# ACETYLATED RIBONUCLEIC ACIDS AND USES THEREOF

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims priority to U.S. Provisional Patent Application 63/338,429 filed on May 4, 2022, the entire contents of which is hereby incorporated by reference.

## BACKGROUND

**[0002]** RNA therapeutics is a new and emerging field.

## SUMMARY

**[0003]** The present disclosure identifies certain challenges with the production of RNA for use in applications such as therapeutic applications, and challenges with the use of RNAs as therapeutics. For example, in some examples, the present disclosure identifies certain problems that can be encountered with using currently available RNAs for therapeutics due to, e.g., the short-lived activity and/or instability of RNAs. In some examples, instability of an RNA can be traced, e.g., to the reactivity of the hydroxyl group on carbon 2 of the ribose of a ribonucleotide.

**[0004]** Among other things, the present disclosure provides technologies related to 2-O-acetylated nucleotides in which a hydroxyl group on carbon 2 of a ribose is acetylated (2-O-acetylated ribose). Without wishing to be bound by theory, in some examples, a ribonucleotide having a 2-O-acetylated ribose can maintain crucial hydrogen bonding interactions necessary for transcription, translation, and duplex formation, while simultaneously having reduced reactivity that would otherwise promote RNA autohydrolysis and nuclease degradation. In some examples, a ribonucleotide having a 2-O-acetylated ribose is more stable than a comparable RNA having fewer 2-O-acetyl groups on a ribose.

**[0005]** Among the technologies provided by this disclosure are technologies for using 2-O-acetylated nucleotide triphosphates as a reagent for in vitro transcription of polyribonucleotides with RNA polymerase. In some examples, RNA polymerase can use 2-O-acetylated nucleotide triphosphates to produce 2-O-acetylated polyribonucleotides which can

be translated by the intracellular machinery of a cell. In some examples, 2-O-acetylated polyribonucleotides can be used for a variety of applications, including therapeutic applications, research applications, diagnostic applications, agricultural applications, and any other suitable applications.

**[0006]** The present disclosure also provides technologies for reducing immunogenicity of RNA therapeutics by providing a polyribonucleotide comprising a modified ribonucleotide, e.g., a 2-O-acetylated ribose. Without wishing to be bound by theory, the present disclosure proposes that a polyribonucleotide comprising a 2-O-acetylated ribose can achieve reduced immunogenicity when administered in a cell, tissue or subject by reduced activation of an innate immune response. In some examples, reduced activation of an innate immune response, e.g., reduced activation of NF-kb or an NF-kb pathway, IRF or an IRF pathway, and/or other inflammatory cytokines; or reduced detection of uncapped RNA by a molecular sensor (e.g., RIG-I), with a polyribonucleotide comprising a 2-O-acetylated ribose or a composition comprising the same allows for repeated dosing of, e.g., at least two doses of, said polyribonucleotide or a composition comprising the same. In some examples, a polyribonucleotide comprising a 2-O-acetylated ribose, or a composition comprising the same can be administered at a higher dose compared to a reference polyribonucleotide that includes fewer 2'-O-acetyl groups on a ribose.

**[0006a]** In an aspect of the present disclosure, there is provided a polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase,

wherein the nucleobase is an adenine, a guanine, a cytosine, or an uracil, and

wherein at least 80% of the ribose moieties of the polyribonucleotide are 2′-O-acetylated, and wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

**[0007]** Also provided herein are technologies for increasing expression from RNA therapeutics by providing a polyribonucleotide comprising a modified ribonucleotide, e.g., a

ribonucleotide comprising a 2-O-acetylated ribose. Without wishing to be bound by theory, the present disclosure proposes that a polyribonucleotide comprising 2-O-acetylated ribose can achieve increased levels of protein or polypeptide expression when administered in a cell, tissue or subject, as compared to administration of a comparable polyribonucleotide having fewer 2'-O-acetyl groups on a ribose.

**[0008]** Further provided herein are technologies for increasing persistence of RNA therapeutics by providing a polyribonucleotide comprising a modified ribonucleotide, e.g., a ribonucleotide comprising a 2-O-acetylated ribose. Without wishing to be bound by theory, the present disclosure proposes that a polyribonucleotide comprising 2-O-acetylated ribose can achieve increased persistence when administered in a cell, tissue or subject, as compared to

administration of a comparable polyribonucleotide having fewer 2'-O-acetyl groups on a ribose. In some examples, increased persistence is a result of increased resistance to one or more nucleases. In some examples, increased persistence may also allow for reduced dosing frequency and/or reduced doses of a polyribonucleotide comprising a modified ribonucleotide, e.g., a ribonucleotide comprising a 2-O-acetylated ribose.

**[0009]** Also provided herein are compositions comprising a polyribonucleotide comprising 2-O-acetylated ribose and methods of making and using the same.

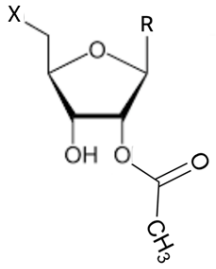
**[0009a]** In another aspect of the present disclosure, there is provided a composition comprising a polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase,

wherein the nucleobase is an adenine, a guanine, a cytosine, or an uracil, and wherein at least 80% of the ribose moieties of the polyribonucleotide are 2′-O-acetylated, and wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

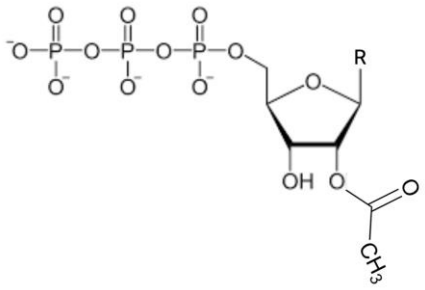
**[0010]** This disclosure provides a modified ribonucleotide comprising a nucleoside, wherein a nucleoside comprises a ribose moiety comprising an acetyl group, wherein a ribose is 2'-O-acetylated. In some examples, a modified ribonucleotide has a structure of:




**[0011]** (a) wherein X is a 5' monophosphate, a 5' diphosphate, or a 5' triphosphate; and

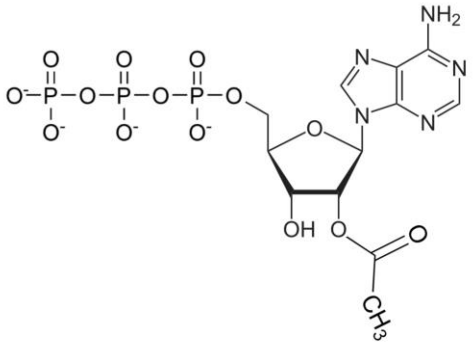
**[0012]** (b) wherein R is a nucleobase chosen from: adenine or a modified version thereof, a guanine or a modified version thereof, a cytosine or a modified version thereof, or a uracil or a modified version thereof.

**[0013]** In some examples, a modified ribonucleotide has a 5’ triphosphate and a structure of:



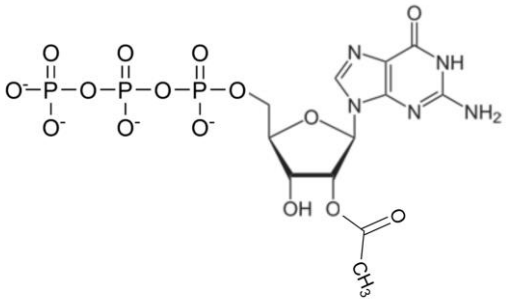
**[0014]** wherein R is a nucleobase chosen from: adenine or a modified version thereof, a guanine or a modified version thereof, a cytosine or a modified version thereof, or a uracil or a modified version thereof.

**[0015]** In some examples, a nucleobase is adenine. In some examples, a polyribonucleotide comprising adenine has a 5’ triphosphate and a structure of:



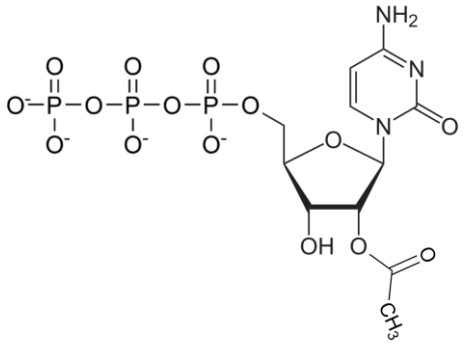
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**[0016]** In some examples, a nucleobase is guanine. In some examples, a polyribonucleotide comprising guanine has a 5’ triphosphate and a structure of:



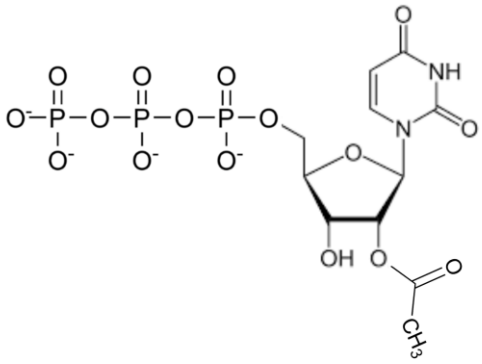
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**[0017]** In some examples, a nucleobase is cytosine. In some examples, a polyribonucleotide comprising cytosine has a 5’ triphosphate and a structure of:



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**[0018]** In some examples, a nucleobase is uracil. In some examples, a polyribonucleotide comprising has a 5’ triphosphate and a structure of and has a structure of:



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**[0019]** Also provided herein is a polyribonucleotide comprising one or more modified ribonucleotides, e.g., a ribonucleotide comprising a 2-O-acetylated ribose. In some examples, a polyribonucleotide comprises a plurality of ribonucleotides chosen from: adenine, guanine, cytosine, or uracil, or any combination thereof.

**[0020]** In some examples, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least about 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% ribose moieties in a polyribonucleotide are acetylated (2’-O-aceylated).

**[0021]** In some examples, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least

about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% of ribose moieties in a polyribonucleotide are acetylated (2'-O-aceylated).

**[0022]** In some examples, at least about 95% of ribose moieties are 2'-O-acetylated.

**[0023]** In some examples, 100% ribose moieties in a polyribonucleotide are acetylated (2'-O-aceylated).

**[0024]** In some examples, polyribonucleotide comprises a cap structure. In some examples, a cap structure does not comprise a 2'-O-acetylated ribose. In some examples, a cap structure comprises a 2'-O-acetylated ribose.

**[0025]** In some examples, a polyribonucleotide comprising a 2-O-acetylated ribose (e.g., comprising an adenine comprising a 2-O-acetylated ribose, a guanine comprising a 2-O-acetylated ribose, a cytosine comprising a 2-O-acetylated ribose, and/or a uracil comprising a 2-O-acetylated ribose) further comprises a modification comprising: a modified backbone, a modified nucleobase, or any combination thereof.

**[0026]** In some examples, a polyribonucleotide comprising a 2-O-acetylated ribose (e.g., comprising an adenine comprising a 2-O-acetylated ribose, a guanine comprising a 2-O-acetylated ribose, a cytosine comprising a 2-O-acetylated ribose, and/or a uracil comprising a 2-O-acetylated ribose) further comprises a modified backbone.

**[0027]** In some examples, a polyribonucleotide comprising a 2-O-acetylated ribose (e.g., comprising an adenine comprising a 2-O-acetylated ribose, a guanine comprising a 2-O-acetylated ribose, a cytosine comprising a 2-O-acetylated ribose, and/or a uracil comprising a 2-O-acetylated ribose) further comprises a modified nucleobase. In some examples, a nucleobase comprising a modification is chosen from adenine, guanine, cytosine, or uracil. In some examples, a nucleobase comprising a modification is an adenine. In some examples, a nucleobase comprising a modification is a cytosine. In some examples, a nucleobase comprising a modification is a guanine. In some examples, a nucleobase comprising a modification is a uracil.

**[0028]** In some examples, a nucleobase modification comprises a modification known in the art or as disclosed herein. In some examples, a nucleobase modification comprises: N4-acetyl-cytidine (ac4C), 5-hydroxymethyluridine (5-hmU), N1-methylpseudouridine, pseudouridine, pyridin-4-one ribonucleoside, 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s2U), 5-methyl cytidine (m5C), 5-aza-cytidine, 6-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine (m3C), 5-formyl-cytidine (f5C), N4-methyl-cytidine (m4C), 2-amino-purine, 2, 6-diaminopurine, 2-amino-6-halo-purine, 6-halo-purine, inosine (I), 1-methyl-inosine (m1 I), wyosine (imG), methylwyosine (mimG), or any combination thereof.

**[0028a]** In a further aspect of the present disclosure, there is provided a polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is N4-acetylcytosine, and

wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

**[0028b]** In another aspect of the present disclosure, there is provided a polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

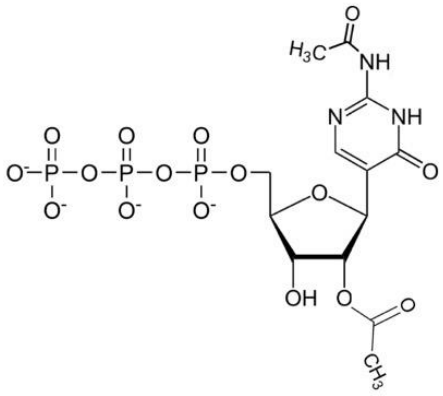
(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is 5-hydroxymethyluracil.

**[0028c]** In a further aspect of the present disclosure, there is provided a polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is N1-methylpseudouracil.

**[0029]** In some examples, a polyribonucleotide comprising a 2-O-acetylated ribose (e.g., comprising an adenine comprising a 2-O-acetylated ribose, a guanine comprising a 2-O-acetylated ribose, a cytosine comprising a 2-O-acetylated ribose, and/or a uracil comprising a 2-O-acetylated ribose) further comprises a nucleoside comprising an acetyl group, wherein the nucleoside is N4-acetylcytidine and the modified ribonucleotide has a structure of:



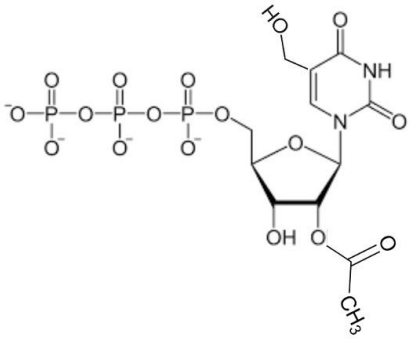
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**[0030]** In some examples, a polyribonucleotide comprises cytidine residues and about 5% to 99%, about 5% to 95%, about 5% to 90%, about 5% to 85%, about 5% to 80%, about 5% to 75%, about 5% to 70%, about 5% to 65 %, about 5% to 60%, about 5% to 55%, about 5% to 50 %, about 5% to45 %, about 5% to 40%, about 5% to 35%, about 5% to 30%, about 5% to 25 %, about 5% to 20%, about 5% to 15 %, about 5% to 10%, about 10% to 99%, about 15% to 99%, about 20% to 99%, about 25% to 99%, about 30% to 99%, about 35% to 99%, about 40% to 99%, about 45% to 99%, about 50% to 99%, about 55% to 99%, about 60%

to 99%, about 65% to 99%, about 70% to 99%, about 80% to 99%, about 85% to 99%, about 90% to 99%, or about 95% to 99% of cytidine residues in the polyribonucleotide comprise N4-acetylcytidine.

**[0031]** In some examples, a polyribonucleotide comprises cytidine residues and 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 at least about 99% of cytidine residues in the polyribonucleotide comprise N4-acetylcytidine.

**[0032]** In some examples, a polyribonucleotide comprising a 2-O-acetylated ribose (e.g., an adenine comprising a 2-O-acetylated ribose, a guanine comprising a 2-O-acetylated ribose, a cytosine comprising a 2-O-acetylated ribose, and/or a uracil comprising a 2-O-acetylated ribose) further comprises one or more modified ribonucleotides comprising a hydroxymethyl group. In some examples, one or more modified ribonucleotides comprises a nucleoside chosen from: an adenosine, a guanosine, a cytidine or a uridine, or any combination thereof. In some examples, a nucleoside of a one or more modified ribonucleotides is 5-hydroxymethyluridine, and the modified ribonucleotide has the structure of:



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**[0033]** In some examples, a polyribonucleotide comprises uridine residues and about 5% to 99%, about 5% to 95%, about 5% to 90%, about 5% to 85%, about 5% to 80%, about 5% to 75%, about 5% to 70%, about 5% to 65 %, about 5% to 60%, about 5% to 55%, about 5% to 50 %, about 5% to45 %, about 5% to 40%, about 5% to 35%, about 5% to 30%, about 5% to 25 %, about 5% to 20%, about 5% to 15 %, about 5% to 10%, about 10% to 99%, about 15% to

99%, about 20% to 99%, about 25% to 99%, about 30% to 99%, about 35% to 99%, about 40% to 99%, about 45% to 99%, about 50% to 99%, about 55% to 99%, about 60% to 99%, about 65% to 99%, about 70% to 99%, about 80% to 99%, about 85% to 99%, about 90% to 99%, or about 95% to 99% of uridine residues in the polyribonucleotide comprise 5-hydroxymethyluridine.

**[0034]** In some examples, a polyribonucleotide comprises uridine residues and 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 at least about 99% of uridine residues in the polyribonucleotide comprise 5-hydroxymethyluridine.

**[0035]** This disclosure also provides methods of making a polyribonucleotide comprising a 2-O- acetylated ribose. In some examples, disclosed herein is method of producing a polyribonucleotide comprising a step of incubating an in vitro transcription mixture, wherein the in vitro transcription mixture comprises: (i) a DNA template; (ii) at least one RNA polymerase or a variant or a fragment thereof; and (iii) a plurality of ribonucleotides comprising at least one modified ribonucleotide comprising a 2'-O-acetylated ribose; thereby producing a polyribonucleotide comprising a 2'-O-acetylated ribose.

**[0036]** Also disclosed herein are in vitro transcription mixtures useful in producing a polyribonucleotide comprising a 2'-O-acetylated ribose. In some examples, disclosed herein is an in vitro transcription mixture comprising: (i) a DNA template; (ii) at least one RNA polymerase or a variant or a fragment thereof; and (iii) a plurality of ribonucleotides comprising at least one modified ribonucleotide comprising a 2'-O-acetylated ribose.

**[0036a]** In a further aspect of the present disclosure, there is provided a method of manufacturing an RNA composition comprising introducing at least one modified ribonucleotide into a polyribonucleotide, wherein the modified ribonucleotide comprises:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase,

wherein the nucleobase is an adenine, a guanine, a cytosine, or an uracil, and

wherein at least 80% of the ribose moieties of the polyribonucleotide are 2′-O-acetylated

**[0037]** In some examples, a method or an in vitro transcription mixture produces a plurality of polyribonucleotides. In some examples, each polyribonucleotide in a plurality of polyribonucleotides comprises a 2’-O-acetylated ribose.

**[0038]** In some examples, a polyribonucleotide produced using a method or an in vitro transcription mixture disclosed herein further comprises one or more modifications, e.g., one or more base modifications.

**[0039]** In some examples, an RNA polymerase is chosen from: a bacteriophage RNA polymerase, a mitochondrial RNA polymerase, a eukaryotic RNA polymerase, a bacterial RNA polymerase, or any combination thereof. In some examples, an RNA polymerase comprises a T7 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase, a viral RNA polymerase, a N4 virion RNA polymerase, or a variant of any of the foregoing.

**[0040]** In some examples, a polyribonucleotide produced by a method disclosed herein or for using in an in vitro transcription mixture comprises a polyribonucleotide comprises a coding region.

**[0041]** In some examples, a polyribonucleotide produced by a method disclosed herein or for using in an in vitro transcription mixture comprises a polyribonucleotide does not comprise a coding region.

**[0042]** In some examples, a polyribonucleotide produced by a method disclosed herein, or using an in vitro transcription mixture comprises a guide RNA, a short hairpin RNA, an siRNA, a microRNA, a long non-coding RNA, or a messenger RNA (mRNA), a circular RNA, or any combination thereof.

**[0043]** In some examples, a polyribonucleotide produced by a method disclosed herein or using an in vitro transcription mixture encodes a payload. In some examples, a payload comprises one or more target polypeptides.

**[0044]** In some examples of a method of producing a ribonucleotide disclosed herein, an incubating step occurs at a temperature of at least 37°C.

**[0045]** In some examples of a method of producing a ribonucleotide disclosed herein, an incubating step occurs at a temperature of about 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, or higher.

**[0046]** In some examples of a method of producing a ribonucleotide disclosed herein, an incubating step is performed for at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours or longer.

**[0047]** Further provided herein are compositions comprising a polyribonucleotide disclosed herein, e.g., a polyribonucleotide made according to a method disclosed herein. In some examples, a composition is or comprises a pharmaceutical composition. In some

examples, a composition is a pharmaceutical composition. In some examples, a pharmaceutical composition is or comprises an immunogenic composition. . In some examples, a pharmaceutical composition is or comprises an antibody therapy. . In some examples, a pharmaceutical composition is or comprises an immune-modulation therapy. In some examples, a pharmaceutical composition is or comprises a vaccine. In some examples, a pharmaceutical composition is or comprises a gene therapy. In some examples, a pharmaceutical composition is or comprises a chemotherapy. In some examples, a pharmaceutical composition is or comprises a protein replacement therapy. In some examples, a pharmaceutical composition is or comprises an immunotherapy. In some examples, a pharmaceutical composition is or comprises a cell engineering therapy.

**[0048]** In some examples, a composition comprises double stranded RNA.

**[0049]** Also provided herein is a method comprising administering a polyribonucleotide disclosed herein, or a composition comprising the same to a cell, tissue, or subject. In some examples, a method further comprises determining cell viability of the cell, tissue or subject.

**[0050]** In some examples, a cell viability is a measure of the length of time one or more cells of a cell, tissue or subject live. In some examples, a cell viability is a measure of a number of cells of a cell, tissue or subject alive at one or more time points. In some examples, a cell, tissue or subject to which a polyribonucleotide or a composition comprising the same has been administered exhibits improved cell viability as compared to a reference cell viability. In some examples, a reference cell viability is a cell viability of a cell, tissue or subject that has been administered a comparable polyribonucleotide or a composition comprising the same that includes fewer 2'-O-acetylated ribose (e.g., does not include any 2'-O-acetylated ribose).

**[0051]** In some examples, a method further comprises comprising determining an immune system response of the cell, tissue or subject to which a polyribonucleotide or a composition comprising the same has been administered. In some examples, an immune response comprises an innate immune system response comprising innate immune system induced toxicity. In some examples, determining an innate immune system response comprises determining a level of NF-κB, IRF, and/or other inflammatory cytokines in the cell, tissue or subject. In some examples, a cell, tissue or subject to which a polyribonucleotide or

a composition comprising the same has been administered exhibits a reduced innate immune system response as compared to a reference. In some examples, a reference is an innate immune system response of a cell, tissue or subject that has been administered a comparable polyribonucleotide or a composition comprising the same that includes fewer 2'-O-acetylated ribose (e.g., does not include any 2'-O-acetylated ribose).

**[0052]** In some examples, a method of administration disclosed herein further comprises determining efficacy of a polyribonucleotide or a composition comprising the same in the cell, tissue or subject to which the polyribonucleotide or a composition comprising the same has been administered. In some examples, determining efficacy comprises determining an antibody response or cellular response in the cell, tissue or subject. In some examples, a cell, tissue or subject to which a polyribonucleotide or a composition comprising the same has been administered exhibits an increased antibody response or cellular response as compared to a reference. In some examples, a reference is an antibody response or cellular response of a cell, tissue or subject that has been administered a comparable polyribonucleotide or a composition comprising the same that includes fewer 2'-O-acetylated ribose (e.g., does not include any 2'-O-acetylated ribose).

**[0053]** In some examples, a method comprises administering the polyribonucleotide or a composition comprising the same to the cell, tissue or subject at least two times. In some examples, a method comprises administering a polyribonucleotide or a composition comprising the same to the cell, tissue or subject at least 2 times, 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 or at least 10 times. In some examples, at least two administrations of a polyribonucleotide or a composition comprising the same to a cell, tissue or subject does not result in reduced efficacy of a polyribonucleotide or a composition comprising the same compared to administration of one dose of a polyribonucleotide or a composition comprising the same.

**[0054]** In some examples, a method comprises administering a polyribonucleotide or a composition comprising the same to a cell, tissue or subject at a higher dose compared to an appropriate reference comparator. In some examples, a reference comparator comprises a comparable polyribonucleotide that includes fewer 2'-O-acetylated ribose (e.g., does not include

any 2’-O-acetylated ribose). In some examples, a cell is a mammalian cell, the tissue is a mammalian tissue, or the subject is a mammal.

**[0055]** In some examples, a polyribonucleotide disclosed herein is or comprises an RNA oligo.

**[0056]** In some examples, a polyribonucleotide disclosed herein comprises a coding region. In some examples, a coding region encodes a gene product. In some examples, a gene product is or comprises a polypeptide. In some examples, a gene product is or comprises a transcript.

**[0057]** In some examples, a polyribonucleotide disclosed herein does not comprise a coding region.

**[0058]** In some examples, a polyribonucleotide disclosed herein is or comprises a messenger RNA (mRNA).

**[0059]** In some examples, a polyribonucleotide disclosed herein is or comprises a gRNA.

**[0060]** In some examples, a polyribonucleotide disclosed herein is or comprises an inhibitory RNA.

**[0061]** In some examples, a polyribonucleotide disclosed herein is or comprises an miRNA or siRNA.

**[0062]** In some examples, a polyribonucleotide disclosed herein is or comprises a long non-coding RNA.

**[0063]** In some examples, a polyribonucleotide disclosed herein is or comprises an antisense oligonucleotide.

**[0064]** In some examples, a method is a method to stimulate an immune response.

**[0065]** In some examples, a method is a vaccination method.

**[0066]** In some examples, a method is an antibody therapy method.

**[0067]** In some examples, a method is an immune-modulation therapy method.

**[0068]** In some examples, a method is a gene therapy method.

**[0069]** In some examples, a method comprises delivery of one or more components of a gene therapy such as a gRNA.

**[0070]** In some examples, a method is a cell therapy engineering method.

**[0071]** In some examples, a method is an immunotherapy method. In some examples, an immunotherapy method comprises delivery of an immune-modulation therapy and/or an immune checkpoint therapy.

**[0072]** In some examples, a method is a protein replacement therapy method. In some examples, a protein replacement therapy method comprises delivery of an enzyme replacement therapy.

**[0073]** In some examples, a method is a chemotherapeutic method.

**[0074]** Also provided herein is a method of vaccination comprising administering one or more polyribonucleotides disclosed herein or a composition comprising the same to a cell, tissue or subject.

**[0075]** Disclosed herein is a method of immunotherapy comprising administering one or more polyribonucleotides disclosed herein or a composition comprising the same to a cell, tissue or subject.

**[0076]** Provided herein is a method of providing an antibody therapy comprising administering one or more polyribonucleotides disclosed herein or a composition comprising the same to a cell, tissue or subject. In some examples, an antibody therapy comprises an antibody, a fragment, a variant, or a fusion thereof. In some examples, an antibody therapy comprises a fragment comprising an antigen-recognition domain (*e.g.*, an scFv, a Fab or other fragments), or an intact antibody, or a polypeptide comprising antigen binding specificity fused to an Fc. In some examples, an antibody therapy comprises a bispecific, a multi-specific, a heterodimer, a Crossmab, a DVD-Ig, a 2 in 1 IgG, an IgG-sc-FV, an scFv-scFv, a BiTE, a DART, a diabody, a Fab-scFv fusion, a Fab-Fab fusion, a tandem antibody, or any other art recognized antibody formats.

**[0077]** Provided herein is a method of providing an immune-modulation therapy comprising administering one or more polyribonucleotides disclosed herein or a composition comprising the same to a cell, tissue or subject. In some examples, an immune-modulation

therapy comprises: a cytokine or a variant or fragment thereof, a chemokine or a variant or fragment thereof, a T-cell modulator, an NK cell modulator, a B cell modulator, a myeloid cell modulator, a modulator of any other immune cell, or any combination thereof. In some examples, an immune-modulation therapy comprises a chimeric antigen receptor (CAR) therapy. In some examples, an immune-modulation therapy, comprises an engineered T cell receptor (TCR) therapy.

**[0078]** Provided herein is a method of gene therapy comprising administering one or more polyribonucleotides disclosed herein or a composition comprising the same to a cell, tissue or subject.

**[0079]** This disclosed provides, a method of protein replacement therapy, comprising administering one or more polyribonucleotides disclosed herein or a composition comprising the same to a cell, tissue or subject.

**[0080]** Also disclosed herein is a method of cell engineering therapy, comprising administering one or more polyribonucleotides disclosed herein or a composition comprising the same to a cell, tissue or subject.

**[0081]** This disclosed provides, a method of obtaining a lower level of immunogenicity in a subject who has received a polyribonucleotide comprising a modified ribonucleotide, or a composition comprising the same, as compared with a subject who has received a comparable unmodified polyribonucleotide. In some examples, a method comprises administering a polyribonucleotide comprising a modified ribonucleotide or a composition comprising the same to a subject.

**[0082]** In some examples of any of the methods, uses or compositions disclosed herein, a polyribonucleotide comprising a modified ribonucleotide does not comprise a 5’ cap, e.g., a 5’-5’ triphosphate linked guanosine. In some examples, a polyribonucleotide comprising a modified ribonucleotide comprises a 5’ phosphate and/or a hydroxyl group at the 5’ terminus of the polyribonucleotide.

**[0083]** In some examples of any of the methods, uses or compositions disclosed herein, a polyribonucleotide comprising a modified ribonucleotide comprises a 5’ cap, e.g., a 5’-5’ triphosphate linked guanosine.

**[0084]** In some examples, a subject who has received a polyribonucleotide comprising a modified ribonucleotide or a composition comprising the same, and a subject who has received a comparable unmodified polyribonucleotide are the same subject.

**[0085]** In some examples, a subject who has received a polyribonucleotide comprising a modified ribonucleotide or a composition comprising the same, and a subject who has received a comparable unmodified polyribonucleotide are different subjects.

**[0086]** Provided herein is a method of manufacturing an RNA composition comprising introducing at least one modified ribonucleotide disclosed herein into a polyribonucleotide. In some examples, a method does not comprise removing double-stranded RNA from the RNA composition.

**[0087]** Disclosed herein is a cell comprising a polyribonucleotide disclosed herein or a composition comprising the same.

**[0087a]** In another aspect of the present disclosure, there is provided a cell comprising a polyribonucleotide as disclosed herein.

**[0088]** Also disclosed herein is use of a modified ribonucleotide disclosed herein in the production of a polyribonucleotide.

**[0088a]** In a further aspect of the present disclosure, there is provided a use of a polyribonucleotide comprising one or more modified ribonucleotides in the manufacture of a medicament, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase,

wherein the nucleobase is an adenine, a guanine, a cytosine, or an uracil, and

wherein at least 80% of the ribose moieties of the polyribonucleotide are 2′-O-acetylated, and wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

**[0088b]** In another aspect of the present disclosure, there is provided a use of a polyribonucleotide comprising one or more modified ribonucleotides in the manufacture of a medicament, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is N4-acetylcytosine, and

wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

**[0088c]** In a further aspect of the present disclosure, there is provided a use of a polyribonucleotide comprising one or more modified ribonucleotides in the manufacture of a medicament, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is 5-hydroxymethyluracil.

**[0089]** This disclosure provides use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for stimulating an immune response.

**[0090]** Provided herein is use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for use as a vaccine.

**[0091]** This disclosure provides use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for use as an immunotherapy.

**[0092]** Provided herein is use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for use as an antibody therapy.

**[0093]** Provided herein is use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for use as an immune-modulation therapy.

**[0094]** Provided herein is use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for use as a gene therapy.

**[0095]** This disclosure provides use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for use as a protein replacement therapy.

**[0096]** Provided herein is use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for use as a cell engineering therapy.

**[0097]** This disclosure provides use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for use as a chemotherapy.

**[0098]** This disclosure provides use of a polyribonucleotide disclosed herein or a composition comprising the same in the preparation of a medicament for stimulating an immune response.

**[0098a]** In another aspect of the present disclosure, there is provided a polyribonucleotide as disclosed herein, or a cell as disclosed herein, or a composition as disclosed herein, for use in therapy.

**[0099]** Provided herein is a composition comprising a polyribonucleotide disclosed herein for use as a vaccine.

**[0100]** This disclosure provides a composition comprising a polyribonucleotide disclosed herein for use as an immunotherapy.

**[0101]** Provided herein is a composition comprising a polyribonucleotide disclosed herein for use as an antibody therapy.

**[0102]** Provided herein is a composition comprising a polyribonucleotide disclosed herein for use as an immune-modulation therapy.

**[0103]** Provided herein is a composition comprising a polyribonucleotide disclosed herein for use as a gene therapy.

**[0104]** This disclosure a composition comprising a polyribonucleotide disclosed herein for use as a protein replacement therapy.

**[0105]** Provided herein is a composition comprising a polyribonucleotide disclosed herein for use as a cell engineering therapy.

**[0106]** This disclosure provides a composition comprising a polyribonucleotide disclosed herein for use as a chemotherapy.

**[0107]** In some examples of any of the uses or methods provided herein, a polyribonucleotide or a composition comprising the same is administered to a cell, tissue or subject.

**[0108]** In some examples of any of the uses or methods provided herein a cell is a mammalian cell, a tissue is a mammalian tissue, or a subject is a mammal. In some examples, a mammal is a human.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0109]** **FIGs. 1A-1D** provide structures of natural base 2-O-acetyl ribonucleotide analogs.

**[0110]** **FIG. 2** is a graph depicting luciferase gene expression normalized to cell viability by dose of RNA synthesized with indicated nucleotide composition.

**[0111]** **FIG. 3** is a graph depicting viability of A549 cells following transfection with respective doses of RNA synthesized with indicated nucleotide composition.

**[0112]** **FIG. 4** is a graph depicting IRF reporter activation by dose of RNA synthesized with indicated nucleotide composition.

**[0113]** **FIG. 5** is a graph depicting NF-KB reporter activation by dose of RNA synthesized with indicated nucleotide composition.

**[0114]** **FIG. 6** is a graph depicting 1H NMR (300 MHz; DMSO-d6) of Silyl-Protected cytidine 2.

**[0115]** **FIG. 7** is a mass spectrum (ESI+, 100% CH3OH, TOF ) of Silyl-Protected cytidine 2.

**[0116]** **FIG. 8** is a HPLC chromatogram (CH3OH) of Silyl-Protected cytidine 2.

**[0117]** **FIG. 9** is a graph depicting 1H NMR (300 MHz, DMSO-d6) of diacetylated cytidine 3.

**[0118]** **FIG. 10** is a mass spectrum ($ESI^+$, 100% $CH_3OH$, TOF ), retention time (4.534) of diacetylated cytidine 3.

## CLAIMS

*What is claimed is:*

1. A polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase,

wherein the nucleobase is an adenine, a guanine, a cytosine, or an uracil, and

wherein at least 80% of the ribose moieties of the polyribonucleotide are 2′-O-acetylated, and wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

2. The polyribonucleotide of claim 1, wherein the polyribonucleotide further comprises one or more ribonucleotides that do not comprise a 2′-O-acetylated ribose.

3. The polyribonucleotide of claim 1, wherein the polyribonucleotide comprises one or more further modifications wherein the one or more further modifications comprise a modified nucleobase, and wherein the modified nucleobase is N4-acetylcytosine, 5-hydroxymethyluracil, or N1-methylpseudouracil.

4. The polyribonucleotide of claim 1, characterized in that when assessed in a cell, tissue or an organism that has been administered the polyribonucleotide:

(i) reduced immunogenicity is observed relative to an appropriate reference comparator;

(ii) increased persistence of the polyribonucleotide is observed related to an appropriate reference comparator;

(iii) increased expression and/or activity of a gene product encoded by the polyribonucleotide is observed related to an appropriate reference comparator; or

(iv) any combination of (i)-(iii).

5. The polyribonucleotide of claim 4, wherein:

(i)reduced immunogenicity comprises reduced activation of an innate immune response induced toxicity;

(ii) persistence of the polyribonucleotide is increased by at least 15%;

(iii) expression and/or activity of a gene product is increased by at least 15; or

(iv) any combination of (i)-(iii).

6. The polyribonucleotide of claim 4, wherein the reference comparator comprises an otherwise similar cell, tissue or organism that has been administered a comparable polyribonucleotide that includes fewer ribose moieties comprising a 2′-O-acetylated ribose than the polyribonucleotide administered to the cell, tissue, or organism.

7. The polyribonucleotide of claim 4, wherein:

(i) the polyribonucleotide comprises a coding region;

(ii) the polyribonucleotide comprises an RNA oligo, a messenger RNA, a gRNA, an inhibitory RNA, an miRNA, an siRNA, an antisense oligonucleotide, a long-non-coding RNA, or a circular RNA; or

(iii) the gene product comprises (a) a polypeptide encoded by the polyribonucleotide comprising one or more modified ribonucleotides, or (b) a polyribonucleotide situated in the polyribonucleotide comprising one or more modified ribonucleotides.

8. The polyribonucleotide of claim 1, wherein the polyribonucleotide further comprises N4-acetyl-cytidine (ac4C), 5-hydroxymethyluridine, N1-methylpseudouridine, pyridin-4-one ribonucleoside, 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s2U), 5-methyl cytidine (m5C), 5-aza-cytidine, 6-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine (m3C), 5-formyl-cytidine (f5C), N4-methyl-cytidine (m4C), 2-amino-purine, 2, 6-diaminopurine, 2-amino-6-halo-purine, 6-halo-purine, inosine (I), 1-methyl-inosine (m1 I), wyosine (imG), methylwyosine (mimG), or any combination thereof.

9. A cell comprising a polyribonucleotide according to claim 1.

10. A composition comprising a polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase,
wherein the nucleobase is an adenine, a guanine, a cytosine, or an uracil, and wherein at least 80% of the ribose moieties of the polyribonucleotide are 2′-O-acetylated, and wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

11. The composition of claim 10, wherein the composition is a pharmaceutical composition.

12. The composition of claim 11, wherein the pharmaceutical composition comprises an immunogenic composition, a vaccine, a gene therapy, a chemotherapy, a protein replacement therapy, an immunotherapy, an antibody therapy, an immune-modulation therapy, a cell engineering therapy, or any combination thereof.

13. Use of a polyribonucleotide comprising one or more modified ribonucleotides in the manufacture of a medicament, wherein the one or more modified ribonucleotides comprise:
(i) a 5′-monophosphate, and
(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase,
wherein the nucleobase is an adenine, a guanine, a cytosine, or an uracil, and
wherein at least 80% of the ribose moieties of the polyribonucleotide are 2′-O-acetylated, and wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

14. The use of claim 13, wherein the medicament is to be administered to a cell, tissue, or subject, and wherein:
(i) cell viability of the cell, tissue or subject;
(ii) an immune system response of the cell, tissue or subject;
(iii) efficacy of the polyribonucleotide in the cell, tissue or subject;
(iv) persistence of the polyribonucleotide in the cell, tissue or subject;
(v) expression and/or activity of a gene product encoded by the polyribonucleotide in the cell, tissue or subject; or
(vi) any combination of (i)-(v),

is further determined.

15. The use of claim 13, wherein the cell is a mammalian cell, the tissue is a mammalian tissue, or the subject is a mammal.

16. The use of claim 13, wherein the medicament is:

(i) to stimulate an immune response;

(ii) an antibody therapy;

(iii) an immune-modulation;

(iv) a vaccination;

(v) a gene therapy;

(vi) a cell therapy engineering;

(vii) an immunotherapy;

(viii) a protein replacement therapy;

(ix) a chemotherapy; or

(x) any combination of (i)-(ix).

17. A polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is N4-acetylcytosine, and

wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

18. The polyribonucleotide of claim 17, wherein the polyribonucleotide comprises ribonucleotides comprising cytidine nucleosides, and wherein at least about 5% of the ribonucleotides comprising cytidine nucleosides comprise an N4-acetylcytosine nucleobase.

19. The polyribonucleotide of claim 17, wherein the polyribonucleotide further comprises one or more modified ribonucleotides comprising:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is 5-hydroxymethyluracil.

20. Use of a polyribonucleotide comprising one or more modified ribonucleotides in the manufacture of a medicament, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is N4-acetylcytosine, and

wherein the polyribonucleotide comprises a cap structure and the cap structure does not comprise a 2′-O-acetylated ribose.

21. A polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is 5-hydroxymethyluracil.

22. The polyribonucleotide of claim 21, wherein the polyribonucleotide comprises ribonucleotides comprising uridine nucleosides, and wherein at least about 5% of the ribonucleotides comprising uridine nucleosides comprise a 5-hydroxymethyluracil nucleobase.

23. Use of a polyribonucleotide comprising one or more modified ribonucleotides in the manufacture of a medicament, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is 5-hydroxymethyluracil.

24. A polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase, wherein the nucleobase is N1-methylpseudouracil.

25. The polyribonucleotide of claim 24, wherein the polyribonucleotide comprises ribonucleotides comprising uridine nucleosides, and wherein at least about 5% of the ribonucleotides comprising uridine nucleosides comprise a N1-methylpseudouracil nucleobase.

26. A method of manufacturing an RNA composition comprising introducing at least one modified ribonucleotide into a polyribonucleotide, wherein the modified ribonucleotide comprises:

(i) a 5′-monophosphate, and

(ii) a nucleoside comprising a 2′-O-acetylated ribose and a nucleobase,

wherein the nucleobase is an adenine, a guanine, a cytosine, or an uracil, and

wherein at least 80% of the ribose moieties of the polyribonucleotide are 2′-O-acetylated.

27. A polyribonucleotide of any one of claims 1-8, 17-19, 21-22 or 24-25, or a cell of claim 9, or a composition of any one of claims 10-12, for use in therapy.