rash, bilirubinemia, thrombocytopenia, nausea, confusion, creatinine increase, anemia, fever, peripheral edema, and malaise (Proleukin® Prescribing Information 2012).

Decades of experience with rIL-2 treatment has improved the management of adverse events. Most side effects are easily managed by experienced personnel with the majority of toxicities being reversible upon treatment discontinuation. In addition, established screening guidelines have reduced the risk of treatment-related mortality to essentially zero (Marabondo S, Kaufman HL, Expert Opin Drug Saf 2017; 16(12): 1347–57).

Summary

The present ~~invention~~disclosure generally embraces the immunotherapeutic treatment of a subject comprising the administration of (i) RNA encoding an amino acid sequence comprising human IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof, and/or (ii) RNA encoding an amino acid sequence comprising human IL2 (hIL2), a functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof. One or both of these RNAs are also designated "immunostimulant RNA" herein. In one ~~embodiment~~example, the immunostimulant, i.e., the hIL, a functional variant thereof, or a functional fragment of the hIL or the functional variant thereof, is fused, either directly or through a linker, to human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof.

In one ~~embodiment~~example, the treatment comprises the administration of (iii) RNA, i.e., vaccine RNA, encoding an amino acid sequence, i.e., a vaccine antigen, comprising a target antigen, an immunogenic variant thereof, or an immunogenic fragment of the target antigen or the immunogenic variant thereof, i.e., an antigenic peptide or protein. Thus, the vaccine antigen comprises an epitope of the target antigen for inducing an immune response against the target antigen or cells expressing the target antigen 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 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 immune response against tumor or cancer cells, in particular tumor or cancer cells expressing a tumor antigen.

The compositions and methods described herein comprise 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 coding sequences, 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). 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, in the vaccine RNA, 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 ~~embodiment~~example, a vaccine antigen comprises an amino acid sequence which breaks immunological tolerance. In one ~~embodiment~~example, 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.

In one ~~embodiment~~example, 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.

The RNA, i.e., immunostimulant RNA and vaccine RNA, may be formulated in lipid particles to generate serum-stable formulations for intravenous (IV) administration. The immunostimulant RNA may be present in lipid nanoparticles (LNP). RNA-nanoparticles may target liver which results in an efficient expression of the encoded protein. In one ~~embodiment~~example, the immunostimulant RNA described herein is N1-methylpseudouridine modified, dsRNA-purified RNA which is formulated as lipid nanoparticles for intravenous administration. The vaccine RNA may be present in RNA-lipoplexes (LPX). RNA-lipoplexes may target antigen-presenting cells (APCs) in lymphoid organs which results in an efficient stimulation of the immune system. Different RNAs may be separately complexed with lipids to generate particulate formulations. In one ~~embodiment~~example, vaccine RNA is co-formulated as particles with an RNA encoding an amino acid sequence which breaks immunological tolerance.

In one aspect, provided herein is a composition or medical preparation comprising at least one RNA, wherein the at least one RNA encodes:

(i) an amino acid sequence comprising human IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof; and/or

(ii) an amino acid sequence comprising human IL2 (hIL2), a functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof.

In one ~~embodiment~~example, the amino acid sequence under (i) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof. In one ~~embodiment~~example, the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused with the hIL7, the functional variant thereof, or the functional fragment of the hIL7 or the functional variant thereof. In one ~~embodiment~~example, the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused to the C-terminus of the hIL7, the functional variant thereof, or the functional fragment of the hIL7 or the functional variant thereof.

In one ~~embodiment~~example, the amino acid sequence under (ii) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof. In one ~~embodiment~~example, the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused with the hIL2, the functional variant thereof, or the functional fragment of the hIL2 or the functional variant thereof. In one ~~embodiment~~example, the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused to the N-terminus of the hIL2, the functional variant thereof, or the functional fragment of the hIL2 or the functional 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: 5, 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: 5; and/or

(ii) the amino acid sequence under (i) comprises the amino acid sequence of SEQ ID NO: 4, 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: 4.

In one ~~embodiment~~example:

(i) the RNA encoding the amino acid sequence under (ii) comprises the nucleotide sequence of SEQ ID NO: 7, 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: 7; and/or

(ii) the amino acid sequence under (ii) comprises the amino acid sequence of SEQ ID NO: 6, 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: 6.

In one ~~embodiment~~example, 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 ~~embodiment~~example, 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 ~~embodiment~~example, at least one RNA comprises the 5' cap $m_2^{7,3'\text{-}O}Gppp(m_1^{2'\text{-}O})ApG$. In one ~~embodiment~~example, each RNA comprises the 5' cap $m_2^{7,3'\text{-}O}Gppp(m_1^{2'\text{-}O})ApG$.

In one ~~embodiment~~example, at least one RNA is a modified RNA, in particular a stabilized mRNA. In one ~~embodiment~~example, at least one RNA comprises a modified nucleoside in place of at least one uridine. In one ~~embodiment~~example, at least one RNA comprises a modified nucleoside in place of each uridine. In one ~~embodiment~~example, each RNA comprises a modified nucleoside in place of at least one uridine. In one ~~embodiment~~example, each RNA comprises a modified nucleoside in place of each uridine. In one ~~embodiment~~example, the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one ~~embodiment~~example, at least one RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 13, 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: 13. In one ~~embodiment~~example, each RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 13, 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: 13.

In one ~~embodiment~~example, at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 14, 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: 14. In one ~~embodiment~~example, each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 14, 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: 14.

In one ~~embodiment~~example, at least one RNA comprises a poly-A sequence. In one ~~embodiment~~example, each RNA comprises a poly-A sequence. In one ~~embodiment~~example, the poly-A sequence comprises at least 100 nucleotides. In one ~~embodiment~~example, the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 15.

In one ~~embodiment~~example, the amino acid sequence under (i), i.e., the amino acid sequence comprising human IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof, comprises from N-terminus to C-terminus: N-hIL7-GS-linker-hAlb-C.

In one ~~embodiment~~example, the amino acid sequence under (ii), i.e., the amino acid sequence comprising human IL2 (hIL2), a functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof, comprises from N-terminus to C-terminus: N-hAlb-GS-linker-hIL2-C.

In one ~~embodiment~~example, the RNA is formulated as a liquid, formulated as a solid, or a combination thereof. In one ~~embodiment~~example, the RNA is formulated for injection. In one ~~embodiment~~example, the RNA is formulated for intravenous administration. In one ~~embodiment~~example, the RNA is formulated or is to be formulated as lipid particles. In one ~~embodiment~~example, the RNA lipid particles are lipid nanoparticles (LNP). In one ~~embodiment~~example, the LNP particles comprise 3D-P-DMA, PEG_{2000}-C-DMA, DSPC, and cholesterol.

In one ~~embodiment~~example, the composition or medical preparation is a pharmaceutical composition. In one ~~embodiment~~example, the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

In one ~~embodiment~~example, the composition or medical preparation is a kit. In one ~~embodiment~~example, the RNA encoding the amino acid sequence under (i) and the RNA encoding the amino acid sequence under (ii) are in separate vials. In one ~~embodiment~~example, the composition or medical preparation comprises instructions for use of the RNAs for treating or preventing cancer.

In one aspect, the present disclosure provides the composition or medical preparation as described herein for use in therapy. In another aspect, the present disclosure provides a use of the composition or medical preparation as described herein in the manufacture of a medicament for therapeutic or prophylactic treatment of a disease or disorder, preferably wherein the therapeutic or prophylactic

treatment of a disease or disorder comprises treating or preventing cancer. In yet another aspect, the present disclosure provides a use of at least one RNA in the manufacture of a medicament for treating cancer in a subject, wherein the use comprises that the at least one RNA is to be administered to the subject, preferably a human subject, wherein the at least one RNA encodes:

(i) an amino acid sequence comprising human IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof; and/or

(ii) an amino acid sequence comprising human IL2 (hIL2), an functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof.

In one ~~aspect~~example, provided herein is the composition or medical preparation described herein for pharmaceutical use. In one ~~embodiment~~example, the pharmaceutical use comprises a therapeutic or

prophylactic treatment of a disease or disorder. In one ~~embodiment~~example, the therapeutic or prophylactic treatment of a disease or disorder comprises treating or preventing cancer.

In one ~~embodiment~~example, the composition or medical preparation is for administration to a human.

In one ~~aspect~~example, provided herein is a method of treating 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 IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof; and/or

(ii) an amino acid sequence comprising human IL2 (hIL2), an functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof.

In one ~~embodiment~~example, the amino acid sequence under (i) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof. In one ~~embodiment~~example, the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused with the hIL7, the functional variant thereof, or the functional fragment of the hIL7 or the functional variant thereof. In one ~~embodiment~~example, the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused to the C-terminus of the hIL7, the functional variant thereof, or the functional fragment of the hIL7 or the functional variant thereof.

In one ~~embodiment~~example, the amino acid sequence under (ii) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof. In one ~~embodiment~~example, the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused with the hIL2, the functional variant thereof, or the functional fragment of the hIL2 or the functional variant thereof. In one ~~embodiment~~example, the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused to the

N-terminus of the hIL2, the functional variant thereof, or the functional fragment of the hIL2 or the functional 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: 5, 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: 5; and/or

(ii) the amino acid sequence under (i) comprises the amino acid sequence of SEQ ID NO: 4, 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: 4.

In one ~~embodiment~~example:

(i) the RNA encoding the amino acid sequence under (ii) comprises the nucleotide sequence of SEQ ID NO: 7, 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: 7; and/or

(ii) the amino acid sequence under (ii) comprises the amino acid sequence of SEQ ID NO: 6, 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: 6.

In one ~~embodiment~~example, 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 ~~embodiment~~example, 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 ~~embodiment~~example, at least one RNA is a modified RNA, in particular a stabilized mRNA. In one ~~embodiment~~example, at least one RNA comprises a modified nucleoside in place of at least one uridine. In one ~~embodiment~~example, at least one RNA comprises a modified nucleoside in place of each uridine. In one ~~embodiment~~example, each RNA comprises a modified nucleoside in place of at least one uridine. In one ~~embodiment~~example, each RNA comprises a modified nucleoside in place of each uridine. In one ~~embodiment~~example, the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one ~~embodiment~~example, at least one RNA comprises the 5' cap $m_2^{7,3'\text{-}O}Gppp(m_1^{2'\text{-}O})ApG$. In one ~~embodiment~~example, each RNA comprises the 5' cap $m_2^{7,3'\text{-}O}Gppp(m_1^{2'\text{-}O})ApG$.

In one ~~embodiment~~example, at least one RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 13, 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: 13. In one ~~embodiment~~example, each RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 13, 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: 13.

In one ~~embodiment~~example, at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 14, 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: 14. In one ~~embodiment~~example, each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 14, 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: 14.

In one ~~embodiment~~example, at least one RNA comprises a poly-A sequence. In one ~~embodiment~~example, each RNA comprises a poly-A sequence. In one ~~embodiment~~example, the poly-A sequence comprises at least 100 nucleotides. In one ~~embodiment~~example, the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 15.

In one ~~embodiment~~example, the amino acid sequence under (i), i.e., the amino acid sequence comprising human IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof, comprises from N-terminus to C-terminus: N-hIL7-GS-linker-hAlb-C.

In one ~~embodiment~~example, the amino acid sequence under (ii), i.e., the amino acid sequence comprising human IL2 (hIL2), a functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof, comprises from N-terminus to C-terminus: N-hAlb-GS-linker-hIL2-C.

In one ~~embodiment~~example, the RNA is formulated as a liquid, formulated as a solid, or a combination thereof. In one ~~embodiment~~example, the RNA is administered by injection. In one ~~embodiment~~example, the RNA is administered by intravenous administration. In one ~~embodiment~~example, the RNA is formulated as lipid particles. In one ~~embodiment~~example, the RNA lipid particles are lipid nanoparticles (LNP). In one ~~embodiment~~example, the LNP particles comprise 3D-P-DMA, PEG_{2000}-C-DMA, DSPC, and cholesterol. In one ~~embodiment~~example, the RNA is formulated as a pharmaceutical composition. In one ~~embodiment~~example, the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

In one ~~embodiment~~example, the subject is a human.

In one ~~embodiment~~example, the composition or medical preparation described herein comprises RNA, which encodes:

(iii) an amino acid sequence comprising a target antigen, an immunogenic variant thereof, or an immunogenic fragment of the target antigen or the immunogenic variant thereof.

In one ~~embodiment~~example, the method described herein comprises administering RNA to the subject, wherein the RNA encodes:

(iii) an amino acid sequence comprising a target antigen, an immunogenic variant thereof, or an immunogenic fragment of the target antigen or the immunogenic variant thereof.

In one ~~embodiment~~example, the target antigen is a tumor antigen.

In one ~~embodiment~~example, the amino acid sequence under (iii) comprises an amino acid sequence enhancing antigen processing and/or presentation.

In one ~~embodiment~~example, 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 ~~embodiment~~example, the amino acid sequence enhancing antigen processing and/or presentation comprises the amino acid sequence of SEQ ID NO: 9, 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: 9.

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

In one ~~embodiment~~example, the secretory signal peptide comprises the amino acid sequence of SEQ ID NO: 8, 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: 8.

In one ~~embodiment~~example, the amino acid sequence under (iii) comprises an amino acid sequence which breaks immunological tolerance and/or the RNA is co-administered with RNA encoding an amino acid sequence which breaks immunological tolerance.

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

In one ~~embodiment~~example, the amino acid sequence which breaks immunological tolerance comprises the amino acid sequence of SEQ ID NO: 10, 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: 10.

In one ~~embodiment~~example the amino acid sequence under (iii) 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 ~~embodiment~~example, the RNA is a modified RNA, in particular a stabilized mRNA. In one ~~embodiment~~example, the RNA comprises a modified nucleoside in place of at least one uridine. In one ~~embodiment~~example, the RNA comprises a modified nucleoside in place of each uridine. In one ~~embodiment~~example, the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one ~~embodiment~~example, the RNA comprises the 5' cap $m_2^{7,2'\text{-}O}Gpp_sp(5')G$.

In one ~~embodiment~~example, the RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 13, 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: 13.

In one ~~embodiment~~example, the RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 14, 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: 14.

In one ~~embodiment~~example, the RNA comprises a poly-A sequence.

In one ~~embodiment~~example, the poly-A sequence comprises at least 100 nucleotides.

In one ~~embodiment~~example, the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 15.

In one ~~embodiment~~example, the amino acid sequence under (iii), i.e., the amino acid sequence comprising a target antigen, an immunogenic variant thereof, or an immunogenic fragment of the target antigen or the immunogenic variant thereof, comprises from N-terminus to C-terminus: N-antigen-amino acid sequence which breaks immunological tolerance-amino acid sequence enhancing antigen processing and/or presentation-C.

In one ~~embodiment~~example, the RNA is formulated as a liquid, formulated as a solid, or a combination thereof.

In one ~~embodiment~~example, the RNA is formulated for injection and/or is administered by injection.

In one ~~embodiment~~example, the RNA is formulated for intravenous administration and/or is administered by intravenous administration.

In one ~~embodiment~~example, the RNA is formulated or is to be formulated as lipoplex particles.

In one ~~embodiment~~example, the RNA lipoplex particles are obtainable by mixing the RNA with liposomes.

In one ~~aspect~~example, provided herein is RNA described herein, e.g.,

(i) RNA encoding an amino acid sequence comprising human IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof; and/or

(ii) RNA encoding an amino acid sequence comprising human IL2 (hIL2), a functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof; and optionally

(iii) RNA encoding an amino acid sequence comprising a target antigen, an immunogenic variant thereof, or an immunogenic fragment of the target antigen or the immunogenic variant thereof,

for use in a method described herein.

In the above and further ~~aspect~~examples, provided herein is a composition comprising lipid nanoparticles (LNP) comprising RNA, 3D-P-DMA, a pegylated lipid, a neutral lipid, in particular a phospholipid, and a steroid such as cholesterol. In one ~~embodiment~~example, the pegylated lipid is PEG_{2000}-C-DMA. In one ~~embodiment~~example, the phospholipid is DSPC. In one ~~embodiment~~example, the pegylated lipid is PEG_{2000}-C-DMA and the phospholipid is DSPC. In some ~~embodiment~~examples, the 3D-P-DMA is present in the LNP in an amount from about 40 to about 60 mole percent, the pegylated lipid such as PEG_{2000}-C-DMA is present in the LNP in an amount from about 1 to about 10 mole percent, the neutral lipid such as DSPC is present in the LNP in an amount from about 5 to about 15 mole percent, and the steroid such as cholesterol is present in the LNP in an amount from about 30 to about 50 mole percent. In some ~~embodiment~~examples, the 3D-P-DMA is present in the LNP in an amount of about 54 mole percent, the pegylated lipid such as PEG_{2000}-C-DMA is present in the LNP in an amount of about 1.6 mole percent, the neutral lipid such as DSPC is present in the LNP in an amount of about 11 mole percent, and the steroid such as cholesterol is present in the LNP in an amount of about 33 mole percent.

In one ~~embodiment~~example, the composition is an aqueous composition. In one ~~embodiment~~example, the composition comprises a Tris/HCl buffer. In one ~~embodiment~~example, the composition comprises sucrose and/or maltose. In one ~~embodiment~~example, the RNA is (i) RNA encoding an amino acid sequence comprising human IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof; and/or (ii) RNA encoding an amino acid sequence comprising human IL2 (hIL2), a functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof. ~~Embodiment~~Examples of this RNA are described herein.

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 IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof; and/or
(ii) an amino acid sequence comprising human IL2 (hIL2), a functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof.

2. The composition or medical preparation of claim 1, wherein the amino acid sequence under (i) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof and/or wherein the amino acid sequence under (ii) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof.

3. The composition or medical preparation of claim 2, wherein the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused with the hIL7 and/or hIL2, the functional variant thereof, or the functional fragment of the hIL7 and/or hIL2 or the functional variant thereof, preferably.

~~4. The composition or medical preparation of claim 3,~~ wherein the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused to the C-terminus of the hIL7 and/or hIL2, the functional variant thereof, or the functional fragment of the hIL7 and/or hIL2 or the functional variant thereof.

~~5. The composition or medical preparation of any one of claims 1 to 4, wherein the amino acid sequence under (ii) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof.~~

~~6. The composition or medical preparation of claim 5, wherein the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused with~~

~~the hIL2, the functional variant thereof, or the functional fragment of the hIL2 or the functional variant thereof.~~

~~7. The composition or medical preparation of claim 6, wherein the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused to the N-terminus of the hIL2, the functional variant thereof, or the functional fragment of the hIL2 or the functional variant thereof.~~

4~~8~~. The composition or medical preparation of any one of claims 1 to 3~~7~~, wherein each of the amino acid sequences under (i), or (ii) is encoded by a separate RNA.

5~~9~~. The composition or medical preparation of any one of claims 1 to 4~~8~~, wherein

(i) the RNA encoding the amino acid sequence under (i) comprises the nucleotide sequence of SEQ ID NO: 5, 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: 5; and/or

(ii) the amino acid sequence under (i) comprises the amino acid sequence of SEQ ID NO: 4, 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: 4; and/or wherein~~.~~

~~10. The composition or medical preparation of any one of claims 1 to 9, wherein~~

(i) the RNA encoding the amino acid sequence under (ii) comprises the nucleotide sequence of SEQ ID NO: 7, 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: 7; and/or

(ii) the amino acid sequence under (ii) comprises the amino acid sequence of SEQ ID NO: 6, 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: 6.

6~~11~~. The composition or medical preparation of any one of claims 1 to 5~~10~~, wherein each or 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.

~~12. The composition or medical preparation of any one of claims 1 to 11, 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.~~

7~~13~~. The composition or medical preparation of any one of claims 1 to 6~~12~~, wherein each or at least one RNA comprises the 5' cap $m_2^{7,3'\text{-O}}Gppp(m_1^{2'\text{-O}})ApG$.

~~14. The composition or medical preparation of any one of claims 1 to 13, wherein each RNA comprises the 5' cap $m_2^{7,3'\text{-O}}Gppp(m_1^{2'\text{-O}})ApG$.~~

8~~15~~. The composition or medical preparation of any one of claims 1 to 7~~14~~, wherein each or at least one RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 13, 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: 13.

~~16. The composition or medical preparation of any one of claims 1 to 15, wherein each RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 13, 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: 13.~~

9~~17~~. The composition or medical preparation of any one of claims 1 to 16, wherein each or at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 14, 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: 14.

~~18. The composition or medical preparation of any one of claims 1 to 17, wherein each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 14, 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: 14.~~

10~~19~~. The composition or medical preparation of any one of claims 1 to 18, wherein each or at least one RNA comprises a poly-A sequence, preferably wherein the poly-A sequence comprises at least 100 nucleotides, optionally wherein the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 15.

~~20. The composition or medical preparation of any one of claims 1 to 19, wherein each RNA comprises a poly-A sequence.~~

~~21. The composition or medical preparation of claim 19 or 20, wherein the poly-A sequence comprises at least 100 nucleotides.~~

~~22. The composition or medical preparation of any one of claims 19 to 21, wherein the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 15.~~

11~~23~~. The composition or medical preparation of any one of claims 1 to 10~~22~~, wherein the RNA is formulated as a liquid, formulated as a solid, or a combination thereof.

12~~24~~. The composition or medical preparation of any one of claims 1 to 11~~23~~, wherein the RNA is formulated for injection or~~.~~

~~25. The composition or medical preparation of any one of claims 1 to 24,~~ wherein the RNA is formulated for intravenous administration.

13~~26~~. The composition or medical preparation of any one of claims 1 to 12~~25~~, wherein the RNA is formulated or is to be formulated as lipid particles, preferably~~.~~

~~27. The composition or medical preparation of claim 26,~~ wherein the RNA lipid particles are lipid nanoparticles (LNP).

14~~28~~. The composition or medical preparation of any one of claims 1 to 13~~27~~, which is a pharmaceutical composition, preferably~~.~~

~~29. The composition or medical preparation of claim 28,~~ wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

15~~30~~. The composition or medical preparation of any one of claims 1 to 14~~27~~, wherein the medical preparation is a kit, preferably~~.~~

~~31. The composition or medical preparation of claim 30,~~ wherein the RNA encoding the amino acid sequence under (i) and the RNA encoding the amino acid sequence under (ii) are in separate vials.

16~~32~~. The composition or medical preparation of claim 15~~30 or 31~~, further comprising instructions for use of the RNAs for treating or preventing cancer.

17~~33~~. The composition or medical preparation of any one of claims 1 to 16~~32~~ for use in therapy~~pharmaceutical use~~.

18~~34~~. Use of t~~T~~he composition or medical preparation of any one of claims 1 to 16~~33, wherein the pharmaceutical use~~ in the manufacture of a medicament for ~~comprises a~~ therapeutic or prophylactic treatment of a disease or disorder, preferably~~.~~

~~35. The composition or medical preparation of claim 34,~~ wherein the therapeutic or prophylactic treatment of a disease or disorder comprises treating or preventing cancer.

19~~36~~. The composition or medical preparation of ~~any one of~~ claim~~s 1 to~~ 17~~35~~ or the use of claim 18, ~~which is for administration~~wherein the composition or medical preparation is to be administered to a human.

20~~37~~. Use of aAt least one RNA for use in ~~A method~~ the manufacture of a medicament for ~~of~~ treating cancer in a subject, wherein the use ~~comprising~~ comprises~~administering~~ that the at least one RNA is to be administered to the subject, preferably a human subject, wherein the at least one RNA encodes:

(i) an amino acid sequence comprising human IL7 (hIL7), a functional variant thereof, or a functional fragment of the hIL7 or the functional variant thereof; and/or
(ii) an amino acid sequence comprising human IL2 (hIL2), an functional variant thereof, or a functional fragment of the hIL2 or the functional variant thereof.

2138. The usemethod of claim 2037, wherein the amino acid sequence under (i) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof and/or wherein the amino acid sequence under (ii) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof.

2239. The usemethod of claim 2138, wherein the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused with the hIL7 and/or the hIL2, the functional variant thereof, or the functional fragment of the hIL7 and/or hIL2 or the functional variant thereof, preferably.

40. The method of claim 39, wherein the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused to the C-terminus of the hIL7 and/or hIL2, the functional variant thereof, or the functional fragment of the hIL7 and/or hIL2 or the functional variant thereof.

41. The method of any one of claims 37 to 40, wherein the amino acid sequence under (ii) comprises human albumin (hAlb), a functional variant thereof, or a functional fragment of the hAlb or the functional variant thereof.

42. The method of claim 41, wherein the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused with the hIL2, the functional variant thereof, or the functional fragment of the hIL2 or the functional variant thereof.

43. The method of claim 42, wherein the hAlb, the functional variant thereof, or the functional fragment of the hAlb or the functional variant thereof is fused to the N-terminus of the hIL2,

~~the functional variant thereof, or the functional fragment of the hIL2 or the functional variant thereof.~~

2344. The use~~method~~ of any one of claims 2037 to 2243, wherein each of the amino acid sequences under (i), or (ii) is encoded by a separate RNA.

2445. The use~~method~~ of any one of claims 2037 to 2344, wherein

(i) the RNA encoding the amino acid sequence under (i) comprises the nucleotide sequence of SEQ ID NO: 5, 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: 5; and/or

(ii) the amino acid sequence under (i) comprises the amino acid sequence of SEQ ID NO: 4, 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: 4; and/or wherein~~.~~

~~46. The method of any one of claims 37 to 45, wherein~~

(i) the RNA encoding the amino acid sequence under (ii) comprises the nucleotide sequence of SEQ ID NO: 7, 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: 7; and/or

(ii) the amino acid sequence under (ii) comprises the amino acid sequence of SEQ ID NO: 6, 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: 6.

2547. The use~~method~~ of any one of claims 2037 to 2446, wherein at each or 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.

~~48. The method of any one of claims 37 to 47, 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.~~

2649. The use~~method~~ of any one of claims 2037 to 2548, wherein each or at least one RNA comprises the 5' cap $m_2^{7,3'\text{-}O}Gppp(m_1^{2'\text{-}O})ApG$.

~~50. The method of any one of claims 37 to 49, wherein each RNA comprises the 5' cap $m_2^{7,3'\text{-}O}Gppp(m_1^{2'\text{-}O})ApG$.~~

2751. The use~~method~~ of any one of claims 2037 to 2650, wherein each or at least one RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 13, 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: 13.

~~52. The method of any one of claims 37 to 51, wherein each RNA comprises a 5' UTR comprising the nucleotide sequence of SEQ ID NO: 13, 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: 13.~~

2853. The use~~method~~ of any one of claims 2037 to 2752, wherein each or at least one RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 14, 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: 14.

~~54. The method of any one of claims 37 to 53, wherein each RNA comprises a 3' UTR comprising the nucleotide sequence of SEQ ID NO: 14, 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: 14.~~

2955. The use~~method~~ of any one of claims 2037 to 2854, wherein each or at least one RNA comprises a poly-A sequence, preferably wherein the poly-A sequence comprises at least 100

nucleotides, optionally wherein the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 15.

~~56. The method of any one of claims 37 to 55, wherein each RNA comprises a poly-A sequence.~~

~~57. The method of claim 55 or 56, wherein the poly-A sequence comprises at least 100 nucleotides.~~

~~58. The method of any one of claims 55 to 57, wherein the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 15.~~

3059. The use~~method~~ of any one of claims 2037 to 2958, wherein the RNA is formulated as a liquid, formulated as a solid, or a combination thereof.

3160. The use~~method~~ of any one of claims 2037 to 3059, wherein the RNA is to be administered by injection or~~.~~

~~61. The method of any one of claims 37 to 60, wherein the RNA is administered~~ by intravenous administration.

3262. The use~~method~~ of any one of claims 2037 to 3161, wherein the RNA is formulated as lipid particles, preferably~~.~~

~~63. The method of claim 62,~~ wherein the RNA lipid particles are lipid nanoparticles (LNP).

3364. The use~~method~~ of any one of claims 2037 to 3263, wherein the RNA is formulated as a pharmaceutical composition, preferably~~.~~

~~65. The method of claim 64,~~ wherein the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

~~66. The method of any one of claims 37 to 65, wherein the subject is a human.~~