

could be an attractive combination partner of treatments involving HPV-based vaccines such as HPV16 E6/E7 RNA-LPX.

In addition, we show that cisplatin augments the antitumor effects of E7 RNA-LPX vaccination. RNA-LPX vaccines and platinum-based chemotherapies can therefore be used as synergistic combination partners for improved cancer therapies.

Summary

The present ~~invention~~disclosure generally embraces the immunotherapeutic treatment of a subject comprising the administration of (i) RNA, i.e., vaccine RNA, encoding an amino acid sequence, i.e., a vaccine antigen, comprising HPV E6 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof, i.e., an antigenic peptide or protein, and (ii) RNA, i.e., vaccine RNA, encoding an amino acid sequence, i.e., a vaccine antigen, comprising HPV E7 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E7 protein or the immunogenic variant thereof, i.e., an antigenic peptide or protein. Thus, the vaccine antigen comprises an epitope of HPV E6 protein or HPV E7 protein for inducing an immune response against HPV E6 protein or HPV E7 protein, in the subject. RNA encoding vaccine antigen is administered to provide (following expression of the polynucleotide by appropriate target cells) antigen for induction, i.e., stimulation, priming and/or expansion, of an immune response, e.g., antibodies and/or immune effector cells, which is targeted to target antigen (HPV E6 protein or HPV E7 protein) or a procession product thereof. In one ~~embodiment~~example, the immune response which is to be induced according to the present disclosure is a B cell-mediated immune response, i.e., an antibody-mediated immune response. Additionally or alternatively, in one ~~embodiment~~example, the immune response which is to be induced according to the present disclosure is a T cell-mediated immune response. In one ~~embodiment~~example, the immune response is an anti-HPV immune response.

The vaccine described herein comprises as the active principle single-stranded RNA that may be translated into the respective protein upon entering cells of a recipient. In addition to

wildtype or codon-optimized sequences encoding the antigen sequence, the RNA may contain one or more structural elements optimized for maximal efficacy of the RNA with respect to stability and translational efficiency (5' cap, 5' UTR, 3' UTR, poly(A)-tail). In one [embodimentexample](#), the RNA contains all of these elements. In one [embodimentexample](#), beta-S-ARCA(D1) ($m_2^{7,2'-O}$ GppSpG) may be utilized as specific capping structure at the 5'-end of the RNA drug substances. As 5'-UTR sequence, the 5'-UTR sequence of the human alpha-globin mRNA, optionally with an optimized 'Kozak sequence' to increase translational efficiency may be used. As 3'-UTR sequence, a combination of two sequence elements (FI element) derived from the "amino terminal enhancer of split" (AES) mRNA (called F) and the mitochondrial encoded 12S ribosomal RNA (called I) placed between the coding sequence and the poly(A)-tail to assure higher maximum protein levels and prolonged persistence of the mRNA may be used. These were identified by an *ex vivo* selection process for sequences that confer RNA stability and augment total protein expression (see WO 2017/060314, herein incorporated by reference). Furthermore, a poly(A)-tail measuring 110 nucleotides in length, consisting of a stretch of 30 adenosine residues, followed by a 10 nucleotide linker sequence (of random nucleotides) and another 70 adenosine residues may be used. This poly(A)-tail sequence was designed to enhance RNA stability and translational efficiency.

Furthermore, sec (secretory signal peptide) and/or MITD (MHC class I trafficking domain) may be fused to the antigen-encoding regions in a way that the respective elements are translated as N or C terminal tag, respectively. Fusion-protein tags derived from the sequence encoding the human MHC class I complex (HLA-B51, haplotype A2, B27/B51, Cw2/Cw3), have been shown to improve antigen processing and presentation. Sec may correspond to the 78 bp fragment coding for the secretory signal peptide, which guides translocation of the nascent polypeptide chain into the endoplasmatic reticulum. MITD may correspond to the transmembrane and cytoplasmic domain of the MHC class I molecule, also called MHC class I trafficking domain. Sequences coding for short linker peptides predominantly consisting of the

amino acids glycine (G) and serine (S), as commonly used for fusion proteins may be used as GS/Linkers.

The antigen may be administered in combination with helper epitopes to break immunological tolerance. The helper epitopes may be tetanus toxoid-derived, e.g., P2P16 amino acid sequences derived from the tetanus toxoid (TT) of *Clostridium tetani*. These sequences may support to overcome tolerance mechanisms by providing tumor-unspecific T-cell help during priming. The tetanus toxoid heavy chain includes epitopes that can bind promiscuously to MHC class II alleles and induce CD4⁺ memory T cells in almost all tetanus vaccinated individuals. In addition, the combination of TT helper epitopes with tumor-associated antigens is known to improve the immune stimulation compared to the application of tumor-associated antigen alone by providing CD4⁺ mediated T-cell help during priming. To reduce the risk of stimulating CD8⁺ T cells, two peptide sequences known to contain promiscuously binding helper epitopes may be used to ensure binding to as many MHC class II alleles as possible, e.g., P2 and P16.

In one [embodimentexample](#), a vaccine antigen comprises an amino acid sequence which breaks immunological tolerance. In one [embodimentexample](#), the amino acid sequence which breaks immunological tolerance comprises helper epitopes, preferably tetanus toxoid-derived helper epitopes. The amino acid sequence which breaks immunological tolerance may be fused to the C-terminus of the vaccine sequence, e.g., antigen sequence, either directly or separated by a linker. Optionally, the amino acid sequence which breaks immunological tolerance may link the vaccine sequence and the MITD. The amino acid sequence which breaks immunological tolerance may be RNA encoded. In one [embodimentexample](#), the antigen-targeting RNAs are applied together with RNA coding for a helper-epitope to boost the resulting immune response. This RNA coding for a helper-epitope may contain structural elements optimized for maximal efficacy of the RNA with respect to stability and translational efficiency (5' cap, 5' UTR, 3' UTR, poly(A)-tail) described above for the antigen-encoding RNA.

The vaccine RNA may be complexed with liposomes to generate serum-stable RNA-lipoplexes (LPX) for intravenous (i.v.) administration. If a combination of different RNAs is used, the RNAs may be separately complexed with liposomes to generate serum-stable RNA-lipoplexes for intravenous (i.v.) administration. RNA-lipoplexes may target antigen-presenting cells (APCs) in lymphoid organs which results in an efficient stimulation of the immune system.

The RNA lipoplex particles may be prepared using liposomes that may be obtained by injecting a solution of lipids in ethanol into water or a suitable aqueous phase. In one [embodimentexample](#), the aqueous phase has an acidic pH. In one [embodimentexample](#), the aqueous phase comprises acetic acid, e.g., in an amount of about 5 mM. Liposomes may be used for preparing RNA lipoplex particles by mixing the liposomes with RNA. In one [embodimentexample](#), the liposomes and RNA lipoplex particles comprise at least one cationic lipid and at least one additional lipid. In one [embodimentexample](#), the at least one cationic lipid comprises 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA). In one [embodimentexample](#), the at least one additional lipid comprises 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE). In one [embodimentexample](#), the at least one cationic lipid comprises 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and the at least one additional lipid comprises 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE). In one [embodimentexample](#), the liposomes and RNA lipoplex particles comprise 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE). In one [embodimentexample](#), the molar ratio of the at least one cationic lipid to the at least one additional lipid is about 2:1. In one [embodimentexample](#), at physiological pH, the charge ratio of positive charges to negative charges in the RNA lipoplex particles is from about 1.6:2 to about 1:2, or about 1.6:2 to about 1.1:2. In specific [embodimentexamples](#), the charge ratio of positive charges to negative charges in the RNA lipoplex particles at physiological pH is about 1.6:2.0, about 1.5:2.0, about 1.4:2.0, about 1.3:2.0, about 1.2:2.0, about 1.1:2.0, or about 1:2.0.

In one [embodimentexample](#), vaccine RNA is co-formulated as lipoplex particles with an RNA encoding an amino acid sequence which breaks immunological tolerance.

In one aspect, the invention disclosure relates to a composition or medical preparation comprising at least one RNA, wherein the at least one RNA encodes:

(i) an amino acid sequence comprising human papillomavirus (HPV) E6 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof; and

(ii) an amino acid sequence comprising HPV E7 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E7 protein or the immunogenic variant thereof.

In one embodiment example, each of the amino acid sequences under (i), or (ii) is encoded by a separate RNA.

In one embodiment example,

(i) the RNA encoding the amino acid sequence under (i) comprises the nucleotide sequence of SEQ ID NO: 2, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 2; and/or

(ii) the amino acid sequence under (i) comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 1.

In one embodiment example,

(i) the RNA encoding the amino acid sequence under (ii) comprises the nucleotide sequence of SEQ ID NO: 4, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 4; and/or

(ii) the amino acid sequence under (ii) comprises the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 3.

In one embodiment example, at least one amino acid sequence under (i), or (ii) comprises an amino acid sequence enhancing antigen processing and/or presentation.

In one embodiment example, each amino acid sequence under (i), or (ii) comprises an amino acid sequence enhancing antigen processing and/or presentation.

In one [embodimentexample](#), the amino acid sequence enhancing antigen processing and/or presentation comprises an amino acid sequence corresponding to the transmembrane and cytoplasmic domain of a MHC molecule, preferably a MHC class I molecule.

In one [embodimentexample](#), the amino acid sequence enhancing antigen processing and/or presentation comprises the amino acid sequence of SEQ ID NO: 26, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 26.

In one [embodimentexample](#), the amino acid sequence enhancing antigen processing and/or presentation further comprises an amino acid sequence coding for a secretory signal peptide.

In one [embodimentexample](#), the secretory signal peptide comprises the amino acid sequence of SEQ ID NO: 25, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 25.

In one [embodimentexample](#), at least one amino acid sequence under (i), or (ii) comprises an amino acid sequence which breaks immunological tolerance and/or at least one RNA is co-administered with RNA encoding an amino acid sequence which breaks immunological tolerance.

In one [embodimentexample](#), each amino acid sequence under (i), or (ii) comprises an amino acid sequence which breaks immunological tolerance and/or each RNA is co-administered with RNA encoding an amino acid sequence which breaks immunological tolerance.

In one [embodimentexample](#), the amino acid sequence which breaks immunological tolerance comprises helper epitopes, preferably tetanus toxoid-derived helper epitopes.

In one [embodimentexample](#), the amino acid sequence which breaks immunological tolerance comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 27.

In one [embodimentexample](#), at least one of the amino acid sequences under (i), or (ii) is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased

compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

In one [embodimentexample](#), each of the amino acid sequences under (i), or (ii) is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

In one [embodimentexample](#), at least one RNA is a modified RNA, in particular a stabilized mRNA. In one [embodimentexample](#), at least one RNA comprises a modified nucleoside in place of at least one uridine. In one [embodimentexample](#), at least one RNA comprises a modified nucleoside in place of each uridine. In one [embodimentexample](#), each RNA comprises a modified nucleoside in place of at least one uridine. In one [embodimentexample](#), each RNA comprises a modified nucleoside in place of each uridine. In one [embodimentexample](#), the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1 ψ), and 5-methyl-uridine (m5U).

In one [embodimentexample](#), at least one RNA comprises the 5' cap $m_2^{7,2'-O}Gpp_p(5')G$.

In one [embodimentexample](#), each RNA comprises the 5' cap $m_2^{7,2'-O}Gpp_p(5')G$.

In one [embodimentexample](#), at least one RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 30, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30.

In one [embodimentexample](#), each RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 30, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30.

In one [embodimentexample](#), at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 31, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 31.

In one [embodimentexample](#), each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 31, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 31.

In one [embodimentexample](#), at least one RNA comprises a poly-A sequence.

In one [embodimentexample](#), each RNA comprises a poly-A sequence.

In one [embodimentexample](#), the poly-A sequence comprises at least 100 nucleotides.

In one [embodimentexample](#), the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 32.

In one [embodimentexample](#), the amino acid sequence under (i), i.e., the amino acid sequence comprising HPV E6 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof, comprises from N-terminus to C-terminus: N-secretory signal peptide-E6-amino acid sequence which breaks immunological tolerance-amino acid sequence enhancing antigen processing and/or presentation-C.

In one [embodimentexample](#), the amino acid sequence under (ii), i.e., the amino acid sequence comprising HPV E7 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E7 protein or the immunogenic variant thereof, comprises from N-terminus to C-terminus: N-secretory signal peptide-E7-amino acid sequence which breaks immunological tolerance-amino acid sequence enhancing antigen processing and/or presentation-C.

In one [embodimentexample](#), the RNA is formulated as a liquid, formulated as a solid, or a combination thereof.

In one [embodimentexample](#), the RNA is formulated for injection.

In one [embodimentexample](#), the RNA is formulated for intravenous administration.

In one [embodimentexample](#), the RNA is formulated or is to be formulated as lipoplex particles.

In one [embodimentexample](#), the RNA lipoplex particles are obtainable by mixing the RNA with liposomes.

In one [embodimentexample](#), the composition or medical preparation further comprises an immune checkpoint inhibitor.

In one embodimentexample, the immune checkpoint inhibitor is selected from a PD-1 inhibitor, and a PD-L1 inhibitor.

In one embodimentexample, the immune checkpoint inhibitor is selected from an anti-PD-1 antibody, and an anti-PD-L1 antibody.

In one embodimentexample, the immune checkpoint inhibitor is an anti-PD-1 antibody.

In one embodimentexample, the anti-PD-1 antibody is pembrolizumab (KEYTRUDA; MK-3475), nivolumab (OPDIVO; BMS-936558), pidilizumab (CT-011), cemiplimab (LIBTAYO, REGN2810), spartalizumab (PDR001), MEDI0680 (AMP-514), dostarlimab (TSR-042), cetrelimab (JNJ 63723283), toripalimab (JS001), AMP-224 (GSK-2661380), PF-06801591, tislelizumab (BGB-A317), ABBV-181, BI 754091, or SHR-1210.

In one embodimentexample, the immune checkpoint inhibitor is an anti-PD-L1 antibody.

In one embodimentexample, the anti-PD-L1 antibody is atezolizumab (TECENTRIQ; RG7446; MPDL3280A; R05541267), durvalumab (MEDI4736), BMS-936559, avelumab (bavencio), lodapolimab (LY3300054), CX-072 (Proclaim-CX-072), FAZ053, KN035, or MDX-1105.

In one embodimentexample, the composition or medical preparation further comprises a platinum compound.

In one embodimentexample, the platinum compound comprises cisplatin.

In one embodimentexample, the composition or medical preparation comprises an immune checkpoint inhibitor and a platinum compound.

In one embodimentexample, the composition or medical preparation is a pharmaceutical composition.

In one embodimentexample, the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

In one embodimentexample, the medical preparation is a kit.

In one embodimentexample, the RNAs and optionally the immune checkpoint inhibitor are in separate vials.

In one embodimentexample, the composition or medical preparation further comprises instructions for use of the RNAs and optionally the immune checkpoint inhibitor for treating or preventing HPV-positive cancer.

In another aspect, the disclosure relates to composition or medical preparation as described herein for use in therapy. In one aspectexample, the inventiondisclosure relates to the composition or medical preparation described herein for pharmaceutical use.

In one embodimentexample, the pharmaceutical use comprises a therapeutic or prophylactic treatment of a disease or disorder.

In one embodimentexample, the therapeutic or prophylactic treatment of a disease or disorder comprises treating or preventing HPV-positive cancer.

In one embodimentexample, the composition or medical preparation described herein is for administration to a human.

In one embodimentexample, the therapeutic or prophylactic treatment of a disease or disorder further comprises administering radiotherapy, preferably local radiotherapy.

In one embodimentexample, the therapeutic or prophylactic treatment of a disease or disorder further comprises administering a further therapy. In one embodimentexample, the further therapy comprises surgery to excise, resect, or debulk a tumor, and/or chemotherapy.

In one embodimentexample, the further therapy comprises administering a further therapeutic agent. In one embodimentexample, the further therapeutic agent comprises an anti-cancer therapeutic agent.

In yet another aspect, the disclosure relates to a use of at least one RNA to the subject in the manufacture of a medicament for treating HPV-positive cancer in a subject, wherein the at least one RNA encodes: (i) an amino acid sequence comprising human papillomavirus (HPV) E6 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof; and (ii) an amino acid sequence comprising HPV E7 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6

protein or the immunogenic variant thereof. In one ~~aspect~~example, the ~~invention~~disclosure

relates to a method of treating HPV-positive cancer in a subject comprising administering at least one RNA to the subject, wherein the at least one RNA encodes:

(i) an amino acid sequence comprising human papillomavirus (HPV) E6 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof; and

(ii) an amino acid sequence comprising HPV E7 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof.

In one embodimentexample, each of the amino acid sequences under (i), or (ii) is encoded by a separate RNA.

In one embodimentexample,

(i) the RNA encoding the amino acid sequence under (i) comprises the nucleotide sequence of SEQ ID NO: 2, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 2; and/or

(ii) the amino acid sequence under (i) comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 1.

In one embodimentexample,

(i) the RNA encoding the amino acid sequence under (ii) comprises the nucleotide sequence of SEQ ID NO: 4, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 4; and/or

(ii) the amino acid sequence under (ii) comprises the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 3.

In one embodimentexample, at least one amino acid sequence under (i), or (ii) comprises an amino acid sequence enhancing antigen processing and/or presentation.

In one embodimentexample, each amino acid sequence under (i), or (ii) comprises an amino acid sequence enhancing antigen processing and/or presentation.

In one embodimentexample, the amino acid sequence enhancing antigen processing and/or presentation comprises an amino acid sequence corresponding to the transmembrane and cytoplasmic domain of a MHC molecule, preferably a MHC class I molecule.

In one embodimentexample, the amino acid sequence enhancing antigen processing and/or presentation comprises the amino acid sequence of SEQ ID NO: 26, or an amino acid sequence

having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 26.

In one embodimentexample, the amino acid sequence enhancing antigen processing and/or presentation further comprises an amino acid sequence coding for a secretory signal peptide.

In one embodimentexample, the secretory signal peptide comprises the amino acid sequence of SEQ ID NO: 25, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 25.

In one embodimentexample, at least one amino acid sequence under (i), or (ii) comprises an amino acid sequence which breaks immunological tolerance and/or at least one RNA is co-administered with RNA encoding an amino acid sequence which breaks immunological tolerance.

In one embodimentexample, each amino acid sequence under (i), or (ii) comprises an amino acid sequence which breaks immunological tolerance and/or each RNA is co-administered with RNA encoding an amino acid sequence which breaks immunological tolerance.

In one embodimentexample, the amino acid sequence which breaks immunological tolerance comprises helper epitopes, preferably tetanus toxoid-derived helper epitopes.

In one embodimentexample, the amino acid sequence which breaks immunological tolerance comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 27.

In one embodimentexample, at least one of the amino acid sequences under (i), or (ii) is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

In one embodimentexample, each of the amino acid sequences under (i), or (ii) is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared

to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

In one [embodimentexample](#), at least one RNA is a modified RNA, in particular a stabilized mRNA. In one [embodimentexample](#), at least one RNA comprises a modified nucleoside in place of at least one uridine. In one [embodimentexample](#), at least one RNA comprises a modified nucleoside in place of each uridine. In one [embodimentexample](#), each RNA comprises a modified nucleoside in place of at least one uridine. In one [embodimentexample](#), each RNA comprises a modified nucleoside in place of each uridine. In one [embodimentexample](#), the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1 ψ), and 5-methyl-uridine (m5U).

In one [embodimentexample](#), at least one RNA comprises the 5' cap $m_2^{7,2'-O}Gpp_p(5')G$.

In one [embodimentexample](#), each RNA comprises the 5' cap $m_2^{7,2'-O}Gpp_p(5')G$.

In one [embodimentexample](#), at least one RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 30, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30.

In one [embodimentexample](#), each RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 30, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30.

In one [embodimentexample](#), at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 31, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 31.

In one [embodimentexample](#), each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 31, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 31.

In one [embodimentexample](#), at least one RNA comprises a poly-A sequence.

In one [embodimentexample](#), each RNA comprises a poly-A sequence.

In one [embodimentexample](#), the poly-A sequence comprises at least 100 nucleotides.

In one [embodimentexample](#), the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 32.

In one [embodimentexample](#), the amino acid sequence under (i), i.e., the amino acid sequence comprising HPV E6 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof, comprises from N-terminus to C-terminus: N-secretory signal peptide-E6-amino acid sequence which breaks immunological tolerance-amino acid sequence enhancing antigen processing and/or presentation-C.

In one [embodimentexample](#), the amino acid sequence under (ii), i.e., the amino acid sequence comprising HPV E7 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E7 protein or the immunogenic variant thereof, comprises from N-terminus to C-terminus: N-secretory signal peptide-E7-amino acid sequence which breaks immunological tolerance-amino acid sequence enhancing antigen processing and/or presentation-C.

In one [embodimentexample](#), the RNA is administered by injection.

In one [embodimentexample](#), the RNA is administered by intravenous administration.

In one [embodimentexample](#), the RNA is formulated as lipoplex particles.

In one [embodimentexample](#), the RNA lipoplex particles are obtainable by mixing the RNA with liposomes.

In one [embodimentexample](#), the method described herein further comprises administering to the subject an immune checkpoint inhibitor.

In one [embodimentexample](#), the immune checkpoint inhibitor is selected from a PD-1 inhibitor, and a PD-L1 inhibitor.

In one [embodimentexample](#), the immune checkpoint inhibitor is selected from an anti-PD-1 antibody, and an anti-PD-L1 antibody.

In one [embodimentexample](#), the immune checkpoint inhibitor is an anti-PD-1 antibody.

In one [embodimentexample](#), the anti-PD-1 antibody is pembrolizumab (KEYTRUDA; MK-3475), nivolumab (OPDIVO; BMS-936558), pidilizumab (CT-011), cemiplimab (LIBTAYO, REGN2810), spartalizumab (PDR001), MEDI0680 (AMP-514), dostarlimab (TSR-042), cetrelimab (JNJ

63723283), toripalimab (JS001), AMP-224 (GSK-2661380), PF-06801591, tislelizumab (BGB-A317), ABBV-181, BI 754091, or SHR-1210.

In one [embodimentexample](#), the immune checkpoint inhibitor is an anti-PD-L1 antibody.

In one [embodimentexample](#), the anti-PD-L1 antibody is atezolizumab (TECENTRIQ; RG7446; MPDL3280A; R05541267), durvalumab (MEDI4736), BMS-936559, avelumab (bavencio), lodapolimab (LY3300054), CX-072 (Proclaim-CX-072), FAZ053, KN035, or MDX-1105.

In one [embodimentexample](#), the subject is a human.

In one [embodimentexample](#), the method described herein further comprises administering to the subject radiotherapy, preferably local radiotherapy.

In one [embodimentexample](#), the method described herein comprises administering to the subject radiotherapy, preferably local radiotherapy, and an immune checkpoint inhibitor.

In one [embodimentexample](#), the method further comprises administering a further therapy.

In one [embodimentexample](#), the further therapy comprises surgery to excise, resect, or debulk a tumor, and/or chemotherapy. In one [embodimentexample](#), the further therapy comprises administering a further therapeutic agent. In one [embodimentexample](#), the further therapeutic agent comprises an anti-cancer therapeutic agent.

In one [embodimentexample](#), the method described herein further comprises administering to the subject a platinum compound.

In one [embodimentexample](#), the platinum compound comprises cisplatin.

In one [embodimentexample](#), the method described herein comprises administering to the subject an immune checkpoint inhibitor and a platinum compound.

In one [embodimentexample](#), the method described herein comprises administering to the subject radiotherapy, preferably local radiotherapy, and a platinum compound.

In one [embodimentexample](#), the method described herein comprises administering to the subject radiotherapy, preferably local radiotherapy, an immune checkpoint inhibitor and a platinum compound.

In one [aspectexample](#), provided herein is RNA described herein, e.g.,

- (i) RNA encoding an amino acid sequence comprising HPV E6 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof; and
 - (ii) RNA encoding an amino acid sequence comprising HPV E7 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E7 protein or the immunogenic variant thereof,
- for use in a method described herein.

Brief description of the drawings

Figure 1: Immunization with E6 and E7 RNA-LPX induces a strong E6 and E7 antigen-specific T cell response in humanized, HLA-transgenic A2DR1 mice

A2DR1 mice (expressing human HLA-A*0201 and HLA-DRB1*01 molecules) (n=3/group) were immunized with 30 µg E6 or E7 RNA-LPX (d0, d7, d14, d21) and spleens excised seven days after the last immunization (d28) to assess T cell reactivities against vaccine-encoded antigens in IFN γ ELISpot. T cell responses of (A) E6 RNA-LPX and (B) E7 RNA-LPX vaccinated A2DR1 mice were analyzed against E6, E7 or control protein (pp65) overlapping peptide pools and IFN γ spots counted. Significance was determined using one-way ANOVA, Tukey's post-test. Mean $\pm$ SEM.

Figure 2: E7 RNA-LPX immunization mediates complete remission of progressing HPV16-positive tumors and establishes protective T cell memory

(A-D) TC-1 luc tumor cells were grafted into the submucosal lining of the tongue. Five days later mice were immunized with E7 RNA-LPX (n=13) or irrelevant (OVA₂₅₇₋₂₆₄) RNA-LPX (n=12). (A) TC-1 luc tumor growth kinetics as measured by *in vivo* bioluminescence. (B-D) Mice were sacrificed 13 days after tumor-challenge and tumor tissues harvested (n=3/group). The proportion of (B) CD45⁺ and (C) E7₄₉₋₅₇ multimer⁺ CD8⁺ T cells in mice treated either with E7

Claims

1. A composition or medical preparation comprising at least one RNA, wherein the at least one RNA encodes:

(i) an amino acid sequence comprising human papillomavirus (HPV) E6 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof; and

(ii) an amino acid sequence comprising HPV E7 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E7 protein or the immunogenic variant thereof.

2. The composition or medical preparation of claim 1, wherein each of the amino acid sequences under (i), or (ii) is encoded by a separate RNA.

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

(i) the RNA encoding the amino acid sequence under (i) comprises the nucleotide sequence of SEQ ID NO: 2, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 2; and/or

(ii) the amino acid sequence under (i) comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 1.

4. The composition or medical preparation of any one of claims 1 to 3, wherein

(i) the RNA encoding the amino acid sequence under (ii) comprises the nucleotide sequence of SEQ ID NO: 4, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 4; and/or

(ii) the amino acid sequence under (ii) comprises the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 3.

5. The composition or medical preparation of any one of claims 1 to 4, wherein at least one amino acid sequence under (i), or (ii) comprises an amino acid sequence enhancing antigen processing and/or presentation.

6. The composition or medical preparation of any one of claims 1 to 5, wherein each amino acid sequence under (i), or (ii) comprises an amino acid sequence enhancing antigen processing and/or presentation.

7. The composition or medical preparation of claim 5 or 6, wherein the amino acid sequence enhancing antigen processing and/or presentation comprises an amino acid sequence corresponding to the transmembrane and cytoplasmic domain of a MHC molecule, preferably a MHC class I molecule.

8. The composition or medical preparation of any one of claims 5 to 7, wherein the amino acid sequence enhancing antigen processing and/or presentation comprises the amino acid sequence of SEQ ID NO: 26, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 26.

9. The composition or medical preparation of any one of claims 1 to 8, wherein at least one amino acid sequence under (i), or (ii) comprises an amino acid sequence which breaks immunological tolerance and/or at least one RNA is to be co-administered with RNA encoding an amino acid sequence which breaks immunological tolerance.

10. The composition or medical preparation of any one of claims 1 to 9, wherein each amino acid sequence under (i), or (ii) comprises an amino acid sequence which breaks immunological tolerance and/or each RNA is to be co-administered with RNA encoding an amino acid sequence which breaks immunological tolerance.

11. The composition or medical preparation of claim 9 or 10, wherein the amino acid sequence which breaks immunological tolerance comprises helper epitopes, preferably tetanus toxoid-derived helper epitopes.

12. The composition or medical preparation of any one of claims 9 to 11, wherein the amino acid sequence which breaks immunological tolerance comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 27.

13. The composition or medical preparation of any one of claims 1 to 12, wherein at least one of the amino acid sequences under (i), or (ii) is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

14. The composition or medical preparation of any one of claims 1 to 13, wherein each of the amino acid sequences under (i), or (ii) is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

15. The composition or medical preparation of any one of claims 1 to 14, wherein at least one RNA comprises the 5' cap $m_2^{7,2'-O}Gppsp(5')G$.

16. The composition or medical preparation of any one of claims 1 to 15, wherein each RNA comprises the 5' cap $m_2^{7,2'-O}Gppsp(5')G$.

17. The composition or medical preparation of any one of claims 1 to 16, wherein at least one RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 30, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30.

18. The composition or medical preparation of any one of claims 1 to 17, wherein each RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 30, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30.

19. The composition or medical preparation of any one of claims 1 to 18, wherein at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 31, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 31.

20. The composition or medical preparation of any one of claims 1 to 19, wherein each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 31, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 31.

21. The composition or medical preparation of any one of claims 1 to 20, wherein at least one RNA comprises a poly-A sequence.

22. The composition or medical preparation of any one of claims 1 to 21, wherein each RNA comprises a poly-A sequence.

23. The composition or medical preparation of claim 21 or 22, wherein the poly-A sequence comprises at least 100 nucleotides.

24. The composition or medical preparation of any one of claims 21 to 23, wherein the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 32.

25. The composition or medical preparation of any one of claims 1 to 24, wherein the RNA is formulated as a liquid, formulated as a solid, or a combination thereof.

26. The composition or medical preparation of any one of claims 1 to 25, wherein the RNA is formulated for injection.

27. The composition or medical preparation of any one of claims 1 to 26, wherein the RNA is formulated for intravenous administration.

28. The composition or medical preparation of any one of claims 1 to 27, wherein the RNA is formulated or is to be formulated as lipoplex particles.

29. The composition or medical preparation of claim 28, wherein the RNA lipoplex particles are obtainable by mixing the RNA with liposomes.

30. The composition or medical preparation of any one of claims 1 to 29, which further comprises an immune checkpoint inhibitor.

31. The composition or medical preparation of claim 30, wherein the immune checkpoint inhibitor is selected from a PD-1 inhibitor, and a PD-L1 inhibitor.

32. The composition or medical preparation of claim 30 or 31, wherein the immune checkpoint inhibitor is selected from an anti-PD-1 antibody, and an anti-PD-L1 antibody.

33. The composition or medical preparation of any one of claims 30 to 32, wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.

34. The composition or medical preparation of claim 33, wherein the anti-PD-1 antibody is pembrolizumab (KEYTRUDA; MK-3475), nivolumab (OPDIVO; BMS-936558), pidilizumab (CT-011), cemiplimab (LIBTAYO, REGN2810), spartalizumab (PDR001), MEDI0680 (AMP-514), dostarlimab (TSR-042), cetrelimab (JNJ 63723283), toripalimab (JS001), AMP-224 (GSK-2661380), PF-06801591, tislelizumab (BGB-A317), ABBV-181, BI 754091, or SHR-1210.

35. The composition or medical preparation of any one of claims 30 to 34, wherein the immune checkpoint inhibitor is an anti-PD-L1 antibody.

36. The composition or medical preparation of claim 35, wherein the anti-PD-L1 antibody is atezolizumab (TECENTRIQ; RG7446; MPDL3280A; R05541267), durvalumab (MEDI4736), BMS-936559, avelumab (bavencio), lodapolimab (LY3300054), CX-072 (Proclaim-CX-072), FAZ053, KN035, or MDX-1105.

37. The composition or medical preparation of any one of claims 1 to 36, which further comprises a platinum compound.

38. The composition or medical preparation of claim 37, wherein the platinum compound comprises cisplatin.

39. The composition or medical preparation of any one of claims 1 to 38, which comprises an immune checkpoint inhibitor and a platinum compound.

40. The composition or medical preparation of any one of claims 1 to 39, which is a pharmaceutical composition.

41. The composition or medical preparation of claim 40, wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

42. The composition or medical preparation of any one of claims 1 to 39, wherein the medical preparation is a kit.

43. The composition or medical preparation of claim 42, wherein the RNAs and optionally the immune checkpoint inhibitor are in separate vials.

44. The composition or medical preparation of claim 42 or 43, further comprising instructions for use of the RNAs and optionally the immune checkpoint inhibitor and/or the platinum compound for treating or preventing HPV-positive cancer.

45. The composition or medical preparation of any one of claims 1 to 44 for use in therapy ~~pharmaceutical use~~.

46. The composition or medical preparation of claim 45, wherein the ~~pharmaceutical use~~therapy comprises a therapeutic or prophylactic treatment of a disease or disorder.

47. The composition or medical preparation of claim 46, wherein the therapeutic or prophylactic treatment of a disease or disorder comprises treating or preventing HPV-positive cancer.

48. The composition or medical preparation of any one of claims 1 to 47, which is to be for administration-administered to a human.

49. The composition or medical preparation of any one of claims 46 to 48, wherein the therapeutic or prophylactic treatment of a disease or disorder further comprises ~~administering~~ radiotherapy, preferably local radiotherapy to be administered.

50. ~~A method of treating HPV-positive cancer in a subject comprising administering~~ Use of at least one RNA to the subject in the manufacture of a medicament for treating HPV-positive cancer in a subject, wherein the at least one RNA encodes:

- (i) an amino acid sequence comprising human papillomavirus (HPV) E6 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof; and
- (ii) an amino acid sequence comprising HPV E7 protein, an immunogenic variant thereof, or an immunogenic fragment of the HPV E6 protein or the immunogenic variant thereof.

51. The ~~method-use~~ of claim 50, wherein each of the amino acid sequences under (i), or (ii) is encoded by a separate RNA.

52. The ~~usemethod~~ of claim 50 or 51, wherein

- (i) the RNA encoding the amino acid sequence under (i) comprises the nucleotide sequence of SEQ ID NO: 2, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 2; and/or
- (ii) the amino acid sequence under (i) comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 1.

53. The ~~usemethod~~ of any one of claims 50 to 52, wherein

- (i) the RNA encoding the amino acid sequence under (ii) comprises the nucleotide sequence of SEQ ID NO: 4, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 4; and/or
- (ii) the amino acid sequence under (ii) comprises the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 3.

54. The ~~use method~~ of any one of claims 50 to 53, wherein at least one amino acid sequence under (i), or (ii) comprises an amino acid sequence enhancing antigen processing and/or presentation.

55. The ~~use method~~ of any one of claims 50 to 54, wherein each amino acid sequence under (i), or (ii) comprises an amino acid sequence enhancing antigen processing and/or presentation.

56 The ~~use method~~ of claim 54 or 55, wherein the amino acid sequence enhancing antigen processing and/or presentation comprises an amino acid sequence corresponding to the transmembrane and cytoplasmic domain of a MHC molecule, preferably a MHC class I molecule.

57. The ~~use method~~ of any one of claims 54 to 56, wherein the amino acid sequence enhancing antigen processing and/or presentation comprises the amino acid sequence of SEQ ID NO: 26, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 26.

58. The ~~use method~~ of any one of claims 50 to 57, wherein at least one amino acid sequence under (i), or (ii) comprises an amino acid sequence which breaks immunological tolerance and/or at least one RNA is to be co-administered with RNA encoding an amino acid sequence which breaks immunological tolerance.

59. The ~~use method~~ of any one of claims 50 to 58, wherein each amino acid sequence under (i), or (ii) comprises an amino acid sequence which breaks immunological tolerance and/or each RNA is to be co-administered with RNA encoding an amino acid sequence which breaks immunological tolerance.

60. The ~~use method~~ of claim 58 or 59, wherein the amino acid sequence which breaks immunological tolerance comprises helper epitopes, preferably tetanus toxoid-derived helper epitopes.

61. The ~~usemethod~~ of any one of claims 58 to 60, wherein the amino acid sequence which breaks immunological tolerance comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 27.

62. The ~~usemethod~~ of any one of claims 50 to 61, wherein at least one of the amino acid sequences under (i), or (ii) is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

63. The ~~usemethod~~ of any one of claims 50 to 62, wherein each of the amino acid sequences under (i), or (ii) is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

64. The ~~usemethod~~ of any one of claims 50 to 63, wherein at least one RNA comprises the 5' cap $m_2^{7,2'-O}Gppp(5')G$.

65. The ~~usemethod~~ of any one of claims 50 to 64, wherein each RNA comprises the 5' cap $m_2^{7,2'-O}Gppp(5')G$.

66. The ~~usemethod~~ of any one of claims 50 to 65, wherein at least one RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 30, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30.

67. The ~~usemethod~~ of any one of claims 50 to 66, wherein each RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 30, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 30.

68. The ~~use~~method of any one of claims 50 to 67, wherein at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 31, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 31.

69. The ~~use~~method of any one of claims 50 to 68, wherein each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 31, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 31.

70. The ~~use~~method of any one of claims 50 to 69, wherein at least one RNA comprises a poly-A sequence.

71. The ~~use~~method of any one of claims 50 to 70, wherein each RNA comprises a poly-A sequence.

72. The ~~use~~method of claim 70 or 71, wherein the poly-A sequence comprises at least 100 nucleotides.

73. The ~~use~~method of any one of claims 70 to 72, wherein the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 32.

74. The ~~method~~use of any one of claims 50 to 73, wherein the RNA is to be administered by injection.

75. The ~~use~~method of any one of claims 50 to 74, wherein the RNA is to be administered by intravenous administration.

76. The ~~use~~method of any one of claims 50 to 75, wherein the RNA is formulated as lipoplex particles.

77. The ~~use~~method of any one of claim 76, wherein the RNA lipoplex particles are obtainable by mixing the RNA with liposomes.

78. The ~~use~~method of any one of claims 50 to 77, which further comprises ~~administering to the subject~~ an immune checkpoint inhibitor to be administered to the subject.

79. The ~~method~~use of claim 78, wherein the immune checkpoint inhibitor is selected from a PD-1 inhibitor, and a PD-L1 inhibitor.

80. The ~~use~~method of claim 78 or 79, wherein the immune checkpoint inhibitor is selected from an anti-PD-1 antibody, and an anti-PD-L1 antibody.

81. The ~~use~~method of any one of claims 78 to 80, wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.

82. The ~~use~~method of claim 81, wherein the anti-PD-1 antibody is pembrolizumab (KEYTRUDA; MK-3475), nivolumab (OPDIVO; BMS-936558), pidilizumab (CT-011), cemiplimab (LIBTAYO, REGN2810), spartalizumab (PDR001), MEDI0680 (AMP-514), dostarlimab (TSR-042), cetrelimab (JNJ 63723283), toripalimab (JS001), AMP-224 (GSK-2661380), PF-06801591, tislelizumab (BGB-A317), ABBV-181, BI 754091, or SHR-1210.

83. The ~~use~~method of any one of claims 78 to 82, wherein the immune checkpoint inhibitor is an anti-PD-L1 antibody.

84. The ~~use~~method of claim 83, wherein the anti-PD-L1 antibody is atezolizumab (TECENTRIQ; RG7446; MPDL3280A; R05541267), durvalumab (MEDI4736), BMS-936559, avelumab (bavencio), lodapolimab (LY3300054),⁷ CX-072 (Proclaim-CX-072), FAZ053, KN035, or MDX-1105.

85. The ~~use~~method of any one of claims 50 to 84, which further comprises ~~administering to the subject~~ a platinum compound to be administered to the subject.

86. The ~~use~~method of claim 85, wherein the platinum compound comprises cisplatin.

87. The ~~use~~method of any one of claims 50 to 86, which comprises ~~administering to the subject~~ an immune checkpoint inhibitor and a platinum compound to be administered to the subject.

88. The ~~use~~method of any one of claims 50 to 87, wherein the subject is a human.

89. The ~~use~~method of any one of claims 50 to 88, which further comprises ~~administering to the subject~~ radiotherapy, preferably local radiotherapy to be administered to the subject.