MODIFIED RIBONUCLEIC ACIDS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application 63/185,925 filed on May 7, 2021, and U.S. Provisional Patent Application 63/273,031 filed on October 28, 2021, the entire contents of each of which is hereby incorporated by reference.

BACKGROUND

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

SUMMARY

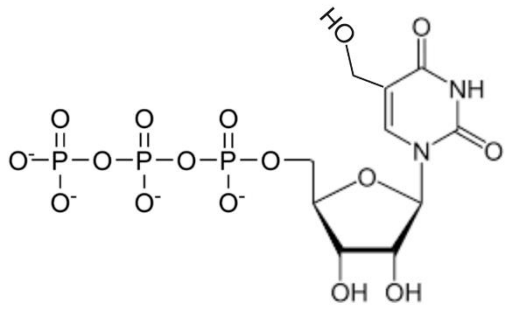
[0002a] In an aspect of the present disclosure, there is provided a polyribonucleotide comprising a nucleotide sequence encoding a payload, wherein the polyribonucleotide comprises a modified ribonucleotide comprising an N4-acetylcytidine nucleoside, and wherein the polyribonucleotide comprises cytidine nucleosides and at least 75% of the cytidine nucleosides comprise N4-acetylcytidine.

[0002b] In a further aspect of the present disclosure, there is provided a composition comprising the polyribonucleotide as disclosed herein.

[0002c] In another aspect of the present disclosure, there is provided a cell comprising a polyribonucleotide as disclosed herein.

[0002d] In a further aspect of the present disclosure, there is provided a polyribonucleotide comprising a nucleotide sequence encoding a payload, wherein the polyribonucleotide comprises one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(I) 5-hydroxymethyluridine nucleoside and: (a) a 5’ monophosphate, (b) a 5’ diphosphate, or (c) a 5’ triphosphate, and



(II) an N4-acetylcytidine nucleoside and: (a) a 5’ monophosphate, (b) a 5’ diphosphate, or (c) a 5’ triphosphate, and

wherein the polyribonucleotide comprises:

(i) cytidine nucleosides and at least 25% of the cytidine nucleosides in the polyribonucleotide comprise N4-acetylcytidine; and

(ii) uridine nucleosides and at least 25% of the uridine nucleosides in the polyribonucleotide comprise 5-hydroxymethyluridine.

CLAIMS

What is claimed is:

1. A polyribonucleotide comprising a nucleotide sequence encoding a payload, wherein the polyribonucleotide comprises a modified ribonucleotide comprising an N4-acetylcytidine nucleoside, and wherein the polyribonucleotide comprises cytidine nucleosides and at least 75% of the cytidine nucleosides comprise N4-acetylcytidine.

2. The polyribonucleotide of claim 1, wherein at least 90% of the cytidine nucleosides in the polyribonucleotide comprise N4-acetylcytidine.

3. The polyribonucleotide of claim 1 or 2, wherein less than 100% of cytidine nucleosides in the polyribonucleotide comprise N4-acetylcytidine.

4. The polyribonucleotide of claim 1 or 2, wherein 100% of the cytidine nucleosides in the polyribonucleotide comprise N4-acetylcytidine.

5. The polyribonucleotide of any one of claims 1-4, wherein the polyribonucleotide further comprises one or more modified ribonucleotides other than N4-acetylcytidine.

6. The polyribonucleotide of claim 5, wherein the nucleoside of the one or more modified ribonucleotides is 5-hydroxymethyluridine.

7. The polyribonucleotide of claim 6, wherein the polyribonucleotide comprises uridine nucleosides and at least 25% of the uridine nucleosides comprise 5-hydroxymethyluridine.

8. The polyribonucleotide of claim 7, wherein at least 50% of the uridine nucleosides in the polyribonucleotide comprise 5-hydroxymethyluridine.

9. The polyribonucleotide of claim 7 or 8, wherein at least 75% of the uridine nucleosides in the polyribonucleotide comprise 5-hydroxymethyluridine.

10. The polyribonucleotide of any one of claims 7-9, wherein less than 100% of uridine nucleosides in the polyribonucleotide comprise 5-hydroxymethyluridine.

11. The polyribonucleotide of any one of claims 7-9, wherein 100% of uridine nucleosides in the polyribonucleotide comprise 5-hydroxymethyluridine.

12. The polyribonucleotide of any one of claims 1-11, characterized in that when assessed in a cell, tissue, or organism that has been administered the polyribonucleotide:
(a) reduced immunogenicity is observed relative to an appropriate reference comparator,
(b) increased cell viability is observed relative to an appropriate reference comparator,
(c) increased expression of the payload is observed relative to an appropriate reference comparator, or
(d) any combination of (a)-(c).

13. The polyribonucleotide of claim 12, wherein reduced immunogenicity comprises:
(i) reduced activation of NF-kb or an NF-kb pathway, IRF or an IRF pathway, and/or cytokines compared to an appropriate reference comparator, and/or
(ii) reduced detection of uncapped RNA by a molecular sensor, optionally wherein the molecular sensor is or comprises RIG-I.

14. The polyribonucleotide of claim 12 or 13, wherein reduced immunogenicity allows for:
(i) repeated dosing of the polyribonucleotide, and/or
(ii) administration of a higher dose of the polyribonucleotide as compared to an appropriate reference comparator.

15. The polyribonucleotide of any one of claims 12-14, wherein the reference comparator comprises an otherwise similar cell, tissue or organism that has been administered a comparable polyribonucleotide that includes:
(i) fewer N4-acetylcytidine nucleosides,
(ii) fewer 5-hydroxymethyluridine nucleosides, or
(iii) both (i) and (ii),
compared to the polyribonucleotide.

16. The polyribonucleotide of any one of claims 12-15, wherein the increase in expression of the payload is 1.2-fold, 1.5-fold, 2-fold, 4-fold, 5-fold, 10-fold or 20-fold compared to the reference comparator.

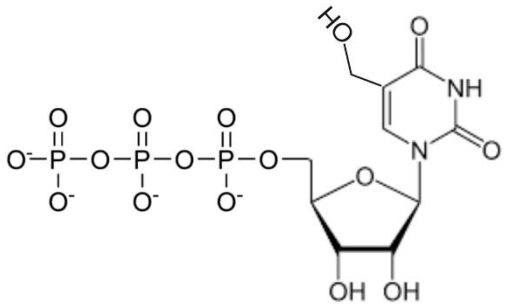
17. A composition comprising the polyribonucleotide of any one of claims 1-16.

18. The composition of claim 17, wherein the composition is a pharmaceutical composition comprising an immunogenic composition, a vaccine, a gene therapy, a chemotherapy, a protein replacement therapy, an immunotherapy, a cell engineering therapy, or a combination thereof.

19. A cell comprising a polyribonucleotide according to any one of claims 1-16.

20. A polyribonucleotide comprising a nucleotide sequence encoding a payload, wherein the polyribonucleotide comprises one or more modified ribonucleotides, wherein the one or more modified ribonucleotides comprise:

(I) a 5-hydroxymethyluridine nucleoside and: (a) a 5' monophosphate, (b) a 5' diphosphate, or (c) a 5' triphosphate, and



(II) an N4-acetylcytidine nucleoside and: (a) a 5' monophosphate, (b) a 5' diphosphate, or (c) a 5' triphosphate, and

wherein the polyribonucleotide comprises:

(i) cytidine nucleosides and at least 25% of the cytidine nucleosides in the polyribonucleotide comprise N4-acetylcytidine, and

(ii) uridine nucleosides and at least 25% of the uridine nucleosides in the polyribonucleotide comprise 5-hydroxymethyluridine.

21. The polyribonucleotide of claim 20, wherein:

(a) the polyribonucleotide comprises cytidine nucleosides and at least 50% of cytidine nucleosides in the polyribonucleotide comprise N4-acetylcytidine, and

(b) the polyribonucleotide comprises uridine nucleosides and at least 50% of uridine nucleosides in the polyribonucleotide comprise 5-hydroxymethyluridine.

22. The polyribonucleotide of claim 20 or 21, characterized in that when assessed in a cell, tissue, or organism that has been administered the polyribonucleotide:

(a) reduced immunogenicity is observed relative to an appropriate reference comparator,

(b) increased cell viability is observed relative to an appropriate reference comparator,

(c) increased expression of the payload is observed relative to an appropriate reference comparator, or

(d) any combination of (a)-(c);

optionally wherein the reference comparator comprises an otherwise similar cell, tissue or organism that has been administered a comparable polyribonucleotide that includes fewer N4-acetylcytidine nucleosides and/or fewer 5-hydroxymethyluridine nucleosides.

23. The polyribonucleotide of claim 22, wherein the increase in expression of the payload is 1.2-fold, 1.5-fold, 2-fold, 4-fold, 5-fold, 10-fold or 20-fold compared to the reference comparator.

24. The polyribonucleotide of any one of claims 20-23, wherein the polyribonucleotide comprises:

(a) cytidine nucleosides and at least 90% of cytidine nucleosides in the polyribonucleotide comprise N4-acetylcytidine,

(b) uridine nucleosides and at least 90% of uridine nucleosides in the polyribonucleotide comprise 5-hydroxymethyluridine, or

(c) both (a) and (b).

25. The polyribonucleotide of any one of claims 20-24, wherein 100% of the cytidine nucleosides in the polyribonucleotide comprise N4-acetylcytidine and 100% of the uridine nucleosides in the polyribonucleotide comprise 5-hydroxymethyluridine.

26. A composition comprising the polyribonucleotide of any one of claims 20-25.

27. The composition of claim 26, wherein the composition is a pharmaceutical composition comprising an immunogenic composition, a vaccine, a gene therapy, a chemotherapy, a protein replacement therapy, an immunotherapy, a cell engineering therapy, or a combination thereof.

28. The polyribonucleotide of any one of claims 1-16 or 20-25, wherein the payload is or comprises:
(i) a polypeptide encoded by the polyribonucleotide, or
(ii) a polyribonucleotide situated in the polyribonucleotide comprising one or more modified ribonucleotides.

29. The polyribonucleotide of claim 28, wherein the polyribonucleotide is or comprises an RNA oligo, a messenger RNA, a gRNA, an inhibitory RNA, an miRNA, an siRNA, an antisense oligonucleotide, or any combination thereof.