VII. Definitions

[00854] At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual subcombination of the members of such groups and ranges. For example, the term "Ci-6 alkyl" is specifically intended to individually disclose methyl, ethyl, C_3 alkyl, C_4 alkyl, C_5 alkyl, and C_6 alkyl.

[00855] *About:* As used herein, the term "about" means +/- 10% of the recited value.

[00856] *Administered in combination:* As used herein, the term "administered in combination" or "combined administration" means that two or more agents are administered to a subject at the same time or within an interval such that there may be an overlap of an effect of each agent on the patient. In some embodiments, they are administered within about 60, 30, 15, 10, 5, or 1 minute of one another. In some embodiments, the administrations of the agents are spaced sufficiently closely together such that a combinatorial *{e.g.,* a synergistic) effect is achieved.

[00857] *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, or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and worms. In some embodiments, the animal is a transgenic animal, genetically-engineered animal, or a clone.

[00858] *Antigens of interest or desired antigens:* As used herein, the terms "antigens of interest" or "desired antigens" include those proteins and other biomolecules provided herein that are immunospecifically bound by the antibodies and fragments, mutants, variants, and alterations thereof described herein. Examples of antigens of interest include, but are not limited to, insulin, insulin-like growth factor, hGH, tPA, cytokines, such as interleukins (IL), e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-1 1, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, interferon (IFN) alpha, IFN beta, IFN gamma, IFN omega or IFN tau, tumor necrosis factor (TNF), such as TNF alpha and TNF beta, TNF gamma, TRAIL; G-CSF, GM-CSF, M-CSF, MCP-1 and VEGF.

[00859] *Approximately:* 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).ASCL1:* As used herein, the term "ASCL1" refers to the achaete-scute complex homolog 1 protein including any variants thereof.

[00860] *Associated with:* As used herein, the terms "associated with," "conjugated," "linked," "attached," and "tethered," when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which the structure is used, *e.g.*, physiological conditions. An "association" need not be strictly through direct covalent chemical bonding. It may also suggest ionic or hydrogen bonding or a hybridization based connectivity sufficiently stable such that the "associated" entities remain physically associated.i?Z¾VF: As used

herein, the term "BDNF" refers to brain-derived neurotrophic factor protein including any variants thereof.

[00861] *Bifunctional:* As used herein, the term "bifunctional" refers to any substance, molecule or moiety which is capable of or maintains at least two functions. The functions may effect the same outcome or a different outcome. The structure that produces the function may be the same or different. For example, bifunctional modified RNAs of the present invention may encode a cytotoxic peptide (a first function) while those nucleosides which comprise the encoding RNA are, in and of themselves, cytotoxic (second function). In this example, delivery of the bifunctional modified RNA to a cancer cell would produce not only a peptide or protein molecule which may ameliorate or treat the cancer but would also deliver a cytotoxic payload of nucleosides to the cell should degradation, instead of translation of the modified RNA, occur.

[00862] *Biocompatible:* As used herein, the term "biocompatible" means compatible with living cells, tissues, organs or systems posing little to no risk of injury, toxicity or rejection by the immune system.

[00863] *Biodegradable:* As used herein, the term "biodegradable" means capable of being broken down into innocuous products by the action of living things.

[00864] *Biologically active:* As used herein, the phrase "biologically active" refers to a characteristic of any substance that has activity in a biological system and/or organism. For instance, a substance that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. In particular embodiments, a cell phenotype altering polynucleotide, primary construct or mmRNA of the present invention may be considered biologically active if even a portion of the cell phenotype altering polynucleotide, primary construct or mmRNA is biologically active or mimics an activity considered biologically relevant. *BRN2:* As used herein, the term "BRN2" refers to the POU class 3 homeobox 2 protein including any variants thereof. BRN2 is also known in the art as OTF7 and POU domain class 3, transcription factor 2 (POU3F2).

[00865] *Cancer stem cells:* As used herein, "cancer stem cells" are cells that can undergo self-renewal and/or abnormal proliferation and differentiation to form a tumor.

[00866] *CEBP-alpha :* As used herein, the term "CEBP-alpha" refers to CCAAT/enhancer binding protein (C/EBP), alpha protein including any variants thereof.

[00867] *Chemical terms:* The following provides the definition of various chemical terms from "acyl" to "thiol."

[00868] The term "acyl," as used herein, represents a hydrogen or an alkyl group (e.g., a haloalkyl group), as defined herein, that is attached to the parent molecular group through a carbonyl group, as defined herein, and is exemplified by formyl (i.e., a carboxyaldehyde group), acetyl, propionyl, butanoyl and the like. Exemplary unsubstituted acyl groups include from 1 to 7, from 1 to 11, or from 1 to 21 carbons. In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein.

[00869] The term "acylamino," as used herein, represents an acyl group, as defined herein, attached to the parent molecular group though an amino group, as defined herein (i.e., -N(R^{N1})-C (0)-R, where R is H or an optionally substituted Ci-6, Ci-io, or Ci-20 alkyl group and R^{N1} is as defined herein). Exemplary unsubstituted acylamino groups include from 1 to 41 carbons (e.g., from 1 to 7, from 1 to 13, from 1 to 21, from 2 to 7, from 2 to 13, from 2 to 21, or from 2 to 41 carbons). In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein, and/or the amino group is -NH_2 or -NHR^{N1}, wherein R^{N1} is, independently, OH, NO2, NH_2, NR^{N2}_2, $S0_2OR^{N2}$, $S0_2R^{N2}$, SOR^{N2}, alkyl, or aryl, and each R^{N2} can be H, alkyl, or aryl.

[00870] The term "acyloxy," as used herein, represents an acyl group, as defined herein, attached to the parent molecular group though an oxygen atom (i.e., -0-C(0)-R, where R is H or an optionally substituted Ci-6, Ci-10, or Ci-20 alkyl group). Exemplary unsubstituted acyloxy groups include from 1 to 21 carbons (e.g., from 1 to 7 or from 1 to 11 carbons). In some embodiments, the alkyl group is further substituted with 1, 2, 3, or 4 substituents as described herein, and/or the amino group is -NH_2 or -NHR^{N1}, wherein R^{N1} is, independently, OH, NO2, NH_2, NR^{N2}_2, $S0_2OR^{N2}$, $S0_2R^{N2}$, SOR^{N2}, alkyl, or aryl, and each R^{N2} can be H, alkyl, or aryl.

[00871] The term "alkaryl," as used herein, represents an aryl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted alkaryl groups are from 7 to 30 carbons (e.g., from 7 to 16 or from 7 to 20 carbons, such as Ci-6 alk-C_6-io aryl, Ci-10 alk-C_6-io aryl, or Ci-20 alk-C_6-io aryl). In some embodiments, the alkylene and the aryl each can be further substituted

with 1, 2, 3, or 4 substituent groups as defined herein for the respective groups. Other groups preceded by the prefix "alk-" are defined in the same manner, where "alk" refers to a Ci-6 alkylene, unless otherwise noted, and the attached chemical structure is as defined herein.

[00872] The term "alkcycloalkyl" represents a cycloalkyl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein (e.g., an alkylene group of from 1 to 4, from 1 to 6, from 1 to 10, or form 1 to 20 carbons). In some embodiments, the alkylene and the cycloalkyl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group.

[00873] The term "alkenyl," as used herein, represents monovalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds and is exemplified by ethenyl, 1-propenyl, 2-propenyl, 2-methyl-l-propenyl, 1-butenyl, 2-butenyl, and the like. Alkenyls include both cis and trans isomers. Alkenyl groups may be optionally substituted with 1, 2, 3, or 4 substituent groups that are selected, independently, from amino, aryl, cycloalkyl, or heterocyclyl (e.g., heteroaryl), as defined herein, or any of the exemplary alkyl substituent groups described herein.

[00874] The term "alkenyloxy" represents a chemical substituent of formula -OR, where R is a C2-20 alkenyl group (e.g., C2-6 or C2-10 alkenyl), unless otherwise specified. Exemplary alkenyloxy groups include ethenyloxy, propenyloxy, and the like. In some embodiments, the alkenyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein (e.g., a hydroxy group).

[00875] The term "alkheteroaryl" refers to a heteroaryl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted alkheteroaryl groups are from 2 to 32 carbons (e.g., from 2 to 22, from 2 to 18, from 2 to 17, from 2 to 16, from 3 to 15, from 2 to 14, from 2 to 13, or from 2 to 12 carbons, such as Ci-6 alk-Ci-12 heteroaryl, Ci-10 alk-Ci-12 heteroaryl, or Ci-20 alk-Ci-12 heteroaryl). In some embodiments, the alkylene and the heteroaryl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group. Alkheteroaryl groups are a subset of alkheterocyclyl groups.

[00876] The term "alkheterocyclyl" represents a heterocyclyl group, as defined herein, attached to the parent molecular group through an alkylene group, as defined herein. Exemplary unsubstituted alkheterocyclyl groups are from 2 to 32 carbons (e.g., from 2 to 22, from 2 to 18, from 2 to 17, from 2 to 16, from 3 to 15, from 2 to 14, from 2 to 13, or from 2 to 12 carbons, such as **Ci-6** alk-**Ci**-12 heterocyclyl, **Ci-10** alk-**Ci**-12 heterocyclyl, or **Ci-20** alk-**Ci**-12 heterocyclyl). In some embodiments, the alkylene and the heterocyclyl each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group.

[00877] The term "alkoxy" represents a chemical substituent of formula -OR, where R is a **Ci-20** alkyl group (e.g., **Ci-6** or **Ci-10** alkyl), unless otherwise specified. Exemplary alkoxy groups include methoxy, ethoxy, propoxy (e.g., n-propoxy and isopropoxy), t-butoxy, and the like. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein (e.g., hydroxy or alkoxy).

[00878] The term "alkoxyalkoxy" represents an alkoxy group that is substituted with an alkoxy group. Exemplary unsubstituted alkoxyalkoxy groups include between 2 to 40 carbons (e.g., from 2 to 12 or from 2 to 20 carbons, such as **Ci-6** alkoxy-**Ci**-6 alkoxy, **Ci-10** alkoxy-**Ci**-10 alkoxy, or **Ci-20** alkoxy-**Ci**-20 alkoxy). In some embodiments, the each alkoxy group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00879] The term "alkoxyalkyl" represents an alkyl group that is substituted with an alkoxy group. Exemplary unsubstituted alkoxyalkyl groups include between 2 to 40 carbons (e.g., from 2 to 12 or from 2 to 20 carbons, such as **Ci-6** alkoxy-**Ci**-6 alkyl, **Ci-10** alkoxy-**Ci**-10 alkyl, or **Ci-20** alkoxy-**Ci**-20 alkyl). In some embodiments, the alkyl and the alkoxy each can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for the respective group.

[00880] The term "alkoxycarbonyl," as used herein, represents an alkoxy, as defined herein, attached to the parent molecular group through a carbonyl atom (e.g., -**C**(0)-OR, where R is H or an optionally substituted **Ci-6**, **Ci**-10, or **Ci-20** alkyl group). Exemplary unsubstituted alkoxycarbonyl include from 1 to 21 carbons (e.g., from 1 to 11 or from 1 to 7 carbons). In some embodiments, the alkoxy group is further substituted with 1, 2, 3, or 4 substituents as described herein.

[00881] The term "alkoxycarbonylalkoxy," as used herein, represents an alkoxy group, as defined herein, that is substituted with an alkoxycarbonyl group, as defined herein (e.g., -0-alkyl-C(0)-OR, where R is an optionally substituted Ci-6, Ci-io, or Ci-20 alkyl group). Exemplary unsubstituted alkoxycarbonylalkoxy include from 3 to 41 carbons (e.g., from 3 to 10, from 3 to 13, from 3 to 17, from 3 to 21, or from 3 to 31 carbons, such as Ci-6 alkoxycarbonyl-Ci-6 alkoxy, Ci-10 alkoxycarbonyl-Ci-10 alkoxy, or Ci-20 alkoxycarbonyl-Ci-20 alkoxy). In some embodiments, each alkoxy group is further independently substituted with 1, 2, 3, or 4 substituents, as described herein (e.g., a hydroxy group).

[00882] The term "alkoxycarbonylalkyl," as used herein, represents an alkyl group, as defined herein, that is substituted with an alkoxycarbonyl group, as defined herein (e.g., -alkyl-C(0)-OR, where R is an optionally substituted Ci-20, Ci-10, or Ci-6 alkyl group). Exemplary unsubstituted alkoxycarbonylalkyl include from 3 to 41 carbons (e.g., from 3 to 10, from 3 to 13, from 3 to 17, from 3 to 21, or from 3 to 31 carbons, such as Ci-6 alkoxycarbonyl-Ci-6 alkyl, Ci-10 alkoxycarbonyl-Ci-10 alkyl, or Ci-20 alkoxycarbonyl-Ci-20 alkyl). In some embodiments, each alkyl and alkoxy group is further independently substituted with 1, 2, 3, or 4 substituents as described herein (e.g., a hydroxy group).

[00883] The term "alkyl," as used herein, is inclusive of both straight chain and branched chain saturated groups from 1 to 20 carbons (e.g., from 1 to 10 or from 1 to 6), unless otherwise specified. Alkyl groups are exemplified by methyl, ethyl, n- and iso-propyl, n-, sec-, iso- and tert-butyl, neopentyl, and the like, and may be optionally substituted with one, two, three, or, in the case of alkyl groups of two carbons or more, four substituents independently selected from the group consisting of: (1) Ci-6 alkoxy; (2) Ci-6 alkylsulfinyl; (3) amino, as defined herein (e.g., unsubstituted amino (i.e., -NH2) or a substituted amino (i.e., -N(R N 1)2, where R^{N1} is as defined for amino); (4) C_{6}-io aryl-Ci-6 alkoxy; (5) azido; (6) halo; (7) (C2-9 heterocyclyl)oxy; (8) hydroxy; (9) nitro; (10) oxo (e.g., carboxyaldehyde or acyl); (11) C1-7 spirocyclyl; (12) thioalkoxy; (13) thiol; (14) -CC"2$R^{A'}$, where $R^{A'}$ is selected from the group consisting of (a) Ci-20 alkyl (e.g., Ci-6 alkyl), (b) C2-20 alkenyl (e.g., C2-6 alkenyl), (c) C_{6}-io aryl, (d) hydrogen, (e) Ci-6 alk-C_{6}-io aryl, (f) amino-Ci-20 alkyl, (g) polyethylene glycol of -(CH2)s2 (OCH_2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3,

independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl, and (h) amino-polyethylene glycol of -NR^{N1}(CH2)s2 (CH$_2$CH$_2$0)si(CH2)s3NR^{N1}, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl; (15) -C(0)NR $^{B'}$R$^{c'}$, where each of R$^{B'}$ and R$^{c'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) Ci-6 alkyl, (c) Ce-io aryl, and (d) Ci-e alk-Ce-io aryl; (16) -S0 $_2$R$^{D'}$, where R$^{D'}$ is selected from the group consisting of (a) Ci-6 alkyl, (b) C$_6$-io aryl, (c) Ci-6 alk-C$_6$-io aryl, and (d) hydroxy; (17) -S0 $_2$NR$^{E'}$R$^{F'}$, where each of R$^{E'}$ and R$^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) Ci-6 alkyl, (c) C$_6$-io aryl and (d) Ci-6 alk-C$_6$-10 aryl; (18) -C(0)R $^{G'}$, where R$^{G'}$ is selected from the group consisting of (a) Ci-20 alkyl (e.g., Ci-6 alkyl), (b) C2-20 alkenyl (e.g., C2-6 alkenyl), (c) C$_6$-io aryl, (d) hydrogen, (e) Ci-6 alk-C$_6$-io aryl, (f) amino-Ci-20 alkyl, (g) polyethylene glycol of -(CH2)s2 (OCH$_2$CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl, and (h) amino-polyethylene glycol of -NR^{N1}(CH$_2$)s2(CH2CH20)si(CH$_2$)s3NR^{N1}, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl; (19) -NR$^{H'}$C(0)R $^{I'}$, wherein R$^{H'}$ is selected from the group consisting of (al) hydrogen and (bl) Ci-6 alkyl, and R$^{1'}$ is selected from the group consisting of (a2) Ci-20 alkyl (e.g., Ci-6 alkyl), (b2) C2-20 alkenyl (e.g., C2-6 alkenyl), (c2) C6-10 aryl, (d2) hydrogen, (e2) Ci-6 alk-C$_6$-io aryl, (f2) amino-Ci-20 alkyl, (g2) polyethylene glycol of -(CH2)s2(OCH2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl, and (h2) amino-polyethylene glycol of -NR^{N1}(CH$_2$)s2(CH2CH20)si(CH$_2$)s3NR^{N1}, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g.,

from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl; (20) $-NR^{J'}C(0)OR^{K'}$, wherein $R^{J'}$ is selected from the group consisting of (al) hydrogen and (bl) Ci-6 alkyl, and $R^{K'}$ is selected from the group consisting of (a2) Ci-20 alkyl (e.g., Ci-6 alkyl), (b2) C2-20 alkenyl (e.g., C2-6 alkenyl), (c2) C_{6}-io aryl, (d2) hydrogen, (e2) Ci-6 alk-C_{6}-io aryl, (f2) amino-Ci-20 alkyl, (g2) polyethylene glycol of -(CH2)s2(OCH2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl, and (h2) amino-polyethylene glycol of $-NR^{N1}(CH_{2})s2(CH2CH20)si(CH_{2})s3NR^{N1}$, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl; and (21) amidine. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a Ci-alkaryl can be further substituted with an oxo group to afford the respective aryloyl substituent.

[00884] The term "alkylene" and the prefix "alk-," as used herein, represent a saturated divalent hydrocarbon group derived from a straight or branched chain saturated hydrocarbon by the removal of two hydrogen atoms, and is exemplified by methylene, ethylene, isopropylene, and the like. The term "C_{x-y} alkylene" and the prefix "C_{x-y} alk-" represent alkylene groups having between x and y carbons. Exemplary values for x are 1, 2, 3, 4, 5, and 6, and exemplary values for y are 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 (e.g., Ci-6, Ci-10, C2-20, C2-6, C2-10, or C2-20 alkylene). In some embodiments, the alkylene can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein for an alkyl group.

[00885] The term "alkylsulfmyl," as used herein, represents an alkyl group attached to the parent molecular group through an -S(O)- group. Exemplary unsubstituted alkylsulfmyl groups are from 1 to 6, from 1 to 10, or from 1 to 20 carbons. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00886] The term "alkylsulfinylalkyl," as used herein, represents an alkyl group, as defined herein, substituted by an alkylsulfmyl group. Exemplary unsubstituted alkylsulfinylalkyl groups are from 2 to 12, from 2 to 20, or from 2 to 40 carbons. In some embodiments, each alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00887] The term "alkynyl," as used herein, represents monovalent straight or branched chain groups from 2 to 20 carbon atoms (e.g., from 2 to 4, from 2 to 6, or from 2 to 10 carbons) containing a carbon-carbon triple bond and is exemplified by ethynyl, 1-propynyl, and the like. Alkynyl groups may be optionally substituted with 1, 2, 3, or 4 substituent groups that are selected, independently, from aryl, cycloalkyl, or heterocyclyl (e.g., heteroaryl), as defined herein, or any of the exemplary alkyl substituent groups described herein.

[00888] The term "alkynyloxy" represents a chemical substituent of formula -OR, where R is a C2-20 alkynyl group (e.g., C2-6 or C2-10 alkynyl), unless otherwise specified. Exemplary alkynyloxy groups include ethynyloxy, propynyloxy, and the like. In some embodiments, the alkynyl group can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein (e.g., a hydroxy group).

[00889] The term "amidine," as used herein, represents a -C(=NH)NH2 group.

[00890] The term "amino," as used herein, represents -N(R^{N1})2, wherein each R^{N1} is, independently, H, OH, NO2, N(R^{N2})$_2$, S0 $_2$OR^{N2}, S0 $_2$$R^{N2}$, SO$R^{N2}$, an *N*-protecting group, alkyl, alkenyl, alkynyl, alkoxy, aryl, alkaryl, cycloalkyl, alkcycloalkyl, carboxyalkyl, sulfoalkyl, heterocyclyl (e.g., heteroaryl), or alkheterocyclyl (e.g., alkheteroaryl), wherein each of these recited R^{N1} groups can be optionally substituted, as defined herein for each group; or two R^{N1} combine to form a heterocyclyl or an *N*-protecting group, and wherein each R^{N2} is, independently, H, alkyl, or aryl. The amino groups of the invention can be an unsubstituted amino (i.e., -NH2) or a substituted amino (i.e., -N(R^{N1})$_2$). In a preferred embodiment, amino is -NH2 or -NHR ^{N1}, wherein R^{N1} is, independently, OH, NO2, NH2, NR^{N2}2, S0 $_2$OR^{N2}, S0 $_2$$R^{N2}$, SO$R^{N2}$, alkyl, carboxyalkyl, sulfoalkyl, or aryl, and each R^{N2} can be H, Ci-20 alkyl (e.g., Ci-6 alkyl), or C_6-io aryl.

[00891] The term "amino acid," as described herein, refers to a molecule having a side chain, an amino group, and an acid group (e.g., a carboxy group of-C02H or a sulfo

group of -SO3H), wherein the amino acid is attached to the parent molecular group by the side chain, amino group, or acid group (e.g., the side chain). In some embodiments, the amino acid is attached to the parent molecular group by a carbonyl group, where the side chain or amino group is attached to the carbonyl group. Exemplary side chains include an optionally substituted alkyl, aryl, heterocyclyl, alkaryl, alkheterocyclyl, aminoalkyl, carbamoylalkyl, and carboxyalkyl. Exemplary amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, hydroxynorvaline, isoleucine, leucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, pyrrolysine, selenocysteine, serine, taurine, threonine, tryptophan, tyrosine, and valine. Amino acid groups may be optionally substituted with one, two, three, or, in the case of amino acid groups of two carbons or more, four substituents independently selected from the group consisting of: (1) Ci-6 alkoxy; (2) Ci-6 alkylsulfinyl; (3) amino, as defined herein (e.g., unsubstituted amino (i.e., -NH2) or a substituted amino (i.e., $-N(R^{N1})_2$, where R^{N1} is as defined for amino); (4) C_{6}-io aryl-Ci-6 alkoxy; (5) azido; (6) halo; (7) (C2-9 heterocyclyl)oxy; (8) hydroxy; (9) nitro; (10) oxo (e.g., carboxyaldehyde or acyl); (11) C1-7 spirocyclyl; (12) thioalkoxy; (13) thiol; (14) -$C0_2R^{A'}$, where $R^{A'}$ is selected from the group consisting of (a) Ci-20 alkyl (e.g., Ci-6 alkyl), (b) C2-20 alkenyl (e.g., C2-6 alkenyl), (c) C_{6}-io aryl, (d) hydrogen, (e) Ci-6 alk-C_{6}-io aryl, (f) amino-Ci-20 alkyl, (g) polyethylene glycol of -(CH_2)s2(OCH2CH2)si(CH_2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R' is H or Ci-20 alkyl, and (h) amino-polyethylene glycol of -$NR^{N1}(CH_2)$s2(CH2CH$_2$0)si(CH2)s3NR^{N1}, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl; (15) -C(0)$NR^{B'}R^{C'}$, where each of $R^{B'}$ and $R^{C'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) Ci-6 alkyl, (c) Ce-io aryl, and (d) Ci-e alk-Ce-io aryl; (16) -$S0_2R^{D'}$, where $R^{D'}$ is selected from the group consisting of (a) Ci-6 alkyl, (b) C_{6}-io aryl, (c) Ci-6 alk-C_{6}-io aryl, and (d) hydroxy; (17) -$S0_2NR^{E'}R^{F'}$, where each of $R^{E'}$ and $R^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) Ci-6 alkyl, (c) C_{6}-io aryl and (d) Ci-6 alk-C_{6}-

10 aryl; (18) -C(0)R G', where R G' is selected from the group consisting of (a) Ci-20 alkyl (e.g., Ci-6 alkyl), (b) C2-20 alkenyl (e.g., C2-6 alkenyl), (c) C_{6}-io aryl, (d) hydrogen, (e) Ci-6 alk-C_{6}-io aryl, (f) amino-Ci-20 alkyl, (g) polyethylene glycol of -(CH2)s2(OCH $_{2}$CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R ' is H or Ci-20 alkyl, and (h) amino-polyethylene glycol of -NR N1(CH $_{2}$)s2(CH2CH20)si(CH $_{2}$)s3NR N1, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R N1 is, independently, hydrogen or optionally substituted Ci-6 alkyl; (19) -NR H'C(0)R I', wherein R H' is selected from the group consisting of (al) hydrogen and (bl) Ci-6 alkyl, and R I' is selected from the group consisting of (a2) Ci-20 alkyl (e.g., Ci-6 alkyl), (b2) C2-20 alkenyl (e.g., C2-6 alkenyl), (c2) C6-io aryl, (d2) hydrogen, (e2) Ci-6 alk-C_{6}-io aryl, (f2) amino-Ci-20 alkyl, (g2) polyethylene glycol of -(CH2)s2(OCH2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R ' is H or Ci-20 alkyl, and (h2) amino-polyethylene glycol of -NR N1(CH $_{2}$)s2(CH2CH20)si(CH $_{2}$)s3NR N1, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R N1 is, independently, hydrogen or optionally substituted Ci-6 alkyl; (20) -NR J'C(0)OR K', wherein R J' is selected from the group consisting of (al) hydrogen and (bl) Ci-6 alkyl, and R K' is selected from the group consisting of (a2) Ci-20 alkyl (e.g., Ci-6 alkyl), (b2) C2-20 alkenyl (e.g., C2-6 alkenyl), (c2) C_{6}-io aryl, (d2) hydrogen, (e2) Ci-6 alk-C_{6}-io aryl, (f2) amino-Ci-20 alkyl, (g2) polyethylene glycol of -(CH2)s2(OCH2CH2)si(CH2)s30R', wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g., from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and R ' is H or Ci-20 alkyl, and (h2) amino-polyethylene glycol of -NR N1(CH $_{2}$)s2(CH2CH20)si(CH $_{2}$)s3NR N1, wherein si is an integer from 1 to 10 (e.g., from 1 to 6 or from 1 to 4), each of s2 and s3, independently, is an integer from 0 to 10 (e.g.,

from 0 to 4, from 0 to 6, from 1 to 4, from 1 to 6, or from 1 to 10), and each R^{N1} is, independently, hydrogen or optionally substituted Ci-6 alkyl; and (21) amidine. In some embodiments, each of these groups can be further substituted as described herein.

[00892] The term "aminoalkoxy," as used herein, represents an alkoxy group, as defined herein, substituted by an amino group, as defined herein. The alkyl and amino each can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for the respective group (e.g., $C0_2R^{A'}$, where $R^{A'}$ is selected from the group consisting of (a) Ci-6 alkyl, (b) C_6-io aryl, (c) hydrogen, and (d) Ci-6 alk-C6-io aryl, e.g., carboxy).

[00893] The term "aminoalkyl," as used herein, represents an alkyl group, as defined herein, substituted by an amino group, as defined herein. The alkyl and amino each can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for the respective group (e.g., $C0_2R^{A'}$, where $R^{A'}$ is selected from the group consisting of (a) Ci-6 alkyl, (b) C_6-io aryl, (c) hydrogen, and (d) Ci-6 alk-C_6-io aryl, e.g., carboxy).

[00894] The term "aryl," as used herein, represents a mono-, bicyclic, or multicyclic carbocyclic ring system having one or two aromatic rings and is exemplified by phenyl, naphthyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, anthracenyl, phenanthrenyl, fluorenyl, indanyl, indenyl, and the like, and may be optionally substituted with 1, 2, 3, 4, or 5 substituents independently selected from the group consisting of: (1) C1-7 acyl (e.g., carboxyaldehyde); (2) Ci-$_2$o alkyl (e.g., Ci-6 alkyl, Ci-6 alkoxy-Ci-6 alkyl, Ci-6 alkylsulfinyl-Ci-6 alkyl, amino-Ci-6 alkyl, azido-Ci-6 alkyl, (carboxyaldehyde)-Ci-6 alkyl, halo-Ci-6 alkyl (e.g., perfluoroalkyl), hydroxy-Ci-6 alkyl, nitro-Ci-6 alkyl, or Ci-6 thioalkoxy-Ci-6 alkyl); (3) Ci-$_2$o alkoxy (e.g., Ci-6 alkoxy, such as perfluoroalkoxy); (4) Ci-6 alkylsulfmyl; (5) C_6-io aryl; (6) amino; (7) Ci-6 alk-C_6-io aryl; (8) azido; (9) C3-8 cycloalkyl; (10) Ci-6 alk-C_{3-8} cycloalkyl; (11) halo; (12) Ci-i$_2$ heterocyclyl (e.g., Ci-i$_2$ heteroaryl); (13) (Ci-i$_2$ heterocyclyl)oxy; (14) hydroxy; (15) nitro; (16) Ci-$_2$o thioalkoxy (e.g., Ci-6 thioalkoxy); (17) $-(CH_2)_qC0_2R^{A'}$, where q is an integer from zero to four, and $R^{A'}$ is selected from the group consisting of (a) Ci-6 alkyl, (b) C_6-io aryl, (c) hydrogen, and (d) Ci-6 alk-C_6-io aryl; (18) $-(CH_2)_qCONR^{B'}R^{C'}$, where q is an integer from zero to four and where $R^{B'}$ and $R^{C'}$ are independently selected from the group consisting of (a) hydrogen, (b) Ci-e alkyl, (c) Ce-io aryl, and (d) Ci-e alk-Ce-io aryl; (19) $-(CH_2)_qS0_2R^{D'}$, where q is an integer from zero to four and where $R^{D'}$ is selected from the group

consisting of (a) alkyl, (b) **Ce-io** aryl, and (c) **alk-Ce-io** aryl; (20) **-(CH** $_2)_q$S0 $_2$NR$^{E'}$R$^{F'}$, where q is an integer from zero to four and where each of R$^{E'}$ and R$^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) **Ci-6** alkyl, (c) **C$_6$-io** aryl, and (d) **Ci-6 alk-C$_6$-io** aryl; (21) thiol; (22) **C$_6$-io** aryloxy; (23) C3-8 cycloalkoxy; (24) **C$_6$-io aryl-Ci-**6 alkoxy; (25) **Ci-6 alk-Ci-i $_2$** heterocyclyl (e.g., **Ci-6 alk-Ci-i $_2$** heteroaryl); (26) **C$_{2-2}$o** alkenyl; and (27) **C$_{2-2}$o** alkynyl. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a **Ci-**alkaryl or a **Ci-**alkheterocyclyl can be further substituted with an oxo group to afford the respective aryloyl and (heterocyclyl)oyl substituent group.

[00895] The term "arylalkoxy," as used herein, represents an alkaryl group, as defined herein, attached to the parent molecular group through an oxygen atom. Exemplary unsubstituted alkoxyalkyl groups include from 7 to 30 carbons (e.g., from 7 to 16 or from 7 to 20 carbons, such as **C$_6$-io** aryl-**Ci-6** alkoxy, **C$_6$-io** aryl-**Ci-10** alkoxy, or **C$_6$-io aryl-Ci-$_2$o** alkoxy). In some embodiments, the arylalkoxy group can be substituted with 1, 2, 3, or 4 substituents as defined herein

[00896] The term "aryloxy" represents a chemical substituent of formula -OR', where R' is an aryl group of 6 to 18 carbons, unless otherwise specified. In some embodiments, the aryl group can be substituted with 1, 2, 3, or 4 substituents as defined herein.

[00897] The term "aryloyl," as used herein, represents an aryl group, as defined herein, that is attached to the parent molecular group through a carbonyl group. Exemplary unsubstituted aryloyl groups are of 7 to 11 carbons. In some embodiments, the aryl group can be substituted with 1, 2, 3, or 4 substituents as defined herein.

[00898] The term "azido" represents an -N3 group, which can also be represented as -N=N=N.

[00899] The term "bicyclic," as used herein, refer to a structure having two rings, which may be aromatic or non-aromatic. Bicyclic structures include spirocyclyl groups, as defined herein, and two rings that share one or more bridges, where such bridges can include one atom or a chain including two, three, or more atoms. Exemplary bicyclic groups include a bicyclic carbocyclyl group, where the first and second rings are carbocyclyl groups, as defined herein; a bicyclic aryl groups, where the first and second rings are aryl groups, as defined herein; bicyclic heterocyclyl groups, where the first ring

is a heterocyclyl group and the second ring is a carbocyclyl (e.g., aryl) or heterocyclyl (e.g., heteroaryl) group; and bicyclic heteroaryl groups, where the first ring is a heteroaryl group and the second ring is a carbocyclyl (e.g., aryl) or heterocyclyl (e.g., heteroaryl) group. In some embodiments, the bicyclic group can be substituted with 1, 2, 3, or 4 substituents as defined herein for cycloalkyl, heterocyclyl, and aryl groups.

[00900] The terms "carbocyclic" and "carbocyclyl," as used herein, refer to an optionally substituted C_{3-12} monocyclic, bicyclic, or tricyclic structure in which the rings, which may be aromatic or non-aromatic, are formed by carbon atoms. Carbocyclic structures include cycloalkyl, cycloalkenyl, and aryl groups.

[00901] The term "carbamoyl," as used herein, represents -C(0)-N(R^{N1})$_2$, where the meaning of each R^{N1} is found in the definition of "amino" provided herein.

[00902] The term "carbamoylalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a carbamoyl group, as defined herein. The alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00903] The term "carbamyl," as used herein, refers to a carbamate group having the structure

-NR^{N1}C(=0)OR or -OC(=0)N(R^{N1})$_2$, where the meaning of each R^{N1} is found in the definition of "amino" provided herein, and R is alkyl, cycloalkyl , alkcycloalkyl, aryl, alkaryl, heterocyclyl (e.g., heteroaryl), or alkheterocyclyl (e.g., alkheteroaryl), as defined herein.

[00904] The term "carbonyl," as used herein, represents a C(O) group, which can also be represented as C=0.

[00905] The term "carboxyaldehyde" represents an acyl group having the structure -CHO.

[00906] The term "carboxy," as used herein, means -CO2H.

[00907] The term "carboxyalkoxy," as used herein, represents an alkoxy group, as defined herein, substituted by a carboxy group, as defined herein. The alkoxy group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for the alkyl group.

[00908] The term "carboxyalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a carboxy group, as defined herein. The alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00909] The term "cyano," as used herein, represents an -CN group.

[00910] The term "cycloalkoxy" represents a chemical substituent of formula -OR, where R is a C3-8 cycloalkyl group, as defined herein, unless otherwise specified. The cycloalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein. Exemplary unsubstituted cycloalkoxy groups are from 3 to 8 carbons. In some embodiment, the cycloalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00911] The term "cycloalkyl," as used herein represents a monovalent saturated or unsaturated non-aromatic cyclic hydrocarbon group from three to eight carbons, unless otherwise specified, and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, bicyclo[2.2.1.]heptyl, and the like. When the cycloalkyl group includes one carbon-carbon double bond, the cycloalkyl group can be referred to as a "cycloalkenyl" group. Exemplary cycloalkenyl groups include cyclopentenyl, cyclohexenyl, and the like. The cycloalkyl groups of this invention can be optionally substituted with: (1) C1-7 acyl (e.g., carboxyaldehyde); (2) Ci-20 alkyl (e.g., Ci-6 alkyl, Ci-6 alkoxy-Ci-6 alkyl, Ci-6 alkylsulfinyl-Ci-6 alkyl, amino-Ci-6 alkyl, azido-Ci-6 alkyl, (carboxyaldehyde)-Ci-6 alkyl, halo-Ci-6 alkyl (e.g., perfluoroalkyl), hydroxy-Ci-6 alkyl, nitro-Ci-6 alkyl, or Ci-6thioalkoxy-Ci-6 alkyl); (3) Ci-20 alkoxy (e.g., Ci-6 alkoxy, such as perfluoroalkoxy); (4) Ci-6 alkylsulfmyl; (5) C_{6}-io aryl; (6) amino; (7) Ci-6 alk-C_{6}-io aryl; (8) azido; (9) C3-8 cycloalkyl; (10) Ci-e alk-Cs-s cycloalkyl; (11) halo; (12) Ci-12 heterocyclyl (e.g., Ci-12 heteroaryl); (13) (Ci-12 heterocyclyl)oxy; (14) hydroxy; (15) nitro; (16) Ci-20 thioalkoxy (e.g., Ci-6 thioalkoxy); (17) -(CH $_{2}$)qC02R $^{A'}$, where q is an integer from zero to four, and R $^{A'}$ is selected from the group consisting of (a) Ci-6 alkyl, (b) Ce-io aryl, (c) hydrogen, and (d) Ci-e alk-Ce-io aryl; (18) -(CH $_{2}$)$_{q}$CONR $^{B'}$R $^{c'}$, where q is an integer from zero to four and where R $^{B'}$ and R $^{c'}$ are independently selected from the group consisting of (a) hydrogen, (b) C_{6}-io alkyl, (c) C_{6}-io aryl, and (d) Ci-6 alk-C_{6}-io aryl; (19) -(CH2)qS02R $^{D'}$, where q is an integer from zero to four and where R $^{D'}$ is selected from the group consisting of (a) C_{6}-io alkyl, (b) C_{6}-io aryl, and (c) Ci-6 alk-C_{6}-io aryl; (20)

-(CH $_2$)qS0 $_2$N R$^{E'}$R$^{F'}$, where q is an integer from zero to four and where each of R$^{E'}$ and R$^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) **C$_6$-io** alkyl, (c) **C6-io** aryl, and (d) **Ci-6 alk-C$_6$-io** aryl; (21) thiol; (22) **C$_6$-io** aryloxy; (23) C3-8 cycloalkoxy; (24) **C$_6$-io** aryl-**Ci**-6 alkoxy; (25) **Ci-6** alk-**Ci**-12 heterocyclyl (e.g., **Ci-6 alk-Ci-**12 heteroaryl); (26) oxo; (27) C2-20 alkenyl; and (28) C2-20 alkynyl. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a **Ci**-alkaryl or a **Ci**-alkheterocyclyl can be further substituted with an oxo group to afford the respective aryloyl and (heterocyclyl)oyl substituent group.

[00912] The term "diastereomer," as used herein means stereoisomers that are not mirror images of one another and are non-superimposable on one another.

[00913] The term "effective amount" of an agent, as used herein, is that amount sufficient to effect beneficial or desired results, for example, clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. For example, in the context of administering an agent that treats cancer, an effective amount of an agent is, for example, an amount sufficient to achieve treatment, as defined herein, of cancer, as compared to the response obtained without administration of the agent.

[00914] The term "enantiomer," as used herein, means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e., at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

[00915] The term "halo," as used herein, represents a halogen selected from bromine, chlorine, iodine, or fluorine.

[00916] The term "haloalkoxy," as used herein, represents an alkoxy group, as defined herein, substituted by a halogen group (i.e., F, CI, Br, or I). A haloalkoxy may be substituted with one, two, three, or, in the case of alkyl groups of two carbons or more, four halogens. Haloalkoxy groups include perfluoroalkoxys (e.g., -OCF3), -OCHF2, -OCH2F, -OCCI3, -OCH $_2$CH$_2$Br, -OCH $_2$CH(CH $_2$CH$_2$Br)CH3, and -OCHICH3. In some embodiments, the haloalkoxy group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkyl groups.

[00917] The term "haloalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a halogen group (i.e., F, CI, Br, or I). A haloalkyl may be substituted with one, two, three, or, in the case of alkyl groups of two carbons or more, four halogens. Haloalkyl groups include perfluoroalkyls (e.g., -CF3), -CHF2, -CH2F, -CCb, $-CH_2CH_2Br$, $-CH_2CH(CH_2CH_2Br)CH_3$, and -CHICH3. In some embodiments, the haloalkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkyl groups.

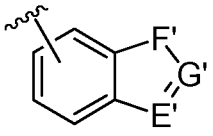
[00918] The term "heteroalkylene," as used herein, refers to an alkylene group, as defined herein, in which one or two of the constituent carbon atoms have each been replaced by nitrogen, oxygen, or sulfur. In some embodiments, the heteroalkylene group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein for alkylene groups.

[00919] The term "heteroaryl," as used herein, represents that subset of heterocyclyls, as defined herein, which are aromatic: i.e., they contain *4n+2* pi electrons within the mono- or multicyclic ring system. Exemplary unsubstituted heteroaryl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. In some embodiment, the heteroaryl is substituted with 1, 2, 3, or 4 substituents groups as defined for a heterocyclyl group.

[00920] The term "heterocyclyl," as used herein represents a 5-, 6- or 7-membered ring, unless otherwise specified, containing one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. The 5-membered ring has zero to two double bonds, and the 6- and 7-membered rings have zero to three double bonds. Exemplary unsubstituted heterocyclyl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term "heterocyclyl" also represents a heterocyclic compound having a bridged multicyclic structure in which one or more carbons and/or heteroatoms bridges two non-adjacent members of a monocyclic ring, e.g., a quinuclidinyl group. The term "heterocyclyl" includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heterocyclic rings is fused to one, two, or three carbocyclic rings, e.g., an aryl ring, a cyclohexane ring, a cyclohexene ring, a cyclopentane ring, a cyclopentene ring, or another monocyclic heterocyclic ring, such as indolyl, quinolyl, isoquinolyl, tetrahydroquinolyl, benzofuryl, benzothienyl and the like.

Examples of fused heterocyclyls include tropanes and l,2,3,5,8,8a-hexahydroindolizine. Heterocyclics include pyrrolyl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, piperidinyl, homopiperidinyl, pyrazinyl, piperazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidiniyl, morpholinyl, thiomorpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, indolyl, indazolyl, quinolyl, isoquinolyl, quinoxalinyl, dihydroquinoxalinyl, quinazolinyl, cinnolinyl, phthalazinyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, benzothiadiazolyl, furyl, thienyl, thiazolidinyl, isothiazolyl, triazolyl, tetrazolyl, oxadiazolyl (e.g., 1,2,3-oxadiazolyl), purinyl, thiadiazolyl (e.g., 1,2,3-thiadiazolyl), tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, dihydrothienyl, dihydroindolyl, dihydroquinolyl, tetrahydroquinolyl, tetrahydroisoquinolyl, dihydroisoquinolyl, pyranyl, dihydropyranyl, dithiazolyl, benzofuranyl, isobenzofuranyl, benzothienyl, and the like, including dihydro and tetrahydro forms thereof, where one or more double bonds are reduced and replaced with hydrogens. Still other exemplary heterocyclyls include: 2,3,4,5-tetrahydro-2-oxo-oxazolyl; 2,3-dihydro-2-oxo-lH-imidazolyl; 2,3,4,5-tetrahydro-5-oxo-lH-pyrazolyl (e.g., 2,3,4,5-tetrahydro-2-phenyl-5-oxo-lH-pyrazolyl); 2,3,4,5-tetrahydro-2,4-dioxo-lH-imidazolyl (e.g., 2,3,4,5-tetrahydro-2,4-dioxo-5-methyl-5-phenyl-lH-imidazolyl); 2,3-dihydro-2-thioxo-l,3,4-oxadiazolyl (e.g., 2,3-dihydro-2-thioxo-5-phenyl-l,3,4-oxadiazolyl); 4,5-dihydro-5-oxo-l *H*-triazolyl (e.g., 4,5-dihydro-3-methyl-4-amino 5-oxo-l*H*-triazolyl); l,2,3,4-tetrahydro-2,4-dioxopyridinyl (e.g., l,2,3,4-tetrahydro-2,4-dioxo-3,3-diethylpyridinyl); 2,6-dioxo-piperidinyl (e.g., 2,6-dioxo-3-ethyl-3-phenylpiperidinyl); l,6-dihydro-6-oxopyridiminyl; l,6-dihydro-4-oxopyrimidinyl (e.g., 2-(methylthio)-l,6-dihydro-4-oxo-5-methylpyrimidin-l-yl); l,2,3,4-tetrahydro-2,4-dioxopyrimidinyl (e.g., 1,2,3,4-tetrahydro-2,4-dioxo-3-ethylpyrimidinyl); 1,6-dihydro-6-oxo-pyridazinyl (e.g., l,6-dihydro-6-oxo-3-ethylpyridazinyl); l,6-dihydro-6-oxo-l,2,4-triazinyl (e.g., 1,6-dihydro-5-isopropyl-6-oxo-l,2,4-triazinyl); 2,3-dihydro-2-oxo-l *H*-indolyl (e.g., 3,3-dimethyl-2,3-dihydro-2-oxo-l *H*-indolyl and 2,3-dihydro-2-oxo-3,3'-spiropropane-l *H*-indol-1-yl); 1,3-dihydro-1-oxo-2*H*-iso-indolyl; 1,3-dihydro-1,3-dioxo-2*H*-iso-indolyl; l*H*-benzopyrazolyl (e.g., l-(ethoxycarbonyl)-l*H*-benzopyrazolyl); 2,3-dihydro-2-oxo-l*H*-benzimidazolyl (e.g., 3-ethyl-2,3-dihydro-2-oxo-l *H*-benzimidazolyl); 2,3-dihydro-2-

oxo-benzoxazolyl (e.g., 5-chloro-2,3-dihydro-2-oxo-benzoxazolyl); 2,3-dihydro-2-oxo-benzoxazolyl; 2-oxo-2H-benzopyranyl; 1,4-benzodioxanyl; 1,3-benzodioxanyl; 2,3-dihydro-3-oxo,4*H*-l,3-benzothiazinyl; 3,4-dihydro-4-oxo-3*H*-quinazolinyl (e.g., 2-methyl-3,4-dihydro-4-oxo-3*H*-quinazolinyl); 1,2,3,4-tetrahydro-2,4-dioxo-3*H*-quinazolyl (e.g., 1-ethyl-1,2,3,4-tetrahydro-2,4-dioxo-3*H*-quinazolyl); l,2,3,6-tetrahydro-2,6-dioxo-7*H*-purinyl (e.g., 1,2,3,6-tetrahydro-l,3-dimethyl-2, 6-dioxo-7*H*-purinyl); 1,2,3,6-tetrahydro-2,6-dioxo-l*H*-purinyl (e.g., l,2,3,6-tetrahydro-3,7-dimethyl-2,6-dioxo-l*H*-purinyl); 2-oxobenz[c,d]indolyl; l,l-dioxo-2H-naphth[l,8-c,d]isothiazolyl; and 1,8-naphthylenedicarboxamido. Additional heterocyclics include 3,3a,4,5,6,6a-hexahydro-pyrrolo[3,4-b]pyrrol-(2H)-yl, and 2,5-diazabicyclo[2.2.1]heptan-2-yl, homopiperazinyl (or diazepanyl), tetrahydropyranyl, dithiazolyl, benzofuranyl, benzothienyl, oxepanyl, thiepanyl, azocanyl, oxecanyl, and thiocanyl. Heterocyclic groups also include groups of the formula



, where E' is selected from the group consisting of -N- and -CH-; F' is selected from the group consisting of -N=CH-, -NH-CH2-, -NH-C(O)-, -NH-, -CH=N-, -CH2-NH-, -C(0)-NH-, -CH=CH-, -CH2-, -CH2CH2-, -CH2O-, -OCH2-, -0-, and -S-; and G' is selected from the group consisting of -CH- and -N-. Any of the heterocyclyl groups mentioned herein may be optionally substituted with one, two, three, four or five substituents independently selected from the group consisting of: (1) C1-7 acyl (e.g., carboxyaldehyde); (2) Ci-20 alkyl (e.g., Ci-6 alkyl, Ci-6 alkoxy-Ci-6 alkyl, Ci-6 alkylsulfinyl-Ci-6 alkyl, amino-Ci-6 alkyl, azido-Ci-6 alkyl, (carboxyaldehyde)-Ci-6 alkyl, halo-Ci-6 alkyl (e.g., perfluoroalkyl), hydroxy-Ci-6 alkyl, nitro-Ci-6 alkyl, or Ci-6 thioalkoxy-Ci-6 alkyl); (3) Ci-20 alkoxy (e.g., Ci-6 alkoxy, such as perfluoroalkoxy); (4) Ci-6 alkylsulfinyl; (5) C_6-io aryl; (6) amino; (7) Ci-6 alk-C6-io aryl; (8) azido; (9) C3-8 cycloalkyl; (10) Ci-6 alk-C_3-8 cycloalkyl; (11) halo; (12) Ci-12 heterocyclyl (e.g., C2-12 heteroaryl); (13) (Ci-12 heterocyclyl)oxy; (14) hydroxy; (15) nitro; (16) Ci-20 thioalkoxy (e.g., Ci-6 thioalkoxy); (17) -(CH2)qC02$R^{A'}$, where q is an integer from zero to four, and $R^{A'}$ is selected from the group consisting of (a) Ci-6 alkyl, (b) C_6-io aryl, (c) hydrogen, and (d) Ci-6 alk-C_6-io aryl;

(18) $-(CH_2)_qCONR^{B'}R^{C'}$, where q is an integer from zero to four and where $R^{B'}$ and $R^{C'}$ are independently selected from the group consisting of (a) hydrogen, (b) Ci-6 alkyl, (c) C_{6}-io aryl, and (d) Ci-6 alk-C_{6}-io aryl; (19) $-(CH_2)_qS0_2R^{D'}$, where q is an integer from zero to four and where $R^{D'}$ is selected from the group consisting of (a) Ci-6 alkyl, (b) C_{6}-io aryl, and (c) Ci-6 alk-C_{6}-io aryl; (20) $-(CH_2)_qS0_2NR^{E'}R^{F'}$, where q is an integer from zero to four and where each of $R^{E'}$ and $R^{F'}$ is, independently, selected from the group consisting of (a) hydrogen, (b) Ci-6 alkyl, (c) C_{6}-io aryl, and (d) Ci-6 alk-C_{6}-io aryl; (21) thiol; (22) C_{6}-io aryloxy; (23) C_{3-8} cycloalkoxy; (24) arylalkoxy; (25) Ci-6 alk-Ci-i$_2$ heterocyclyl (e.g., Ci-6 alk-Ci-i$_2$ heteroaryl); (26) oxo; (27) (Ci-i$_2$ heterocyclyl)imino; (28) C_{2-2}o alkenyl; and (29) C_{2-2}o alkynyl. In some embodiments, each of these groups can be further substituted as described herein. For example, the alkylene group of a Ci-alkaryl or a Ci-alkheterocyclyl can be further substituted with an oxo group to afford the respective aryloyl and (heterocyclyl)oyl substituent group.

[00921] The term "(heterocyclyl)imino," as used herein, represents a heterocyclyl group, as defined herein, attached to the parent molecular group through an imino group. In some embodiments, the heterocyclyl group can be substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00922] The term "(heterocyclyl)oxy," as used herein, represents a heterocyclyl group, as defined herein, attached to the parent molecular group through an oxygen atom. In some embodiments, the heterocyclyl group can be substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00923] The term "(heterocyclyl)oyl," as used herein, represents a heterocyclyl group, as defined herein, attached to the parent molecular group through a carbonyl group. In some embodiments, the heterocyclyl group can be substituted with 1, 2, 3, or 4 substituent groups as defined herein.

[00924] The term "hydrocarbon," as used herein, represents a group consisting only of carbon and hydrogen atoms.

[00925] The term "hydroxy," as used herein, represents an -OH group.

[00926] The term "hydroxyalkenyl," as used herein, represents an alkenyl group, as defined herein, substituted by one to three hydroxy groups, with the proviso that no more

than one hydroxy group may be attached to a single carbon atom of the alkyl group, and is exemplified by dihydroxypropenyl, hydroxyisopentenyl, and the like.

[00927] The term "hydroxyalkyl," as used herein, represents an alkyl group, as defined herein, substituted by one to three hydroxy groups, with the proviso that no more than one hydroxy group may be attached to a single carbon atom of the alkyl group, and is exemplified by hydroxymethyl, dihydroxypropyl, and the like.

[00928] The term "isomer," as used herein, means any tautomer, stereoisomer, enantiomer, or diastereomer of any compound of the invention. It is recognized that the compounds of the invention can have one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric E/Z isomers) or diastereomers (e.g., enantiomers (i.e., (+) or (-)) or cis/trans isomers). According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all of the corresponding stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, e.g., racemates. Enantiomeric and stereoisomeric mixtures of compounds of the invention can typically be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

[00929] The term "*N*-protected amino," as used herein, refers to an amino group, as defined herein, to which is attached one or two *N*-protecting groups, as defined herein.

[00930] The term "*N*-protecting group," as used herein, represents those groups intended to protect an amino group against undesirable reactions during synthetic procedures. Commonly used *N*-protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis," 3rd Edition (John Wiley & Sons, New York, 1999), which is incorporated herein by reference. *N*-protecting groups include acyl, aryloyl, or carbamyl groups such as formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoroacetyl, trichloroacetyl, phthalyl, o-nitrophenoxyacetyl, a-

chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and chiral auxiliaries such as protected or unprotected D, L or D, L-amino acids such as alanine, leucine, phenylalanine, and the like; sulfonyl-containing groups such as benzenesulfonyl, p-toluenesulfonyl, and the like; carbamate forming groups such as benzyloxycarbonyl, p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 3,5-dimethoxybenzyloxycarbonyl, 2,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, l-(p-biphenylyl)-l-methylethoxycarbonyl, α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxy carbonyl, t-butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2,-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxy carbonyl, fluorenyl-9-methoxycarbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl, and the like, alkaryl groups such as benzyl, triphenylmethyl, benzyloxymethyl, and the like and silyl groups, such as trimethylsilyl, and the like. Preferred *N*-protecting groups are formyl, acetyl, benzoyl, pivaloyl, t-butylacetyl, alanyl, phenylsulfonyl, benzyl, t-butyloxycarbonyl (Boc), and benzyloxycarbonyl (Cbz).

[00931] The term "nitro," as used herein, represents an -NO2 group.

[00932] The term "oxo" as used herein, represents **=0.**

[00933] The term "perfluoroalkyl," as used herein, represents an alkyl group, as defined herein, where each hydrogen radical bound to the alkyl group has been replaced by a fluoride radical. Perfluoroalkyl groups are exemplified by trifluoromethyl, pentafluoroethyl, and the like.

[00934] The term "perfluoroalkoxy," as used herein, represents an alkoxy group, as defined herein, where each hydrogen radical bound to the alkoxy group has been replaced by a fluoride radical. Perfluoroalkoxy groups are exemplified by trifluoromethoxy, pentafluoroethoxy, and the like.

[00935] The term "spirocyclyl," as used herein, represents a C2-7 alkylene diradical, both ends of which are bonded to the same carbon atom of the parent group to form a spirocyclic group, and also a Ci-6 heteroalkylene diradical, both ends of which are bonded

to the same atom. The heteroalkylene radical forming the spirocyclyl group can containing one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. In some embodiments, the spirocyclyl group includes one to seven carbons, excluding the carbon atom to which the diradical is attached. The spirocyclyl groups of the invention may be optionally substituted with 1, 2, 3, or 4 substituents provided herein as optional substituents for cycloalkyl and/or heterocyclyl groups.

[00936] The term "stereoisomer," as used herein, refers to all possible different isomeric as well as conformational forms which a compound may possess (e.g., a compound of any formula described herein), in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers and/or conformers of the basic molecular structure. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

[00937] The term "sulfoalkyl," as used herein, represents an alkyl group, as defined herein, substituted by a sulfo group of -SO3H. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00938] The term "sulfonyl," as used herein, represents an **-S(0)** $_2$**-** group.

[00939] The term "thioalkaryl," as used herein, represents a chemical substituent of formula -SR, where R is an alkaryl group. In some embodiments, the alkaryl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00940] The term "thioalkheterocyclyl," as used herein, represents a chemical substituent of formula -SR, where R is an alkheterocyclyl group. In some embodiments, the alkheterocyclyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00941] The term "thioalkoxy," as used herein, represents a chemical substituent of formula -SR, where R is an alkyl group, as defined herein. In some embodiments, the alkyl group can be further substituted with 1, 2, 3, or 4 substituent groups as described herein.

[00942] The term "thiol" represents an -SH group.

[00943] *Compound:* As used herein, the term "compound," is meant to include all stereoisomers, geometric isomers, tautomers, and isotopes of the structures depicted.

[00944] The compounds described herein can be asymmetric *{e.g.*, having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

[00945] Compounds of the present disclosure also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge. Examples prototropic tautomers include ketone - enol pairs, amide - imidic acid pairs, lactam - lactim pairs, amide - imidic acid pairs, enamine - imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, such as, IH- and 3H-imidazole, 1H-, 2H- and 4H- 1,2,4-triazole, IH- and 2H- isoindole, and IH- and 2H-pyrazole. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution.

[00946] Compounds of the present disclosure also include all of the isotopes of the atoms occurring in the intermediate or final compounds. "Isotopes" refers to atoms having the same atomic number but different mass numbers resulting from a different number of neutrons in the nuclei. For example, isotopes of hydrogen include tritium and deuterium.

[00947] The compounds and salts of the present disclosure can be prepared in combination with solvent or water molecules to form solvates and hydrates by routine

methods. *CNTF:* As used herein, the term "CNTF" refers to ciliary neurotrophic factor including any variants thereof.

[00948] *Committed:* As used herein, the term "committed" means, when referring to a cell, when the cell is far enough into the differentiation pathway where, under normal circumstances, it will continue to differentiate into a specific cell type or subset of cell type instead of into a different cell type or reverting to a lesser differentiated cell type.

[00949] *Conserved:* As used herein, the term "conserved" refers to nucleotides or amino acid residues of a polynucleotide sequence or polypeptide sequence, respectively, that are those that occur unaltered in the same position of two or more sequences being compared. Nucleotides or amino acids that are relatively conserved are those that are conserved amongst more related sequences than nucleotides or amino acids appearing elsewhere in the sequences.

[00950] In some embodiments, two or more sequences are said to be "completely conserved" if they are 100% identical to one another. In some embodiments, two or more sequences are said to be "highly conserved" if they are at least 70%> identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some embodiments, two or more sequences are said to be "highly conserved" if they are about **70%o** identical, about 80%> identical, about 90%> identical, about 95%, about 98%>, or about 99% identical to one another. In some embodiments, two or more sequences are said to be "conserved" if they are at least 30% identical, at least 40% identical, at least 50% identical, at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some embodiments, two or more sequences are said to be "conserved" if they are about 30%> identical, about 40%> identical, about 50%> identical, about 60%> identical, about 70%> identical, about 80%> identical, about 90%> identical, about 95% identical, about 98%> identical, or about 99% identical to one another. Conservation of sequence may apply to the entire length of an oligonucleotide or polypeptide or may apply to a portion, region or feature thereof.

[00951] *Controlled Release:* As used herein, the term "controlled release" refers to a pharmaceutical composition or compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome.

[00952] *Cyclic or Cyclized:* As used herein, the term "cyclic" refers to the presence of a continuous loop. Cyclic molecules need not be circular, only joined to form an unbroken chain of subunits. Cyclic molecules such as the engineered RNA or mRNA of the present invention may be single units or multimers or comprise one or more components of a complex or higher order structure.

[00953] *Cytostatic:* As used herein, "cytostatic" refers to inhibiting, reducing, suppressing the growth, division, or multiplication of a cell *{e.g.,* a mammalian cell *{e.g.,* a human cell)), bacterium, virus, fungus, protozoan, parasite, prion, or a combination thereof.

[00954] *Cytotoxic:* As used herein, "cytotoxic" refers to killing or causing injurious, toxic, or deadly effect on a cell *{e.g.,* a mammalian cell *{e.g.,* a human cell)), bacterium, virus, fungus, protozoan, parasite, prion, or a combination thereof.

[00955] *Delivery:* As used herein, "delivery" refers to the act or manner of delivering a compound, substance, entity, moiety, cargo or payload.

[00956] *Delivery Agent:* As used herein, "delivery agent" refers to any substance which facilitates, at least in part, the *in vivo* delivery of a cell phenotype altering polynucleotide, primary construct or mmRNA to targeted cells.

[00957] *Destabilized:* As used herein, the term "destable," "destabilize," or "destabilizing region" means a region or molecule that is less stable than a starting, wild-type or native form of the same region or molecule.

[00958] *Detectable label:* As used herein, "detectable label" refers to one or more markers, signals, or moieties which are attached, incorporated or associated with another entity that is readily detected by methods known in the art including radiography, fluorescence, chemiluminescence, enzymatic activity, absorbance and the like. Detectable labels include radioisotopes, fluorophores, chromophores, enzymes, dyes, metal ions, ligands such as biotin, avidin, streptavidin and haptens, quantum dots, and the like. Detectable labels may be located at any position in the peptides or proteins disclosed herein. They may be within the amino acids, the peptides, or proteins, or located at the N- or C- termini.

[00959] *Developmental Potential:* As used herein, "developmental potential" or "developmental potency" refers to the total of all developmental cell fates or cell types that can be achieved by a cell upon differentiation.

[00960] *Developmental Potential Altering Factor.* As used herein, "developmental potential altering factor" refers to a protein or RNA which can alter the developmental potential of a cell.

[00961] *Digest:* As used herein, the term "digest" means to break apart into smaller pieces or components. When referring to polypeptides or proteins, digestion results in the production of peptides.

[00962] *Differentiated cell:* As used herein, the term "differentiated cell" refers to any somatic cell that is not, in its native form, pluripotent. Differentiated cell also encompasses cells that are partially differentiated.

[00963] *Differentiation:* As used herein, the term "differentiation factor" refers to a developmental potential altering factor such as a protein, RNA or small molecule that can induce a cell to differentiate to a desired cell-type.

[00964] *Differentiate:* As used herein, "differentiate" refers to the process where an uncommitted or less committed cell acquires the features of a committed cell.

[00965] *Distal:* As used herein, the term "distal" means situated away from the center or away from a point or region of interest.

[00966] *Dose splitting factor (DSF)*-ratio of PUD of dose split treatment divided by PUD of total daily dose or single unit dose. The value is derived from comparison of dosing regimens groups.

[00967] *EGF:* As used herein, the term "EGF" refers to epidermal growth factor including any variants thereof.

[00968] *Embryonic stem cell:* As used herein, the term "embryonic stem cell" refers to naturally occurring pluripotent stem cells of the inner cell mass of the embryonic blastocyst.

[00969] *Encapsulate:* As used herein, the term "encapsulate" means to enclose, surround or encase.

[00970] *Encoded protein cleavage signal:* As used herein, "encoded protein cleavage signal" refers to the nucleotide sequence which encodes a protein cleavage signal.

[00971] *Engineered:* As used herein, embodiments of the invention are "engineered" when they are designed to have a feature or property, whether structural or chemical, that varies from a starting point, wild type or native molecule.

[00972] *Exosome:* As used herein, "exosome" is a vesicle secreted by mammalian cells or a complex involved in RNA degradation.

[00973] *Expression:* As used herein, "expression" of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence *(e.g.,* by transcription); (2) processing of an RNA transcript *(e.g.,* by splicing, editing, 5' cap formation, and/or 3' end processing); (3) translation of an RNA into a polypeptide or protein; and (4) post-translational modification of a polypeptide or protein.

[00974] *FGF:* As used herein, the term "FGF" refers to the fibroblast growth factor protein family including any variants thereof.

[00975] *FGF-8:* As used herein, the term "FGF-8" refers to fibroblast growth factor-8 protein including any variants thereof.

[00976] *Feature:* As used herein, a "feature" refers to a characteristic, a property, or a distinctive element.

[00977] *Formulation:* As used herein, a "formulation" includes at least a cell phenotype altering polynucleotide, primary construct or mmRNA and a delivery agent.

[00978] *Fragment:* A "fragment," as used herein, refers to a portion. For example, fragments of proteins may comprise polypeptides obtained by digesting full-length protein isolated from cultured cells.

[00979] *Functional:* As used herein, a "functional" biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.

[00980] *HNF4-alpha:* As used herein, the term "FiNF4-alpha" refers to hepatocyte nuclear factor 4, alpha protein including any variants thereof.

[00981] *Homology:* As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, *e.g.* between nucleic acid molecules *(e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%,

75%, 80%, 85%, 90%, 95%, or 99% identical or similar. The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences). In accordance with the invention, two polynucleotide sequences are considered to be homologous if the polypeptides they encode are at least about 50%>, 60%, 70%, 80%, 90%, 95%, or even 99% for at least one stretch of at least about 20 amino acids. In some embodiments, homologous polynucleotide sequences are characterized by the ability to encode a stretch of at least 4-5 uniquely specified amino acids. For polynucleotide sequences less than 60 nucleotides in length, homology is determined by the ability to encode a stretch of at least 4-5 uniquely specified amino acids. In accordance with the invention, two protein sequences are considered to be homologous if the proteins are at least about 50%>, 60%>, 70%>, 80%>, or 90%> identical for at least one stretch of at least about 20 amino acids.

[00982] *Identity:* As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, *e.g.,* between oligonucleotide molecules *{e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes *{e.g.,* gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%>, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using methods such as those described in Computational

Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; each of which is incorporated herein by reference. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4:1 1-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Methods commonly employed to determine percent identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM J Applied Math., 48:1073 (1988); incorporated herein by reference. Techniques for determining identity are codified in publicly available computer programs. Exemplary computer software to determine homology between two sequences include, but are not limited to, GCG program package, Devereux, J., *et al., Nucleic Acids Research,* 12(1), 387 (1984)), BLASTP, BLASTN, and FASTA Altschul, S. F. *et al., J. Molec. Biol,* 215, 403 (1990)).

[00983] *Inhibit expression of a gene:* As used herein, the phrase "inhibit expression of a gene" means to cause a reduction in the amount of an expression product of the gene. The expression product can be an RNA transcribed from the gene *{e.g.*, an mRNA) or a polypeptide translated from an mRNA transcribed from the gene. Typically a reduction in the level of an mRNA results in a reduction in the level of a polypeptide translated therefrom. The level of expression may be determined using standard techniques for measuring mRNA or protein.

[00984] *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, in a Petri dish, *etc.*, rather than within an organism *(e.g.*, animal, plant, or microbe).

[00985] *In vivo:* As used herein, the term *"in vivo"* refers to events that occur within an organism *(e.g.*, animal, plant, or microbe or cell or tissue thereof).

[00986] *Isolated:* As used herein, the term "isolated" refers to a substance or entity that has been separated from at least some of the components with which it was associated (whether in nature or in an experimental setting). Isolated substances may have varying levels of purity in reference to the substances from which they have been associated. Isolated substances and/or entities may be separated from at least about 10%, about 20%>, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated agents are more than about 80%>, about 85%, about 90%>, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. *Substantially isolated:* By "substantially isolated" is meant that the compound is substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched in the compound of the present disclosure. Substantial separation can include compositions containing at least about 50%, at least about 60%, at least about 70%, at least about 80%>, at least about 90%>, at least about 95%, at least about 97%, or at least about 99% by weight of the compound of the present disclosure, or salt thereof. Methods for isolating compounds and their salts are routine in the art.

[00987] *KLF:* As used herein, the term "KLF" refers to the kruppel-like factor protein family including any variants thereof.

[00988] *KLF I :* As used herein, the term "KLF1" refers to the protein kruppel-like factor 1 including any variants thereof.

[00989] *KLF2:* As used herein, the term "KLF2" refers to the protein kruppel-like factor 2 including any variants thereof.

[00990] *KLF4:* As used herein, the term "KLF4" refers to the protein kruppel-like factor 4 including any variants thereof.

[00991] *LIN28:* As used herein, the term "LIN28" refers to the lin-28 homolog protein including any variants thereof.

[00992] *Linker:* As used herein, a linker refers to a group of atoms, e.g., 10-1,000 atoms, and can be comprised of the atoms or groups such as, but not limited to, carbon, amino, alkylamino, oxygen, sulfur, sulfoxide, sulfonyl, carbonyl, and imine. The linker

can be attached to a modified nucleoside or nucleotide on the nucleobase or sugar moiety at a first end, and to a payload, e.g., a detectable or therapeutic agent, at a second end. The linker may be of sufficient length as to not interfere with incorporation into a nucleic acid sequence. The linker can be used for any useful purpose, such as to form mmRNA multimers (e.g., through linkage of two or more cell phenotype altering polynucleotides, primary constructs, or mmRNA molecules) or mmRNA conjugates, as well as to administer a payload, as described herein. Examples of chemical groups that can be incorporated into the linker include, but are not limited to, alkyl, alkenyl, alkynyl, amido, amino, ether, thioether, ester, alkylene, heteroalkylene, aryl, or heterocyclyl, each of which can be optionally substituted, as described herein. Examples of linkers include, but are not limited to, unsaturated alkanes, polyethylene glycols (e.g., ethylene or propylene glycol monomeric units, e.g., diethylene glycol, dipropylene glycol, triethylene glycol, tripropylene glycol, tetraethylene glycol, or tetraethylene glycol), and dextran polymers, Other examples include, but are not limited to, cleavable moieties within the linker, such as, for example, a disulfide bond (-S-S-) or an azo bond (-N=N-), which can be cleaved using a reducing agent or photolysis. Non-limiting examples of a selectively cleavable bond include an amido bond can be cleaved for example by the use of tris(2-carboxyethyl)phosphine (TCEP), or other reducing agents, and/or photolysis, as well as an ester bond can be cleaved for example by acidic or basic hydrolysis.

[00993] *MicroRNA (miRNA) binding site:* As used herein, a microRNA (miRNA) binding site represents a nucleotide location or region of a nucleic acid transcript to which at least the "seed" region of a miRNA binds.

[00994] *Modified:* As used herein "modified" refers to a changed state or structure of a molecule of the invention. Molecules may be modified in many ways including chemically, structurally, and functionally. In one embodiment, the mRNA molecules of the present invention are modified by the introduction of non-natural nucleosides and/or nucleotides, e.g., as it relates to the natural ribonucleotides A, U, G, and C. Noncanonical nucleotides such as the cap structures are not considered "modified" although they differ from the chemical structure of the A, C, G, U ribonucleotides.

[00995] *Mucus:* As used herein, "mucus" refers to the natural substance that is viscous and comprises mucin glycoproteins.

[00996] *Multipotent:* As used herein, "multipotent" or "partially differentiated cell" when referring to a cell refers to a cell that has a developmental potential to differentiate into cells of one or more germ layers, but not all three germ layers.

[00997] *MYC:* As used herein, the term "MYC" refers to the v-myc myelocytomatosis viral oncogene protein family including any variants thereof.

[00998] *c-MYC:* As used herein, the term "c-MYC" refers to the protein v-myc myelocytomatosis viral oncogene homolog (avian) including any variants thereof.

[00999] *n-MYC:* As used herein, the term "n-MYC" refers to the protein v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian) including any variants thereof.

[001000] *MYOD1* : As used herein, the term "MYOD1" refers to the myogenic differentiation 1 protein including any variants thereof.

[001001] *MYT1L:* As used herein, the term "MYT1L" refers to the myelin transcription factor 1-like protein including any variants thereof.

[001002] *NANOG:* As used herein, the term "NANOG" refers to the protein Nanog homeobox including any variants thereof.

[001003] *Naturally occurring:* As used herein, "naturally occurring" means existing in nature without artificial aid.

[001004] *NGF:* As used herein, the term "NGF" refers to nerve growth factor protein family including any variants thereof.

[001005] *Non-human vertebrate:* As used herein, a "non human vertebrate" includes all vertebrates except *Homo sapiens,* including wild and domesticated species. Examples of non-human vertebrates include, but are not limited to, mammals, such as alpaca, banteng, bison, camel, cat, cattle, deer, dog, donkey, gayal, goat, guinea pig, horse, llama, mule, pig, rabbit, reindeer, sheep water buffalo, and yak.

[001006] *NR5A2:* As used herein, the term "NR5A2" refers to the protein nuclear receptor subfamily 5, group A, member 1 including any variants thereof.

[001007] *NTF*: As used herein, the term "NTF" refers to the neurotrophin protein family including any variants thereof.

[001008] *NTF3:* As used herein, the term "NTF3" refers to neurotrophin 3 including any variants thereof.

[001009] *NTF4:* As used herein, the term "NTF4" refers to neurotrophin 4 including any variants thereof.

[001010] *OCT:* As used herein, the term "OCT" refers to the octamer-binding protein family including any variants thereof.

[001011] *OCT4:* As used herein, the term "OCT4" refers to the ocatmer-binding protein 4, including any variants thereof. OCT4 is also known in the art as POU class 5 homeobox 1 (POU5F1) and octamer-binding protein 3 (OCT3).

[001012] *Off-target:* As used herein, "off target" refers to any unintended effect on any one or more target, gene, or cellular transcript.

[001013] *Oligopotent:* As used herein, "oligopotent" when referring to a cell means to give rise to a more restricted subset of cell lineages than multipotent stem cells..

[001014] *Open reading frame:* As used herein, "open reading frame" or "ORF" refers to a sequence which does not contain a stop codon in a given reading frame.

[001015] *Operably linked:* As used herein, the phrase "operably linked" refers to a functional connection between two or more molecules, constructs, transcripts, entities, moieties or the like.

[001016] *Optionally substituted:* Herein a phrase of the form "optionally substituted X" *(e.g.*, optionally substituted alkyl) is intended to be equivalent to "X, wherein X is optionally substituted" *(e.g.*, "alkyl, wherein said alkyl is optionally substituted"). It is not intended to mean that the feature "X" *(e.g.* alkyl) *per se* is optional.

[001017] *Peptide:* As used herein, "peptide" is less than or equal to 50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[001018] *Paratope:* As used herein, a "paratope" refers to the antigen-binding site of an antibody.

[001019] *Patient:* As used herein, "patient" refers to a subject who may seek or be in need of treatment, requires treatment, is receiving treatment, will receive treatment, or a subject who is under care by a trained professional for a particular disease or condition.

[001020] *Pharmaceutically acceptable:* The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, 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.

[001021] *Pharmaceutically acceptable excipients:* The phrase "pharmaceutically acceptable excipient," as used herein, refers any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being substantially nontoxic and non-inflammatory in a patient. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

[001022] *Pharmaceutically acceptable salts:* The present disclosure also includes pharmaceutically acceptable salts of the compounds described herein. As used herein, "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form (e.g., by reacting the free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate,

glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present disclosure can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences,* 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418, *Pharmaceutical Salts: Properties, Selection, and Use,* P.H. Stahl and C.G. Wermuth (eds.), Wiley-VCH, 2008, and Berge et al., *Journal of Pharmaceutical Science,* 66, 1-19 (1977), each of which is incorporated herein by reference in its entirety.

[001023] *Pharmacokinetic:* As used herein, "pharmacokinetic" refers to any one or more properties of a molecule or compound as it relates to the determination of the fate of substances administered to a living organism. Pharmacokinetics is divided into several areas including the extent and rate of absorption, distribution, metabolism and excretion. This is commonly referred to as ADME where: (A) Absorption is the process of a substance entering the blood circulation; (D) Distribution is the dispersion or dissemination of substances throughout the fluids and tissues of the body; (M) Metabolism (or Biotransformation) is the irreversible transformation of parent

compounds into daughter metabolites; and (E) Excretion (or Elimination) refers to the elimination of the substances from the body. In rare cases, some drugs irreversibly accumulate in body tissue.

[001024] *Pharmaceutically acceptable solvate:* The term "pharmaceutically acceptable solvate," as used herein, means a compound of the invention wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. For example, solvates may be prepared by crystallization, recrystallization, or precipitation from a solution that includes organic solvents, water, or a mixture thereof. Examples of suitable solvents are ethanol, water (for example, mono-, di-, and tri-hydrates), *N*-methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), *N*,*N*'-dimethylformamide (DMF), *N*,*N*'-dimethylacetamide (DMAC), l,3-dimethyl-2-imidazolidinone (DMEU), l,3-dimethyl-3,4,5,6-tetrahydro-2-(lH)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-pyrrolidone, benzyl benzoate, and the like. When water is the solvent, the solvate is referred to as a "hydrate."

[001025] *Physicochemical:* As used herein, "physicochemical" means of or relating to a physical and/or chemical property.

[001026] *Pluripotent:* As used herein, "pluripotent" refers to a cell with the developmental potential, under different conditions, to differentiate to cell types characteristic of all three germ layers.

[001027] *Pluripotency:* As used herein, "pluripotency" or "pluripotent state" refers to the developmental potential of a cell where the cell has the ability to differentitate into all three embryonic germ layers (endoderm, mesoderm and ectoderm).

[001028] *PRDM16:* As used herein, the term "PRDM16" refers to PR domain containing 16 protein including any variants thereof.

[001029] *Preventing:* As used herein, the term "preventing" refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying

progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition.

[001030] *Prodrug:* The present disclosure also includes prodrugs of the compounds described herein. As used herein, "prodrugs" refer to any substance, molecule or entity which is in a form predicate for that substance, molecule or entity to act as a therapeutic upon chemical or physical alteration. Prodrugs may by covalently bonded or sequestered in some way and which release or are converted into the active drug moiety prior to, upon or after administered to a mammalian subject. Prodrugs can be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved, either in routine manipulation or *in vivo,* to the parent compounds. Prodrugs include compounds wherein hydroxyl, amino, sulfhydryl, or carboxyl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino, sulfhydryl, or carboxyl group respectively. Preparation and use of prodrugs is discussed in T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in *Bioreversible Carriers in Drug Design,* ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are hereby incorporated by reference in their entirety.

[001031] *Proliferate:* As used herein, the term "proliferate" means to grow, expand or increase or cause to grow, expand or increase rapidly. "Proliferative" means having the ability to proliferate. "Anti-proliferative" means having properties counter to or inapposite to proliferative properties.

[001032] *Progenitor cell:* As used herein, the term "progenitor cell" refers to cells that have greater developmental potential relative to a cell which it can give rise to by differentiation.

[001033] *Protein cleavage site:* As used herein, "protein cleavage site" refers to a site where controlled cleavage of the amino acid chain can be accomplished by chemical, enzymatic or photochemical means.

[001034] *Protein cleavage signal:* As used herein "protein cleavage signal" refers to at least one amino acid that flags or marks a polypeptide for cleavage.

[001035] *Protein of interest:* As used herein, the terms "proteins of interest" or "desired proteins" include those provided herein and fragments, mutants, variants, and alterations thereof.

[001036] *Proximal:* As used herein, the term "proximal" means situated nearer to the center or to a point or region of interest.

[001037] *PU.l:* As used herein, the term "PU.l" refers to spleen focus forming virus (SFFV) proviral integration oncogene spil protein including any variants thereof.

[001038] *Purified:* As used herein, "purify," "purified," "purification" means to make substantially pure or clear from unwanted components, material defilement, admixture or imperfection.

[001039] *REM2:* As used herein, the term "REM2" refers to the protein RAS (RAD and GEM)-like GTP binding 2 protein including any variants thereof.

[001040] *Repeated transfection:* As used herein, the term "repeated transfection" refers to transfection of the same cell culture with a cell phenotype altering polynucleotide, primary construct or mmRNA a plurality of times. The cell culture can be transfected at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, at least 10 times, at least 11 times, at least 12 times, at least 13 times, at least 14 times, at least 15 times, at least 16 times, at least 17 times at least 18 times, at least 19 times, at least 20 times, at least 25 times, at least 30 times, at least 35 times, at least 40 times, at least 45 times, at least 50 times or more.

[001041] *Reprogramming:* As used herein, "reprogramming" refers to a process that reverses the developmental potential of a cell or population of cells.

[001042] *Reprogramming factor:* As used herein, the term "reprogramming factor" refers to a developmental potential altering factor such as a protein, RNA or small molecule, the expression of which contributes to the reprogramming of a cell to a less differentiated or undifferentiated state.

[001043] *Sample:* As used herein, the term "sample" or "biological sample" refers to a subset of its tissues, cells or component parts (e.g. body fluids, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). A sample further may include a homogenate, lysate or extract prepared from a whole organism or a subset of its

tissues, cells or component parts, or a fraction or portion thereof, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs. A sample further refers to a medium, such as a nutrient broth or gel, which may contain cellular components, such as proteins or nucleic acid molecule.

[001044] *Signal Sequences:* As used herein, the phrase "signal sequences" refers to a sequence which can direct the transport or localization of a protein.

[001045] *Single unit dose:* As used herein, a "single unit dose" is a dose of any therapeutic administed in one dose/at one time/single route/single point of contact, i.e., single administration event.

[001046] *Similarity:* As used herein, the term "similarity" refers to the overall relatedness between polymeric molecules, *e.g.* between polynucleotide molecules *{e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art.

[001047] *Sonic hedgehog:* As used herein, the phrase "sonic hedgehog" refers to the sonic hedgehog protein including any variants thereof.

[001048] *Somatic cell:* As used herein, "somatic cells" refers to any cell other than a germ cell, a cell present in or obtained from a pre-implantation embryo, or a cell resulting from proliferation of such a cell *in vitro.*

[001049] *Somatic stem cell:* As used herein, a "somatic stem cell" refers to any pluripotent or multipotent stem cell derived from non-embryonic tissue including fetal, juvenile and adult tissue.

[001050] *Somatic pluripotent cell:* As used herein, a "somatic pluripotent cell" refers to a somatic cell that has had its developmental potential altered to that of a pluripotent state.

[001051] *SOX:* As used herein, the term "SOX" refers to the SRY (sex determining region Y)-box protein family including any variants thereof.

[001052] *SOX1 :* As used herein, the term "SOX1" refers to the protein SRY (sex determining region Y)-box 1 including any variants thereof.

[001053] *SOX2:* As used herein, the term "SOX2" refers to the protein SRY (sex determining region Y)-box 2 including any variants thereof.

[001054] *SOX3:* As used herein, the term "SOX3" refers to the protein SRY (sex determining region Y)-box 3 including any variants thereof.

[001055] *SOX15:* As used herein, the term "SOX15" refers to the protein SRY (sex determining region Y)-box 15 including any variants thereof.

[001056] *SOX18:* As used herein, the term "SOX18" refers to the protein SRY (sex determining region Y)-box 18 including any variants thereof.

[001057] *Split dose:* As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses.

[001058] *Stable:* As used herein "stable" refers to a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and preferably capable of formulation into an efficacious therapeutic agent.

[001059] *Stabilized:* As used herein, the term "stabilize", "stabilized," "stabilized region" means to make or become stable.

[001060] *Stem cell:* As used herein, the term "stem cell" refers to a cell in an undifferentiated or partially differentiated state that has the property of self-renewal and ahs the developmental potential to differentiate into multiple cell types, without a specific developmental potential. A stem cell may be able capable of proliferation and giving rise to more such stem cells while maintaining its developmental potential.

[001061] *Subject:* As used herein, the term "subject" or "patient" refers to any organism to which a composition in accordance with the invention may be administered, *e.g.,* for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals *{e.g.,* mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants.

[001062] *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.

[001063] *Substantially equal:* As used herein as it relates to time differences between doses, the term means plus/minus 2%.

[001064] *Substantially simultaneously:* As used herein and as it relates to plurality of doses, the term means within 2 seconds.

[001065] *Suffering from:* An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of a disease, disorder, and/or condition.

[001066] *Susceptible to:* An individual who is "susceptible to" a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition but harbors a propensity to develop a disease or its symptoms. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition (for example, cancer) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein and/or nucleic acid associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or condition; and (6) exposure to and/or infection with a microbe associated with development of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[001067] *Sustained release:* As used herein, the term "sustained release" refers to a pharmaceutical composition or compound release profile that conforms to a release rate over a specific period of time.

[001068] *Synthetic:* The term "synthetic" means produced, prepared, and/or manufactured by the hand of man. Synthesis of polynucleotides or polypeptides or other molecules of the present invention may be chemical or enzymatic.

[001069] *Targeted Cells:* As used herein, "targeted cells" refers to any one or more cells of interest. The cells may be found *in vitro, in vivo, in situ* or in the tissue or organ of an organism. The organism may be an animal, preferably a mammal, more preferably a human and most preferably a patient.

[001070] *TGF:* As used herein, the term "TGF" refers to the transforming growth factor protein family including any variants thereof.

[001071] *TGF-alpha:* As used herein, the term "TGF-alpha" refers to transforming growth factor, alpha protein including any variants thereof.

[001072] *TGF-beta:* As used herein, the term "TGF-beta" refers to transforming growth factor, beta protein including any variants thereof.

[001073] *TERT:* As used herein, the term "TERT" refers to the protein telomerase reverse transcriptase protein including any variants thereof.

[001074] *Therapeutic Agent:* The term "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect.

[001075] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" means an amount of an agent to be delivered *{e.g.,* nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.)* that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[001076] *Therapeutically effective outcome:* As used herein, the term "therapeutically effective outcome" means an outcome that is sufficient in a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[001077] *Total daily dose:* As used herein, a "total daily dose" is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose.

[001078] *Totipotency:* As used herein, "totipotency" refers to a cell with a developmental potential to make all of the cells found in the adult body as well as the extra-embryonic tissues, including the placenta.

[001079] *Transcription factor:* As used herein, the term "transcription factor" refers to a DNA-binding protein that regulates transcription of DNA into RNA, for example, by activation or repression of transcription. Some transcription factors effect regulation of transcription alone, while others act in concert with other proteins. Some transcription factor can both activate and repress transcription under certain conditions. In general, transcription factors bind a specific target sequence or sequences highly similar to a specific consensus sequence in a regulatory region of a target gene. Transcription factors may regulate transcription of a target gene alone or in a complex with other molecules.

[001080] *Transcription:* As used herein, the term "transcription" refers to methods to introduce exogenous nucleic acids into a cell. Methods of transfection include, but are not limited to, chemical methods, plysical treatments and cationic lipids or mixtures.

[001081] *Transdifferentiation:* As used herein, "transdifferentiation" refers to the capacity of differentiated cells of one type to lose identifying characteristics and to change their phenotype to that of other fully differentiated cells.

[001082] *Treating:* As used herein, the term "treating" refers to partially or completely alleviating, ameliorating, improving, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular infection, disease, disorder, and/or condition. For example, "treating" cancer may refer to inhibiting survival, growth, and/or spread of a tumor. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[001083] *Unmodified:* As used herein, "unmodified" refers to any substance, compound or molecule prior to being changed in any way. Unmodified may, but does not always, refer to the wild type or native form of a biomolecule. Molecules may undergo a series of modifications whereby each modified molecule may serve as the "unmodified" starting molecule for a subsequent modification.

[001084] *Unipotent:* As used herein, "unipotent" when referring to a cell means to give rise to a single cell lineage.

Equivalents and Scope

[001085] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with 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 appended claims.

[001086] In the claims, articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[001087] It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of is thus also encompassed and disclosed.

[001088] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[001089] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention *(e.g.,* any nucleic acid or protein encoded thereby; any method of production; any method of use; *etc.)* can be

excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[001090] All cited sources, for example, references, publications, databases, database entries, and art cited herein, are incorporated into this application by reference, even if not expressly stated in the citation. In case of conflicting statements of a cited source and the instant application, the statement in the instant application shall control.

[001091] Section and table headings are not intended to be limiting.

EXAMPLES

Example 1. Modified mRNA Production

[001092] Modified mRNAs (mmRNA) according to the invention may be made using standard laboratory methods and materials. The open reading frame (ORF) of the gene of interest may be flanked by a 5' untranslated region (UTR) which may contain a strong Kozak translational initiation signal and/or an alpha-globin 3' UTR which may include an oligo(dT) sequence for templated addition of a poly-A tail. The modified mRNAs may be modified to reduce the cellular innate immune response. The modifications to reduce the cellular response may include pseudouridine (ψ) and 5-methyl-cytidine (5meC, 5mc or m^5C). (See, Kariko K et al. Immunity 23: 165-75 (2005), Kariko K et al. Mol Ther 16:1833-40 (2008), Anderson BR et al. NAR (2010); each of which is herein incorporated by reference in their entirety).

[001093] The ORF may also include various upstream or downstream additions (such as, but not limited to, β-globin, tags, etc.) may be ordered from an optimization service such as, but limited to, DNA2.0 (Menlo Park, CA) and may contain multiple cloning sites which may have Xbal recognition. Upon receipt of the construct, it may be reconstituted and transformed into chemically competent *E. coli.*

[001094] For the present invention, NEB DH5-alpha Competent *E. coli* are used. Transformations are performed according to NEB instructions using 100 ng of plasmid. The protocol is as follows:

1. Thaw a tube of NEB 5-alpha Competent *E. coli* cells on ice for 10 minutes.
2. Add 1-5 μï containing 1 pg-100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.
3. Place the mixture on ice for 30 minutes. Do not mix.

4. Heat shock at 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µï of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting.

[001095] Alternatively, incubate at 30°C for 24-36 hours or 25°C for 48 hours.

[001096] A single colony is then used to inoculate 5 ml of LB growth media using the appropriate antibiotic and then allowed to grow (250 RPM, 37° C) for 5 hours. This is then used to inoculate a 200 ml culture medium and allowed to grow overnight under the same conditions.

[001097] To isolate the plasmid (up to 850 µg), a maxi prep is performed using the Invitrogen PURELINK™ HiPure Maxiprep Kit (Carlsbad, CA), following the manufacturer's instructions.

[001098] In order to generate cDNA for *In Vitro* Transcription (IVT), the plasmid (an Example of which is shown in Figure 3) is first linearized using a restriction enzyme such as Xbal. A typical restriction digest with Xbal will comprise the following: Plasmid 1.0 µg; lOx Buffer 1.0 µï; Xbal 1.5 µï; dH_20 up to 10 µï; incubated at 37° C for 1 hr. If performing at lab scale (< 5µg), the reaction is cleaned up using Invitrogen' s PURELINK™ PCR Micro Kit (Carlsbad, CA) per manufacturer's instructions. Larger scale purifications may need to be done with a product that has a larger load capacity such as Invitrogen's standard PURELINK™ PCR Kit (Carlsbad, CA). Following the cleanup, the linearized vector is quantified using the NanoDrop and analyzed to confirm linearization using agarose gel electrophoresis.

[001099] As a non-limiting example, G-CSF may represent the polypeptide of interest. Sequences used in the steps outlined in Examples 1-5 are shown in Table 11. It should be noted that the start codon (ATG) has been underlined in each sequence of Table 11.

Table 11. G-CSF Sequences

SEQ ID NO	Description

405	cDNAsequence: ATGGCTGGACCTGCCACCCAGAGCCCCATGAAGCTGATGGCCCTGCAGCTGCT GCTGTGGCACAGTGCACTCTGGACAGTGCAGGAAGCCACCCCCCTGGGCCCTG CCAGCTCCCTGCCCCAGAGCTTCCTGCTCAAGTGCTTAGAGCAAGTGAGGAAG ATCCAGGGCGATGGCGCAGCGCTCCAGGAGAAGCTGTGTGCCACCTACAAGCT GTGCCACCCCGAGGAGCTGGTGCTGCTCGGACACTCTCTGGGCATCCCCTGGG CTCCCCTGAGCAGCTGCCCCAGCCAGGCCCTGCAGCTGGCAGGCTGCTTGAGC CAACTCCATAGCGGCCTTTTCCTCTACCAGGGGCTCCTGCAGGCCCTGGAAGGG ATCTCCCCCGAGTTGGGTCCCACCTTGGACACACTGCAGCTGGACGTCGCCGAC TTTGCCACCACCATCTGGCAGCAGATGGAAGAACTGGGAATGGCCCCTGCCCT GCAGCCCACCCAGGGTGCCATGCCGGCCTTCGCCTCTGCTTTCCAGCGCCGGGC AGGAGGGGTCCTGGTTGCCTCCCATCTGCAGAGCTTCCTGGAGGTGTCGTACCG CGTTCTACGCCACCTTGCCCAGCCCTGA
406	cDNA having T7 polymerase site, AfeI and Xba restriction site: TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC ATGGCTGGACCTGCCACCCAGAGCCCCATGAAGCTGATGGCCCTGCAGCTGCT GCTGTGGCACAGTGCACTCTGGACAGTGCAGGAAGCCACCCCCCTGGGCCCTG CCAGCTCCCTGCCCCAGAGCTTCCTGCTCAAGTGCTTAGAGCAAGTGAGGAAG ATCCAGGGCGATGGCGCAGCGCTCCAGGAGAAGCTGTGTGCCACCTACAAGCT GTGCCACCCCGAGGAGCTGGTGCTGCTCGGACACTCTCTGGGCATCCCCTGGG CTCCCCTGAGCAGCTGCCCCAGCCAGGCCCTGCAGCTGGCAGGCTGCTTGAGC CAACTCCATAGCGGCCTTTTCCTCTACCAGGGGCTCCTGCAGGCCCTGGAAGGG ATCTCCCCCGAGTTGGGTCCCACCTTGGACACACTGCAGCTGGACGTCGCCGAC TTTGCCACCACCATCTGGCAGCAGATGGAAGAACTGGGAATGGCCCCTGCCCT GCAGCCCACCCAGGGTGCCATGCCGGCCTTCGCCTCTGCTTTCCAGCGCCGGGC AGGAGGGGTCCTGGTTGCCTCCCATCTGCAGAGCTTCCTGGAGGTGTCGTACCG CGTTCTACGCCACCTTGCCCAGCCCTGA AGCGCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGC ACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTCGA GCATGCATCTAGA
407	Optimized sequence; containing T7 polymerase site, AfeI and Xba restriction site TAATACGACTCACTATA GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC ATGGCCGGTCCCGCGACCCAAAGCCCCATGAAACTTATGGCCCTGCAGTTGCT GCTTTGGCACTCGGCCCTCTGGACAGTCCAAGAAGCGACTCCTCTCGGACCTGC CTCATCGTTGCCGCAGTCATTCCTTTTGAAGTGTCTGGAGCAGGTGCGAAAGAT TCAGGGCGATGGAGCCGCACTCCAAGAGAAGCTCTGCGCGACATACAAACTTT GCCATCCCGAGGAGCTCGTACTGCTCGGGCACAGCTTGGGGATTCCCTGGGCT CCTCTCTCGTCCTGTCCGTCGCAGGCTTTGCAGTTGGCAGGGTGCCTTTCCCAG CTCCACTCCGGTTTGTTCTTGTATCAGGGACTGCTGCAAGCCCTTGAGGGAATC TCGCCAGAATTGGGCCCGACGCTGGACACGTTGCAGCTCGACGTGGCGGATTT CGCAACAACCATCTGGCAGCAGATGGAGGAACTGGGGATGGCACCCGCGCTGC AGCCCACGCAGGGGGCAATGCCGGCCTTTGCGTCCGCGTTTCAGCGCAGGGCG GGTGGAGTCCTCGTAGCGAGCCACCTTCAATCATTTTTGGAAGTCTCGTACCGG

	GTGCTGAGACATCTTGCGCAGCCGTGA AGCGCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGC ACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGGCGGCCGCTCGA GCATGCATCTAGA
408	mRNA sequence (transcribed) GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC AUGGCCGGUCCCGCGACCCAAAGCCCCAUGAAACUUAUGGCCCUGCAGUUGC UGCUUUGGCACUCGGCCCUCUGGACAGUCCAAGAAGCGACUCCUCUCGGACC UGCCUCAUCGUUGCCGCAGUCAUUCCUUUUGAAGUGUCUGGAGCAGGUGCG AAAGAUUCAGGGCGAUGGAGCCGCACUCCAAGAGAAGCUCUGCGCGACAUA CAAACUUUGCCAUCCCGAGGAGCUCGUACUGCUCGGGCACAGCUUGGGGAUU CCCUGGGCUCCUCUCUCGUCCUGUCCGUCGCAGGCUUUGCAGUUGGCAGGGU GCCUUUCCCAGCUCCACUCCGGUUUGUUCUUGUAUCAGGGACUGCUGCAAGC CCUUGAGGGAAUCUCGCCAGAAUUGGGCCCGACGCUGGACACGUUGCAGCUC GACGUGGCGGAUUUCGCAACAACCAUCUGGCAGCAGAUGGAGGAACUGGGG AUGGCACCCGCGCUGCAGCCCACGCAGGGGGCAAUGCCGGCCUUUGCGUCCG CGUUUCAGCGCAGGGCGGGUGGAGUCCUCGUAGCGAGCCACCUUCAAUCAUU UUUGGAAGUCUCGUACCGGGUGCUGAGACAUCUUGCGCAGCCGUGA AGCGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCU UGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGAAG

Example 2: PCR for cDNA Production

[001100] PCR procedures for the preparation of cDNA are performed using 2x KAPA HIFI™ HotStart Ready Mix by Kapa Biosystems (Woburn, MA). This system includes 2x KAPA ReadyMixl2.5 μï; Forward Primer (10 uM) 0.75 μï; Reverse Primer (10 uM) 0.75 μï; Template cDNA 100 ng; and dH_20 diluted to 25.0 μï. The reaction conditions are at 95° C for 5 min. and 25 cycles of 98° C for 20 sec, then 58° C for 15 sec, then 72° C for 45 sec, then 72° C for 5 min. then 4° C to termination.

[001101] The reverse primer of the instant invention incorporates a poly-Ti_{20} for a poly-Ai_{20} in the mRNA. Other reverse primers with longer or shorter poly(T) tracts can be used to adjust the length of the poly(A) tail in the mRNA.

[001102] The reaction is cleaned up using Invitrogen's PURELINK™ PCR Micro Kit (Carlsbad, CA) per manufacturer's instructions (up to 5 μg). Larger reactions will require a cleanup using a product with a larger capacity. Following the cleanup, the cDNA is quantified using the NanoDrop and analyzed by agarose gel electrophoresis to confirm the cDNA is the expected size. The cDNA is then submitted for sequencing analysis before proceeding to the *in vitro* transcription reaction.

Example 3. *In vitro* Transcription (IVT)

[001103] The *in vitro* transcription reaction generates mRNA containing modified nucleotides or modified RNA. The input nucleotide triphosphate (NTP) mix is made in-house using natural and un-natural NTPs.

[001104] A typical *in vitro* transcription reaction includes the following:

1. Template cDNA 1.0 µg
2. lOx transcription buffer (400 mM Tris-HCl pH 8.0, 190 mM MgCk, 50 mM DTT, 10 mM Spermidine) 2.0 µï
3. Custom NTPs (25mM each) 7.2 µï
4. RNase Inhibitor 20 U
5. T7 RNA polymerase 3000 U
6. dH_20 Up to 20.0 µï. and
7. Incubation at 37° C for 3 hr-5 hrs.

[001105] The crude IVT mix may be stored at 4° C overnight for cleanup the next day. 1 U of RNase-free DNase is then used to digest the original template. After 15 minutes of incubation at 37° C, the mRNA is purified using Ambion's MEGACLEAR™ Kit (Austin, TX) following the manufacturer's instructions. This kit can purify up to 500 µg of RNA. Following the cleanup, the RNA is quantified using the NanoDrop and analyzed by agarose gel electrophoresis to confirm the RNA is the proper size and that no degradation of the RNA has occurred.

Example 4. Enzymatic Capping of mRNA

[001106] Capping of the mRNA is performed as follows where the mixture includes: IVT RNA 60 µg-180 µg and dH_20 up to 72 µï. The mixture is incubated at 65° C for 5 minutes to denature RNA, and then is transferred immediately to ice.

[001107] The protocol then involves the mixing of lOx Capping Buffer (0.5 M Tris-HCl (pH 8.0), 60 mM KC1, 12.5 mM $MgCl_2$) (10.0 µï); 20 mM GTP (5.0 µï); 20 mM S-Adenosyl Methionine (2.5 µï); RNase Inhibitor (100 U); 2'-0-Methyltransferase (400U); Vaccinia capping enzyme (Guanylyl transferase) (40 U); dH_20 (Up to 28 µï); and incubation at 37° C for 30 minutes for 60 µg RNA or up to 2 hours for 180 µg of RNA.

[001108] The mRNA is then purified using Ambion's MEGACLEAR™ Kit (Austin, TX) following the manufacturer's instructions. Following the cleanup, the RNA is quantified using the NANODROP™ (ThermoFisher, Waltham, MA) and analyzed by agarose gel electrophoresis to confirm the RNA is the proper size and that no degradation of the RNA has occurred. The RNA product may also be sequenced by running a reverse-transcription-PCR to generate the cDNA for sequencing.

Example 5. PolyA Tailing Reaction

[001109] Without a poly-T in the cDNA, a poly-A tailing reaction must be performed before cleaning the final product. This is done by mixing Capped IVT RNA (100 µï); RNase Inhibitor (20 U); lOx Tailing Buffer (0.5 M Tris-HCl (pH 8.0), 2.5 M NaCl, 100 mM $MgCl_2$)(12.0 µï); 20 mM ATP (6.0 µï); Poly-A Polymerase (20 U); dH_20 up to 123.5 µï and incubation at 37° C for 30 min. If the poly-A tail is already in the transcript, then the tailing reaction may be skipped and proceed directly to cleanup with Ambion's MEGACLEAR™ kit (Austin, TX) (up to 500 µg). Poly-A Polymerase is preferably a recombinant enzyme expressed in yeast.

[001110] For studies performed and described herein, the poly-A tail is encoded in the IVT template to comprise 160 nucleotides in length. However, it should be understood that the processivity or integrity of the polyA tailing reaction may not always result in exactly 160 nucleotides. Hence polyA tails of approximately 160 nucleotides, e.g, about 150-165, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164 or 165 are within the scope of the invention.

Example 6. Natural 5′ Caps and 5′ Cap Analogues

[001111] 5'-capping of modified RNA may be completed concomitantly during the *in* vzYro-transcription reaction using the following chemical RNA cap analogs to generate the 5'-guanosine cap structure according to manufacturer protocols: 3′-O-Me-m7G(5*)ppp(5*) G [the ARCA cap];G(5*)ppp(5*)A; G(5*)ppp(5*)G; m7G(5*)ppp(5*)A; m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). 5'-capping of modified RNA may be completed post-transcriptionally using a Vaccinia Virus Capping Enzyme to generate the "Cap 0" structure: m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). Cap 1 structure may be generated using both Vaccinia Virus Capping Enzyme and a 2'-0 methyl-transferase to generate: m7G(5')ppp(5')G-2'-0-methyl. Cap 2 structure may be

generated from the Cap 1 structure followed by the 2'-0-methylation of the 5'-antepenultimate nucleotide using a 2'-0 methyl-transferase. Cap 3 structure may be generated from the Cap 2 structure followed by the 2'-0-methylation of the 5'-preantepenultimate nucleotide using a 2'-0 methyl-transferase. Enzymes are preferably derived from a recombinant source.

[001112] When transfected into mammalian cells, the modified mRNAs have a stability of between 12-18 hours or more than 18 hours, e.g., 24, 36, 48, 60, 72 or greater than 72 hours.

Example 7. Capping

A. Protein Expression Assay

[001113] Synthetic mRNAs encoding human G-CSF (cDNA shown in SEQ ID NO: 405; mRNA sequence fully modified with 5-methylcytosine at each cytosine and pseudouridine replacement at each uridine site shown in SEQ ID NO: 408 with a polyA tail approximately 160 nucletodies in length not shown in sequence) containing the ARCA (3' 0-Me-m7G(5')ppp(5')G) cap analog or the Capl structure can be transfected into human primary keratinocytes at equal concentrations. 6, 12, 24 and 36 hours post-transfection the amount of G-CSF secreted into the culture medium can be assayed by ELISA. Synthetic mRNAs that secrete higher levels of G-CSF into the medium would correspond to a synthetic mRNA with a higher translationally-competent Cap structure.

B. Purity Analysis Synthesis

[001114] Synthetic mRNAs encoding human G-CSF (cDNA shown in SEQ ID NO: 405; mRNA sequence fully modified with 5-methylcytosine at each cytosine and pseudouridine replacement at each uridine site shown in SEQ ID NO: 408 with a polyA tail approximately 160 nucletodies in length not shown in sequence) containing the ARCA cap analog or the Capl structure crude synthesis products can be compared for purity using denaturing Agarose-Urea gel electrophoresis or HPLC analysis. Synthetic mRNAs with a single, consolidated band by electrophoresis correspond to the higher purity product compared to a synthetic mRNA with multiple bands or streaking bands. Synthetic mRNAs with a single HPLC peak would also correspond to a higher purity product. The capping reaction with a higher efficiency would provide a more pure mRNA population.

C. Cytokine Analysis

[001115] Synthetic mRNAs encoding human G-CSF (cDNA shown in SEQ ID NO: 405; mRNA sequence fully modified with 5-methylcytosine at each cytosine and pseudouridine replacement at each uridine site shown in SEQ ID NO: 408 with a polyA tail approximately 160 nucletodies in length not shown in sequence) containing the ARCA cap analog or the Capl structure can be transfected into human primary keratinocytes at multiple concentrations. 6, 12, 24 and 36 hours post-transfection the amount of pro-inflammatory cytokines such as TNF-alpha and IFN-beta secreted into the culture medium can be assayed by ELISA. Synthetic mRNAs that secrete higher levels of pro-inflammatory cytokines into the medium would correspond to a synthetic mRNA containing an immune-activating cap structure.

D. Capping Reaction Efficiency

[001116] Synthetic mRNAs encoding human G-CSF (cDNA shown in SEQ ID NO: 405; mRNA sequence fully modified with 5-methylcytosine at each cytosine and pseudouridine replacement at each uridine site shown in SEQ ID NO: 408 with a polyA tail approximately 160 nucletodies in length not shown in sequence) containing the ARCA cap analog or the Capl structure can be analyzed for capping reaction efficiency by LC-MS after capped mRNA nuclease treatment. Nuclease treatment of capped mRNAs would yield a mixture of free nucleotides and the capped 5'-5-triphosphate cap structure detectable by LC-MS. The amount of capped product on the LC-MS spectra can be expressed as a percent of total mRNA from the reaction and would correspond to capping reaction efficiency. The cap structure with higher capping reaction efficiency would have a higher amount of capped product by LC-MS.

Example 8. Agarose Gel Electrophoresis of Modified RNA or RT PCR Products

[001117] Individual modified RNAs (200-400 ng in a 20 µï volume) or reverse transcribed PCR products (200-400 ng) are loaded into a well on a non-denaturing 1.2% Agarose E-Gel (Invitrogen, Carlsbad, CA) and run for 12-15 minutes according to the manufacturer protocol.

Example 9. Nanodrop Modified RNA Quantification and UV Spectral Data

[001118] Modified RNAs in TE buffer (1 µï) are used for Nanodrop UV absorbance readings to quantitate the yield of each modified RNA from an *in vitro* transcription reaction.

Example 10. Method of Screening for Protein Expression

A. Electrospray Ionization

[001119] A biological sample which may contain proteins encoded by modified RNA administered to the subject is prepared and analyzed according to the manufacturer protocol for electrospray ionization (ESI) using 1, 2, 3 or 4 mass analyzers. A biologic sample may also be analyzed using a tandem ESI mass spectrometry system.

[001120] Patterns of protein fragments, or whole proteins, are compared to known controls for a given protein and identity is determined by comparison.

B. Matrix-Assisted Laser Desorption/Ionization

[001121] A biological sample which may contain proteins encoded by modified RNA administered to the subject is prepared and analyzed according to the manufacturer protocol for matrix-assisted laser desorption/ionization (MALDI).

[001122] Patterns of protein fragments, or whole proteins, are compared to known controls for a given protein and identity is determined by comparison.

C. Liquid Chromatography-Mass spectrometry-Mass spectrometry

[001123] A biological sample, which may contain proteins encoded by modified RNA, may be treated with a trypsin enzyme to digest the proteins contained within. The resulting peptides are analyzed by liquid chromatography-mass spectrometry-mass spectrometry (LC/MS/MS). The peptides are fragmented in the mass spectrometer to yield diagnostic patterns that can be matched to protein sequence databases via computer algorithms. The digested sample may be diluted to achieve 1 ng or less starting material for a given protein. Biological samples containing a simple buffer background (e.g. water or volatile salts) are amenable to direct in-solution digest; more complex backgrounds (e.g. detergent, non-volatile salts, glycerol) require an additional clean-up step to facilitate the sample analysis.

[001124] Patterns of protein fragments, or whole proteins, are compared to known controls for a given protein and identity is determined by comparison.

Example 11. Chemical Modification Ranges of Modified mRNA

[001125] Modified nucleosides such as, but not limited to, the chemical modifications 5-methylcytosine and pseudouridine have been shown to lower the innate immune response and increase expression of RNA in mammalian cells. Surprisingly and not previously known, the effects manifested by these chemical modifications can be titrated when the amount of chemical modification of a particular nucleotide is less than 100%. Previously, it was believed that the benefit of chemical modification could be derived using less than complete replacement of a modified nucleoside and published reports suggest no loss of benefit until the level of substitution with a modified nucleoside is less than 50% (Kariko et al, Immunity (2005) 23:165-175).

[001126] However, it has now been shown that the benefits of chemical modification are directly correlated with the degree of chemical modification and must be considered in view of more than a single measure of immune response. Such benefits include enhanced protein production or mRNA translation and reduced or avoidance of stimulating the innate immune response as measured by cytokine profiles and metrics of immune response triggers.

[001127] Enhanced mRNA translation and reduced or lack of innate immune stimulation are seen with ***100%*** substitution with a modified nucleoside. Lesser percentages of substitution result in less mRNA translation and more innate immune stimulation, with unmodified mRNA showing the lowest translation and the highest innate immune stimulation.

***In Vitro* PBMC Studies: Percent modification**

[001128] 480 ng of G-CSF mRNA modified with 5-methylcytosine (5mC) and pseudouridine (pseudoU) or unmodified G-CSF mRNA was transfected with 0.4 uL of Lipofectamine 2000 into peripheral blood mononuclear cells (PBMC) from three normal blood donors (Dl, D2, and D3). The G-CSF mRNA (SEQ ID NO: 408; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) was completely modified with 5mC and pseudo ***(100%*** modification), not modified with 5mC and pseudo (0% modification) or was partially modified with 5mC and pseudoU so the mRNA would contain 75% modification, 50% modification or 25% modification. A control sample of Luciferase (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately

160 nucleotides not shown in sequence; 5'cap, Capl; fully modified 5meC and pseudoU) was also analyzed for G-CSF expression. For TNF-alpha and IFN-alpha control samples of Lipofectamine2000, LPS, R-848, Luciferase (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl; fully modified 5mC and pseudo), and P(I)P(C) were also analyzed. The supernatant was harvested and run by ELISA 22 hours after transfection to determine the protein expression. The expression of G-CSF is shown in Table 12 and the expression of IFN-alpha and TNF-alpha is shown in Table 8. The expression of IFN-alpha and TNF-alpha may be a secondary effect from the transfection of the G-CSF mRNA. Tables 12, 13 show that the amount of chemical modification of G-CSF, interferon alpha (IFN-alpha) and tumor necrosis factor-alpha (TNF-alpha) is titratable when the mRNA is not fully modified and the titratable trend is not the same for each target.

[001129] As mentioned above, using PBMC as an in vitro assay system it is possible to establish a correlation between translation (in this case G-CSF protein production) and cytokine production (in this case exemplified by IFN-alpha protein production). Better protein production is correlated with lower induction of innate immune activation pathway, and the percentage modification of a chemistry can be judged favorably based on this ratio (Table 14). As calculated from Tables 12 and 13 and shown in Table 14, full modification with 5-methylcytidine and pseudouridine shows a much better ratio of protein/cytokine production than without any modification (natural G-CSF mRNA) (100-fold for IFN-alpha and 27-fold for TNF-alpha). Partial modification shows a linear relationship with increasingly less modification resulting in a lower protein/cytokine ratio.

Table 12. G-CSF Expression

	G-CSF Expression (pg/ml)		
	D1	**D2**	**D3**
100% modification	1968.9	2595.6	2835.7
75% modification	566.7	631.4	659.5
50% modification	188.9	187.2	191.9
25% modification	139.3	126.9	102.0
0% modification	194.8	182.0	183.3
Luciferase	90.2	0.0	22.1

Table 13. IFN-alpha and TNF-alpha Expression

	IFN-alpha Expression (pg/ml)			TNF-alpha Expression (pg/ml)		
	D1	D2	D3	D1	D2	D3
100% modification	336.5	78.0	46.4	115.0	15.0	11.1
75% modification	339.6	107.6	160.9	107.4	21.7	11.8
50% modification	478.9	261.1	389.7	49.6	24.1	10.4
25% modification	564.3	400.4	670.7	85.6	26.6	19.8
0% modification	1421.6	810.5	1260.5	154.6	96.8	45.9
LPS	0.0	0.6	0.0	0.0	12.6	4.3
R-848	0.5	3.0	14.1	655.2	989.9	420.4
P(I)P(C)	130.8	297.1	585.2	765.8	2362.7	1874.4
Lipid only	1952.2	866.6	855.8	248.5	82.0	60.7

Table 14. PC Ratio and Effect of Percentage of Modification

% Modification	Average G-CSF (pg/ml)	Average IFN-a (pg/ml)	Average TNF-a (pg/ml)	G-CSF/ IFN-alpha (PC ratio)	G-CSF/TNF-alpha (PC ratio)
100	2466	153	47	16	52
75	619	202	47	3.1	13
50	189	376	28	0.5	6.8
25	122	545	44	0.2	2.8
0	186	1164	99	0.16	1.9

Example 12. Toxicity of Nucleoside Triphosphates (NTPs)

[001130] The cytotoxicity of natural and modified nucleoside triphosphates (NTPs) alone or in combination with other bases, was analyzed in human embryonic kidney 293 (HEK293) cells in the absence of transfection reagent. HEK293 cells were seeded on 96-well plates at a density of 30,000 cells per well having 0.75ul of RNAiMAX ™ (Invitrogen, Carlsbad, CA) per well at a total well volume of lOOul. 10 ul of the NTPs outlined in Table 12 were combined with 10 ul of lipid dilution and incubated for 30 minutes to form a complex before 80 ul of the HEK293 cell suspension was added to the NTP complex.

[001131] Natural and modified NTPs were transfected at a concentration of 2.1 nM, 21 nM, 210 nM, 2.1 um, 21 uM, 210 um or 2.1 mM. NTPs in combination were transfected at a total concentration of NTPs of 8.4 nM, 84 nM, 840 nM, 8.4 uM, 84 uM, 840 uM and 8.4 mM. As a control modified G-CSF mRNA (SEQ ID NO: 408; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl; fully modified 5-

methylcytosine and pseudouridine) was transfected in HEK293 cells at a concentration of 8.4 nM. The cytotoxicity of the NTPs and the modified G-CSF mRNA was assayed at 4, 24, 48 and 72 hours post addition to the HEK293 cells using a CYTO TOX-GLO™ assay from Promega (Madison, WI) following the manufacturer protocol except pippeting was used for lysing the cells instead of shaking the plates.

[001132] Table 15 and 16 show the percent of viable cells for each of the NTPs, NTP combinations and controls tested. There was no toxicity seen with the individual NTPs as compared to the untreated cells. These data demonstrate that introduction of individual NTPs, including 5- methylcytidine, pseudouridine, andNl-methylpseudouridine, into mammalian cells is not toxic at doses 1,000,000 times an effective dose when introduced as a modified mRNA.

Table 15. Cytotoxicity of Individual NTPs

Individual NTP Cytotoxicity								
	Time	Dose						
		2.1 mM	210 uM	21 uM	2.1 uM	210 nM	21 nM	2.1 nM
Adenine	4 hr	90.03	85.97	91.20	90.23	90.36	93.21	93.48
	24 hr	88.42	87.31	86.86	86.81	86.94	87.19	86.44
	48 hr	93.71	90.55	89.94	89.80	89.17	91.13	92.12
	72 hr	97.49	94.81	93.83	94.58	92.22	93.88	95.74
Cytosine	4 hr	90.51	89.88	91.41	90.49	88.95	93.11	93.34
	24 hr	86.92	86.33	85.72	86.70	86.12	86.16	85.78
	48 hr	94.23	87.81	87.28	87.73	85.36	88.95	88.99
	72 hr	97.15	92.34	92.22	88.93	88.22	91.80	94.22
Guanine	4 hr	90.96	90.14	91.36	90.60	90.00	92.84	93.33
	24 hr	86.37	85.86	85.93	86.13	86.35	85.50	85.41
	48 hr	93.83	87.05	88.18	87.89	85.31	87.92	89.57
	72 hr	97.04	91.41	92.39	92.30	92.19	92.55	93.72
Uracil	4 hr	90.97	89.60	91.95	90.90	91.05	92.90	93.15
	24 hr	87.68	86.48	85.89	86.75	86.52	87.23	87.63
	48 hr	94.39	88.98	89.11	89.44	88.33	88.89	91.28
	72 hr	96.82	93.45	93.63	94.60	94.50	94.53	95.51
Pseudouridine	4 hr	92.09	92.37	91.35	92.02	92.84	91.96	92.26
	24 hr	88.38	86.68	86.05	86.75	85.91	87.59	87.31
	48 hr	88.62	87.79	87.73	87.66	87.82	89.03	91.99
	72 hr	96.87	89.82	94.23	93.54	92.37	94.26	94.25
5-methyl cytosine	4 hr	92.01	91.54	91.16	91.31	92.31	91.40	92.23
	24 hr	87.97	85.76	84.72	85.14	84.71	86.37	86.35
	48 hr	87.29	85.94	85.74	86.18	86.44	87.10	88.18
	72 hr	96.08	88.10	92.26	90.92	89.97	92.10	91.93
N1-methyl pseudouridine	4 hr	92.45	91.43	91.48	90.41	92.15	91.44	91.89
	24 hr	88.92	86.48	85.17	85.72	85.89	86.85	87.79
	48 hr	89.84	86.02	87.52	85.85	87.38	86.72	87.81
	72 hr	96.80	93.03	93.83	92.25	92.40	92.84	92.98

Untreated	4 hr	92.77	_	_	_	_	_	_
	24 hr	87.52	_	_	_	_	_	_
	48 hr	92.95	_	_	_	_	_	_
	72 hr	96.97	_	_	_	_	_	_

Table 16. Cytotoxicity of NTPs in Combination

NTP Combination Cytotoxicity								
	Time	**Dose**						
		8.4 mM	**840 uM**	**84 uM**	**8.4 uM**	**840 nM**	**84 nM**	**8.4 nM**
Pseudouridine/ 5-methylcytosine/ Adenine/ Guanine	4 hr	92.27	92.04	91.47	90.86	90.87	91.10	91.50
	24 hr	88.51	86.90	86.43	88.15	88.46	86.28	87.51
	48 hr	88.30	87.36	88.58	88.13	87.39	88.72	90.55
	72 hr	96.53	94.42	94.31	94.53	94.38	94.36	93.65
N1-methyl pseudouridine/5-methylcytosine/ Adenine/ Guanine	4 hr	92.31	91.71	91.36	91.15	91.30	90.86	91.38
	24 hr	88.19	87.07	86.46	87.70	88.13	85.30	87.21
	48 hr	87.17	86.53	87.51	85.85	84.69	87.73	86.79
	72 hr	96.40	94.88	94.40	93.65	94.82	92.72	93.10
G-CSF modified mRNA	4 hr	na	na	na	na	na	na	92.63
	24 hr	na	na	na	na	na	na	87.53
	48 hr	na	na	na	na	na	na	91.70
	72 hr	na	na	na	na	na	na	96.36

Example 13. Chemical Modification: *In vitro* studies

A. *In vitro* Screening in PBMC

[001133] 500 ng of G-CSF (mRNA sequence shown in SEQ ID NO: 408; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) mRNA fully modified with the chemical modification outlined Tables 17 and 18 was transfected with 0.4 uL Lipofectamine 2000 into peripheral blood mononuclear cells (PBMC) from three normal blood donors. Control samples of LPS, R848, P(I)P(C) and mCherry (mRNA sequence shown in SEQ ID NO: 410; polyA tail of approximately 160 nucleotides not shown in sequence, 5'cap, Capl; fully modified with 5-methylcytosine and pseudouridine) were also analyzed. The supernatant was harvested and stored frozen until analyzed by ELISA to determine the G-CSF protein expression, and the induction of the cytokines interferon-alpha (IFN-a) and tumor necrosis factor alpha (TNF-a). The protein expression of G-CSF is shown in Table 17, the expression of IFN-a and TNF-a is shown in Table 18.

[001134] The data in Table 17 demonstrates that many, but not all, chemical modifications can be used to productively produce human G-CSF in PBMC. Of note,

100% Nl-methylpseudouridine substitution demonstrates the highest level of human G-CSF production (almost 10-fold higher than pseudouridine itself). When Nl-methylpseudouridine is used in combination with 5-methylcytidine a high level of human G-CSF protein is also produced (this is also higher than when pseudouridine is used in combination with 5 methylcytidine).

[001135] Given the inverse relationship between protein production and cytokine production in PBMC, a similar trend is also seen in Table 18, where 100% substitution with Nl-methylpseudouridine results no cytokine induction (similar to transfection only controls) and pseudouridine shows detectable cytokine induction which is above background.

[001136] Other modifications such as N6-methyladenosine and a-thiocytidine appear to increase cytokine stimulation.

Table 17. Chemical Modifications and G-CSF Protein Expression

Chemical Modifications	G-CSF Protein Expression (pg/ml)		
	Donor 1	Donor 2	Donor 3
Pseudouridine	2477	1,909	1,498
5-methyluridine	318	359	345
N1-methylpseudouridine	21,495	16,550	12,441
2-thiouridine	932	1,000	600
4-thiouridine	5	391	218
5-methoxyuridine	2,964	1,832	1,800
5-methylcytosine and pseudouridine (1st set)	2,632	1,955	1,373
5-methylcytosine and N1-methylpseudouridine (1st set)	10,232	7,245	6,214
2'Fluoroguanosine	59	186	177
2'Fluorouridine	118	209	191
5-methylcytosine and pseudouridine (2nd set)	1,682	1,382	1,036
5-methylcytosine and N1-methylpseudouridine (2nd set)	9,564	8,509	7,141
5-bromouridine	314	482	291
5-(2-carbomethoxyvinyl)uridine	77	286	177
5-[3(1-E-propenylamino)uridine	541	491	550
α-thiocytidine	105	264	245
5-methylcytosine and pseudouridine (3rd set)	1,595	1,432	955
N1-methyladenosine	182	177	191
N6-methyladenosine	100	168	200
5-methylcytidine	291	277	359
N4-acetylcytidine	50	136	36
5-formylcytidine	18	205	23
5-methylcytosine and pseudouridine (4th set)	264	350	182

5-methylcytosine and N1-methylpseudouridine (4th set)	9,505	6,927	5,405
LPS	1,209	786	636
mCherry	5	168	164
R848	709	732	636
P(I)P(C)	5	186	182

Table 18. Chemical Modifications and Cytokine Expression

Chemical Modifications	IFN-α Expression (pg/ml)			TNF-α Expression (pg/ml)		
	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
Pseudouridine	120	77	171	36	81	126
5-methyluridine	245	135	334	94	100	157
N1-methylpseudouridine	26	75	138	101	106	134
2-thiouridine	100	108	154	133	133	141
4-thiouridine	463	258	659	169	126	254
5-methoxyuridine	0	64	133	39	74	111
5-methylcytosine and pseudouridine (1st set)	88	94	148	64	89	121
5-methylcytosine and N1-methylpseudouridine (1st set)	0	60	136	54	79	126
2'Fluoroguanosine	107	97	194	91	94	141
2'Fluorouridine	158	103	178	164	121	156
5-methylcytosine and pseudouridine (2nd set)	133	92	167	99	111	150
5-methylcytosine and N1-methylpseudouridine (2nd set)	0	66	140	54	97	149
5-bromouridine	95	86	181	87	106	157
5-(2-carbomethoxyvinyl)uridine	0	61	130	40	81	116
5-[3(1-E-propenylamino)uridine	0	58	132	71	90	119
α-thiocytidine	1,138	565	695	300	273	277
5-methylcytosine and pseudouridine (3rd set)	88	75	150	84	89	130
N1-methyladenosine	322	255	377	256	157	294
N6-methyladenosine	1,935	1,065	1,492	1,080	630	857
5-methylcytidine	643	359	529	176	136	193
N4-acetylcytidine	789	593	431	263	67	207
5-formylcytidine	180	93	88	136	30	40
5-methylcytosine and pseudouridine (4th set)	131	28	18	53	24	29
5-methylcytosine and N1-methylpseudouridine (4th set)	0	0	0	36	14	13
LPS	0	67	146	7,004	3,974	4,020
mCherry	100	75	143	67	100	133

R848	674	619	562	11,179	8,546	9,907
Pd)P(C)	470	117	362	249	177	197

B. *In vitro* Screening in HeLa Cells

[001137] The day before transfection, 20,000 HeLa cells (ATCC no. CCL-2; Manassas, VA) were harvested by treatment with Trypsin-EDTA solution (LifeTechnologies, Grand Island, NY) and seeded in a total volume of lOOul EMEM medium (supplemented with 10%FCS and lx Glutamax) per well in a 96-well cell culture plate (Corning, Manassas, VA). The cells were grown at 37oG in 5% **CO2** atmosphere overnight. Next day, 83 ng of Luciferase modified RNA (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) with the chemical modification described in Table 19, were diluted in lOul final volume of OPTI-MEM (LifeTechnologies, Grand Island, NY). Lipofectamine 2000 (LifeTechnologies, Grand Island, NY) was used as transfection reagent and 0.2 ul were diluted in 10 ul final volume of OPTI-MEM. After 5 minutes of incubation at room temperature, both solutions were combined and incubated an additional 15 minute at room temperature. Then the 20ul combined solution was added to the lOOul cell culture medium containing the HeLa cells and incubated at room temperature.

[001138] After 18 to 22 hours of incubation cells expressing luciferase were lysed with 100 ul of Passive Lysis Buffer (Promega, Madison, WI) according to manufacturer instructions. Aliquots of the lysates were transferred to white opaque polystyrene 96-well plates (Corning, Manassas, VA) and combined with 100 ul complete luciferase assay solution (Promega, Madison, WI). The lysate volumes were adjusted or diluted until no more than 2 mio relative light units (RLU) per well were detected for the strongest signal producing samples and the RLUs for each chemistry tested are shown in Table 19. The plate reader was a BioTek Synergy H I (BioTek, Winooski, VT).The background signal of the plates without reagent was about 200 relative light units per well.

[001139] These results demonstrate that many, but not all, chemical modifications can be used to productively produce human G-CSF in HeLa cells. Of note, 100% Nl-methylpseudouridine substitution demonstrates the highest level of human G-CSF production.

Table 19. Relative Light Units of Luciferase

Chemical Modification	RLU
N6-methyladenosine (m6a)	534
5-methylcytidine (m5c)	138,428
N4-acetylcytidine (ac4c)	235,412
5-formylcytidine (f5c)	436
5-methylcytosine/pseudouridine, test A1	48,659
5-methylcytosine/N1-methylpseudouridine, test A1	190,924
Pseudouridine	655,632
1-methylpseudouridine (m1u)	1,517,998
2-thiouridine (s2u)	3387
5-methoxyuridine (mo5u)	253,719
5-methylcytosine/pseudouridine, test B1	317,744
5-methylcytosine/N1-methylpseudouridine, test B1	265,871
5-Bromo-uridine	43,276
5 (2 carbovinyl) uridine	531
5 (3-1E propenyl Amino) uridine	446
5-methylcytosine/pseudouridine, test A2	295,824
5-methylcytosine/N1-methylpseudouridine, test A2	233,921
5-methyluridine	50,932
α-Thio-cytidine	26,358
5-methylcytosine/pseudouridine, test B2	481,477
5-methylcytosine/N1-methylpseudouridine, test B2	271,989
5-methylcytosine/pseudouridine, test A3	438,831
5-methylcytosine/N1-methylpseudouridine, test A3	277,499
Unmodified Luciferase	234,802

C. *In vitro* Screening in Rabbit Reticulocyte Lysates

[001 140] Luciferase mRNA (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) was modified with the chemical modification listed in Table 20 and were diluted in sterile nuclease-free water to a final amount of 250 ng in 10 ul. The diluted luciferase was added to 40 ul of freshly prepared Rabbit Reticulocyte Lysate and the *in vitro* translation reaction was done in a standard 1.5 mL polypropylene reaction tube (Thermo Fisher Scientific, Waltham, MA) at 30°C in a dry heating block. The translation assay was done with the Rabbit

Reticulocyte Lysate (nuclease-treated) kit (Promega, Madison, WI) according to the manufacturer's instructions. The reaction buffer was supplemented with a one-to-one blend of provided amino acid stock solutions devoid of either Leucine or Methionine resulting in a reaction mix containing sufficient amounts of both amino acids to allow effective *in vitro* translation.

[001141] After 60 minutes of incubation, the reaction was stopped by placing the reaction tubes on ice. Aliquots of the *in vitro* translation reaction containing luciferase modified RNA were transferred to white opaque polystyrene 96-well plates (Corning, Manassas, VA) and combined with lOOul complete luciferase assay solution (Promega, Madison, WI). The volumes of the *in vitro* translation reactions were adjusted or diluted until no more than 2 mio relative light units (RLUs) per well were detected for the strongest signal producing samples and the RLUs for each chemistry tested are shown in Table 20. The plate reader was a BioTek Synergy H I (BioTek, Winooski, VT).The background signal of the plates without reagent was about 200 relative light units per well.

[001142] These cell-free translation results very nicely correlate with the protein production results in HeLa, with the same modifications generally working or not working in both systems. One notable exception is 5-formylcytidine modified luciferase mRNA which worked in the cell-free translation system, but not in the HeLa cell-based transfection system. A similar difference between the two assays was also seen with 5-formylcytidine modified G-CSF mRNA.

Table 20. Relative Light Units of Luciferase

Chemical Modification	RLU
N6-methyladenosine (m6a)	398
5-methylcytidine (m5c)	152,989
N4-acetylcytidine (ac4c)	60,879
5-formylcytidine (f5c)	55,208
5-methylcytosine/pseudouridine, test A1	349,398
5-methylcytosine/N1-methylpseudouridine, test A1	205,465
Pseudouridine	587,795
1-methylpseudouridine (m1u)	589,758
2-thiouridine (s2u)	708

5-methoxyuridine (mo5u)	288,647
5-methylcytosine/pseudouridine, test B1	454,662
5-methylcytosine/N1-methylpseudouridine, test B1	223,732
5-Bromo-uridine	221,879
5 (2 carbovinyl) uridine	225
5 (3- IE propenyl Amino) uridine	211
5-methylcytosine/pseudouridine, test A2	558,779
5-methylcytosine/N1-methylpseudouridine, test A2	333,082
5-methyluridine	214,680
a-Thio-cytidine	123,878
5-methylcytosine/pseudouridine, test B2	487,805
5-methylcytosine/N1-methylpseudouridine, test B2	154,096
5-methylcytosine/pseudouridine, test A3	413,535
5-methylcytosine/N1-methylpseudouridine, test A3	292,954
Unmodified Luciferase	225,986

Example 14. Chemical Modification: *In vivo* studies

A. *In vivo* Screening of G-CSF Modified mRNA

[001143] Balb-C mice (n=4) are intramuscularly injected in each leg with modified G-CSF mRNA (mRNA sequence shown in SEQ ID NO: 401; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl), fully modified with the chemical modifications outlined in Table 21, is formulated in IxPBS. A control of luciferase modified mRNA (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl; fully modified with pseudouridine and 5-methylcytosine) and a control of PBS are also tested. After 8 hours serum is collected to determine G-CSF protein levels cytokine levels by ELISA.

Table 21. G-CSF

mRNA	Chemical Modifications
G-CSF	Pseudouridine
G-CSF	5-methyluridine
G-CSF	2-thiouridine
G-CSF	4-thiouridine
G-CSF	5-methoxyuridine
G-CSF	2'-fluorouridine
G-CSF	5-bromouridine
G-CSF	5-[3(1-E-propenylamino)uridine)
G-CSF	alpha-thio-cytidine

G-CSF	5-methylcytidine
G-CSF	N4-acetylcytidine
G-CSF	Pseudouridine and 5-methylcytosine
G-CSF	Nl-methylpseudouridine and 5-methylcytosine
Luciferase	Pseudouridine and 5-methylcytosine
PBS	None

B. *In vivo* Screening of Luciferase Modified mRNA

[001144] Balb-C mice (n=4) were subcutaneously injected with 200ul containing 42 to 103 ug of modified luciferase mRNA (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl), fully modified with the chemical modifications outlined in Table 22, was formulated in lxPBS. A control of PBS was also tested. The dosages of the modified luciferase mRNA is also outlined in Table 22. 8 hours after dosing the mice were imaged to determine luciferase expression. Twenty minutes prior to imaging, mice were injected intraperitoneally with a D-luciferin solution at 150 mg/kg. Animals were then anesthetized and images were acquired with an IVIS Lumina II imaging system (Perkin Elmer). Bioluminescence was measured as total flux (photons/second) of the entire mouse.

[001145] As demonstrated in Table 22, all luciferase mRNA modified chemistries demonstrated *in vivo* activity, with the exception of 2'-fluorouridine. In addition 1-methylpseudouridine modified mRNA demonstrated very high expression of luciferase (5-fold greater expression than pseudouridine containing mRNA).

[001146]

Table 22. Luciferase Screening

mRNA	Chemical Modifications	Dose (ug) of mRNA	Dose volume (ml)	Luciferase expression (photon/second)
Luciferase	5-methylcytidine	83	0.72	1.94E+07
Luciferase	N4-acetylcytidine	76	0.72	1.11E07
Luciferase	Pseudouridine	95	1.20	1.36E+07
Luciferase	1-methylpseudouridine	103	0.72	7.40E+07
Luciferase	5-methoxyuridine	95	1.22	3.32+07
Luciferase	5-methyluridine	94	0.86	7.42E+06
Luciferase	5-bromouridine	89	1.49	3.75E+07
Luciferase	2'-fluoroguanosine	42	0.72	5.88E+05
Luciferase	2'-fluorocytidine	47	0.72	4.21E+05

Luciferase	2'-flurorouridine	59	0.72	3.47E+05
PBS	None	-	0.72	3.16E+05

Example 15. *In vivo* Screening of Combination Luciferase Modified mRNA

[001147] Balb-C mice (n=4) were subcutaneously injected with 200ul of 100 ug of modified luciferase mRNA (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl), fully modified with the chemical modifications outlined in Table 23, was formulated in 1x PBS. A control of PBS was also tested. The dosages of the modified luciferase mRNA is also outlined in Table 22. 8 hours after dosing the mice were imaged to determine luciferase expression. Twenty minutes prior to imaging, mice were injected intraperitoneally with a D-luciferin solution at 150 mg/kg. Animals were then anesthetized and images were acquired with an IVIS Lumina II imaging system (Perkin Elmer). Bioluminescence was measured as total flux (photons/second) of the entire mouse.

[001148] As demonstrated in Table 23, all luciferase mRNA modified chemistries (in combination) demonstrated *in vivo* activity. In addition the presence of Nl-methylpseudouridine in the modified mRNA (with N4-acetylcytidine or 5 methylcytidine) demonstrated higher expression than when the same combinations where tested using with pseudouridine. Taken together, these data demonstrate that Nl-methylpseudouridine containing luciferase mRNA results in improved protein expression in vivo whether used alone (Table 22) or when used in combination with other modified nulceotides (Table 23).

Table 23. Luciferase Screening Combinations

mRNA	**Chemical Modifications**	**Luciferase expression (photon/second)**
Luciferase	N4-acetylcytidine/pseudouridine	4.18E+06
Luciferase	N4-acetylcytidine/N1-methylpseudouridine	2.88E+07
Luciferase	5-methylcytidine/5-methoxyuridine	3.48E+07
Luciferase	5-methylcytidine/5-methyluridine	1.44E+07
Luciferase	5-methylcytidine/where 50% of the uridine is replaced with 2-thiouridine	2.39E+06
Luciferase	5-methylcytidine/pseudouridine	2.36E+07
Luciferase	5-methylcytidine/N1-methyl-pseudouridine	4.15E+07
PBS	None	3.59E+05

Example 16. 2'O-methyl and 2'Fluoro compounds

[001149] Luciferase mRNA (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) were produced as fully modified versions with the chemistries in Table 24 and transcribed using mutant T7 polymerase (Durascribe® T7 Transcription kit (Cat. No. DS0 10925) (Epicentre®, Madison, WI). 2' fluoro-containing mRNA were made using Durascribe T7, however, 2'Omethyl-containing mRNA could not be transcribed using Durascribe T7.

[001150] Incorporation of 2'Omethyl modified mRNA might possibly be accomplished using other mutant T7 polymerases (Nat Biotechnol. (2004) 22:1 155-1 160; Nucleic Acids Res. (2002) 30:el38). Alternatively, 2'OMe modifications could be introduced post-transcriptionally using enzymatic means.

[001151] Introduction of modifications on the 2' group of the sugar has many potential advantages. 2'OMe substitutions, like 2' fluoro substitutions are known to protect against nucleases and also have been shown to abolish innate immune recognition when incorporated into other nucleic acids such as siRNA and anti-sense (incorporated in its entirety, Crooke, ed. Antisense Drug Technology, 2nd edition; Boca Raton: CRC press).

[001152] The 2'Fluoro-modified mRNA were then transfected into HeLa cells to assess protein production in a cell context and the same mRNA were also assessed in a cell-free rabbit reticulocyte system. A control of unmodified luciferase (natural luciferase) was used for both transcription experiments, a control of untreated and mock transfected (Lipofectamine 2000 alone) were also analyzed for the HeLa transfection and a control of no RNA was analyzed for the rabbit reticulysates.

[001153] For the HeLa transfection experiments, the day before transfection, 20,000 HeLa cells (ATCC no. CCL-2; Manassas, VA) were harvested by treatment with Trypsin-EDTA solution (LifeTechnologies, Grand Island, NY) and seeded in a total volume of lOOul EMEM medium (supplemented with 10%FCS and lx Glutamax) per well in a 96-well cell culture plate (Corning, Manassas, VA). The cells were grown at 37oG in 5% CO2 atmosphere overnight. Next day, 83 ng of the 2'fluoro-containing luciferase modified RNA (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) with the chemical

modification described in Table 24, were diluted in lOul final volume of OPTI-MEM (LifeTechnologies, Grand Island, NY). Lipofectamine 2000 (LifeTechnologies, Grand Island, NY) was used as transfection reagent and 0.2 ul were diluted in 10 ul final volume of OPTI-MEM. After 5 minutes of incubation at room temperature, both solutions were combined and incubated an additional 15 minute at room temperature. Then the 20ul combined solution was added to the lOOul cell culture medium containing the HeLa cells and incubated at room temperature. After 18 to 22 hours of incubation cells expressing luciferase were lysed with 100 ul of Passive Lysis Buffer (Promega, Madison, WI) according to manufacturer instructions. Aliquots of the lysates were transferred to white opaque polystyrene 96-well plates (Corning, Manassas, VA) and combined with 100 ul complete luciferase assay solution (Promega, Madison, WI). The lysate volumes were adjusted or diluted until no more than 2 mio relative light units (RLU) per well were detected for the strongest signal producing samples and the RLUs for each chemistry tested are shown in Table 24. The plate reader was a BioTek Synergy HI (BioTek, Winooski, VT).The background signal of the plates without reagent was about 200 relative light units per well.

[001154] For the rabbit reticulocyte lysate assay, 2'-fluoro-containing luciferase mRNA were diluted in sterile nuclease-free water to a final amount of 250 ng in 10 ul and added to 40 ul of freshly prepared Rabbit Reticulocyte Lysate and the *in vitro* translation reaction was done in a standard 1.5 mL polypropylene reaction tube (Thermo Fisher Scientific, Waltham, MA) at 30°C in a dry heating block. The translation assay was done with the Rabbit Reticulocyte Lysate (nuclease-treated) kit (Promega, Madison, WI) according to the manufacturer's instructions. The reaction buffer was supplemented with a one-to-one blend of provided amino acid stock solutions devoid of either Leucine or Methionine resulting in a reaction mix containing sufficient amounts of both amino acids to allow effective *in vitro* translation. After 60 minutes of incubation, the reaction was stopped by placing the reaction tubes on ice.

[001155] Aliquots of the *in vitro* translation reaction containing luciferase modified RNA were transferred to white opaque polystyrene 96-well plates (Corning, Manassas, VA) and combined with lOOul complete luciferase assay solution (Promega, Madison, WI). The volumes of the *in vitro* translation reactions were adjusted or diluted until no

more than 2 mio relative light units (RLUs) per well were detected for the strongest signal producing samples and the RLUs for each chemistry tested are shown in Tables 24 and 25. The plate reader was a BioTek Synergy HI (BioTek, Winooski, VT). The background signal of the plates without reagent was about 160 relative light units per well.

[001156] As can be seen in Table 24 and 25, multiple 2'Fluoro-containing compounds are active in vitro and produce luciferase protein.

Table 24. HeLa Cells

Chemical Modification	Concentration (ug/ml)	Volume (ul)	Yield (ug)	RLU
2'Fluoroadenosine	381.96	500	190.98	388.5
2'Fluorocytosine	654.56	500	327.28	2420
2'Fluoroguanine	541,795	500	270.90	11,705.5
2'Flurorouridine	944.005	500	472.00	6767.5
Natural luciferase	N/A	N/A	N/A	133,853.5
Mock	N/A	N/A	N/A	340
Untreated	N/A	N/A	N/A	238

Table 25. Rabbit Reticulysates

Chemical Modification	RLU
2'Fluoroadenosine	162
2'Fluorocytosine	208
2'Fluoroguanine	371,509
2'Flurorouridine	258
Natural luciferase	2,159,968
No RNA	156

Example 17. Luciferase in HeLa Cells using a combination of modifications

[001157] To evaluate using of 2'fluoro-modified mRNA in combination with other modification a series of mRNA were transcribed using either wild-type T7 polymerase (non-fluoro-containing compounds) or using mutant T7 polymerases (fluyoro-containing compounds) as described in Example 86. All modified mRNA were tested by in vitro transfection in HeLa cells.

[001158] The day before transfection, 20,000 HeLa cells (ATCC no. CCL-2; Manassas, VA) were harvested by treatment with Trypsin-EDTA solution (LifeTechnologies, Grand Island, NY) and seeded in a total volume of lOOul EMEM medium (supplemented with 10%FCS and lx Glutamax) per well in a 96-well cell culture plate (Corning, Manassas,

VA). The cells were grown at 37oG in 5% CO2 atmosphere overnight. Next day, 83 ng of Luciferase modified RNA (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) with the chemical modification described in Table 44, were diluted in lOul final volume of OPTI-MEM (LifeTechnologies, Grand Island, NY). Lipofectamine 2000 (LifeTechnologies, Grand Island, NY) was used as transfection reagent and 0.2 ul were diluted in 10 ul final volume of OPTI-MEM. After 5 minutes of incubation at room temperature, both solutions were combined and incubated an additional 15 minute at room temperature. Then the 20ul combined solution was added to the lOOul cell culture medium containing the HeLa cells and incubated at room temperature.

[001159] After 18 to 22 hours of incubation cells expressing luciferase were lysed with 100 ul of Passive Lysis Buffer (Promega, Madison, WI) according to manufacturer instructions. Aliquots of the lysates were transferred to white opaque polystyrene 96-well plates (Corning, Manassas, VA) and combined with 100 ul complete luciferase assay solution (Promega, Madison, WI). The lysate volumes were adjusted or diluted until no more than 2 mio relative light units (RLU) per well were detected for the strongest signal producing samples and the RLUs for each chemistry tested are shown in Table 26. The plate reader was a BioTek Synergy H I (BioTek, Winooski, VT).The background signal of the plates without reagent was about 200 relative light units per well.

[001160] As evidenced in Table 26, most combinations of modifications resulted in mRNA which produced functional luciferase protein, including all the non-flouro containing compounds and many of the combinations containing 2'fluro modifications.

Table 26. Luciferase

Chemical Modification	RLU
N4-acetylcytidine/pseudouridine	113,796
N4-acetylcytidine/N1-methylpseudouridine	316,326
5-methylcytidine/5-methoxyuridine	24,948
5-methylcytidine/5-methyluridine	43,675
5-methylcytidine/half of the uridines modified with 50% 2-thiouridine	41,601
5-methylcytidine/2-thiouridine	1,102
5-methylcytidine/pseudouridine	51,035
5-methylcytidine/N1 methyl pseudouridine	152,151
N4-acetylcytidine/2'Fluorouridine triphosphate	288
5-methylcytidine/2'Fluorouridine triphosphate	269

2'Fluorocytosine triphosphate /pseudouridine	260
2'Fluorocytosine triphosphate /Nl-methylpseudouridine	412
2'Fluorocytosine triphosphate/2-thiouridine	427
2'Fluorocytosine triphosphate/5-bromouridine	253
2'Fluorocytosine triphosphate /2'Fluorouridine triphosphate	184
2'Fluoroguanine triphosphate/5-methylcytidine	321
2'Fluoroguanine triphosphate/5-methylcytidine/Pseudouridine	207
2'Fluoroguanine /5-methylcytidine/Nl methylpsuedouridine	235
2'Fluoroguanine/pseudouridine	218
2'Fluoroguanine/N 1-methylpsuedouridine	247
5-methylcytidine/pseudouridine, test A	13,833
5-methylcytidine/N-methylpseudouridine, test A	598
2'Fluorocytosine triphosphate	201
2'F luorouridine triphosphate	305
5-methylcytidine/pseudouridine, test B	115,401
5-methylcytidine/N-methylpseudouridine, test B	21,034
Natural luciferase	30,801
Untreated	344
Mock	262

Example 18. G-CSF *In Vitro* Transcription

[001161] To assess the activity of all our different chemical modifications in the context of a second open reading frame, we replicated experiments previously conducted using luciferase mRNA, with human G-CSF mRNA. G-CSF mRNA (mRNA sequence shown in SEQ ID NO: 408; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) were fully modified with the chemistries in Tables 27 and 28 using wild-type T7 polymerase (for all non-fluoro-containing compounds) or mutant T7 polymerase (for all fluoro-containing compounds). The mutant T7 polymerase was obtained commercially (Durascribe® T7 Transcription kit (Cat. No. DS0 10925) (Epicentre®, Madison, WI).

[001162] The modified RNA in Tables 27 and 28 were transfected *in vitro* in HeLa cells or added to rabbit reticulysates (250ng of modified mRNA) as indicated. A control of untreated, mock transfected (transfection reagent alone), G-CSF fully modified with 5-methylcytosine and Nl-methylpseudouridine or luciferase control (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) fully modified with 5-methylcytosine and Nl-methylpseudouridine were also analyzed. The expression of G-CSF protein was

determined by ELISA and the values are shown in Tables 27 and 28. In Table 27, "NT" means not tested.

[001163] As shown in Table 27, many, but not all, chemical modifications resulted in human G-CSF protein production. These results from cell-based and cell-free translation systems correlate very nicely with the same modifications generally working or not working in both systems. One notable exception is 5-formylcytidine modified G-CSF mRNA which worked in the cell-free translation system, but not in the HeLa cell-based transfection system. A similar difference between the two assays was also seen with 5-formylcytidine modified luciferase mRNA.

[001 164] As demonstrated in Table 28, many, but not all, G-CSF mRNA modified chemistries (when used in combination) demonstrated *in vivo* activity. In addition the presence of Nl-methylpseudouridine in the modified mRNA (with N4-acetylcytidine or 5 methylcytidine) demonstrated higher expression than when the same combinations where tested using with pseudouridine. Taken together, these data demonstrate that Nl-methylpseudouridine containing G-CSF mRNA results in improved protein expression *in vitro.*

Table 27. G-CSF Expression

Chemical Modification	**G-CSF protein (pg/ml) HeLa cells**	**G-CSF protein (pg/ml) Rabbit reticulysates cells**
Pseudouridine	1,150,909	147,875
5-methyluridine	347,045	147,250
2-thiouridine	417,273	18,375
N1-methylpseudouridine	NT	230,000
4-thiouridine	107,273	52,375
5-methoxyuridine	1,715,909	201,750
5-methylcytosine/pseudouridine, Test A	609,545	119,750
5-methylcytosine/N1-methylpseudouridine , Test A	1,534,318	110,500
2'-Fluoro-guanosine	11,818	0
2'-Fluoro-uridine	60,455	0
5-methylcytosine/pseudouridine, Test B	358,182	57,875
5-methylcytosine/N1-methylpseudouridine , Test B	1,568,636	76,750
5-Bromo-uridine	186,591	72,000
5-(2carbomethoxyvinyl) uridine	1,364	0
5-[3(1-E-propenylamino) uridine	27,955	32,625

α-thio-cytidine	120,455	42,625
5-methylcytosine/pseudouridine, Test C	882,500	49,250
N 1-methyl-adenosine	4,773	0
N6-methyl-adenosine	1,591	0
5-methyl-cytidine	646,591	79,375
N4-acetylcytidine	39,545	8,000
5-formyl-cytidine	0	24,000
5-methylcytosine/pseudouridine, Test **D**	87,045	47,750
5-methylcytosine/Nl-methylpseudouridine , Test **D**	1,168,864	97,125
Mock	909	682
Untreated	0	0
5-methylcytosine/Nl-methylpseudouridine , Control	1,106,591	NT
Luciferase control	NT	0

Table 28. Combination Chemistries in HeLa cells

Chemical Modification	**G-CSF protein (pg/ml) HeLa cells**
N4-acetylcytidine/pseudouridine	537,273
N4-acetylcytidine/N1-methylpseudouridine	1,091,818
5-methylcytidine/5-methoxyuridine	516,136
5-methylcytidine/5-bromouridine	48,864
5-methylcytidine/5-methyluridine	207,500
5-methylcytidine/2-thiouridine	33,409
N4-acetylcytidine/5-bromouridine	211,591
N4-acetylcytidine/2-thiouridine	46,136
5-methylcytosine/pseudouridine	301,364
5-methylcytosine/N1-methylpseudouridine	1,017,727
N4-acetylcytidine/2'Fluorouridine triphosphate	62,273
5-methylcytidine/2'Fluorouridine triphosphate	49,318
2'Fluorocytosine triphosphate/pseudouridine	7,955
2'Fluorocytosine triphosphate/N1-methylpseudouridine	1,364
2'Fluorocytosine triphosphate/2-thiouridine	0
2'Fluorocytosine triphosphate/5-bromouridine	1,818
2'Fluorocytosine triphosphate/2'Fluorouridine triphosphate	909
2'Fluoroguanine triphosphate/5-methylcytidine	0
2'Fluoroguanine triphosphate/5-methylcytidine/pseudouridine	0
2'Fluoroguanine triphosphate /5-methylcytidine/N1 methylpseudouridine	1,818
2'Fluoroguanine triphosphate/pseudouridine	1,136
2'Fluoroguanine triphosphate/2'Fluorocytosine triphosphate/N1-methylpseudouridine	0
5-methylcytidine/pseudouridine	617,727
5-methylcytidine/N1-methylpseudouridine	747,045
5-methylcytidine/pseudouridine	475,455

5-methylcytidine/N 1-methylpseudouridine	689,091
5-methylcytosine/Nl-methylpseudouridine, Control 1	848,409
5-methylcytosine/Nl -methylpseudouridine, Control 2	581,818
Mock	682
Untreated	0
Luciferase 2'Fluorocytosine triphosphate	0
Luciferase 2'Fluorouridine triphosphate	0

Example 19. Screening of Chemistries

[001165] The tables listed in below (Tables 29-3 1) summarize much of the *in vitro* and *in vitro* screening data with the different compounds presented in the previous examples. A good correlation exists between cell-based and cell-free translation assays. The same chemistry substitutions generally show good concordance whether tested in the context of luciferase or G-CSF mRNA. Lastly, Nl-methylpseudouridine containing mRNA show a very high level of protein expression with little to no detectable cytokine stimulation in vitro and in vivo, and is superior to mRNA containing pseudouridine both *in vitro* and *in vivo.*

[001166] Luciferase mRNA (mRNA sequence shown in SEQ ID NO: 409; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) and G-CSF mRNA (mRNA sequence shown in SEQ ID NO: 408; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) were modified with naturally and non-naturally occurring chemistries described in Tables 29 and 30 or combination chemistries described in Table 30 and tested using methods described herein.

[001167] In Tables 29 and 30, "*" refers to *in vitro* transcription reaction using a mutant T7 polymerase (Durascribe® T7 Transcription kit (Cat. No. DS0 10925) (Epicentre®, Madison, WI); "**" refers to the second result *in vitro* transcription reaction using a mutant T7 polymerase (Durascribe® T7 Transcription kit (Cat. No. DS0 10925) (Epicentre®, Madison, WI); "***" refers to production seen in cell free translations (rabbit reticulocyte lysates); the protein production of HeLa is judged by "+," "+/-" and "-"; when referring to G-CSF PBMC "++++" means greater than 6,000 pg/ml G-CSF, "+++" means greater than 3,000 pg/ml G-CSF, "++" means greater than 1,500 pg/ml G-CSF, "+" means greater than 300 pg/ml G-CSF, "+/-" means 150-300 pg/ml G-CSF and the background was about 110 pg/ml; when referring to cytokine PBMC "++++" means

greater than 1,000 pg/ml interferon-alpha (IFN-alpha), "+++" means greater than 600 pg/ml IFN-alpha, "++" means greater than 300 pg/ml IFN-alpha, "+" means greater than 100 pg/ml IFN-alpha, "-" means less than 100 pg/ml and the background was about 70 pg/ml; and "NT" means not tested. In Table 30, the protein production was evaluated using a mutant T7 polymerase (Durascribe® T7 Transcription kit (Cat. No. DSO 10925) (Epicentre®, Madison, WI).

Table 29. Naturally Occurring

Common Name (symbol)	IVT (Luc)	IVT (G-CSF)	Protein (Luc; HeLa)	Protein (G-CSF; HeLa)	Protein (G-CSF; PBMC)	Cytokines (G-CSF; PBMC)	In Vivo Protein (Luc)	In Vivo Protein (G-CSF)
1-methyladenosine (m^1A)	Fail	Pass	NT	-	+/-	++	NT	NT
N^6-methyladenosine (m^6A)	Pass	Pass	-	-	+/-	++++	NT	NT
2'-O-methyladenosine (Am)	Fail*	Not Done	NT	NT	NT	NT	NT	NT
5-methylcytidine (m^5C)	Pass	Pass	+	+	+	++	+	NT
2'-O-methylcytidine (Cm)	Fail*	Not Done	NT	NT	NT	NT	NT	NT
2-thiocytidine (s^2C)	Fail	Fail	NT	NT	NT	NT	NT	NT
N^4-acetylcytidine (ac^4C)	Pass	Pass	+	+	+/-	+++	+	NT
5-formylcytidine (f^5C)	Pass	Pass	-***	-***	-	+	NT	NT
2'-O-methylguanosine (Gm)	Fail*	Not Done	NT	NT	NT	NT	NT	NT
inosine (I)	Fail	Fail	NT	NT	NT	NT	NT	NT
pseudouridine (Y)	Pass	Pass	+	+	++	+	+	NT
5-methyluridine (m^5U)	Pass	Pass	+	+	+/-	+	NT	NT
2'-O-methyluridine (Um)	Fail*	Not Done	NT	NT	NT	NT	NT	NT
1-methylpseudouridine (m^1Y)	Pass	Pass	+	Not Done	++++	-	+	NT
2-thiouridine (s^2U)	Pass	Pass	-	+	+	+	NT	NT
4-thiouridine (s^4U)	Fail	Pass		+	+/-	++	NT	NT
5-methoxyuridine (mo^5U)	Pass	Pass	+	+	++	-	+	NT
3-methyluridine (m^3U)	Fail	Fail	NT	NT	NT	NT	NT	NT

Table 30. Non-Naturally Occurring

Common Name	IVT (Luc)	IVT (G-CSF)	Protein (Luc; HeLa)	Protein (G-CSF; HeLa)	Protein (G-CSF; PBMC)	Cytokines (G-CSF; PBMC)	In Vivo Protein (Luc)	In Vivo Protein (G-CSF)
2'-F-ara-guanosine	Fail	Fail	NT	NT	NT	NT	NT	NT
2'-F-ara-adenosine	Fail	Fail	NT	NT	NT	NT	NT	NT
2'-F-ara-cytidine	Fail	Fail	NT	NT	NT	NT	NT	NT
2'-F-ara-uridine	Fail	Fail	NT	NT	NT	NT	NT	NT
2'-F-guanosine	Fail/Pass**	Pass/Fail**	+**	+/-	-	+	+	NT
2'-F-adenosine	Fail/Pass**	Fail/Fail**	-**	NT	NT	NT	NT	NT
2'-F-cytidine	Fail/Pass**	Fail/Pass**	+**	NT	NT	NT	+	NT
2'-F-uridine	Fail/Pass**	Pass/Pass**	+**	+	+/-	+	-	NT
2'-OH-ara-guanosine	Fail	Fail	NT	NT	NT	NT	NT	NT
2'-OH-ara-adenosine	Not Done	Not Done	NT	NT	NT	NT	NT	NT
2'-OH-ara-cytidine	Fail	Fail	NT	NT	NT	NT	NT	NT
2'-OH-ara-uridine	Fail	Fail	NT	NT	NT	NT	NT	NT
5-Br-Uridine	Pass	Pass	+	+	+	+	+	
5-(2-carbomethoxyvinyl) Uridine	Pass	Pass	-	-	+/-	-		
5-[3-(1-E-Propenylamino) Uridine (aka Chem 5)	Pass	Pass	-	+	+	-		
N6-(19-Amino-pentaoxanonadecyl) A	Fail	Fail	NT	NT	NT	NT	NT	NT
2-Dimethylamino guanosine	Fail	Fail	NT	NT	NT	NT	NT	NT
6-Aza-cytidine	Fail	Fail	NT	NT	NT	NT	NT	NT
a-Thio-cytidine	Pass	Pass	+	+	+/-	+++	NT	NT
Pseudo-isocytidine	NT	NT	NT	NT	NT	NT	NT	NT

5-Iodo-uridine	NT	NT	NT	NT	NT	NT	NT	NT
a-Thio-uridine	NT	NT	NT	NT	NT	NT	NT	NT
6-Aza-uridine	NT	NT	NT	NT	NT	NT	NT	NT
Deoxy-thymidine	NT	NT	NT	NT	NT	NT	NT	NT
a-Thio guanosine	NT	NT	NT	NT	NT	NT	NT	NT
8-Oxo-guanosine	NT	NT	NT	NT	NT	NT	NT	NT
06-Methyl-guanosine	NT	NT	NT	NT	NT	NT	NT	NT
7-Deaza-guanosine	NT	NT	NT	NT	NT	NT	NT	NT
6-Chloro-purine	NT	NT	NT	NT	NT	NT	NT	NT
a-Thio-adenosine	NT	NT	NT	NT	NT	NT	NT	NT
7-Deaza-adenosine	NT	NT	NT	NT	NT	NT	NT	NT
5-iodo-cytidine	NT	NT	NT	NT	NT	NT	NT	NT

[001168] In Table 31, the protein production of HeLa is judged by "+," "+/-" and "-"; when referring to G-CSF PBMC "++++" means greater than 6,000 pg/ml G-CSF, "+++" means greater than 3,000 pg/ml G-CSF, "++" means greater than 1,500 pg/ml G-CSF, "+" means greater than 300 pg/ml G-CSF, "+/-" means 150-300 pg/ml G-CSF and the background was about 110 pg/ml; when referring to cytokine PBMC "++++" means greater than 1,000 pg/ml interferon-alpha (IFN-alpha), "+++" means greater than 600 pg/ml IFN-alpha, "++" means greater than 300 pg/ml IFN-alpha, "+" means greater than 100 pg/ml IFN-alpha, "-" means less than 100 pg/ml and the background was about 70 pg/ml; "WT" refers to the wild type T7 polymerase, "MT" refers to mutant T7 polymerase (Durascribe® T7 Transcription kit (Cat. No. DSO 10925) (Epicentre®, Madison, WI) and "NT" means not tested.

Table 31. Combination Chemistry

Cytidine analog	Uridine analog	Purine	IVT Luc	IVT (G-CSF)	Protein (Luc; HeLa)	Protein (G-CSF; HeLa)	Protein (G-CSF; PBMC)	Cytokines (G-CSF; PBMC)	In Vivo Protein (Luc)
N4-acetylcytidine	pseudouridine	A,G	Pass WT	Pass WT	+	+	NT	NT	+
N4-acetylcytidine	N1-methylpseudouridin	A,G	Pass WT	Pass WT	+	+	NT	NT	+

	e								
5-methylcytidine	5-methoxyuridine	A,G	Pass WT	Pass WT	+	+	NT	NT	+
5-methylcytidine	5-bromouridine	A,G	Pass WT	Pass WT	Not Done	+	NT	NT	
5-methylcytidine	5-methyluridine	A,G	Pass WT	Pass WT	+	+	NT	NT	+
5-methylcytidine	50% 2-thiouridine; 50% uridine	A,G	Pass WT	Pass WT	+	NT	NT	NT	+
5-methylcytidine	100% 2-thiouridine	A,G	Pass WT	Pass WT	-	+	NT	NT	
5-methylcytidine	pseudouridine	A,G	Pass WT	Pass WT	+	+	++	+	+
5-methylcytidine	N1-methylpseudouridine	A,G	Pass WT	Pass WT	+	+	++++	-	+
N4-acetylcytidine	2-thiouridine	A,G	Not Done	Pass WT	Not Done	+	NT	NT	NT
N4-acetylcytidine	5-bromouridine	A, G	Not Done	Pass WT	Not Done	+	NT	NT	NT
N4-acetylcytidine	2 Fluorouridine triphosphate	A,G	Pass	Pass	-	+	NT	NT	NT
5-methylcytidine	2 Fluorouridine triphosphate	A,G	Pass	Pass	-	+	NT	NT	NT
2 Fluorocytosine triphosphate	pseudouridine	A,G	Pass	Pass	-	+	NT	NT	NT
2 Fluorocytosine triphosphate	N1-methylpseudouridine	A,G	Pass	Pass	-	+/-	NT	NT	NT
2 Fluorocytosine triphosphat	2-thiouridine	A,G	Pass	Pass	-	-	NT	NT	NT

e									
2 Fluorocytosine triphosphate	5-bromouridine	A,G	Pass	Pass	-	+/-	NT	NT	NT
2 Fluorocytosine triphosphate	2 Fluorouridine triphosphate	A,G	Pass	Pass	-	+/-	NT	NT	NT
5-methylcytidine	uridine	A,2 Fluoro GTP	Pass	Pass	-	-	NT	NT	NT
5-methylcytidine	pseudouridine	A,2 Fluoro GTP	Pass	Pass	-	-	NT	NT	NT
5-methylcytidine	Nl-methylpseudouridine	A,2 Fluoro GTP	Pass	Pass	-	+/-	NT	NT	NT
2 Fluorocytosine triphosphate	pseudouridine	A,2 Fluoro GTP	Pass	Pass	-	+/-	NT	NT	NT
2 Fluorocytosine triphosphate	Nl-methylpseudouridine	A,2 Fluoro GTP	Pass	Pass	-	-	NT	NT	NT

Example 20. 2'Fluoro Chemistries in PBMC

[001 169] The ability of G-CSF modified mRNA (mRNA sequence shown in SEQ ID NO: 408; polyA tail of approximately 160 nucleotides not shown in sequence; 5'cap, Capl) to trigger innate an immune response was determined by measuring interferon-alpha (IFN-alpha) and tumor necrosis factor-alpha (TNF-alpha) production. Use of *in vitro* PBMC cultures is an accepted way to measure the immunostimulatory potential of oligonucleotides (Robbins et al, Oligonucleotides 2009 19:89-102) and transfection methods are described herein. Shown in Table 32 are the average from 2 or 3 separate PBMC donors of the interferon-alpha (IFN-alpha) and tumor necrosis factor alpha (TNF-alpha) production over time as measured by specific ELISA. Controls of R848, P(I)P(C), LPS and Lipofectamine 2000 (L2000) were also analyzed.

[001170] With regards to innate immune recognition, while both modified mRNA chemistries largely prevented IFN-alpha and TNF-alpha production relative to positive controls (R848, P(I)P(C)), 2'fluoro compounds reduce IFN-alpha and TNF-alpha production even lower than other combinations and N4-acetylcytidine combinations raised the cytokine profile.

Table 32. IFN-alpha and TNF-alpha

	IFN-alpha: 3 Donor Average (pg/ml)	TNF-alpha: 2 Donor Average (pg/ml)
L2000	1	361
P(I)P(C)	482	544
R848	45	8,235
LPS	0	6,889
N4-acetylcytidine/pseudouridine	694	528
N4-acetylcytidine/N1-methylpseudouridine	307	283
5-methylcytidine/5-methoxyuridine	0	411
5-methylcytidine/5-bromouridine	0	270
5-methylcytidine/5-methyluridine	456	428
5-methylcytidine/2-thiouridine	274	277
N4-acetylcytidine/2-thiouridine	0	285
N4-acetylcytidine/5-bromouridine	44	403
5-methylcytidine/pseudouridine	73	332
5-methylcytidine/N1-methylpseudouridine	31	280
N4-acetylcytidine/2'fluorouridine triphosphate	35	32
5-methylcytodine /2'fluorouridine triphosphate	24	0
2'fluorocytidine triphosphate/N1-methylpseudouridine	0	11
2'fluorocytidine triphosphate/2-thiouridine	0	0
2'fluorocytidine/ triphosphate5-bromouridine	12	2
2'fluorocytidine triphosphate/2'fluorouridine triphosphate	11	0
2'fluorocytidine triphosphate/5-methylcytidine	14	23
2'fluorocytidine triphosphate/5-methylcytidine/pseudouridine	6	21
2'fluorocytidine triphosphate/5-methylcytidine/N1-methylpseudouridine	3	15
2'fluorocytidine triphosphate/pseudouridine	0	4
2'fluorocytidine triphosphate/N1-methylpseudouridine	6	20
5-methylcytidine/pseudouridine	82	18
5-methylcytidien/N1-methylpseudouridine	35	3

Example 21. Directed SAR of Pseudouridine and Nl-methyl PseudoUridine

[001171] With the recent focus on the pyrimidine nucleoside pseudouridine, a series of structure-activity studies were designed to investigate mRNA containing modifications to pseudouridine or Nl-methyl-pseudourdine and their effects on reprogramming.

[001172] The study was designed to explore the effect of chain length, increased lipophilicity, presence of ring structures, and alteration of hydrophobic or hydrophilic interactions when modifications were made at the N1 position, C6 position, the 2-position, the 4-position and on the phosphate backbone. Stability is also investigated.

[001173] To this end, modifications involving alkylation, cycloalkylation, alkyl-cycloalkylation, arylation, alkyl-arylation, alkylation moieties with amino groups, alkylation moieties with carboxylic acid groups, and alkylation moieties containing amino acid charged moieties are investigated. The degree of alkylation is generally ***Ci-Ce.*** Examples of the chemistry modifications include those listed in Table 33.

Table 33. Pseudouridine and Nl-methyl Pseudo Uridine SAR

Chemistry Modification	**Compound #**	**Naturally occuring**
N1-Modifications		
N1-Ethyl-pseudo-UTP	1	N
N1-Propyl-pseudo-UTP	2	N
N1-*iso*-propyl-pseudo-UTP	3	N
N1-(2,2,2-Trifluoroethyl)-pseudo-UTP	4	N
N1-Cyclopropyl-pseudo-UTP	5	N
N1-Cyclopropylmethyl-pseudo-UTP	6	N
N1-Phenyl-pseudo-UTP	7	N
N1-Benzyl-pseudo-UTP	8	N
N1-Aminomethyl-pseudo-UTP	9	N
P seudo-UTP-N1-2-ethanoic acid	10	N
N 1-(3-Amino-3-carboxypropyl)pseudo-UTP	11	N
N1-Methyl-3-(3-amino-3-carboxypropyl)pseudo-UTP	12	Y
C-6 Modifications		
6-Methyl-pseudo-UTP	13	N
6-Trifluoromethyl-pseudo-UTP	14	N
6-Methoxy-pseudo-UTP	15	N
6-Phenyl-pseudo-UTP	16	N
6-Iodo-pseudo-UTP	17	N

6-Bromo-pseudo-UTP	18	N
6-Chloro-pseudo-UTP	19	N
6-Fluoro-pseudo-UTP	20	N
2- or 4-position Modifications		
4-Thio-pseudo-UTP	21	N
2-Thio-pseudo-UTP	22	N
Phosphate backbone Modifications		
Alpha-thio-pseudo-UTP	23	N
N1-Me-alpha-thio-pseudo-UTP	24	N

Table 34. Pseudouridine and Nl-methyl Pseudo Uridine SAR

Chemistry Modification	Compound #	Naturally occuring
N1-Methyl-pseudo-UTP	1	Y
Nl-Butyl-pseudo-UTP	2	N
N1-*tert*-Butyl-pseudo-UTP	3	N
N1-Pentyl-pseudo-UTP	4	N
N1-Hexyl-pseudo-UTP	5	N
N1-Trifluoromethyl-pseudo-UTP	6	Y
N1-Cyclobutyl-pseudo-UTP	7	N
N1-Cyclopentyl-pseudo-UTP	8	N
N1-Cyclohexyl-pseudo-UTP	9	N
N1-Cycloheptyl-pseudo-UTP	10	N
N1-Cyclooctyl-pseudo-UTP	11	N
N1-Cyclobutylmethyl-pseudo-UTP	12	N
N1-Cyclopentylmethyl-pseudo-UTP	13	N
N1-Cyclohexylmethyl-pseudo-UTP	14	N
N1-Cycloheptylmethyl-pseudo-UTP	15	N
N1-Cyclooctylmethyl-pseudo-UTP	16	N
N1-p-tolyl-pseudo-UTP	17	N
N1-(2,4,6-Trimethyl-phenyl)pseudo-UTP	18	N
N1-(4-Methoxy-phenyl)pseudo-UTP	19	N
N1-(4-Amino-phenyl)pseudo-UTP	20	N
N1(4-Nitro-phenyl)pseudo-UTP	21	N
Pseudo-UTP-Nl-/?-benzoic acid	22	N
N1-(4-Methyl-benzyl)pseudo-UTP	24	N
N1-(2,4,6-Trimethyl-benzyl)pseudo-UTP	23	N
N1-(4-Methoxy-benzyl)pseudo-UTP	25	N
N1-(4-Amino-benzyl)pseudo-UTP	26	N
N1-(4-Nitro-benzyl)pseudo-UTP	27	N
Pseudo-UTP-N1-methyl-p-benzoic acid	28	N

N1-(2-Amino-ethyl)pseudo-UTP	29	N
N1-(3-Amino-propyl)pseudo-UTP	30	N
N1-(4-Amino-butyl)pseudo-UTP	31	N
N1-(5-Amino-pentyl)pseudo-UTP	32	N
N1-(6-Amino-hexyl)pseudo-UTP	33	N
Pseudo-UTP-N1-3-propionic acid	34	N
Pseudo-UTP-Nl-4-butanoic acid	35	N
Pseudo-UTP-N1-5-pentanoic acid	36	N
Pseudo-UTP-N1-6-hexanoic acid	37	N
Pseudo-UTP-N1-7-heptanoic acid	38	N
N1-(2-Amino-2-carboxyethyl)pseudo-UTP	39	N
N1-(4-Amino-4-carboxybutyl)pseudo-UTP	40	N
N3-Alkyl-pseudo-UTP	41	N
6-Ethyl-pseudo-UTP	42	N
6-Propyl-pseudo-UTP	43	N
6-iso-Propyl-pseudo-UTP	44	N
6-Butyl-pseudo-UTP	45	N
6-tert-Butyl-pseudo-UTP	46	N
6-(2,2,2-Trifluoroethyl)-pseudo-UTP	47	N
6-Ethoxy-pseudo-UTP	48	N
6-Trifluoromethoxy-pseudo-UTP	49	N
6-Phenyl-pseudo-UTP	50	N
6-(Substituted-Phenyl)-pseudo-UTP	51	N
6-Cyano-pseudo-UTP	52	N
6-Azido-pseudo-UTP	53	N
6-Amino-pseudo-UTP	54	N
6-Ethylcarboxylate-pseudo-UTP	54b	N
6-Hydroxy-pseudo-UTP	55	N
6-Methylamino-pseudo-UTP	55b	N
6-Dimethylamino-pseudo-UTP	57	N
6-Hydroxyamino-pseudo-UTP	59	N
6-Formyl-pseudo-UTP	60	N
6-(4-Morpholino)-pseudo-UTP	61	N
6-(4-Thiomorpholino)-pseudo-UTP	62	N
N1-Me-4-thio-pseudo-UTP	63	N
N1-Me-2-thio-pseudo-UTP	64	N
1,6-Dimethyl-pseudo-UTP	65	N
l-Methyl-6-trifluoromethyl-pseudo-UTP	66	N
1-Methyl-6-ethyl-pseudo-UTP	67	N
1-Methyl-6-propyl-pseudo-UTP	68	N
l-Methyl-6-iso-propyl-pseudo-UTP	69	N
1-Methyl-6-butyl-pseudo-UTP	70	N

1-Methyl-6-tert-butyl-pseudo-UTP	71	N
l-Methyl-6-(2,2,2-Trifiuoroethyl)pseudo-UTP	72	N
1-Methyl-6-iodo-pseudo-UTP	73	N
1-Methyl-6-bromo-pseudo-UTP	74	N
1-Methyl-6-chloro-pseudo-UTP	75	N
1-Methyl-6-fluoro-pseudo-UTP	76	N
1-Methyl-6-methoxy-pseudo-UTP	77	N
1-Methyl-6-ethoxy-pseudo-UTP	78	N
l-Methyl-6-trifluoromethoxy-pseudo-UTP	79	N
1-Methyl-6-phenyl-pseudo-UTP	80	N
1-Methyl-6-(substituted phenyl)pseudo-UTP	81	N
1-Methyl-6-cyano-pseudo-UTP	82	N
1-Methyl-6-azido-pseudo-UTP	83	N
1-Methyl-6-amino-pseudo-UTP	84	N
l-Methyl-6-ethylcarboxylate-pseudo-UTP	85	N
1-Methyl-6-hydroxy-pseudo-UTP	86	N
l-Methyl-6-methylamino-pseudo-UTP	87	N
l-Methyl-6-dimethylamino-pseudo-UTP	88	N
l-Methyl-6-hydroxyamino-pseudo-UTP	89	N
1-Methyl-6-formyl-pseudo-UTP	90	N
l-Methyl-6-(4-morpholino)-pseudo-UTP	91	N
l-Methyl-6-(4-thiomorpholino)-pseudo-UTP	92	N
1-Alkyl-6-vinyl-pseudo-UTP	93	N
1-Alkyl-6-allyl-pseudo-UTP	94	N
1-Alkyl-6-homoallyl-pseudo-UTP	95	N
1-Alkyl-6-ethynyl-pseudo-UTP	96	N
l-Alkyl-6-(2-propynyl)-pseudo-UTP	97	N
1-Alkyl-6-(l -propynyl)-pseudo-UTP	98	N

Example 22. Incorporation of naturally and non-naturally occuring nucleosides

[001174] Naturally and non-naturally occurring nucleosides are incorporated into mRNA encoding a polypeptide of interest. Examples of these are given in Tables 35 and 36. Certain commercially available nucleoside triphosphates (NTPs) are investigated in the polynucleotides of the invention. A selection of these are given in Table 36. The resultant mRNA are then examined for their ability to produce protein, induce cytokines, and/or produce a therapeutic outcome.

Table 35. Naturally and non-naturally occurring nucleosides

Chemistry Modification	Compound	Naturally

	#	occuring
N4-Methyl-Cytosine	1	Y
N4,N4-Dimethyl-2 '-OMe-Cytosine	2	Y
5-Oxyacetic acid-methyl ester-Uridine	3	Y
N3-Methyl-pseudo-Uridine	4	Y
5-Hydroxymethy 1-Cytosine	5	Y
5-Trifluoromethyl-Cytosine	6	N
5-Trifluoromethy 1-Uridine	7	N
5-Methyl-amino-methyl-Uridine	8	Y
5-Carboxy-methyl-amino-methyl-Uridine	9	Y
5-Carboxymethylaminomethyl-2 '-OMe-Uridine	10	Y
5-Carboxymethylaminomethyl-2-thio-Uridine	11	Y
5-Methylaminomethyl-2-thio-Uridine	12	Y
5-Methoxy-carbonyl-methyl-Uridine	13	Y
5-Methoxy-carbonyl-methyl-2 '-OMe-Uridine	14	Y
5-Oxyacetic acid- Uridine	15	Y
3-(3-Amino-3-carboxypropyl)-Uridine	16	Y
5-(carboxyhydroxymethyl)uridine methyl ester	17	Y
5-(carboxyhydroxymethyl)uridine	18	Y

Table 36. Non-naturally occurring nucleoside triphosphates

Chemistry Modification	Compound #	Naturally occuring
N1-Me-GTP	1	N
2'-OMe-2-Amino-ATP	2	N
2'-OMe-pseudo-UTP	3	Y
2'-OMe-6-Me-UTP	4	N
2'-Azido-2'-deoxy-ATP	5	N
2'-Azido-2'-deoxy-GTP	6	N
2'-Azido-2'-deoxy-UTP	7	N
2'-Azido-2'-deoxy-CTP	8	N
2'-Amino-2'-deoxy-ATP	9	N
2'-Amino-2'-deoxy-GTP	10	N
2'-Amino-2'-deoxy-UTP	11	N
2'-Amino-2'-deoxy-CTP	12	N
2-Amino-ATP	13	N

8-Aza-ATP	14	N
Xanthosine-5'-TP	15	N
5-Bromo-CTP	16	N
2'-F-5-Methyl-2'-deoxy-UTP	17	N
5-Aminoallyl-CTP	18	N
2-Amino-riboside-TP	19	N

Example 23. Incorporation of modifications to the nucleobase and carbohydrate (sugar)

[001175] Naturally and non-naturally occurring nucleosides are incorporated into mRNA encoding a polypeptide of interest. Commercially available nucleosides and NTPs having modifications to both the nucleobase and carbohydrate (sugar) are examined for their ability to be incorporated into mRNA and to produce protein, induce cytokines, and/or produce a therapeutic outcome. Examples of these nucleosides are given in Tables 37 and 38.

Table 37. Combination modifications

Chemistry Modification	Compound #
5-iodo-2'-fluoro-deoxyuridine	1
5-iodo-cytidine	6
2'-bromo-deoxyuridine	7
8-bromo-adenosine	8
8-bromo-guanosine	9
2,2'-anhydro-cytidine hydrochloride	10
2,2'-anhydro-uridine	11
2'-Azido-deoxyuridine	12
2-amino-adenosine	13
N4-Benzoyl-cytidine	14
N4-Amino-cytidine	15
2'-O-Methyl-N4-Acetyl-cytidine	16
2'Fluoro-N4-Acetyl-cytidine	17
2'Fluor-N4-Bz-cytidine	18
2'O-methyl-N4-Bz-cytidine	19

2'0-methyl-N6-Bz-deoxyadenosine	20
2'Fluoro-N6-Bz-deoxyadenosine	21
N2-isobutyl-guanosine	22
2'Fluro-N2-isobutyl-guanosine	23
2'0-methyl-N2-isobutyl-guanosine	24

Table 38. Naturally occuring combinations

Name	Compound #	Naturally occurring
5-Methoxycarbonylmethyl-2-thiouridine TP	1	Y
5-Methylaminomethyl-2-thiouridine TP	2	Y
5-Crbamoylmethyluridine TP	3	Y
5-Carbamoylmethyl-2'-O-methyluridine TP	4	Y
1-Methyl-3-(3-amino-3-carboxypropyl) pseudouridine TP	5	Y
5-Methylaminomethyl-2-selenouridine TP	6	Y
5-Carboxymethyluridine TP	7	Y
5-Methyldihydrouridine TP	8	Y
lysidine TP	9	Y
5-Taurinomethyluridine TP	10	Y
5-Taurinomethyl-2-thiouridine TP	11	Y
5-(*iso*-Pentenylaminomethyl)uridine TP	12	Y
5-(*iso*-Pentenylaminomethyl)- 2-thiouridine TP	13	Y
5-(*iso*-Pentenylaminomethyl)-2'-O-methyluridine TP	14	Y
N4-Acetyl-2'-O-methylcytidine TP	15	Y
N4,2'-O-Dimethylcytidine TP	16	Y
5-Formyl-2'-O-methylcytidine TP	17	Y
2'-O-Methylpseudouridine TP	18	Y
2-Thio-2'-O-methyluridine TP	19	Y
3,2'-O-Dimethyluridine TP	20	Y

[001176] In the tables "UTP" stands for uridine triphosphate, "GTP" stands for guanosine triphosphate, "ATP" stands for adenosine triphosphate, "CTP" stands for cytosine triphosphate, "TP" stands for triphosphate and "Bz" stands for benzyl.

OTHER EMBODIMENTS

[001177] It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit of the invention in its broader aspects.

[001178] While the present invention has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the invention.

[001179] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

WHAT IS CLAIMED IS:

1. A method for altering cell phenotype comprising contacting a cell with a composition comprising at least a first and a second cell phenotype altering polynucleotide, wherein each of said first cell phenotype altering polynucleotide and said second cell phenotype altering polynucleotide comprise:
   (a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide selected from the group consisting of SEQ ID NOs: 269-394;
   (b) a first flanking region located at the 5' terminus of said first region comprising;
      (i) a sequence of linked nucleosides selected from the group consisting of the native 5' untranslated region (UTR) of any of SEQ ID NOs: 269-394, SEQ ID NO: 1 and functional variants thereof;
   (c) a second flanking region located at the 3' terminus of said first region comprising;
      (i') a sequence of linked nucleosides selected from the group consisting of the native 3' UTR of any of SEQ ID NOs: 269-394, SEQ ID NOs 2-7 and functional variants thereof; and
      (ii') a 3' tailing sequence of linked nucleosides;

   wherein the first region of linked nucleosides comprises at least a first modified nucleoside.
2. The method of claim 1 wherein each of the first cell phenotype altering polynucleotide and the second cell phenotype altering polynucleotide further comprise a poly-A tail.
3. The method of claim 2, wherein each of the first cell phenotype altering polynucleotide and the second cell phenotype altering polynucleotide further comprise at least one 5' cap structure.
4. The method of claim 3, wherein the first region of the first cell phenotype altering polynucleotide encodes a first cell phenotype altering polypeptide selected from the group consisting of OCT4, SOXl, SOX2, SOX3, SOX15, SOX18, NANOG,

KLFl, KLF2, KLF4, NR5A2, c-MYC, 1-MYC, n-MYC, REM2, TERT, LIN28 and variants thereof.

5. The composition of claim 4, wherein the first region of the second cell phenotype altering polynucleotide encodes a second cell phenotype altering polypeptide is selected from the group consisting of OCT4, SOX1, SOX2, SOX3, SOX15, SOX18, NANOG, KLFl, KLF2, KLF4, NR5A2, c-MYC, 1-MYC, n-MYC, REM2, TERT, LIN28 and variants thereof

6. The composition of claim 5, wherein the first region of the first cell phenotype altering polynucleotide encodes OCT4 and the first region of the second cell phenotype altering polynucleotide encodes SOX2.

7. The composition of claim 6, wherein OCT4 has a sequence selected from the group consisting of SEQ ID NO: 269-294 and functional variants thereof.

8. The composition of claim 6, wherein SOX2 has a sequence selected from the group consisting of SEQ ID NO: 296 and 297 and functional variants thereof.

9. The method of claim 1, wherein the cell is a human cell.

10. The method of claim 1, wherein the cell is contacted at least twice.

11. The method of claim 1, wherein the cell is contacted a plurality of times.

12. The method of claim 1, wherein the composition further comprises a third and a fourth cell phenotype altering polynucleotide, wherein each of said third cell phenotype altering polynucleotide and said fourth cell phenotype altering polynucleotide comprise:

(a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide selected from the group consisting of SEQ ID NOs: 269-394;

(b) a first flanking region located at the 5' terminus of said first region comprising;

(i) a sequence of linked nucleosides selected from the group consisting of the native 5' untranslated region (UTR) of any of SEQ ID NOs: 269-394, SEQ ID NO: 1 and functional variants thereof;

(c) a second flanking region located at the 3' terminus of said first region comprising;

(i') a sequence of linked nucleosides selected from the group consisting of the native 3' UTR of any of SEQ ID NOs: 269-394, SEQ ID NOs 2-7 and functional variants thereof; and

(ii') a 3' tailing sequence of linked nucleosides;

wherein the first region of linked nucleosides comprises at least a first modified nucleoside.

13. The method of claim 12, wherein the first region of the third cell phenotype altering polynucleotide encodes a third cell phenotype altering polypeptide is selected from the group consisting of OCT4, SOX1, SOX2, SOX3, SOX15, SOX18, NANOG, KLF1, KLF2, KLF4, NR5A2, c-MYC, 1-MYC, n-MYC, REM2, TERT, LIN28 and variants thereof.

14. The method of claim 13, wherein the first region of the fourth cell phenotype altering polynucleotide encodes a fourth cell phenotype altering polypeptide is selected from the group consisting of OCT4, SOX1, SOX2, SOX3, SOX15, SOX18, NANOG, KLF1, KLF2, KLF4, NR5A2, c-MYC, 1-MYC, n-MYC, REM2, TERT, LIN28 and variants thereof.

15. The method of claim 14, wherein the first region of the first cell phenotype altering polynucleotide encodes OCT4, the first region of the second cell phenotype altering polynucleotide encodes SOX2, the first region of the third cell phenotype altering polynucleotide encodes KLF4 and the first region of the fourth cell phenotype altering polynucleotide encodes c-MYC.

16. The method of claim 14, wherein the first region of the first cell phenotype altering polynucleotide encodes OCT4, the first region of the second cell phenotype altering polynucleotide encodes SOX2, the first region of the third cell phenotype altering polynucleotide encodes LIN28 and the first region of the fourth cell phenotype altering polynucleotide encodes NANOG.

17. A composition comprising at least a first and a second cell phenotype altering polynucleotide, wherein each of said first cell phenotype altering polynucleotide and said second cell phenotype altering polynucleotide comprise:

(a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide selected from the group consisting of SEQ ID NOs: 269-394;

(b) a first flanking region located at the 5' terminus of said first region comprising;

(i) a sequence of linked nucleosides selected from the group consisting of the native 5' untranslated region (UTR) of any of SEQ ID NOs: 269-394, SEQ ID NO: 1 and functional variants thereof;

(c) a second flanking region located at the 3' terminus of said first region comprising;

(i') a sequence of linked nucleosides selected from the group consisting of the native 3' UTR of any of SEQ ID NOs: 269-394, SEQ ID NOs 2-7 and functional variants thereof; and

(ii') a 3' tailing sequence of linked nucleosides;

wherein the first region of linked nucleosides comprises at least a first modified nucleoside.

18. The composition of claim 17, further comprising a third and a fourth cell phenotype altering polynucleotide, wherein each of said third cell phenotype altering polynucleotide and said fourth cell phenotype altering polynucleotide comprise:

(a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide selected from the group consisting of SEQ ID NOs: 269-394;

(b) a first flanking region located at the 5' terminus of said first region comprising;

(i) a sequence of linked nucleosides selected from the group consisting of the native 5' untranslated region (UTR) of any of SEQ ID NOs: 269-394, SEQ ID NO: 1 and functional variants thereof;

(c) a second flanking region located at the 3' terminus of said first region comprising;

(i') a sequence of linked nucleosides selected from the group consisting of the native 3' UTR of any of SEQ ID NOs: 269-394, SEQ ID NOs 2-7 and functional variants thereof; and

(ii') a 3' tailing sequence of linked nucleosides;

wherein the first region of linked nucleosides comprises at least a first modified nucleoside.

19. A kit comprising the composition of claim 17.

20. The kit of claim 19, wherein the composition further comprises a third and a fourth cell phenotype altering polynucleotide, wherein each of said third cell phenotype altering polynucleotide and said fourth cell phenotype altering polynucleotide comprise:

(a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide selected from the group consisting of SEQ ID NOs: 269-394;

(b) a first flanking region located at the 5' terminus of said first region comprising;

(i) a sequence of linked nucleosides selected from the group consisting of the native 5' untranslated region (UTR) of any of SEQ ID NOs: 269-394, SEQ ID NO: 1 and functional variants thereof;

(c) a second flanking region located at the 3' terminus of said first region comprising;

(i') a sequence of linked nucleosides selected from the group consisting of the native 3' UTR of any of SEQ ID NOs: 269-394, SEQ ID NOs 2-7 and functional variants thereof; and

(ii') a 3' tailing sequence of linked nucleosides;

wherein the first region of linked nucleosides comprises at least a first modified nucleoside.

Figure 1

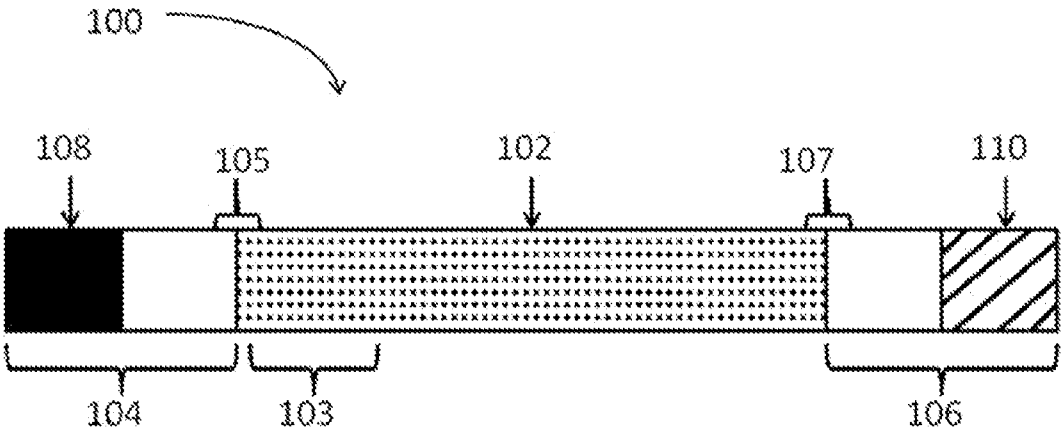
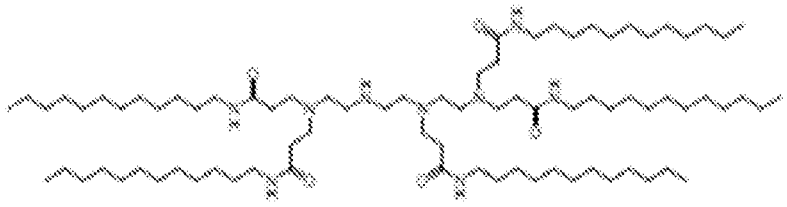
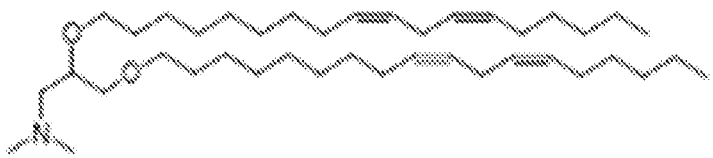


Figure 2

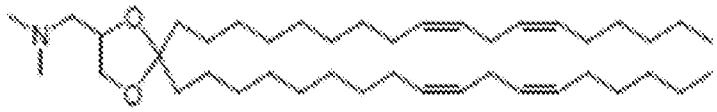
98N12-5 (TETA5-LAP)



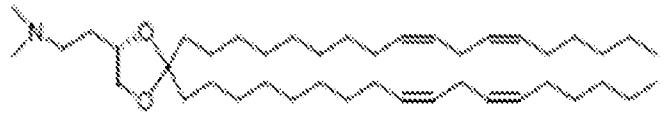
DLin-DMA



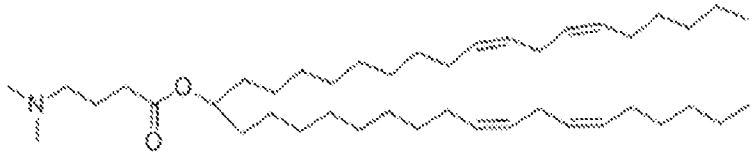
DLin-K-DMA (2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane)



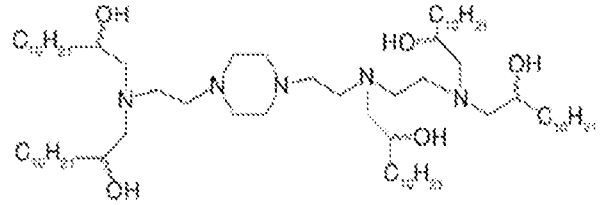
DLin-KC2-DMA



DLin-MC3-DMA



C12-200



PRIOR ART

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US201 3/074560

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 19/34 (2014.01)
USPC - 514/44R
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/7105; C07H 21/02; C12N 15/11; C12P 19/34 (2014.01)
USPC - 435/6.1, 6.11, 69.1, 91.1; 514/44R; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 31/7105; C07H 21/02; C12N 15/11; C12P 19/34 (2014.02)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2012/0046346 A1 (ROSSI et al) 23 February 2012 (23.02.2012) entire document	1-20
A	WO 2000/50586 A2 (GEBAUER et al) 31 August 2000 (31.08.2000) entire document	1-20
A	US 2009/0286852 A1 (KARIKO et al) 19 November 2009 (19.11.2009) entire document	1-20
A	US 8,183,217 B2 (WATERHOUSE et al) 22 May 2012 (22.05.2012) entire document	1-20
A	US 2012/0270273 A1 (ZHANG et al) 25 October 2012 (25.10.2012) entire document	1-20
P, X	WO 2013/151666 A2 (BANCEL et al) 10 October 2013 (10.10.2013) entire document	1-20

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 May 2014	**09 MAY 2014**
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/074560

Box No. I Nucleotide and/or amino acid scquence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)
   ☐ on paper
   ☒ in electronic form

   b. (time)
   ☒ in the international application as filed
   ☐ together with the international application in electronic form
   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NO:269 was searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/074560

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. Ill Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheets

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20, limited to a polypeptide selected to be SEQ ID NO: 269.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-20 are drawn to a method for altering cell phenotype comprising contacting a cell with a composition comprising at least a first and a second cell phenotype altering polynucleotide, wherein each of said first cell phenotype altering polynucleotide and said second cell phenotype altering polynucleotide comprise: (a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide selected from the group consisting of SEQ ID NOs: 269-394;(b) a first flanking region located at the 5' terminus of said first region comprising; (i) a sequence of linked nucleosides selected from the group consisting of the native 5' untranslated region (UTR) of any of SEQ ID NOs: 269-394, SEQ ID NO: 1 and functional variants thereof; (c) a second flanking region located at the 3' terminus of said first region comprising; (i) a sequence of linked nucleosides selected from the group consisting of the native 3' UTR of any of SEQ ID NOs: 269-394, SEQ ID NOs 2-7 and functional variants thereof; and (ii) a 3' tailing sequence of linked nucleosides; wherein the first region of linked nucleosides comprises at least a first modified nucleoside and a composition thereof.

The first invention of Group I+ is restricted to a method for altering cell phenotype comprising contacting a cell with a composition comprising at least a first cell phenotype altering polynucleotide, wherein said first cell phenotype altering polynucleotide consists of: (a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide selected to be SEQ ID NO: 269; b) a first flanking region located at the 5' terminus of said first region consisting of; (i) a sequence of linked nucleosides selected to be the native 5' untranslated region (UTR) of SEQ ID NO: 269; (c) a second flanking region located at the 3' terminus of said first region consisting of; (i) a sequence of linked nucleosides selected to be the native 3' UTR of SEQ ID NO: 269; and (ii) a 3' tailing sequence of linked nucleosides; wherein the first region of linked nucleosides comprises at least a first modified nucleoside and a composition thereof. It is believed that claims 1-20 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NO: 269.

Applicant is invited to elect additional cell phenotype altering polynucleotides with specified SEQ ID NOs for each polynucleotide to be searched. An exemplary election would be a method for altering cell phenotype comprising contacting a cell with a composition comprising at least a first cell phenotype altering polynucleotide, wherein said first cell phenotype altering polynucleotide consists of: (a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide selected to be SEQ ID NO: 270; b) a first flanking region located at the 5' terminus of said first region consisting of; (i) a sequence of linked nucleosides selected to be the native 5' untranslated region (UTR) of SEQ ID NO: 270; (c) a second flanking region located at the 3' terminus of said first region consisting of; (i) a sequence of linked nucleosides selected to be the native 3' UTR of SEQ ID NO: 270; and (ii) a 3' tailing sequence of linked nucleosides; wherein the first region of linked nucleosides comprises at least a first modified nucleoside and a composition thereof. Additional cell phenotype altering polynucleotides will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13:1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for producing an alteration in a cell phenotype requiring the selection of alternatives for the first phenotype altering polynucleotide(s) selected from SEQ ID NOs: 1-7, 269-394, and functional variants thereof.

The Groups I+ share the technical features of a method and composition for altering cell phenotype comprising contacting a cell with a composition comprising at least a first and a second cell phenotype altering polynucleotide wherein each of said first cell phenotype altering polynucleotide and said second cell phenotype altering polynucleotide comprise: (a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide; (b) a first flanking region located at the 5' terminus of said first region comprising; (i) a sequence of linked nucleosides consisting of the native 5' untranslated region (UTR) (c) a second flanking region located at the 3' terminus of said first region comprising; (i) a sequence of linked nucleosides consisting of the native 3' UTR and (ii) a 3' tailing sequence of linked nucleosides wherein the first region of linked nucleosides comprises at least a first modified nucleoside. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2012/0046346 A1 to Rossi et al. discloses a method and composition for altering cell phenotype (Also provided herein are kits for altering the phenotype or developmental potential of a cell, Para. [0117]) comprising contacting a cell with a composition comprising at least a first and a second cell phenotype altering polynucleotide (Also provided herein are kits for altering the phenotype or developmental potential of a cell. In one aspect, provided herein is a kit comprising: a) a container with at least one synthetic, modified RNA molecule, Para. [0117]), wherein each of said first cell phenotype altering polynucleotide and said second cell phenotype altering polynucleotide comprise: (a) a first region of linked nucleosides, said first region encoding a cell phenotype altering polypeptide (It is also contemplated herein that a first and second synthetic, modified RNA are administered in a separate and temporally distinct manner. Thus, each of a plurality of synthetic, modified RNAs can be administered at a separate time or at a different frequency interval to achieve the desired expression of a polypeptide, Para. [0252]); (b) a first flanking region located at the 5' terminus of said first region comprising; (i) a sequence of linked nucleosides consisting of the native 5' untranslated region (UTR) (c) a second flanking region located at the 3' terminus of said first region comprising; (i) a sequence of linked nucleosides consisting of the native 3' UTR (the synthetic, modified RNAs described herein can further comprise a 5' and/or 3' untranslated region (UTR). Untranslated regions are regions of the RNA before the start codon (5') and after the stop codon (3'), and are therefore not translated by the translation machinery. Para. [0228]; in some embodiments, synthetic, modified RNAs as described herein include in vitro transcribed RNAs including modifications as found in eukaryotic/mammalian/human RNA in vivo. Other modifications that mimic such naturally occurring modifications can also be helpful in producing a synthetic, modified RNA molecule that will be tolerated by a cell, Para. [0209]; modified RNAs described herein can be naturally occurring, Para. [0288]) and (ii) a 3' tailing sequence of linked nucleosides wherein the first region of linked nucleosides comprises at least a first modified nucleoside (the synthetic modified RNA molecule encoding the reprogramming factor further comprises a poly(A) tail, a Kozak sequence, a 3' untranslated region, a 5' untranslated region, or any combination thereof; In one embodiment, the poly(A) tail, the Kozak sequence, the 3' untranslated region, the 5' untranslated region, or the any combination thereof comprises one or more modified nucleosides, Para. [0084]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature