CLAIMS

We claim:

1. A method for reducing an immunogenicity of a gene therapy vector comprising an in vitro-synthesized RNA molecule that comprises an open reading frame that encodes a functional protein, the method comprising introducing a modified nucleotide that contains a modified nucleoside into said gene therapy vector during in vitro transcription,

whereby the gene therapy vector comprising the in vitro-synthesized RNA molecule is significantly less immunogenic based on a detectable decrease in immunogenicity compared to an unmodified in vitro-synthesized RNA molecule with the same sequence, but without the modified nucleotide, and

wherein said gene therapy vector comprising the in vitro-synthesized RNA molecule is purified to remove contaminants that are toxic to a cell by inducing an innate immune response, inducing RNA interference, or directly or indirectly decreasing translation of the in vitro-synthesized mRNA in the cell, and

such that an effective amount of the gene therapy vector comprising the in vitro-synthesized RNA molecule can be administered without triggering a detectable immune response, or can be repeatedly administered or delivered without eliciting an immune response sufficient to detectably reduce expression of the recombinant protein or inducing signaling pathways though RNA sensors,

thereby reducing an immunogenicity of a gene therapy vector.

2. The method of claim 1, wherein said modified nucleotide contains 1-methylpseudouridine or pseudouridine that is not further modified.

3. The method of claim 2, wherein said introducing additionally comprises introducing a modified nucleotide that contains the modified nucleoside 5-methylcytidine.

4. The method of claim 1, wherein said introducing comprises introducing a modified nucleotide that contains a modified nucleoside selected from the group consisting of pseudouridine (Ψ), 1-methylpseudouridine ($m^1\Psi$) or 5-methyluridine (m^5U) in place of 100% of said uridine nucleotides, and/or introducing a modified nucleotide that contains the modified nucleoside 5-methylcytidine in place of 100% of said cytidine nucleotides.

5. The method of claim 1, wherein said introducing comprises introducing a modified nucleotide that contains a modified nucleoside selected from the group consisting of pseudouridine (Ψ), 1-methylpseudouridine ($m^1\Psi$), 5-methyluridine (m^5U), 2-thiouridine (s^2U) and 2'-O-methyluridine (Um) in place of 0.2% to 100% of the uridine nucleotides; and/or introducing a modified nucleotide that contains the modified nucleoside 5-methylcytidine (m^5C) in place of 0.2% to 100% of the cytidine nucleotides.

6. The method of any of claims 1 to 5, wherein said gene therapy vector that comprises an in vitro-synthesized RNA molecule comprises:

(i) a poly-A tail, preferably having 50-200 nucleotides, and most preferably, greater than 150 nucleotides;

(ii) a cap nucleotide;

(iii) a cap-independent translational enhancer;

(iv) a 5′ UTR; and/or

(v) a 3′ UTR.

7. The method of any of claims 1 to 6, wherein said gene therapy vector comprising an in vitro-synthesized RNA molecule is purified using HPLC or a gravity flow column.

8. The method of any of claims 1 to 6, wherein said gene therapy vector comprising an in vitro-synthesized RNA molecule is purified using a process comprising treating the in vitro-synthesized RNA molecule with one or more enzymes that specifically digest one or more RNA contaminant molecules and/or DNA contaminant molecules.

9. The method of any of claims 1 to 6, wherein said gene therapy vector comprising an in vitro-synthesized RNA molecule is purified using a process comprising treating the in vitro-synthesized RNA molecule with a ribonuclease III (RNase III) enzyme such that short RNase III digestion products are generated, and purifying said short RNase III digestion products away from said in vitro-synthesized RNA molecule.

10. The method of any of claims 1 to 9, wherein said detectable decrease in immunogenicity of the gene therapy vector comprising the in vitro-synthesized RNA molecule comprising pseudouridine or a modified nucleotide compared to said unmodified in vitro-synthesized RNA molecule is detected by measuring secretion of cytokines, selected from the group consisting of IL-12, IFN-α, TNF-α, RANTES, MIP-1α, MIP-1β, IL-6, IFN-β, and IL-8, or measuring expression of dendritic cell (DC) activation markers, selected from the group consisting of CD83, HLA-DR, CD80 and CD86.

11. The method of any of Claims 1 to 9, wherein said gene therapy vector comprising the in vitro-synthesized RNA molecule is free of double-stranded RNA molecules.

12. The gene therapy vector obtained by the method of any of claims 1 to 9 for use in a method of delivering a recombinant protein to a subject, the method comprising the step of contacting the subject with said gene therapy vector comprising said RNA molecule, thereby delivering said recombinant protein to a subject.

13. Use of the gene therapy vector obtained by the method of any claims 1 to 9 in the preparation of a medicament for delivering a recombinant protein to a subject, wherein the medicament is suitable for contacting the subject with said gene therapy vector comprising said RNA molecule, thereby delivering said recombinant protein to a subject.

14. The method of any of claims 1 to 9, wherein said reducing an immunogenicity of a gene therapy vector comprises decreasing TNF-α production in a subject in response to the gene therapy vector or decreasing IL-12 production in a subject in response to the gene therapy vector.

15. The method of any of claims 1 to 9, wherein the detectable decrease in immunogenicity is detected by at least one method selected from the group consisting of:

(i) detecting a decrease in immunogenicity such that the RNA can be repeatedly administered without eliciting an immune response sufficient to detectably reduce expression of said a protein encoded by said polyribonucleotide molecule or said RNA molecule comprised in said gene therapy vector;

(ii) measuring secretion of a cytokine secreted in response to administration of the gene therapy vector comprising the in vitro-synthesized RNA molecule comprising the modified nucleotide relative to the same quantity of the unmodified in vitro-synthesized RNA molecule, wherein said cytokine is selected from the group consisting of IL-12, IFN-α, TNF-α, RANTES; and

(iii) measuring expression of a dendritic cell activation marker selected from the group consisting of CD83, HLA-DR, CD80 and CD86; or MIP-1α, MIP-1β, IL-6, IFN-β, and IL-8.

16. The method of any of claims 1 to 9, wherein the method further comprises inducing a mammalian cell that resides in vivo in a subject to produce a recombinant protein for gene therapy, the method comprising: contacting the mammalian cell with the gene therapy vector comprising the in vitro-synthesized RNA encoding the recombinant protein, the in vitro-synthesized RNA comprising 1-methyl-pseudouridine (m1Ψ) or pseudouridine that is not further modified (Ψ),

such that an effective amount of the gene therapy vector comprising said in vitro-synthesized RNA molecule is administered without triggering a detectable immune response or eliciting an immune response sufficient to detectably reduce expression of the recombinant protein upon repeatedly being administered,

thereby inducing the mammalian cell to produce the recombinant protein.

17. The method of claim 16, wherein said protein encoded by said gene therapy vector comprising said in vitro-synthesized RNA molecule is selected from the group consisting of: erythropoietin (EPO); a detectable enzyme selected from firefly luciferase, Renilla luciferase, bacterial beta-galactosidase (lacZ), and green fluorescent protein (GFP); a transcription factor selected from MYC and SRY or MCOP; a growth factor or cytokine selected from the group consisting of platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta1 (TGF-beta1), insulin-like growth factor (IGF), alpha-melanocyte-stimulating hormone (alpha-MSH); insulin-like growth factor-I (IGF-I); IL-4; IL-13; and IL-10; inducible nitric oxide synthase (iNOS); a heat shock protein; Cystic Fibrosis Transmembrane Conductance Regulator (CFTR); an enzyme with antioxidant activity selected from among catalase, phospholipid hydroperoxide glutathione peroxidase, superoxide dismutase-1, and superoxide dismutase-2; Bruton's tyrosine kinase; adenosine deaminase; and ecto-nucleoside triphosphate diphosphydrolase.

18. The method of claim 16, wherein said mammalian cell is an antigen-presenting cell, a dendritic cell, a macrophage, a neural cell, a brain cell, an astrocyte, a microglial cell, and a neuron, a spleen cell, a lymphoid cell, a lung cell, a lung epithelial cell, a skin cell, a keratinocyte, an endothelial cell, an alveolar cell, an alveolar macrophage, an alveolar pneumocyte, a vascular endothelial cell, a mesenchymal cell, an epithelial cell, a colonic epithelial cell, a hematopoietic cell, a bone marrow cell, a Claudius' cell, a Hensen cell, a Merkel cell, a Müller cell, a Paneth cell, a Purkinje cell, a Schwann cell, a Sertoli cell, an acidophil cell, an acinar cell, an adipoblast, an adipocyte, a brown or white alpha cell, an amacrine cell, a beta cell, a capsular cell, a cementocyte, a chief cell, a chondroblast, a chondrocyte, a chromaffin cell, a chromophobic cell, a corticotroph, a delta cell, a Langerhans cell, a follicular dendritic cell, an enterochromaffin cell, an ependymocyte, an epithelial cell, a basal cell, a squamous cell, an endothelial cell, a transitional cell, an erythroblast, an erythrocyte, a fibroblast, a fibrocyte, a follicular cell, a germ cell, a gamete, an ovum, a spermatozoon, an oocyte, a primary oocyte, a secondary oocyte, a spermatid, a spermatocyte, a primary spermatocyte, a secondary spermatocyte, a germinal epithelium, a giant cell, a glial cell, an astroblast, an astrocyte, an oligodendroblast, an oligodendrocyte, a glioblast, a goblet cell, a gonadotroph, a granulosa cell, a haemocytoblast, a hair cell, a hepatoblast, a hepatocyte, a hyalocyte, a interstitial cell, a juxtaglomerular cell, a keratinocyte, a keratocyte, a lemmal cell, a leukocyte, a granulocyte, a basophil, an eosinophil, a neutrophil, a lymphoblast, a B-lymphoblast, a T-lymphoblast, a lymphocyte, a B-lymphocyte, a T-lymphocyte, a helper induced T-lymphocyte, a Th1 T-lymphocyte, a Th2 T-lymphocyte, a natural killer cell, a thymocyte, a Kupffer cell, an alveolar macrophage, a foam cell, a histiocyte, a luteal cell, a lymphocytic stem cell, a lymphoid cell, a lymphoid stem cell, a macroglial cell, a mammotroph, a mast cell, a medulloblast, a megakaryoblast, a megakaryocyte, a melanoblast, a melanocyte, a mesangial cell, a mesothelial cell, a metamyelocyte, a monoblast, a monocyte, a mucous neck cell, a myoblast, a muscle cell, a cardiac muscle cell, a skeletal muscle cell, a smooth muscle cell, a myelocyte, a myeloid cell, a myeloid stem cell, a myoblast, a myoepithelial cell, a myofibrobast, a neuroblast, a neuroepithelial cell, a neuron, an odontoblast, an osteoblast, an osteoclast, an osteocyte, an oxyntic cell, a parafollicular cell, a paraluteal cell, a peptic cell, a pericyte, a peripheral blood mononuclear cell, a phaeochromocyte, a phalangeal cell, a pinealocyte, a pituicyte, a plasma cell, a platelet, a podocyte, a proerythroblast, a promonocyte, a promyeloblast, a promyelocyte, a pronormoblast, a reticulocyte, a retinal pigment epithelial cell, a retinoblast, a small cell, a somatotroph, a stem cell, a sustentacular cell, a teloglial cell, and a zymogenic cell.

19. The method of any of claims 1 to 9, wherein said RNA sensors are selected from the group consisting of RNA-dependent protein kinase (PKR), retinoic acid-inducible gene-I (RIG-I), Toll-like receptor 3 (TLR3), Toll-like receptor 7 (TLR7), Toll-like receptor 8 (TLR8), and oligoadenylate synthetase (OAS).

20. A method for reducing an immunogenicity of a gene therapy vector comprising an in vitro-synthesized polyribonucleotide or RNA molecule, the method comprising:

(i) generating a gene therapy vector comprising an in vitro-synthesized polyribonucleotide or RNA molecule in a process comprising replacing a nucleotide of the RNA molecule with a modified nucleotide that contains a pseudouridine or a modified nucleoside by in vitro transcription in which the

basic NTP is replaced with or substituted by the corresponding triphosphate-derivative of the pseudouridine or other modified nucleotide,

whereby the gene therapy vector comprising the in vitro-synthesized polyribonucleotide or RNA molecule is significantly less immunogenic based on a detectable decrease in immunogenicity compared to an unmodified in vitro-synthesized polyribonucleotide or RNA molecule with the same sequence, but without the modified nucleotide; and

(ii) purifying the gene therapy vector comprising an in vitro-synthesized polyribonucleotide or RNA molecule to remove contaminants that are toxic to a cell by inducing an innate immune response, inducing RNA interference, or directly or indirectly decreasing translation of the in vitro-synthesized mRNA in the cell such that an effective amount of the purified gene therapy vector comprising the in vitro-synthesized polyribonucleotide or RNA molecule can be administered to a human or mammalian cell or subject without triggering a detectable immune response, or can be repeatedly administered or delivered without eliciting an immune response sufficient to detectably reduce expression of the recombinant protein or inducing signaling pathways through RNA sensors;

thereby reducing an immunogenicity of a gene therapy vector.

21. The method of claim 20, wherein said RNA sensors are selected from the group consisting of RNA-dependent protein kinase (PKR), retinoic acid-inducible gene-I (RIG-I), Toll-like receptor 3 (TLR3), Toll-like receptor 7 (TLR7), Toll-like receptor 8 (TLR8), and oligoadenylate synthetase (OAS).

22. The method of any of claims 1 to 21, wherein said method results in increasing in enhancing in vivo translation of said gene therapy vector.

23. A purified RNA preparation comprising an in vitro-synthesized RNA molecule encoding a recombinant protein,

wherein said in vitro-synthesized RNA comprises pseudouridine (Ψ) or 1-methyl-pseudouridine (m1Ψ) in place of uridine; and

wherein said purified RNA preparation is free or substantially free of contaminant RNA molecules that are able to activate RNA sensors in a human or animal cell, and

wherein said modified mRNA molecules exhibit a cap with a cap 0 or cap1 structure.

24. Use of the purified RNA preparation of claim 23 in the preparation of a medicament for inducing a mammalian cell to produce a recombinant protein of interest in gene therapy, wherein said therapy comprises contacting said mammalian cell with said purified RNA preparation comprising said in vitro-synthesized RNA molecule.

CLAIMS

We claim:

1. A method for reprogramming a human or animal cell that exhibits a first differentiated state or phenotype to a cell that exhibits a second differentiated state or phenotype comprising: introducing into the cell that exhibits a first differentiated state a purified RNA preparation comprising modified mRNA molecules that encode at least one reprogramming factor and culturing the cell under conditions wherein the cell exhibits a second differentiated state.

2. The method of Claim 1, wherein the modified mRNA molecules contain at least one modified nucleoside selected from the group consisting of: pseudouridine (Ψ), 5-methylcytosine (m^5C), 5-methyluridine (m^5U), 2'-O-methyluridine (Um or $m^{2'\text{-}O}U$), 2-thiouridine (s^2U), and N^6-methyladenosine (m^6A)) in place of at least a portion of the corresponding unmodified canonical nucleoside.

3. The method of Claim 2, wherein the at least one modified nucleoside is present in place of substantially all of the corresponding unmodified canonical nucleoside.

4. The method of any of Claims 1-3, wherein said purified RNA preparation:

i) comprises at least one single-stranded mRNA that encodes an iPS cell induction factor, wherein substantially all of said first single-stranded mRNAs comprise at least one pseudouridine residue and/or at least one 5-methylcytidine residue, and

ii) is substantially free of RNA contaminant molecules which are able to activate RNA sensors in said somatic cell.

5. The method of Claim 4, wherein said RNA contaminant molecules are selected from the group consisting of: partial mRNAs encoding only a portion of said reprogramming factor, RNA molecules that are smaller than the full-length mRNA, RNA molecules that are larger than the full-length mRNA, double-stranded mRNA molecules, and un-capped mRNA molecules.

6. The method of any of Claims 1-5, wherein said cell that exhibits a second differentiated state is an iPS cell or dedifferentiated cell.

7. The method of any of Claims 1-5, wherein said cell that exhibits a second differentiated state is a transdifferentiated cell.

8. The method of any of Claim 1-5 wherein said reprogrammed cell that exhibits a second differentiated state is a redifferentiated cell.

9. The method of any of Claims 1-8, wherein said modified mRNA molecules that encode at least one reprogramming factor is selected from the group consisting of KLF4, LIN28, c-MYC, NANOG, OCT4, and SOX2.

10. The method of any of Claims 1-9, wherein the method further comprises contacting said cell that exhibits a first differentiated state or phenotype with at least one growth factor and/or cytokine.

11. The method of any of Claims 1-10, wherein all of the uridine nucleosides in said modified mRNA are replaced by pseudouridine nucleosides.

12. The method of any of Claims 1-11, wherein all of the cytidine nucleosides in said modified mRNA are replaced by 5-methylcytidine nucleosides.

13. The method of any of Claims 1-12, wherein said purified RNA preparation is free of an amount of RNA contaminant molecules that would activate an immune response in said cell that exhibits a first differentiated state to prevent said cell that exhibits a second differentiated state from surviving at least 10 days in culture.

14. The method of any of Claims 1-13, wherein said cell that exhibits a second differentiated state is able to form a cell line.

15. The method of Claim 14, wherein said dedifferentiated cell expresses NANOG and TRA-1-60.

16. A composition comprising a purified RNA preparation, wherein said purified RNA preparation:

i) comprise single-stranded mRNA molecules that encode at least one reprogramming factor, wherein said single-stranded mRNA molecules comprise at least one nucleoside selected from the group consisting of pseudouridine (Ψ), 5-methylcytosine (m^5C), 5-methyluridine (m^5U), 2'-O-methyluridine (Um or $m^{2'-O}U$), 2-thiouridine (s^2U), and N^6-methyladenosine (m^6A), and

ii) is substantially free of RNA contaminant molecules which are able to activate RNA sensors in a human or animal cell.

17. The composition of Claim 16, wherein said purified RNA preparation comprises single-stranded mRNA molecules that encode at least one reprogramming factor selected from the group consisting of KLF4, LIN28, c-MYC, NANOG, OCT4, and SOX2.

18. A method for inducing a cell to produce the MYC protein comprising: contacting a cell with an in vitro-synthesized mRNA encoding the MYC gene, wherein the in vitro-synthesized mRNA comprises at least one pseudouridine residue and/or at least one 5-methylcytidine residue, thereby inducing the mammalian cell to produce the MYC protein.