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 5'-cap structure and the 5'-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

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.

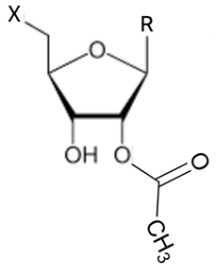
[0009a] In another aspect of the present disclosure, there is provided a composition comprising a polyribonucleotide comprising one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(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 5'-cap structure and the 5'-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.

[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.

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.

[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.

[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 for:

(i) stimulating an immune response;

(ii) use as an antibody therapy;

(iii) use as an immune modulation therapy;

(iv) use as a vaccine;

(v) use as a gene therapy;

(vi) use as a cell engineering therapy;

(vii) use as an immunotherapy;

(viii) use as a protein replacement therapy;

(ix) use as a chemotherapy; or

(x) any combination of (i) – (ix),

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 5’-cap structure and the 5’-cap structure does not comprise a 2′-O-acetylated ribose.

[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.

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 5'-cap structure and the 5'-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),

wherein the appropriate reference comparator comprises an otherwise similar cell, tissue or organism that has been administered a comparable polyribonucleotide that includes less than 80% ribose moieties comprising a 2′-O-acetylated ribose than the polyribonucleotide administered to the cell, tissue, or organism.

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:

(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.

7. 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.

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

9. 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 5’-cap structure and the 5’-cap structure does not comprise a 2′-O-acetylated ribose.

10. The composition of claim 9, wherein the composition is a pharmaceutical composition.

11. The composition of claim 10, 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.

12. Use of a polyribonucleotide comprising one or more modified ribonucleotides in the manufacture of a medicament for:
(i) stimulating an immune response;
(ii) use as an antibody therapy;
(iii) use as an immune modulation therapy;
(iv) use as a vaccine;
(v) use as a gene therapy;
(vi) use as a cell engineering therapy;
(vii) use as an immunotherapy;
(viii) use as a protein replacement therapy;
(ix) use as a chemotherapy; or
(x) any combination of (i) – (ix),
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 5’-cap structure and the 5’-cap structure does not comprise a 2′-O-acetylated ribose.

13. The use of claim 12, 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.

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

15. A polyribonucleotide of any one of claims 1-7, or a cell of claim 8, or a composition of any one of claims 9-11, for use in therapy.