RNA CONSTRUCTS AND USES THEREOF

PRIORITY CLAIM

This application claims priority under 35 U.S.C. § 119 to each of the following applications, the disclosure of each of which is hereby incorporated by reference in its entirety: International Application No. PCT/EP20/61239, filed April 22, 2020; and International Application No. PCT/EP20/66968, filed June 18, 2020.

BACKGROUND

Use of RNA polynucleotides as therapeutics is a new and emerging field.

SUMMARY

The present disclosure identifies certain challenges that can be associated with RNA therapeutics.

For example, in some examples, the present disclosure identifies the source of certain problems that can be encountered with expression of polypeptides encoded by RNA therapeutics. Among other things, the present disclosure provides technologies for improving translation efficiency of an RNA encoding a payload, and/or expression of a polypeptide payload encoded by an RNA. In some examples, translation efficiency and/or expression of an RNA-encoded payload can be improved with an RNA polynucleotide comprising: a Cap1 structure (e.g., a $m_2^{7,3'\text{-}O}Gppp(m_1^{2'\text{-}O})ApG$ cap); a 5' UTR comprising a cap proximal sequence disclosed herein, and a sequence encoding a payload. Without wishing to be bound by theory, the present disclosure proposes that improved translation efficiency and/or polypeptide payload expression can be achieved through preferential binding of eukaryotic translation initiation factor 4E (eIF4E), rather than IFN-induced protein with tetratricopeptide repeats-1 (IFIT1) to an RNA comprising a Cap1 structure, e.g., a $m_2^{7,3'\text{-}O}Gppp(m_1^{2'\text{-}O})ApG$ cap, and/or a 5' UTR comprising a cap proximal sequence disclosed herein. For example, in some examples, it is proposed that eIF4E may compete with IFIT1 for binding to an RNA polynucleotide based on a 5' cap structure. Among other things, the present disclosure provides certain technologies that may favor eIF4E binding, at least relative to IFIT1 binding, and/or may otherwise enhance translation.

In some examples, the present disclosure teaches that identity of particular sequence(s) proximal to a 5' cap (e.g., a 5' Cap1 structure) can influence translation efficiency of an associated payload. Without wishing to be bound by any particular theory, the present disclosure proposes that eIF4E competes with IFIT1 for binding to an RNA polynucleotide based on the identity of one or more nucleotides downstream of a 5' cap, e.g., a cap proximal sequence as disclosed herein. In some examples, the present disclosure demonstrates that an AGAAU or an AGCAC sequence downstream of a 5' cap (e.g., a 5' Cap 1 structure) can improve translation. The present disclosure proposes that presence of such sequence (e.g., AGAAU or an AGCAC can increase eIF4E binding, at least relative to IFIT1.

Alternatively or additionally, the present disclosure documents certain advantages of avoiding (e.g., ensuring that absence of) a self-hybridizing sequence (which may, in some instances, be referred to as a self-complementary sequence) in an RNA polynucleotide encoding a payload, For example, the present disclosure demonstrates that such absence can improve, and/or be required for, translation (e.g., translation efficiency) of an associated (e.g., RNA-encoded) payload, and/or otherwise for expression of a polypeptide encoded thereby. Without wishing to be bound by theory, it is believed that a self-hybridizing sequence (and in particular a sequence that hybridizes with sequences within or comprising one or more of a Kozak sequence, a 5' UTR element, and/or a 3' UTR element) interfere with one or more examples of translation. For example, in some examples, it is proposed that such self-hybridization may inhibit binding of transcription and/or translation factors to an RNA polynucleotide by self-hybridizing to a complementary sequence in said RNA polynucleotide.

Still further alternatively or additionally, in some examples, the present disclosure defines particular lipid components, and/or ratios thereof, that may be especially useful or effective in delivering nucleic acids, and in particular RNAs (e.g., therapeutic RNAs or other RNAs encoding a polypeptide) upon administration (e.g., by injection, such as by intramuscular injection or by intravenous injection) to a subject. For example, in some examples, the present disclosure demonstrates that lipid ALC-0315 is unusually and particularly useful for delivery as described herein.

Disclosed herein, *inter alia*, is a composition or medical preparation comprising an RNA polynucleotide, comprising: (i) a 5' cap that is or comprises a cap1 structure, e.g., as disclosed herein; (ii) a 5' UTR sequence comprising a cap proximal sequence, e.g., as disclosed herein;

and (iii) a sequence encoding a payload. Also disclosed herein are methods of making and using the same to, e.g., induce an immune response in a subject.

In one aspect, provided herein is a composition or medical preparation comprising an RNA polynucleotide comprising:

a 5' cap comprising a Cap1 structure; a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of the RNA polynucleotide; and a sequence encoding a payload, wherein:

(i) the Cap1 structure comprises m7G(5')ppp(5')(2'OMeN_1)pN_2, wherein N_1 is position +1 of the RNA polynucleotide, and N_2 is position +2 of the RNA polynucleotide, and wherein N_1 and N_2 are each independently chosen from: A, C, G, or U; and

(ii) the cap proximal sequence comprises N_1 and N_2 of the Cap1 structure, and:

(a) a sequence selected from the group consisting of: $A_3A_4X_5$ (SEQ ID NO: 1); $C_3A_4X_5$ (SEQ ID NO: 2); $A_3C_4A_5$ (SEQ ID NO: 3) and $A_3U_4G_5$ (SEQ ID NO: 4); or

(b) a sequence comprising: $X_3Y_4X_5$ (SEQ ID NO: 7);

wherein X_3 (nucleotide X at position +3 in SEQ ID NO: 7) or X_5 (nucleotide X at position +5 in SEQ ID NO: 1 or SEQ ID NO: 2) is each independently chosen from A, G, C, or U; and

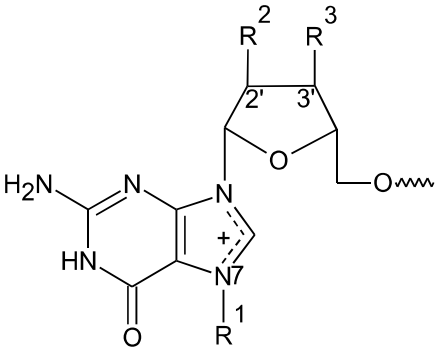
wherein Y_4 (nucleotide Y at position +4 in SEQ ID NO: 7) is not C.

In another aspect, this disclosure also provides a composition or medical preparation comprising an RNA polynucleotide comprising: a 5' cap; a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of an RNA polynucleotide; and a sequence encoding a payload, wherein:

(i) the 5' cap comprises a Cap1 structure comprising G*ppp($m_1^{2'\text{-}O}$)N_1pN_2, wherein:

N1 is position +1 of the RNA polynucleotide, and N_2 is position +2 of the RNA polynucleotide, and wherein N_1 and N_2 are each independently chosen from: A, C, G, or U; and

G* comprises the following structure:



wherein ~~~ represents the bond by which G* is bound to the first phosphor atom of the ppp group, R^1 is CH_3, R^2 is OH or O-CH_3, and R^3 is O-CH_3; and

(ii) the cap proximal sequence comprises N_1 and N_2 of the Cap1 structure, and:

(a) a sequence selected from the group consisting of: $A_3A_4X_5$ (SEQ ID NO: 1); $C_3A_4X_5$ (SEQ ID NO: 2); $A_3C_4A_5$ (SEQ ID NO: 3) and $A_3U_4G_5$ (SEQ ID NO: 4); or

(b) a sequence comprising: $X_3Y_4X_5$ (SEQ ID NO: 7);

wherein X_3 (nucleotide X at position +3 in SEQ ID NO: 7) or X_5 (nucleotide X at position +5 in SEQ ID NO: 1 or SEQ ID NO: 2) is each independently chosen from A, G, C, or U; and

wherein Y_4 (nucleotide Y at position +4 in SEQ ID NO: 7) is not C.

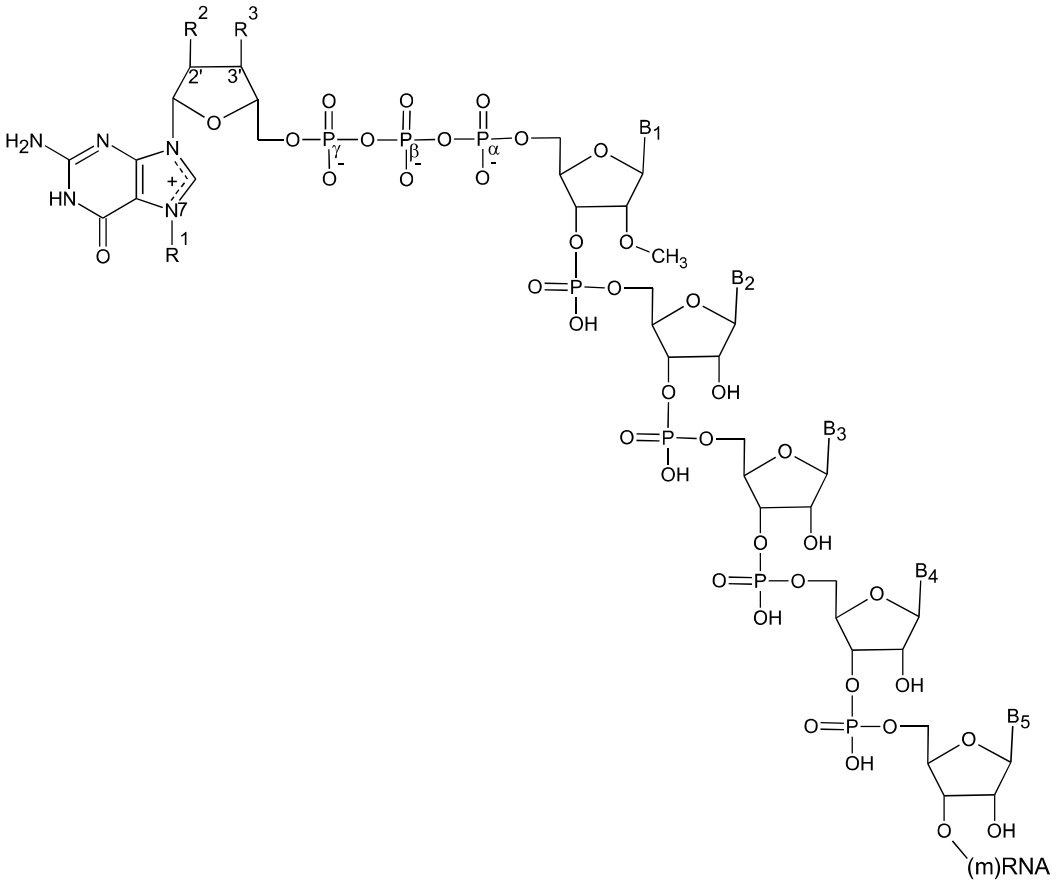
In another aspect, also provided herein is a composition or medical preparation comprising an RNA polynucleotide comprising:

a 5' cap comprising a Cap1 structure; a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of the RNA polynucleotide; and a sequence encoding a payload, wherein:

(i) the Cap1 structure comprises m7(3'OMeG)(5')ppp(5')(2'OMeA_1)pG_2, wherein A_1 is position +1 of the RNA polynucleotide, and G_2 is position +2 of the RNA polynucleotide; and

(ii) the cap proximal sequence comprises A_1 and G_2 of the Cap1 structure, and a sequence comprising: $A_3A_4U_5$ at positions +3, +4 and +5 respectively of the RNA polynucleotide.

In another aspect, this disclosure provides a composition or medical preparation comprising a capped RNA polynucleotide encoding a gene product, which RNA polynucleotide comprises the formula:



wherein R^1 is CH_3, R^2 is OH or O-CH_3, and R^3 is O-CH_3 ,

wherein B_1 is any nucleobase, preferably A; B_2 is any nucleobase, preferably G; B_3 is any nucleobase, preferably A or C; B_4 is any nucleobase; and B_5 is any nucleobase, and

wherein, when the RNA polynucleotide is administered to a subject, the levels of expression of the encoded gene product at about 6 hours after administration and at about 48 hours after administration do not differ by more than 5-fold.

In yet another aspect, the present disclosure provides a pharmaceutical composition comprising an RNA polynucleotide as described herein, formulated as a particle. Provided herein is a pharmaceutical composition comprising an RNA polynucleotide disclosed herein. In some examples, a pharmaceutical composition comprises a composition or a medical preparation disclosed herein.

In yet another aspect, also provided herein is a method of manufacturing a pharmaceutical composition, e.g., comprising an RNA polynucleotide disclosed herein, by

combining an RNA polynucleotide with lipids to form lipid nanoparticles that encapsulate said RNA.

In a further aspect, this disclosure provides a nucleic acid template suitable to produce a cap1-capped RNA, in which the first five nucleotides transcribed from the template strand of the nucleic acid template comprise the sequence $N_1pN_2pN_3pN_4pN_5$, wherein N_1 is any nucleotide, preferably T; N_2 is any nucleotide, preferably C; N_3 is any nucleotide, preferably T or G; N_4 is any nucleotide; and N_5 is any nucleotide. In some examples, a DNA template comprises: a 5' UTR, a sequence encoding a payload, a 3' UTR and a polyA sequence.

In a further aspect, provided herein is an vitro transcription reaction comprising:

(i) a template DNA comprising a polynucleotide sequence complementary to an RNA polynucleotide sequence disclosed herein;

(ii) a polymerase; and

(iii) an RNA polynucleotide.

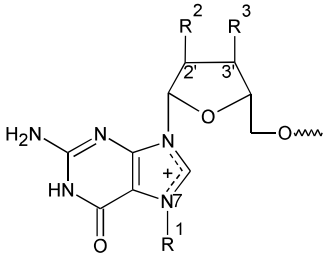
In a further aspect, also provided herein is an RNA polynucleotide isolated from an in vitro transcription reaction provided.

In a further aspect, this disclosure provides, a method for producing a capped RNA comprising, transcribing a nucleic acid template in the presence of a cap structure, wherein the cap structure comprises $G^*ppp(m_1^{2'\text{-}O})N_1pN_2$,

wherein N_1 is complementary to position +1 of the nucleic acid template and N_2 is complementary to position +2 of the nucleic acid template, and N_1 and N_2 are independently chosen from A, C, G or U,

wherein position +3 of the nucleic acid template is any nucleotide, preferably T or G; position +4 of the nucleic acid template is any nucleotide; and position +5 of the nucleic acid template is any nucleotide,

wherein G* comprises the following structure:



wherein ~~~ represents the bond by which G* is bound to the first phosphor atom of the ppp group, R^1 is CH_3, R^2 is OH or O-CH_3, and R^3 is O-CH_3.

In a further aspect, also provided herein is a composition comprising a DNA polynucleotide comprising a sequence complementary to an RNA polynucleotide sequence provided. In some examples, a DNA polynucleotide disclosed herein can be used to transcribe an RNA polynucleotide disclosed herein.

In yet another aspect, the present disclosure provides a pharmaceutical composition comprising an RNA polynucleotide formulated in a lipid nanoparticle (LNP) or a lipoplex (LPX) particle as described herein for use in therapy. In yet another aspect, the present disclosure provides a use of a pharmaceutical composition comprising an RNA polynucleotide formulated in a lipid nanoparticle (LNP) or a lipoplex (LPX) particle as described herein in the manufacture of a medicament for inducing an immune response in a subject. In yet another aspect, the present disclosure provides a use of a pharmaceutical composition comprising an RNA polynucleotide formulated in a lipid nanoparticle (LNP) or a lipoplex (LPX) particle as described herein in the manufacture of a medicament for vaccination of a subject. This disclosure provides, a method comprising: administering to a subject, a pharmaceutical composition comprising an RNA polynucleotide disclosed herein formulated in a lipid nanoparticle (LNP) or a lipoplex (LPX) particle, e.g., as disclosed herein.

Also provided herein is a method of inducing an immune response in a subject, comprising administering to a subject, a pharmaceutical composition comprising an RNA polynucleotide disclosed herein formulated in a lipid nanoparticle (LNP) or a lipoplex (LPX) particle, e.g., as disclosed herein

Provided herein is a method of vaccination of a subject by administering a pharmaceutical composition comprising an RNA polynucleotide disclosed herein formulated in a lipid nanoparticle (LNP) or a lipoplex (LPX) particle, e.g., as disclosed herein.

In another aspect, this disclosure provides, a method of decreasing interaction with IFIT1 of an RNA polynucleotide that comprises a 5' cap and a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of the RNA polynucleotide, the method comprising a step of:

providing a variant of an RNA polynucleotide that differs from a parental RNA polynucleotide by substitution of one or more residues within a cap proximal sequence, and

determining that interaction of a variant with IFIT1 is decreased relative to that of a parental RNA polynucleotide.

In another aspect, the present disclosure provides a method of producing a polypeptide comprising a step of: providing an RNA polynucleotide that comprises a 5' cap, a cap proximal sequence that comprises positions +1, +2, +3, +4, and +5 of the RNA polynucleotide, and a sequence encoding a payload; wherein the RNA polynucleotide is characterized in that when assessed in an organism to be administered the RNA polynucleotide or a composition comprising the same, elevated expression and/or increased duration of expression of the payload is observed relative to an appropriate reference comparator. Also provided herein is a method of producing a polypeptide comprising a step of:

providing an RNA polynucleotide that comprises a 5' cap, a cap proximal sequence that comprises positions +1, +2, +3, +4, and +5 of the RNA polynucleotide, and a sequence encoding a payload;

wherein an RNA polynucleotide is characterized in that when assessed in an organism administered an RNA polynucleotide or a composition comprising the same, elevated expression and/or increased duration of expression of a payload is observed relative to an appropriate reference comparator.

In another aspect, provided herein is a method of increasing translatability of an RNA polynucleotide that comprises a 5' cap, a cap proximal sequence that comprises positions +1, +2, +3, +4, and +5 of the RNA polynucleotide and a sequence encoding a payload, the method comprising a step of:

providing a variant of an RNA polynucleotide that differs from a parental RNA polynucleotide by substitution of one or more residues within a cap proximal sequence; and

determining that expression of a variant is increased relative to that of a parental RNA polynucleotide.

In another aspect, the present disclosre provides in a therapeutic RNA comprising a 5' cap that includes a Cap1 structure, a cap proximal sequence and a sequence encoding a payload, the improvement that comprises: including one or more of the following residues in a cap proximal sequence: A at position +1 of the RNA polynucleotide, G at position +2 of the RNA polynucleotide, A at position +3 of the RNA polynucleotide, A at position +4 of the RNA polynucleotide, and U at position +5 of the RNA polynucleotide, demonstrated to increase expression of the RNA when to be administered to a subject in an LNP formulation.

Provided herein is a therapeutic RNA comprising a 5' cap that includes a Cap1 structure, a cap proximal sequence and a sequence encoding a payload, the improvement that comprises: including one or more of the following residues in a cap proximal sequence: X at position +1 of an RNA polynucleotide, X at position +2 of an RNA polynucleotide, A at position +3 of an RNA polynucleotide, A at position +4 of an RNA polynucleotide, and X at position +5 of an RNA polynucleotide, demonstrated to increase expression of an RNA when administered to a subject in an LNP formulation. In some examples, X is chosen from A, C, G or U.

This disclosed provides, a therapeutic RNA comprising a 5' cap that includes a Cap1 structure, a cap proximal sequence and a sequence encoding a payload, the improvement that comprises: including one or more of the following residues in a cap proximal sequence: X at position +1 of an RNA polynucleotide, X at position +2 of an RNA polynucleotide, C at

position +3 of an RNA polynucleotide, A at position +4 of an RNA polynucleotide, and X at position +5 of an RNA polynucleotide, demonstrated to increase expression of an RNA when administered to a subject in an LNP formulation. In some examples, X is chosen from A, C, G or U.

Also provided herein is therapeutic RNA comprising a 5' cap that includes a Cap1 structure, a cap proximal sequence and a sequence encoding a payload, the improvement that comprises:including one or more of the following residues in a cap proximal sequence: A at position +1 of an RNA polynucleotide, G at position +2 of an RNA polynucleotide, A at position

+3 of an RNA polynucleotide, A at position +4 of an RNA polynucleotide, and U at position +5 of an RNA polynucleotide, demonstrated to increase expression of an RNA when administered to a subject in an LNP formulation.

This discosure provides, a method of increasing translation of an RNA polynucleotide comprising a 5' cap that includes a Cap1 structure, a cap proximal sequence and a sequence encoding a payload, the improvement that comprises: including one or more of the following residues in a cap proximal sequence: X at position +1 of an RNA polynucleotide, X at position +2 of an RNA polynucleotide, A at position +3 of an RNA polynucleotide, A at position +4 of an RNA polynucleotide, and X at position +5 of an RNA polynucleotide. In some examples, X is chosen from A, C, G or U.

Provided herein is a method of increasing translation of an RNA polynucleotide comprising a 5' cap that includes a Cap1 structure, a cap proximal sequence and a sequence encoding a payload, the improvement that comprises: including one or more of the following residues in a cap proximal sequence: X at position +1 of an RNA polynucleotide, X at position +2 of an RNA polynucleotide, C at position +3 of an RNA polynucleotide, A at position +4 of an RNA polynucleotide, and X at position +5 of an RNA polynucleotide. In some examples, X is chosen from A, C, G or U.

In another aspect, also provided herein is a method of increasing translation of an RNA polynucleotide comprising a 5' cap that includes a Cap1 structure, a cap proximal sequence and a sequence encoding a payload, the improvement that comprises: including one or more of the following residues in a cap proximal sequence: A at position +1 of an RNA polynucleotide, G at position +2 of an RNA polynucleotide, A at position +3 of an RNA polynucleotide, A at position +4 of an RNA polynucleotide, and U at position +5 of an RNA polynucleotide.

In another aspect, also provided herein is a method of providing a framework for an RNA polynucleotide that comprises a 5' cap, a cap proximal sequence, and a payload sequence, the method comprising a step of:

assessing at least two variants of an RNA polynucleotide, wherein:

each variant includes a same 5' cap and payload sequence; and

the variants differ from one another at one or more specific residues of a cap proximal sequence;

wherein the assessing comprises determining expression levels and/or duration of expression of a payload sequence; and

selecting at least one combination of 5' cap and a cap proximal sequence that displays elevated expression relative to at least one other combination.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 demonstrates plasma levels of EPO at 6, 24, 48 and 72 hours after intravenous administration of mice with murine EPO (mEPO) mRNA constructs with or without a Lig3 presence in the 3'UTR sequence. Blood was collected 6, 24, 48 and 72 hours after administration and samples were analyzed for mEPO levels via ELISA.

Figures 2A-2C are schematics of a Lig3 sequence self-hybridizing to a 5' UTR (FIG. 2A-2B) or a 3' UTR (FIG. 2C)/

Figures 3A-3I are structures of 5' caps that can be incorporated into mRNAs.

Figure 4 demonstrates plasma levels of mEPO at 6, 24, 48 and 72 hours after intravenous administration of mice with mEPO mRNA constructs having different nucleotides at the +3, +4 or +5 positions. Blood was collected 6, 24, 48 and 72 hours after administration and samples were analyzed for mEPO levels via ELISA.

Figure 5 demonstrates an anti-S protein IgG response 7, 14, 21 and 28 d after immunization with BNT162a1. BALB/c mice were immunized IM once with 1, 5 or 10 µg of LNP-formulated RBL063.3. On day 7, 14, 21 and 28 after immunization, animals were bled and the serum samples were analyzed for total amount of anti-S1 (left) and anti-RBD (right) antigen-specific immunoglobulin G (IgG) measured via ELISA. For day 7, day 14, day 21 and day 28, values for a serum dilution of 1:100 were included in the graph. One point in the graph stands for one mouse, every mouse sample was measured in duplicates (group size n=8; mean + SEM is included for the groups).

Figure 6 demonstrates Anti-S protein IgG response 7, 14, 21 and 28 d after immunization with BNT162b1. BALB/c mice were immunized IM once with 0.2, 1 or 5µg of LNP-formulated RBP020.3. On day 7, 14, 21 and 28 after immunization, animals were bled and the serum samples were analyzed for total amount of anti-S1 (left) and anti-RBD (right) antigen-specific

CLAIMS

What is claimed is:

1. A composition or medical preparation comprising an RNA polynucleotide comprising:

a 5’ cap comprising a Cap1 structure; a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of the RNA polynucleotide; and a sequence encoding a payload, wherein:

(i) the Cap1 structure comprises m7G(5')ppp(5')(2'OMeN_1)pN_2, wherein N_1 is position +1 of the RNA polynucleotide, and N_2 is position +2 of the RNA polynucleotide, and wherein N_1 and N_2 are each independently chosen from: A, C, G, or U; and

(ii) the cap proximal sequence comprises N_1 and N_2 of the Cap1 structure, and:

(a) a sequence selected from the group consisting of: $A_3A_4X_5$ (SEQ ID NO: 1); $C_3A_4X_5$ (SEQ ID NO: 2); $A_3C_4A_5$ (SEQ ID NO: 3) and $A_3U_4G_5$ (SEQ ID NO: 4); or

(b) a sequence comprising: $X_3Y_4X_5$ (SEQ ID NO: 7);

wherein X_3 (nucleotide X at position +3 in SEQ ID NO: 7) or X_5 (nucleotide X at position +5 in SEQ ID NO: 1 or SEQ ID NO: 2) is each independently chosen from A, G, C, or U; and

wherein Y_4 (nucleotide Y at position +4 in SEQ ID NO: 7) is not C.

2. The composition or medical preparation of claim 1, wherein:

(i) the N_1 position is A and the N_2 position is G, and the Cap1 structure comprises m7G(5')ppp(5')(2'OMeA_1)pG_2;

(ii) the N_1 position is A and the N_2 position is U, and the Cap1 structure comprises m7G(5')ppp(5')(2'OMeA_1)pU_2; or

(iii) the N_1 position is G and the N_2 position is G, and the Cap1 structure comprises m7G(5')ppp(5')(2'OMeG_1)pG_2.

3. The composition or medical preparation of claim 1 or 2, wherein a methylated Guanosine (m7G) in the Cap1 structure further comprises one or more modifications.

4. The composition or medical preparation of claim 3, wherein the m7G in the Cap1 structure comprises a methylated ribose.

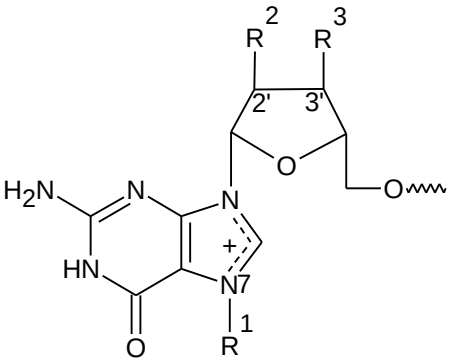
5. The composition or medical preparation of claim 3 or 4, wherein the m7G in the Cap1 comprises a 3'O methylation (m7(3'OMeG)).

6. A composition or medical preparation comprising an RNA polynucleotide comprising: a 5' cap; a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of an RNA polynucleotide; and a sequence encoding a payload, wherein:

(i) the 5' cap comprises a Cap1 structure comprising G*ppp($m_1^{2'-O}$)N_1pN_2, wherein:

N1 is position +1 of the RNA polynucleotide, and N_2 is position +2 of the RNA polynucleotide, and wherein N_1 and N_2 are each independently chosen from: A, C, G, or U; and

G* comprises the following structure:



wherein ~~~ represents the bond by which G* is bound to the first phosphor atom of the ppp group, R^1 is CH_3, R^2 is OH or O-CH_3, and R^3 is O-CH_3; and

(ii) a cap proximal sequence comprises N_1 and N_2 of the Cap1 structure, and:

(a) a sequence chosen from the group consisting of: $A_3A_4X_5$ (SEQ ID NO: 1); $C_3A_4X_5$ (SEQ ID NO: 2); $A_3C_4A_5$ (SEQ ID NO: 3) or $A_3U_4G_5$ (SEQ ID NO: 4); or

(b) a sequence comprising $X_3Y_4X_5$ (SEQ ID NO: 7);

wherein X_3 (nucleotide X at position +3 in SEQ ID NO: 7) or X_5 (nucleotide X at position +5 in SEQ ID NO: 1 or SEQ ID NO: 2) is each independently chosen from A, G, C, or U; and

wherein Y_4 (nucleotide Y at position +4 in SEQ ID NO: 7) is not C.

7. The composition or medical preparation of claim 6, wherein:

(i) the N_1 position is A and the N_2 position is G, and the Cap1 structure comprises m7G(5')ppp(5')(2'OMeA_1)pG_2;

(ii) the N_1 position is A and the N_2 position is U, and the Cap1 structure comprises m7G(5')ppp(5')(2'OMeA_1)pU_2; or

(iii) the N_1 position is G and the N_2 position is G, and the Cap1 structure comprises m7G(5')ppp(5')(2'OMeG_1)pG_2.

8. The composition or medical preparation of any one of the preceding claims, wherein the cap proximal sequence comprises N_1 and N_2 of the Cap1 structure, and a sequence comprising: $A_3A_4X_5$ (SEQ ID NO: 1).

9. The composition or medical preparation of claim 8, wherein:

(i) X_5 is A, C, G, or U; or

(ii) X_5 is U.

10. The composition or medical preparation of any one of claims 1-8, wherein the cap proximal sequence comprises N_1 and N_2 of the Cap1 structure, and a sequence comprising $C_3A_4X_5$ (SEQ ID NO: 2)

11. The composition or medical preparation of claim 10, wherein X_5 is A, C, G, or U.

12. The composition or medical preparation of any one of claims 1-8, wherein the cap proximal sequence comprises N_1 and N_2 of the Cap1 structure, and a sequence comprising $X_3Y_4X_5$ (SEQ ID NO: 7), wherein X_3 or X_5 are each independently chosen from A, G, C, or U, and Y_4 is not C.

13. The composition or medical preparation of any one of claims 1-8, wherein the cap proximal sequence comprises N_1 and N_2 of the Cap1 structure, and a sequence comprising $A_3C_4A_5$ (SEQ ID NO: 3).

14. The composition or medical preparation of any one of claims 1-8, wherein the cap proximal sequence comprises N_1 and N_2 of the Cap1 structure, and a sequence comprising $A_3U_4G_5$ (SEQ ID NO: 4).

15. A composition or medical preparation comprising an RNA polynucleotide comprising:

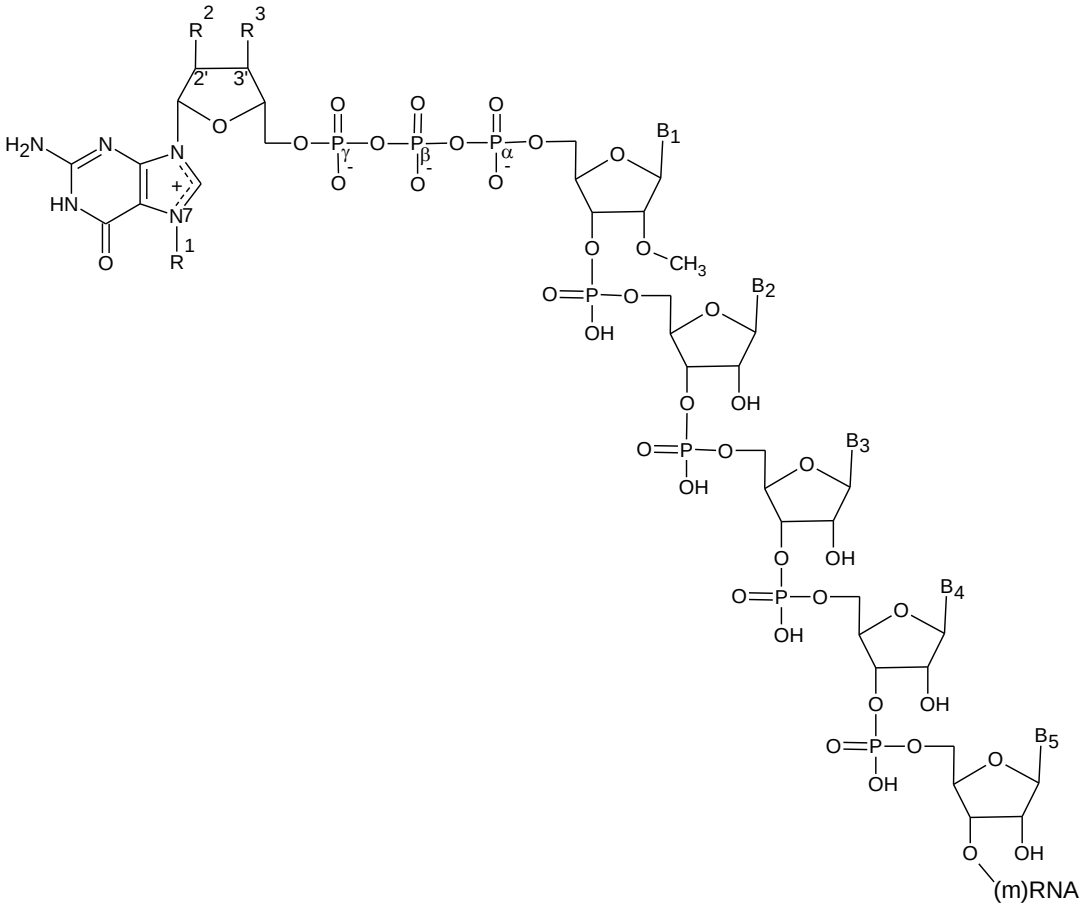
a 5’ cap comprising a Cap1 structure; a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of the RNA polynucleotide; and a sequence encoding a payload, wherein:

(i) the Cap1 structure comprises m7(3’OMeG)(5')ppp(5')(2'OMeA_1)pG_2, wherein A_1 is position +1 of the RNA polynucleotide, and G_2 is position +2 of the RNA polynucleotide; and

(ii) the cap proximal sequence comprises A_1 and G_2 of the Cap1 structure, and a sequence comprising: $A_3A_4U_5$ at positions +3, +4 and +5 respectively of the RNA polynucleotide.

16. The composition or medical preparation of any one of the preceding claims, wherein the RNA polynucleotide comprises a 5’ UTR and the cap proximal sequence is situated in a 5’ UTR.

17. A composition or medical preparation comprising a capped RNA polynucleotide encoding a gene product, which RNA polynucleotide comprises the formula:



wherein R^1 is CH_3, R^2 is OH or O-CH_3, and R^3 is O-CH_3 ,
wherein B_1 is any nucleobase, preferably A; B_2 is any nucleobase, preferably G; B_3 is any nucleobase, preferably A or C; B_4 is any nucleobase; and B_5 is any nucleobase, and
wherein, when the RNA polynucleotide is to be administered to a subject, the levels of expression of the encoded gene product at about 6 hours after administration and at about 48 hours after administration do not differ by more than 5-fold.

18. The composition or medical preparation of claim 17, wherein, when the RNA polynucleotide is to be administered to a subject, the expression of the gene product is detectable at least 72 hours after administration.

19. The composition or medical preparation of claim 17 or 18, wherein the RNA polynucleotide comprises a 5’ UTR.

20. The composition or medical preparation of any one of the preceding claims, wherein the RNA polynucleotide further comprises a 3’ UTR sequence; and/or a polyA sequence.

21. The composition or medical preparation of any one of the preceding claims, wherein the RNA polynucleotide does not comprise a self-hybridizing sequence.

22. The composition or medical preparation of 21, wherein a self-hybridizing sequence is a sequence that can hybridize to a sequence in a 5’ UTR or 3’ UTR of the RNA polynucleotide.

23. The composition or medical preparation of claim 21 or 22, wherein the self-hybridizing sequence is or comprises SEQ ID NO: 8.

24. The composition or medical preparation of any one of the preceding claims, wherein a 5’ UTR comprises a human alpha globin (hAg) 5’UTR or a fragment thereof, a TEV 5’ UTR or a fragment thereof, a HSP70 5’ UTR or a fragment thereof, or a c-Jun 5’ UTR or a fragment thereof.

25. The composition or medical preparation of any one of the preceding claims, wherein the 5’ UTR comprises a human alpha globin 5’ UTR provided in SEQ ID NO: 11 or SEQ ID NO: 12, or a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity thereto.

26. The composition or medical preparation of any one of the preceding claims, wherein the 5’ UTR further comprises a T7 RNA polymerase promoter sequence.

27. The composition or medical preparation of any one of the preceding claims, wherein the 5’ cap structure is added co-transcriptionally.

28. The composition or medical preparation of any one of the preceding claims, wherein the 5’cap structure is not added enzymatically.

29. The composition or medical preparation of any one of the preceding claims, wherein the RNA polynucleotide comprises a 3’ UTR or a fragment thereof.

30. The composition or medical preparation of any one of the preceding claims, wherein the 3’ UTR sequence comprises SEQ ID NO: 13, or a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity thereto.

31. The composition or medical preparation of any one of claims 18-30, wherein the 3’ UTR or a proximal sequence thereto comprises a restriction site.

32. The composition or medical preparation of claim 31, wherein the restriction site is a *BamHI* site or a *XhoI* site.

33. The composition or medical preparation of any one of the preceding claims, wherein a PolyA sequence comprises at least 100 nucleotides.

34. The composition or medical preparation of claim 33, wherein:

(i) a polyA sequence is an interrupted sequence of adenine nucleotides; and/or

(ii) a polyA sequence comprises 30 adenine nucleotides followed by 70 adenine nucleotides, wherein the 30 adenine nucleotides and 70 adenine nucleotides are separated by a linker sequence.

35. The composition or medical preparation of claim 34, wherein a polyA sequence comprises the sequence of SEQ ID NO: 14.

36. The composition or medical preparation of any one of claims 18-35, wherein the 5’ cap, the 5’ UTR, the sequence encoding the payload, the 3’ UTR and the polyA sequence are situated disposed in a 5’ to 3’ orientation.

37. The composition or medical preparation of any one of the preceding claims, wherein the RNA polynucleotide comprises a Kozak sequence upstream of the sequence encoding a payload.

38. The composition or medical preparation of any one of the preceding claims, wherein the sequence encoding a payload comprises a promoter sequence.

39. The composition or medical preparation of any one of the preceding claims, wherein the sequence encoding a payload comprises a sequence encoding a secretory signal peptide.

40. The composition or medical preparation of any one of the preceding claims, wherein an RNA polynucleotide comprises a sequence encoding a payload chosen from: a protein replacement polypeptide; an antibody agent; a cytokine; an antigenic polypeptide; a gene editing component; a regenerative medicine component or combinations thereof.

41. The composition or medical preparation of any one of the preceding claims, characterized in that, when assessed in an organism to be administered the composition or medical preparation comprising the RNA polynucleotide, elevated expression and/or increased duration of expression of the payload is observed relative to an appropriate reference comparator.

42. The composition or medical preparation of claim 40, wherein a payload is or comprises a protein replacement polypeptide.

43. The composition or medical preparation of claim 42, wherein a protein replacement polypeptide comprises a polypeptide with aberrant expression in a disease or disorder.

44. The composition or medical preparation of claim 43, wherein a protein replacement polypeptide comprises an intracellular protein, an extracellular protein, or a transmembrane protein.

45. The composition or medical preparation of claim 43 or 44, wherein a protein replacement polypeptide comprises an enzyme.

46. The composition or medical preparation of claim 43, wherein a disease or disorder with aberrant expression of a polypeptide includes but is not limited to: a rare disease, a metabolic disorder, a muscular dystrophy, a cardiovascular disease, or a monogenic disease.

47. The composition or medical preparation of claim 40, wherein a payload is or comprises an antibody agent.

48. The composition or medical preparation of claim 47, wherein an antibody agent binds to a polypeptide expressed on a cell.

49. The composition or medical preparation of claim 47 or 48, wherein an antibody agent comprises a CD3 antibody, a Claudin 6 antibody, or a combination thereof.

50. The composition or medical preparation of claim 40, wherein a payload is or comprises a cytokine or a fragment or a variant thereof.

51. The composition or medical preparation of claim 50, wherein a cytokine comprises: IL-12 or a fragment or variant or a fusion thereof, IL-15 or a fragment or a variant or a fusion thereof, GMCSF or a fragment or a variant thereof; or IFN-alpha or a fragment or a variant thereof.

52. The composition or medical preparation of claim 40, wherein a payload is or comprises an antigenic polypeptide or an immunogenic variant or an immunogenic fragment thereof.

53. The composition or medical preparation of claim 52, wherein an antigenic polypeptide comprises one epitope from an antigen.

54. The composition or medical preparation of claim 52, wherein an antigenic polypeptide comprises a plurality of distinct epitopes from an antigen.

55. The composition or medical preparation of claim 54, wherein an antigenic polypeptide comprising a plurality of distinct epitopes from an antigen is polyepitopic.

56. The composition or medical preparation of any one of claims 52-55, wherein an antigenic polypeptide comprises: an antigenic polypeptide from an allergen, a viral antigenic polypeptide, a bacterial antigenic polypeptide, a fungal antigenic polypeptide, a parasitic antigenic polypeptide, an antigenic polypeptide from an infectious agent, an antigenic polypeptide from a pathogen, a tumor antigenic polypeptide, or a self-antigenic polypeptide.

57. The composition or medical preparation of claim 56, wherein a viral antigenic polypeptide comprises an HIV antigenic polypeptide, an influenza antigenic polypeptide, a Coronavirus antigenic polypeptide, a Rabies antigenic polypeptide, or a Zika virus antigenic polypeptide.

58. The composition or medical preparation of claim 56 or 57, wherein a viral antigenic polypeptide is or comprises a Coronavirus antigenic polypeptide.

59. The composition or medical preparation of claim 58, wherein a Coronavirus antigen is or comprises a SARS-CoV-2 protein.

60. The composition or medical preparation of claim 59, wherein a SARS-CoV-2 protein comprises a SARS-CoV-2 Spike (S) protein, or an immunogenic variant or an immunogenic fragment thereof.

61. The composition or medical preparation of claim 60, wherein the SARS-CoV-2 protein, or immunogenic variant or immunogenic fragment thereof, comprises proline residues at positions 986 and 987.

62. The composition or medical preparation of claim 60 or 61, wherein a SARS-CoV-2 S polypeptide:

(i) has at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 9; or

(ii) is encoded by an RNA having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 10.

63. The composition or medical preparation of claim 56, wherein a payload is or comprises a tumor antigenic polypeptide or an immunogenic variant or an immunogenic fragment thereof.

64. The composition or medical preparation of claim 63, wherein a tumor antigenic polypeptide comprises a tumor specific antigen, a tumor associated antigen, a tumor neoantigen, or a combination thereof.

65. The composition or medical preparation of claim 63 or 64,wherein a tumor antigenic polypeptide comprises p53, ART-4, BAGE, ss-catenin/m, Bcr-abL CAMEL, CAP-1, CASP-8, CDC27/m, CDK4/m, CEA, CLAUDIN-12, c-MYC, CT, Cyp-B, DAM, ELF2M, ETV6-AML1, G250, GAGE, GnT-V, Gap100, HAGE, HER-2/neu, HPV-E7, HPV-E6, HAST-2, hTERT (or hTRT), LAGE, LDLR/FUT, MAGE-A, preferably MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, or MAGE-A12, MAGE-B, MAGE-C, MART-1/Melan-A, MC1R, Myosin/m, MUC1, MUM-1, -2, -3, NA88-A, NF1, NY-ESO-1, NY-BR-1, p190 minor BCR-abL, Plac-1, Pm1/RARa, PRAME, proteinase 3, PSA, PSM, RAGE, RU1 or RU2, SAGE, SART-1 or SART-3, SCGB3A2, SCP1, SCP2, SCP3, SSX, SURVIVIN, TEL/AML1, TPI/m, TRP-1, TRP-2, TRP-2/INT2, TPTE, WT, WT-1, or a combination thereof.

66. The composition or medical preparation of any one of claims 63-65, wherein a tumor antigenic polypeptide comprises a tumor antigen from a carcinoma, a sarcoma, a melanoma, a lymphoma, a leukemia, or a combination thereof.

67. The composition or medical preparation of claim 66, wherein a tumor antigenic polypeptide comprises a melanoma tumor antigen.

68. The composition or medical preparation of claim 66, wherein a tumor antigenic polypeptide comprises a prostate cancer antigen.

69. The composition or medical preparation of claim 66, wherein a tumor antigenic polypeptide comprises a HPV16 positive head and neck cancer antigen.

70. The composition or medical preparation of claim 66, wherein a tumor antigenic polypeptide comprises a breast cancer antigen.

71. The composition or medical preparation of claim 66, wherein a tumor antigenic polypeptide comprises an ovarian cancer antigen.

72. The composition or medical preparation of claim 66, wherein a tumor antigenic polypeptide comprises a lung cancer antigen.

73. The composition or medical preparation of claim 72, wherein a tumor antigenic polypeptide comprises an NSCLC antigen.

74. The composition or medical preparation of claim 56, wherein a payload is or comprises a self-antigenic polypeptide or an immunogenic variant or an immunogenic fragment thereof.

75. The composition or medical preparation of claim 74, wherein a self-antigenic polypeptide comprises an antigen that is typically expressed on cells and is recognized as a self-antigen by an immune system.

76. The composition or medical preparation of claim 74 or 75, wherein a self-antigenic polypeptide comprises: a multiple sclerosis antigenic polypeptide, a Rheumatoid arthritis antigenic polypeptide, a lupus antigenic polypeptide, a celiac disease antigenic polypeptide, a Sjogren's syndrome antigenic polypeptide, or an ankylosing spondylitis antigenic polypeptide, or a combination thereof.

77. The composition or medical preparation of any one of the preceding claims, wherein an RNA polynucleotide comprises a modified nucleoside in place of uridine.

78. The composition or medical preparation of claim 77, wherein a modified nucleoside is selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

79. The composition or medical preparation of any one of the preceding claims, wherein an RNA polynucleotide further comprises one or more additional sequences, e.g., one or more additional payloads, and/or one or more regulatory elements.

80. The composition or medical preparation of any one of the preceding claims, wherein an RNA polynucleotide is characterized in that upon administration to an organism it increases translation efficiency of a payload, compared to administration of an otherwise similar RNA polynucleotide without a m7(3'OMeG)(5')ppp(5')(2'OMeA_1)pG_2 Cap.

81. The composition or medical preparation of any one of the preceding claims, wherein an RNA polynucleotide is characterized in that upon administration to an organism it increases the expression level and/or duration of expression of an encoded payload, compared to administration of an otherwise similar RNA polynucleotide without a m7(3'OMeG)(5')ppp(5')(2'OMeA_1)pG_2 cap, without a cap proximal sequence disclosed herein, and/or with a self-hybridizing sequence, optionally wherein:

(i) increased expression is determined at least 6 hours, at least 24 hours, at least 48 hours at least 72 hours, at least 96 hours or at least 120 hours after the administration, optionally wherein the increase in expression is at least 2-fold to 10-fold; or

(ii) elevated expression persists for at least 24 hours, at least 48 hours, at least 72 hours, at least 96 hours, or at least 120 hours.

82. The composition or medical preparation of any one of the preceding claims, wherein the RNA is formulated or is to be formulated as a liquid, a solid, or a combination thereof.

83. The composition or medical preparation of any one of the preceding claims, wherein the RNA is formulated or is to be formulated for injection.

84. The composition or medical preparation of any one of the preceding claims, wherein the RNA is formulated or is to be formulated for intramuscular administration.

85. A pharmaceutical composition comprising an RNA polynucleotide of any one of the preceding claims, formulated as a particle.

86. The pharmaceutical composition of claim 85, wherein a particle is or comprises a lipid nanoparticle (LNP) or a lipoplex (LPX) particle.

87. The pharmaceutical composition of claim 86, wherein a lipid nanoparticle comprises each of: a cationic lipid; a sterol; a neutral lipid; and a lipid conjugate.

88. The pharmaceutical composition of claim 87, wherein the cationic lipid is or comprises ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), the sterol is a cholesterol, the neutral lipid is or comprises a phospholipid, and the lipid conjugate is or comprises a polyethylene glycol (PEG)-lipid.

89. The pharmaceutical composition of claim 88, wherein the phospholipid is or comprises distearoylphosphatidylcholine (DSPC).

90. The pharmaceutical composition of claim 88, wherein the (PEG)-lipid is or comprises 2-[(polyethylene glycol)-2000]-*N*,*N*-ditetradecylacetamide.

91. The pharmaceutical composition of any one of claims 87-90, wherein the lipid nanoparticle comprises each of:a. ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate); b. a cholesterol; c. distearoylphosphatidylcholine (DSPC); and d. 2-[(polyethylene glycol)-2000]-*N*,*N*-ditetradecylacetamide.

92. The pharmaceutical composition of any one of claims 87-91-G7, wherein a neutral lipid is present in a concentration ranging from 5 to 15 mol percent of total lipids.

93. The pharmaceutical composition of any one of claims 87-92, wherein a cationically ionizable lipid is present in a concentration ranging from 40 to 55 mol percent of total lipids.

94. The pharmaceutical composition of any one of claims 87-93, wherein a steroid is present in a concentration ranging from 30 to 50 mol percent of total lipids.

95. The pharmaceutical composition of any one of claims 87-94, wherein a pegylated lipid is present in a concentration ranging from 1 to 10 mol percent of total lipids.

96. The pharmaceutical composition of any one of claims 87-95, wherein a lipid nanoparticle comprise from 40 to 55 mol percent of a cationically ionizable lipid; from 5 to 15 mol percent of a neutral lipid; from 30 to 50 mol percent of a steroid; and from 1 to 10 mol percent of a pegylated lipid.

97. The pharmaceutical composition of claim 86, wherein an RNA lipoplex particle is obtainable by mixing an RNA polynucleotide with liposomes.

98. The composition or medical preparation of any one of claims 1-84, or a pharmaceutical composition of any one of claims 85-97, wherein the RNA is mRNA or saRNA.

99. The pharmaceutical composition of any one of claims 85-98, wherein a pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

100. The pharmaceutical composition of any one of claims 85-99, which is packaged as a kit.

101. The pharmaceutical composition of claim 100, further comprising instructions for use of said pharmaceutical composition for inducing an immune response in a subject.

102. A method of manufacturing a pharmaceutical composition of any one of claims 85-101, by combining an RNA polynucleotide with lipids to form lipid nanoparticles that encapsulate said RNA.

103. A nucleic acid template suitable to produce a cap1-capped RNA, in which the first five nucleotides transcribed from the template strand of the nucleic acid template comprise the sequence $N_1pN_2pN_3pN_4pN_5$, wherein N_1 is any nucleotide, preferably T; N_2 is any nucleotide, preferably C; N_3 is any nucleotide, preferably T or G; N_4 is any nucleotide; and N_5 is any nucleotide.

104. The nucleic acid template of claim 103, wherein the DNA template comprises: a 5' UTR, a sequence encoding a payload, a 3' UTR and a polyA sequence.

105. An in vitro transcription reaction comprising:

(i) a template DNA comprising a polynucleotide sequence complementary to an RNA polynucleotide sequence provided in any one of claims 1-84;

(ii) a polymerase; and

(iii) an RNA polynucleotide.

106. The in vitro transcription reaction of claim 105, wherein the polymerase is or comprises a T7 polymerase.

107. The in vitro transcription reaction of claim 106, further comprising a 5' cap analog.

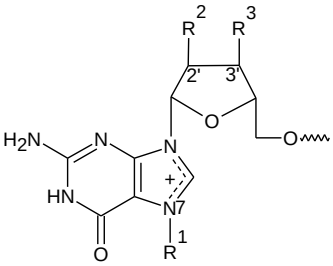
108. The in vitro transcription reaction of claim 107, wherein a 5' cap analog is or comprises a Cap1 structure.

109. The in vitro transcription reaction of claim 108, wherein the RNA polynucleotide comprises a cap comprising a Cap1 structure; and a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of the RNA polynucleotide; and a sequence encoding a payload.

110. The in vitro transcription reaction of claim 109, the Cap1 structure comprises $m7G(5')ppp(5')(2'OMeN_1)pN_2$, wherein N_1 is position +1 of the RNA polynucleotide, and N_2 is position +2 of the RNA polynucleotide, and wherein N_1 and N_2 are each independently chosen from: A, C, G, or U.

111. An RNA polynucleotide isolated from an in vitro transcription reaction provided in any one of claims 105-110.

112. A method for producing a capped RNA comprising, transcribing a nucleic acid template in the presence of a cap structure, wherein the cap structure comprises G*ppp($m_1^{2'-O}$)N_1pN_2, wherein N_1 is complementary to position +1 of the nucleic acid template and N_2 is complementary to position +2 of the nucleic acid template, and N_1 and N_2 are independently chosen from A, C, G or U,
wherein position +3 of the nucleic acid template is any nucleotide, preferably T or G; position +4 of the nucleic acid template is any nucleotide; and position +5 of the nucleic acid template is any nucleotide,
wherein G* comprises the following structure:



wherein ∿∿∿ represents the bond by which G* is bound to the first phosphor atom of the ppp group, R^1 is CH_3, R^2 is OH or O-CH_3, and R^3 is O-CH_3.

113. A composition comprising a DNA polynucleotide comprising a sequence complementary to an RNA polynucleotide sequence provided in any one of claims 1-84.

114. The DNA polynucleotide composition of claim 113, which can be used to transcribe an RNA polynucleotide.

115. The DNA polynucleotide composition of claim 113 or 114, which is disposed in a vector.

116. A pharmaceutical composition comprising an RNA polynucleotide formulated in a lipid nanoparticle (LNP) or a lipoplex (LPX) particle of any one of claims 85-101 for use in therapy.

117. Use of a pharmaceutical composition comprising an RNA polynucleotide formulated in a lipid nanoparticle (LNP) or a lipoplex (LPX) particle of any one of claims 85-101 in the manufacture of a medicament for inducing an immune response in a subject.

118. The use of claim 117, wherein an immune response is induced in a subject.

119. The use of claim 117 or 118, wherein an immune response is a prophylactic immune response or a therapeutic immune response.

120. The use of any one of claims 116-119, wherein the subject is a mammal.

121. The use of claim 120, wherein the subject is a human.

122. The use of any one of claims 116-121, wherein the subject has a disease or disorder disclosed herein.

123. Use of a pharmaceutical composition comprising an RNA polynucleotide formulated in a lipid nanoparticle (LNP) or a lipoplex (LPX) particle of any one of claims 85-101 in the manufacture of a medicament for vaccination of a subject.

124. The use of claim 123, wherein the vaccination generates an immune response to an agent.

125. The use of claim 124, wherein an immune response is a prophylactic immune response.

126. The use of any one of claims 123-125, wherein the subject is a mammal.

127. The use of claim 126, wherein the subject is a human.

128. The use of any one of claims 116-127, wherein the subject has a disease or disorder disclosed herein.

129. The use of any one of claims 116-128, wherein one dose of a pharmaceutical composition is to be administered.

130. The use of any one of claims 116-128, wherein a plurality of doses of a pharmaceutical composition is to be administered.

131. The use of any one of claims 116-130, further comprising that one or more therapeutic agents is to be administered.

132. The use of claim 131, wherein one or more therapeutic agents are to be administered before, after, or concurrently with a pharmaceutical composition comprising an RNA polynucleotide to be administered.

133. A method of decreasing interaction with IFIT1 of an RNA polynucleotide that comprises a 5’ cap and a cap proximal sequence comprising positions +1, +2, +3, +4, and +5 of the RNA polynucleotide, the method comprising a step of:

providing a variant of the RNA polynucleotide that differs from the parental RNA polynucleotide by substitution of one or more residues within the cap proximal sequence, and

determining that interaction of the variant with IFIT1 is decreased relative to that of the parental RNA polynucleotide.

134. The method of claim 133, wherein the determining comprises that the RNA polynucleotide or a composition comprising the same is to be administered to a cell or an organism.

135. A method of producing a polypeptide comprising a step of:

providing an RNA polynucleotide that comprises a 5’ cap, a cap proximal sequence that comprises positions +1, +2, +3, +4, and +5 of the RNA polynucleotide, and a sequence encoding a payload;

wherein the RNA polynucleotide is characterized in that when assessed in an organism to be administered the RNA polynucleotide or a composition comprising the same, elevated

expression and/or increased duration of expression of the payload is observed relative to an appropriate reference comparator.

136. A method of increasing translatability of an RNA polynucleotide that comprises a 5’ cap, a cap proximal sequence that comprises positions +1, +2, +3, +4, and +5 of the RNA polynucleotide and a sequence encoding a payload, the method comprising a step of:

providing a variant of the RNA polynucleotide that differs from a parental RNA polynucleotide by substitution of one or more residues within the cap proximal sequence; and

determining that expression of the variant is increased relative to that of the parental RNA polynucleotide.

137. The method of claim 138, wherein the determining comprises that the RNA polynucleotide or a composition comprising the same is to be administered to a cell or an organism.

138. The method of claim 136 or 137, wherein the increased translatability is assessed by increased expression and/or a persistence of expression of the payload.

139. The method of claim 138, wherein:

(i) increased expression is determined at least 6 hours, at least 24 hours, at least 48 hours at least 72 hours, at least 96 hours or at least 120 hours after the administration, optionally wherein the increase in expression is at least 2-fold to 10-fold; or

(ii) elevated expression persists for at least 24 hours, at least 48 hours, at least 72 hours, at least 96 hours, or at least 120 hours.

140. In a therapeutic RNA comprising a 5’ cap that includes a Cap1 structure, a cap proximal sequence and a sequence encoding a payload, the improvement that comprises:

including one or more of the following residues in a cap proximal sequence: A at position +1 of the RNA polynucleotide, G at position +2 of the RNA polynucleotide, A at position +3 of the RNA polynucleotide, A at position +4 of the RNA polynucleotide, and U at position +5 of the RNA polynucleotide,

demonstrated to increase expression of the RNA when to be administered to a subject in an LNP formulation.

141. A method of increasing translation of an RNA polynucleotide comprising a 5’ cap that includes a Cap1 structure, a cap proximal sequence and a sequence encoding a payload, the improvement that comprises:

including one or more of the following residues in a cap proximal sequence: A at position +1 of the RNA polynucleotide, G at position +2 of the RNA polynucleotide, A at position +3 of the RNA polynucleotide, A at position +4 of the RNA polynucleotide, and U at position +5 of the RNA polynucleotide.

142. A method of providing a framework for an RNA polynucleotide that comprises a 5’ cap, a cap proximal sequence, and a payload sequence, the method comprising a step of:

assessing at least two variants of the RNA polynucleotide, wherein:

each variant includes the same 5’ cap and payload sequence; and

the variants differ from one another at one or more specific residues of the cap proximal sequence;

wherein the assessing comprises determining expression levels and/or duration of expression of the payload sequence; and

selecting at least one combination of 5’ cap and a cap proximal sequence that displays elevated expression relative to at least one other combination.

143. The method of claim 142, wherein the assessing comprises that the RNA construct or a composition comprising the same is to be administered to a cell or an organism:

144. The method of claim 142 or 143, wherein the elevated expression is detected at a time point at least 6 hours, at least 24 hours, at least 48 hours, at least 72 hours, at least 96 hours, or at least 120 hours after the RNA construct or a composition comprising the same is to be administered, optionally wherein the elevated expression is at least 2-fold to 10-fold.

145. The method of any one of claims 142-144, wherein the elevated expression persists for at least 24 hours, at least 48 hours, at least 72 hours, at least 96 hours, or at least 120 hours.

146. The method of any one of claims 116-145, wherein the RNA polynucleotide comprises one or more features of an RNA polynucleotide provided in any one of claims 1-84.

147. The method of any one of claims 134-146, wherein the composition comprises a pharmaceutical composition of any one of claims 85-101.