PAN-RAS mRNA CANCER VACCINES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 63/091,711, filed October 14, 2020, the contents of which are incorporated by reference in its entirety into the present application.

FIELD OF THE DISCLOSURE

[0002] This disclosure is related to therapeutic vaccines for treating cancer patients bearing mutations in tumor-suppressor genes such as the Pan-RAS family of genes.

BACKGROUND

[0003] Cancer is a family of genetic disorders that changes of genetic material drive a normal cell into a dysregulated state that manifests as malignant growth of tumor tissues. With aging of the society, cancer poses an increasing burden both in mortality and healthcare cost. According to data from the National Cancer Institute (NCI), in 2020, roughly 1.8 million people will be diagnosed with cancer and an estimated 606,520 people will die of cancer in the United States. Of all different types of cancers, lung cancer is responsible for the most deaths with 135,720 people expected to die from this disease. That is nearly three times the 53,200 deaths due to colorectal cancer, which is the second most common cause of cancer death. Pancreatic cancer is the third deadliest cancer, causing 47,050 deaths.

[0004] Thus, a need exists to treat a cancer effectively. This disclosure satisfies this need and provides related advantages as well.

SUMMARY

[0005] In one aspect, the present disclosure provides an isolated ribonucleic acid (RNA) comprising an open reading frame (ORF) encoding a ras derived peptide having the amino acid sequence of SEQ ID NO: 101, SEQ ID NO: 103, or SEQ ID NO: 104, wherein the encoded ras derived peptide comprises the following mutations:

an aspartic acid (D) aligned to the 12th amino acid residue; and

an aspartic acid (D) aligned to the 13th amino acid residue; and

a phenylalanine (F) aligned to the 19th amino acid residue; and

optionally the peptide having any one or more of:

a threonine (T) aligned to the 59th amino acid residue;

an aspartic acid (D) aligned to the 60th amino acid;

an H aligned to the 61st amino acid residue;

an asparagine (N) aligned to the 117th amino acid residue–

a T aligned to the 146th amino acid residue

and wherein the RNA is encapsulated in a nanoparticle.

[0005a] In another aspect, the present disclosure provides a method of producing an RNA, comprising culturing the cell as described herein under conditions suitable for transcribing a DNA encoding the RNA to the RNA or contacting the polynucleotide as described herein or the vector as described herein with an RNA polymerase, adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine-5'-triphosphate (GTP), and uridine triphosphate (UTP) or a chemically modified UTP under conditions suitable for transcribing the polynucleotide or the vector to the RNA, and optionally further isolating the RNA.

[0005b] In another aspect, the present disclosure provides an immunogenic composition comprising an effective amount of the RNA as described herein formulated in a pharmaceutically acceptable carrier, and optionally wherein the pharmaceutically acceptable carrier comprises a polymeric nanoparticle or a liposomal nanoparticle or both and further optionally wherein the polymeric lipid nanoparticle comprises a Histidine-Lysine co-polymer (HKP) further optionally wherein the HKP comprises H3K(+H)4b or H3k(+H)4b or both.

[0005c] In another aspect, the present disclosure provides a method of producing a composition, comprising contacting the RNA as described herein with an HKP, thereby the RNA and the HKP are self-assembled into nanoparticles and optionally wherein the mass ratio of HKP and the RNA in the contacting step is 10:1 to 1: 10, optionally 2.5:1, and further optionally comprising contacting the HKP and RNA with cationic lipid, optionally wherein the cationic lipid comprises Dlin-MC3-DMA (MC3) or DOTAP (dioleoyloxy-3-(trimethylammonio)propane) or both, and optionally wherein the mass ratio of the cationic lipid and the RNA in the contacting step is 10:1

to 1:10, optionally 1:1, and further optionally wherein the mass ratio of HKP and the RNA in the contacting step is 10:1 to 1: 10, optionally 2.5:1.

[0005d] In yet another aspect, the present disclosure provides a use of the RNA as described herein, the polynucleotide as described herein, the vector as described herein, the cell as described herein, or the composition as described herein in the manufacture of a medicament for treating a subject having a cancer.

[0005e] In a further aspect, the present disclosure provides a kit comprising instructions for use and one or more of: the RNA as described herein, the polynucleotide as described herein, the vector as described herein, the cell as described herein, or the composition as described herein.

[0005f] In one ~~aspect~~example, provided herein is a composition or a vaccine that comprises, or consists essentially of, or yet further consists of a messenger ribonucleic acid (mRNA) molecule that expresses cancer neoantigens that are derived from mutated human ras genes. In another ~~aspect~~example, they are formulated with a carrier, e.g., they are formulated with a pharmaceutically acceptable carrier. Suitable carriers include, but are not limited to, Histidine-Lysine Co-polymers (HKP), 4-(dimethylamino)-butanoic acid, (10Z,13Z)-1-(9Z,12Z)-9,12-octadecadien-1-yl-10,13-nonadecadien-1-yl ester (DLIN-MC3-DMA or MC3), 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP),

or any combination thereof, which in some ~~aspect~~examples, can serve as an adjuvant for amplifying an immune response against cancer cells that harbor mutations in ras. Methods also are provided for using these pharmaceutical compositions, including methods of treatment, process development, and specific delivery routes.

[0006] In one ~~aspect~~example, provided is a ribonucleic acid (RNA) comprising, or consisting essentially of, or yet further consisting of an open reading frame (ORF) encoding one or more ras derived peptides. In some ~~embodiment~~examples, each of the one or more ras derived peptides consists of between 23 and 29 amino acid residues, for example 25 amino acid residues. Additionally or alternatively, the encoded peptides are selected from the group as set forth in SEQ ID NOs:1 to 69, or an equivalent of each thereof. In some ~~embodiment~~examples, the one or more ras derived peptides do not comprise or alternatively consist essentially of, or alternatively consisting of any one or more of SEQ ID NOs: 1-18, 32-49 or 53-68.

[0007] In another ~~aspect~~example, provided is an isolated ribonucleic acid (RNA) comprising, or consisting essentially of, or yet further consisting of an open reading frame (ORF) encoding a ras derived peptide. In some ~~embodiment~~examples, the encoded ras derived peptide comprises one or more (for example, any one, or any two, or any three, or any four, or all five) of the following mutations: a phenylalanine (F) aligned to the 19th amino acid residue of SEQ ID NO: 70 (referred to herein as L19F); a threonine (T) aligned to the 59th amino acid residue of SEQ ID NO: 70 (referred to herein as A59T); an aspartic acid (D) aligned to the 60th amino acid residue of SEQ ID NO: 70 (referred to herein as G60D); an asparagine (N) aligned to the 117th amino acid residue of SEQ ID NO: 70 (referred to herein as K117N); or a T aligned to the 146th amino acid residue of SEQ ID NO: 70 (referred to herein as A146T). In some ~~embodiment~~examples, the encoded ras derived peptide further comprises any one or more (for example, any one, or any two, or any three) of the following mutations: a D aligned to the 12th amino acid residue of SEQ ID NO: 70 (referred to herein as a G12D); a D aligned to the 13th amino acid residue of SEQ ID NO: 70 (referred to herein as G13D); or a histidine (H) aligned to the 61th amino acid residue of SEQ ID NO: 70 (referred to herein as Q61H). In some ~~embodiment~~examples, the encoded ras derived peptide comprises the following mutations: G12D, G13D, L19F, A59T, G60D, Q61H, K117N, and A146T. In some ~~embodiment~~examples, the encoded ras derived peptide comprises, or consists essentially of, or yet further consists of the polypeptide as set

forth in SEQ ID NO: 70 or an equivalent thereof, with the proviso that the equivalent retains the eight mutations of G12D, G13D, L19F, A59T, G60D, Q61H, K117N, and A146T. In some ~~embodiment~~examples, the RNA comprises, or consists essentially of, or yet further consists of the polynucleotide as set forth in SEQ ID NO: 88 or nucleotide (nt) 1 to nt 612 of SEQ ID NO: 88. In further ~~embodiment~~examples, the RNA is formulated in a pharmaceutically acceptable carrier, such as encapsulated in a nanoparticle.

[0008] In a further aspect, provided is a polynucleotide (such as a DNA) encoding an RNA as disclosed herein, or a polynucleotide complementary thereto, or both. In yet a further aspect, provided is a vector comprising, or consisting essentially of, or yet further consisting of a polynucleotide as disclosed herein. In some ~~embodiment~~examples, the vector further comprises a regulatory sequence operatively linked to the polynucleotide to direct the replication or transcription thereof, such as a promoter. In some ~~embodiment~~examples, the vector is a non-viral vector, such as a plasmid, a liposome, or a micelle. In further ~~embodiment~~examples, the vector is pUC57, or pSFV1, or pcDNA3, or pTK126. In yet further ~~embodiment~~examples, the vector comprises, or consists essentially of, or yet further consist of the polynucleotide as set forth in SEQ ID NO: 91 or an equivalent thereof which transcribes to the same RNA. In some ~~embodiment~~examples, the vector is a viral vector, such as an adenoviral vector, or an adeno-associated viral vector, or a retroviral vector, or a lentiviral vector, or a plant viral vector.

[0009] In one aspect, provided is a cell comprising one or more of: an RNA as disclosed herein, a polynucleotide as disclosed herein, or a vector as disclosed herein. In one ~~aspect~~example, the cell is a prokaryotic cell. In another ~~aspect~~example, the cell is a eukaryotic cell.

[0010] In a further aspect, provided is a composition comprising, or consisting essentially of, or yet further consisting of a carrier (e.g., a pharmaceutically acceptable carrier) and one or more of: an RNA as disclosed herein, a polynucleotide as disclosed herein, a vector as disclosed herein, or a cell as disclosed herein.

[0011] In yet a further ~~aspect~~example, provided is a method of producing an RNA of this disclosure. In some ~~embodiment~~examples, the method comprises, or consists essentially of, or yet further consists of culturing a cell as disclosed herein under conditions suitable for expressing the RNA (such as transcribing a DNA to the RNA). In one ~~aspect~~example, the cell comprises the DNA encoding the RNA of this disclosure. In some ~~embodiment~~examples, the

method comprises, or consists essentially of, or yet further consists of contacting a polynucleotide as disclosed herein or a vector as disclosed herein with an RNA polymerase, adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine-5'-triphosphate (GTP), and uridine triphosphate (UTP) or a chemically modified UTP under conditions suitable for expressing the RNA (such as transcribing a DNA to the RNA). In further ~~embodiment~~examples, the method further comprises isolating the RNA. Additionally provided is an RNA produced by a method as disclosed herein.

[0012] Additionally provided is a composition (such as an immunogenic composition) comprising, or consisting essentially of, or yet further consisting of an effective amount of an RNA as disclosed herein formulated in a carrier, e.g., a pharmaceutically acceptable carrier, such as a nanoparticle. In some ~~embodiment~~examples, the nanoparticle is a polymeric nanoparticle carrier, for example those comprising, or consisting essentially of, or yet further consisting of a Histidine-Lysine co-polymer (HKP), such as H3K(+H)4b or H3k(+H)4b or both. In some ~~embodiment~~examples, the nanoparticle is a lipid nanoparticle, for example, 9-Heptadecanyl 8-{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate (SM-102), 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), or an equivalent of each thereof.

[0013] In one ~~aspect~~example, provided is a method of producing a composition (such as an immunogenic composition) as disclosed herein. The method comprises, or consists essentially of, or yet further consists of contacting an RNA as disclosed herein with an HKP or a lipid or both, thereby the RNA and the HKP or lipid or both HKP and lipid are self-assembled into nanoparticles.

[0014] In another ~~aspect~~example, provided is a method of treating a subject having a cancer or suspect of having a cancer, or at risk or alternatively a high risk of having a cancer. The method comprises, or consists essentially of, or yet further consists of administering to the subject, for example a pharmaceutically effective amount of, any one or more of: an RNA as disclosed herein, a polynucleotide as disclosed herein, a vector as disclosed herein, a cell as disclosed herein, or a composition (such as an immunogenic composition) as disclosed herein. In some ~~embodiment~~examples, the cancer

comprises (such as expresses) one or more mutations (also referred to herein as neoantigens) expressed by an RNA as disclosed herein, such as a ras mutation. In some ~~embodiment~~examples, the cancer comprises a mutated ras gene encoding a neoantigen as disclosed herein. In further ~~embodiment~~examples, the method further comprises, or consists essentially of, or yet further consists of administering to the subject an additional anti-cancer therapy.

[0015] In yet another ~~aspect~~example, provided is a kit for use in a method as disclosed herein. The kit comprises, or consists essentially of, or yet further consists of instructions for use and one or more of: an RNA as disclosed herein, a polynucleotide as disclosed herein, a vector as disclosed herein, a cell as disclosed herein, a composition as disclosed herein, a pharmaceutically acceptable carrier as disclosed herein, or an anti-cancer therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **FIG 1** illustrates the major protein domains of ras.

[0017] **FIG 2** lists advantages and disadvantages of viral vectored vaccines, DNA vaccines and RNA vaccines.

[0018] **FIG 3** illustrates screening neoantigen by in silico prediction and in vitro assays.

[0019] **FIG 4** illustrates a minigene structure of an mRNA vaccine. An exemplified amino acid sequence encoded by the minigene, QNAADSYSWVPEQAESRAMENQYSP, is provided herein as SEQ ID NO: 71.

[0020] **FIG 5** provides a schematic representation of an optimized mRNA vaccine expression structure. This structure can be contained and/or transcribed in a linear in vitro transcription (IVT) expression system or plasmid DNA delivery vector.

[0021] **FIG 6** illustrates plasmid vectors which can be used for mRNA production. The commonly used plasmids are pSFV1, pcDNA3 and pTK126.

[0022] **FIG 7** illustrates polylipid nanoparticle (PLNP) and lipid nanoparticle (LNP) structures.

[0023] **FIG 8** shows that H3K(+H)4b is a significantly better carrier than H3K4b in mRNA delivery. To form polyplexes, the mRNA (1 g) was mixed with 3 different

WHAT IS CLAIMED IS:

1. An isolated ribonucleic acid (RNA) comprising an open reading frame (ORF) encoding a ras derived peptide having the amino acid sequence of SEQ ID NO: 101, SEQ ID NO: 103, or SEQ ID NO: 104, wherein the encoded ras derived peptide comprises ~~any one or more of~~ the following mutations:

an aspartic acid (D) aligned to the 12th amino acid residue; and

an aspartic acid (D) aligned to the 13th amino acid residue; and

a phenylalanine (F) aligned to the 19th amino acid residue; and

optionally the peptide having any one or more of:

~~of SEQ ID NO: 70;~~

a threonine (T) aligned to the 59th amino acid residue ~~of SEQ ID NO: 70~~;

an aspartic acid (D) aligned to the 60th amino acid ~~residue of SEQ ID NO: 70~~;

an H aligned to the 61st amino acid residue;

an asparagine (N) aligned to the 117th amino acid residue ~~of SEQ ID NO: 70; or~~

a T aligned to the 146th amino acid residue ~~of SEQ ID NO: 70~~

and wherein the RNA is encapsulated in a nanoparticle.

~~2. The isolated RNA of claim 1, wherein the encoded ras derived peptide further comprises any one or more of the following mutations:~~

~~a D aligned to the 12th amino acid residue of SEQ ID NO: 70,~~

~~a D aligned to the 13th amino acid residue of SEQ ID NO: 70; or~~

~~a histidine (H) aligned to the 61th amino acid residue of SEQ ID NO: 70.~~

~~3. The isolated RNA of claim 2, wherein the encoded ras derived peptide comprises the following mutations:~~

~~D aligned to the 12th amino acid residue of SEQ ID NO: 70,~~

~~D aligned to the 13th amino acid residue of SEQ ID NO: 70;~~

~~F aligned to the 19th amino acid residue of SEQ ID NO: 70;~~

~~T aligned to the 59th amino acid residue of SEQ ID NO: 70;~~

~~D aligned to the 60th amino acid residue of SEQ ID NO: 70;~~

~~H aligned to the 61th amino acid residue of SEQ ID NO: 70;~~

~~N aligned to the 117th amino acid residue of SEQ ID NO: 70; or~~

~~T aligned to the 146th amino acid residue of SEQ ID NO: 70.~~

~~4~~2. The isolated RNA of claim ~~3~~1, wherein the ras derived peptide comprises the polypeptide as set forth in ~~SEQ ID NO: 70~~SEQ ID NO: 101, SEQ ID NO: 103, or SEQ ID NO: 104, or an equivalent thereof retaining the following mutations:

D aligned to the 12th amino acid residue ~~of SEQ ID NO: 70~~,

D aligned to the 13th amino acid residue ~~of SEQ ID NO: 70~~;

F aligned to the 19th amino acid residue ~~of SEQ ID NO: 70~~;

T aligned to the 59th amino acid residue ~~of SEQ ID NO: 70~~;

D aligned to the 60th amino acid residue ~~of SEQ ID NO: 70~~;

H aligned to the 61th amino acid residue ~~of SEQ ID NO: 70~~;

N aligned to the 117th amino acid residue ~~of SEQ ID NO: 70~~; or

T aligned to the 146th amino acid residue ~~of SEQ ID NO: 70~~.

~~5. A ribonucleic acid (RNA) comprising an open reading frame (ORF) encoding one or more ras derived peptides, wherein each of the one or more ras derived peptides consists of between 23 and 29 amino acid residues, and wherein the encoded peptides are selected from the group as set forth in SEQ ID NOs:1-69, or an equivalent of each thereof.~~

~~6. The RNA of claim 5, wherein the ras derived peptides are selected from the group as set forth in SEQ ID NOs:1-31, or an equivalent of each thereof.~~

~~7. The RNA of claim 5, wherein the ras derived peptides are selected from the group as set forth in SEQ ID NOs :32 – 52, or an equivalent of each thereof.~~

~~8. The RNA of claim 5, wherein the ras derived peptides are selected from the group as set forth in SEQ ID NOs: 53 - 69 or an equivalent of each thereof.~~

~~9. The RNA of claim 5, wherein the ras derived peptides are selected from the group as set forth in SEQ ID NOs: 19-31, 50-52 or 69.~~

~~10. The RNA of claim 9, wherein the ORF encodes the polypeptide as set forth in SEQ ID NO: 70, or an equivalent thereof retaining the following mutations:~~

~~D aligned to the 12th amino acid residue of SEQ ID NO: 70,~~

~~D aligned to the 13th amino acid residue of SEQ ID NO: 70;~~

~~F aligned to the 19th amino acid residue of SEQ ID NO: 70;~~

~~T aligned to the 59th amino acid residue of SEQ ID NO: 70;~~

~~D aligned to the 60th amino acid residue of SEQ ID NO: 70;~~

~~H aligned to the 61th amino acid residue of SEQ ID NO: 70;~~

~~N aligned to the 117th amino acid residue of SEQ ID NO: 70; or~~

~~T aligned to the 146th amino acid residue of SEQ ID NO: 70.~~

~~11~~3. The RNA of claim ~~4 or 10~~2, wherein the ORF comprises the polynucleotide as set forth in

AUGUUUGUUUUUCUUGUUUUAUUGCCACUAGUCUCUAGUCAGUGUAUGACUGAA
UAUAAACUUGUGGUAGUUGGAGCUGAUGACGUAGGCAAGAGUGCCUUUACGAUA
CAGCUAAUUCAGAAUCAUUUUGUGGACGAAUAUGAUCCAACAAUAGAGGAUUCC
UACAGGAAGCAAGUAGUAAUUGAUGGAGAAACCUGUCUCUUGGAUAUUCUCGAC
ACAACAGAUCACGAGGAGUACAGUGCAAUGAGGGACCAGUACAUGAGGACUGGG
GAGGGCUUUCUUUGUGUAUUUGCCAUAAAUAAUACUAAAUCAUUUGAAGAUAUU
CACCAUUAUAGAGAACAAAUUAAAAGAGUUAAGGACUCUGAAGAUGUACCUAUG
GUCCUAGUAGGAAAUAAUUGUGAUUUGCCUUCUAGAACAGUAGACACAAAACAG
GCUCAGGACUUAGCAAGAAGUUAUGGAAUUCCUUUUAUUGAAACAUCAACAAAG
ACAAGACAGAGAGUGGAGGAUGCUUUUUAUACAUUGGUGAGAGAGAUCCGACAA
UACAGAUUGAAAAAAAUCAGCAAAGAAGAAAAGACUCCUGGCUGUGUGAAAAUU

AAAAAAUGCAUUAUAAUGUAA (SEQ ID NO: 88) or nucleotide (nt) 1 to nt 612 of SEQ ID NO: 88, or an equivalent thereof encoding the same ras derived peptide.

~~12. The RNA of any one of claims 5 to 9, wherein the ORF encodes a polypeptide comprising two or more ras derived peptides and a peptide linker between any two adjacent ras derived peptides.~~

~~13. The RNA of any one of claims 1, 2, or 5 to 9, wherein the encoded ras derived peptide or peptides comprise a wildtype residue aligned to the 12th amino acid residue of SEQ ID NO: 70, or a wildtype residue aligned to the 13th amino acid residue of SEQ ID NO: 70, or both.~~

~~14~~4. The RNA of claim 1 or 2~~any one of claims 1 to 13~~, wherein the ORF further encodes a signal peptide, optionally wherein the single peptide comprises MFVFLVLLPLVSSQC (SEQ ID NO: 87).

~~15~~5. The RNA of any one of claims 1 to ~~14~~3, further comprising a 3'-UTR and a 5'-UTR, and optionally wherein the 5'-UTR comprises an m7G cap structure and a start codon, optionally wherein the 5'-UTR comprises AGGacaUUUgcUUcUgacacaacUgUgUUcacUagcaaccUcaaacagacaCCGCCACC (SEQ ID NO: 89) or an equivalent thereof.

~~16. The RNA of claim 15, wherein the 5'-UTR comprises an m7G cap structure and a start codon, optionally wherein the 5'-UTR comprises AGGacaUUUgcUUcUgacacaacUgUgUUcacUagcaaccUcaaacagacaCCGCCACC (SEQ ID NO: 89) or an equivalent thereof.~~

~~17~~6. The RNA of claim 4 or 5~~15 or 16~~, wherein the 3'-UTR comprises a stop codon and a polyA tail, optionally wherein the 3'-UTR comprises GCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUUCCCUAAGUCCAACUACUAAACUGGGGGAUAUUAUGAAGGGCCUUGAGCAUCUGGAUUCUGCCUAAUAAAAAACAUUUAUUUUCAUUGCCAAUAGGCCGAAAUCGGCAAGCGCGAUCGC (SEQ ID NO: 90) or an equivalent thereof.

~~18~~7. The RNA of any one of claim~~s~~ 1 or 6 ~~to 17~~, prepared by transcribing a polynucleotide encoding the RNA in an in vitro transcription (IVT) system.

~~1~~98. The RNA of any one of claims 1 to ~~1~~86, prepared by transcribing a plasmid DNA (pDNA) vector, optionally a pUC57 plasmid, encoding the RNA, optionally wherein the plasmid comprises SEQ ID NO: 91 or an equivalent thereof.

~~2~~09. The RNA of any one of claim~~s~~ 1 or 8~~to 19~~, wherein the GC content of the full-length RNA is ~~about~~ 35 % to ~~about~~ 70 % of the total RNA content.

~~21~~10. The RNA of any one of claim~~s~~ 1 or 9~~to 20~~, wherein the RNA is chemically modified, optionally wherein the chemical modification comprising one or both of the incorporation of an N1-methyl-pseudouridine residue or a pseudouridine residue, further optionally wherein at least ~~about~~ 50% to ~~about~~ 100% of the uridine residues in the RNA are N1-methyl pseudouridine or pseudouridine.

~~22~~11. A polynucleotide encoding the RNA of any one of claims 1 to 6, 9 or 10~~to 21~~, or a polynucleotide complementary thereto, or both.

~~23~~12. The polynucleotide of claim ~~22~~11, wherein the polynucleotide is selected from the group of: a deoxyribonucleic acid (DNA), an RNA, a hybrid of DNA and RNA, or an analog of each thereof.

~~24~~13. A vector comprising the polynucleotide of claim 11 or 12~~22 or 23~~.

~~25~~14. The vector of claim ~~24~~13, further comprising a regulatory sequence operatively linked to the polynucleotide to direct the transcription thereof.

~~26. The vector of claim 25, wherein the regulatory sequence comprises a promotor.~~

~~27. The vector of claim 26, wherein the promoter comprises: a bacteriophage RNA polymerase promoter, optionally a T7 promoter, or a SP6 promoter, or a T3 promoter.~~

~~28. The vector of any one of claims 24 to 27, further comprising a marker selected from a detectable marker, a purification marker, or a selection marker.~~

~~29. The vector of any one of claims 24 to 28, wherein the vector is a non-viral vector, optionally a plasmid, or a liposome, or a micelle.~~

~~30~~15. The vector of claim 13 or 14~~29~~, wherein the plasmid comprises or consists of SEQ ID NO: 91 or an equivalent thereof.

~~31. The vector of any one of claims 24 to 28, wherein the vector is a viral vector, optionally an adenoviral vector, or an adeno-associated viral vector, or a retroviral vector, or a lentiviral vector, or a plant viral vector.~~

~~32~~16. A cell comprising one or more of: the RNA of any one of claims 1 to 10~~to 21~~, the polynucleotide of claim ~~22~~11 or ~~23~~12, or the vector of any one of claims ~~24 to 31~~13 to 15.

~~33. The cell of claim 32, wherein the cell is a prokaryotic cell, optionally an *Escherichia coli* cell.~~

~~34. The cell of claim 32, wherein the cell is a eukaryotic cell, optionally a mammal cell, an insect cell, or a yeast cell.~~

~~35~~17. A composition comprising a carrier, optionally a pharmaceutically acceptable carrier, and one or more of: the RNA of any one of claims 1 to 10~~to 21~~, the polynucleotide of claim ~~22~~ 11 or ~~23~~12, the vector of any one of claims 13 to 15~~24 to 31~~, or the cell ~~of any one~~ of claim~~s~~ ~~32~~ 16~~to 34~~.

~~36~~18. A method of producing an RNA, comprising culturing the cell of ~~any one of~~ claim~~s~~ ~~32 to 34~~16 under conditions suitable for transcribing a DNA encoding the RNA to the RNA or contacting the polynucleotide of claim 11 or 12 or the vector of any one of claims 13 to 15 with an RNA polymerase, adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine-5'-triphosphate (GTP), and uridine triphosphate (UTP) or a chemically modified UTP under conditions suitable for transcribing the polynucleotide or the vector to the RNA, and optionally further isolating the RNA.

~~37. A method of producing an RNA, comprising contacting the polynucleotide of claim 22 or 23 or the vector of any one of claims 24 to 31 with an RNA polymerase, adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine-5'-triphosphate (GTP), and uridine triphosphate (UTP) or a chemically modified UTP under conditions suitable for transcribing the polynucleotide or the vector to the RNA.~~

~~38. The method of claim 36 or 37, further comprising isolating the RNA.~~

~~39. An RNA produced by the method of any one of claims 36 to 38.~~

~~40~~19. An immunogenic composition comprising an effective amount of the RNA of any one of claims 1 to 10~~to 21~~ formulated in a pharmaceutically acceptable carrier, and optionally wherein the pharmaceutically acceptable carrier comprises a polymeric nanoparticle or a liposomal nanoparticle or both and further optionally wherein the polymeric lipid nanoparticle comprises a Histidine-Lysine co-polymer (HKP) further optionally wherein the HKP comprises H3K(+H)4b or H3k(+H)4b or both.

~~41. The composition according to claim 40, wherein the pharmaceutically acceptable carrier comprises a polymeric nanoparticle or a liposomal nanoparticle or both.~~

~~42. The composition of claim 41, wherein the polymeric nanoparticle carrier comprises a Histidine-Lysine co-polymer (HKP).~~

~~43. The composition of claim 42, wherein the HKP comprises H3K(+H)4b or H3k(+H)4b or both.~~

~~44. The composition of claim 42 or 43, wherein the polymeric nanoparticle carrier further comprises a lipid, optionally a cationic lipid.~~

~~45. The composition of claim 44, wherein the cationic lipid is ionizable.~~

~~46. The composition of claim 44 or 45, wherein the cationic lipid comprises Dlin-MC3-DMA (MC3) or dioleoyloxy-3-(trimethylammonio)propane (DOTAP) or both.~~

~~47. The composition of any one of claims 44 to 46, wherein the lipid further comprises one or more of: a helper lipid, a cholesterol, or a PEGylated lipid.~~

~~48. The composition of any one of claims 44 to 47, wherein the lipid further comprises PLA or PLGA.~~

~~49. The composition of any one of claims 42 to 48, wherein the HKP and the mRNA self-assemble into nanoparticles upon admixture.~~

~~50. The composition of any one of claims 41 to 49, wherein the liposomal nanoparticle carrier comprises a Spermine-Lipid-Cholesterol (SLiC).~~

~~51. The composition of claim 50, wherein the SLiC is selected from the group consisting of TM1-TM5.~~

~~52~~20. The composition of claim ~~40~~19, wherein the pharmaceutical acceptable carrier is a lipid nanoparticle (LNP).

~~53. The composition of claim 52, wherein the LNP comprises a lipid, optionally a cationic lipid, optionally wherein the cationic lipid is ionizable, and optionally wherein the LNP comprises one or more of: 9-Heptadecanyl 8-{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate (SM-102), 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), or an equivalent of each thereof.~~

~~54. The composition of claim 53, wherein the LNP further comprises one or more of: a helper lipid, a cholesterol, or a PEGylated lipid.~~

~~55. The composition of claim 47 or 54, wherein the helper lipid comprises one or more of: distearoylphosphatidyl choline (DSPC), Dipalmitoylphosphatidylcholine (DPPC), (2*R*)-3-(Hexadecanoyloxy)-2-{[(9Z)-octadec-9-enoyl]oxy}propyl 2-(trimethylazaniumyl)ethyl phosphate (POPC), or dioleoyl phosphatidylethanolamine (DOPE).~~

~~56. The composition of any one of claims 47, 54 or 55, wherein the cholesterol comprises a plant cholesterol or an animal cholesterol or both.~~

~~57. The composition of any one of claims 47 or 54 to 56, wherein the PEGylated lipid comprises one or more of: PEG-c-DOMG (R-3-[(ω-methoxy-poly(ethyleneglycol)2000)carbamoyl)]-1,2-dimyristyloxypropyl-3-amine), PEG-DSG (1,2-Distearoyl-sn-glycerol, methoxypolyethylene glycol), PEG-DMG (1,2-Dimyristoyl-sn-glycerol) optionally PEG2000-DMG ((1,2-dimyristoyl-sn-glycero-3-phophoethanolamine-N-[methoxy(polyethylene glycol)-2000)], or PEG-DPG (1,2-Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol).~~

~~58. The composition of any one of claims 52 to 57, wherein the LNP comprises SM-102, DSPC, cholesterol and PEG2000-DMG.~~

~~59. The composition of claim 58, wherein the mass ratio of the SM-102, DSPC, cholesterol and PEG200-DMG is about 1:1:1:1 and/or wherein the molar ratio of the SM-102, DSPC, cholesterol and PEG2000-DMG is about 50:10:38.5:1.5.~~

~~60~~21. A method of producing ~~the~~ a composition ~~of any one of claims 40 to 51 or 55 to 57~~, comprising contacting the RNA of any one of claims 1 to 8 ~~to 21~~ with an HKP, thereby the RNA and the HKP are self-assembled into nanoparticles and optionally wherein the mass ratio of HKP and the RNA in the contacting step is 10:1 to 1: 10, optionally 2.5:1, and further optionally comprising contacting the HKP and RNA with cationic lipid, optionally wherein the cationic lipid comprises Dlin-MC3-DMA (MC3) or DOTAP (dioleoyloxy-3-(trimethylammonio)propane) or both, and optionally wherein the mass ratio of the cationic lipid and the RNA in the contacting step is 10:1 to 1:10, optionally 1:1, and further optionally wherein the mass ratio of HKP and the RNA in the contacting step is 10:1 to 1: 10, optionally 2.5:1.

~~61. The method of claim 60, wherein the mass ratio of HKP and the RNA in the contacting step is about 10:1 to about 1: 10, optionally 2.5:1.~~

~~62. The method of claim 60 or 61, further comprising contacting the HKP and RNA with cationic lipid, optionally wherein the cationic lipid comprises Dlin-MC3-DMA (MC3) or DOTAP (dioleoyloxy-3-(trimethylammonio)propane) or both.~~

~~63. The method of claim 62, wherein the mass ratio of the cationic lipid and the RNA in the contacting step is about 10:1 to about 1: 10, optionally 1:1.~~

~~64. The method of claim 62 or 63, wherein the mass ratio of the HKP, the mRNA and the cationic lipid in the contacting step is about 4:1:1.~~

~~65. A method of producing the composition of any one of claims 40 or 52 to 59, comprising contacting the RNA of any one of claims 1to 21 with a lipid, thereby the RNA and the lipid are self-assembled into lipid nanoparticles (LNPs).~~

~~66. The method of claim 65, wherein the LNPs comprise one or more of: 9-Heptadecanyl 8-{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate (SM-102), 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), or an equivalent of each thereof.~~

~~67. The method of claim 66, wherein the LNPs further comprise one or more of: a helper lipid, a cholesterol, or a PEGylated lipid, optionally wherein the helper lipid comprises one or more of: distearoylphosphatidyl choline (DSPC), Dipalmitoylphosphatidylcholine (DPPC), (2*R*)-~~

3-(Hexadecanoyloxy)-2-{[(9Z)-octadec-9-enoyl]oxy}propyl 2-(trimethylazaniumyl)ethyl phosphate (POPC), or dioleoyl phosphatidylethanolamine (DOPE), optionally wherein the cholesterol comprises a plant cholesterol or an animal cholesterol or both, and optionally wherein the PEGylated lipid comprises one or more of: PEG-c-DOMG (R-3-[(ω-methoxy-poly(ethyleneglycol)2000)carbamoyl)]-1,2-dimyristyloxypropyl-3-amine), PEG-DSG (1,2-Distearoyl-sn-glycerol, methoxypolyethylene glycol), PEG-DMG (1,2-Dimyristoyl-sn-glycerol) optionally PEG2000-DMG ((1,2-dimyristoyl-sn-glycero-3-phophoethanolamine-N-[methoxy(polyethylene glycol)-2000)], or PEG-DPG (1,2-Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol).

68. The method of any one of claims 65 to 67, wherein the LNPs comprise SM-102, DSPC, cholesterol and PEG2000-DMG, optionally wherein the mass ratio of the SM-102, DSPC, cholesterol and PEG200-DMG is about 1:1:1:1 or optionally wherein the molar ratio of the SM-102, DSPC, cholesterol and PEG2000-DMG is about 50:10:38.5:1.5.

69. The method of any one of claims 60 to 68, wherein the contacting step is performed in a microfluidic mixer, optionally selected from a slit interdigitial micromixer, or a staggered herringbone micromixer (SHM).

7022. A method of treating a subject having a cancer, the method comprising administering to said subject a pharmaceutically effective amount of any one or more of: Use of the RNA of any one of claims 1 to 21 and 3910, the polynucleotide of claim 11 or 12 22 or 23, the vector of any one of claims 13 to 1524 to 31, the cell of any one of claims 32 to 34 claim 16, or the composition according to claim 17, 19 or 20 in the manufacture of a medicament for treating a subject having a cancerany one of claims 35 or 40 to 59.

71. The method of claim 70, wherein the administration is intratumoral, or intravenous, or intramuscular, or intradermal, or subcutaneous.

72. The method of claim 70 or 71, wherein the subject is a mammal, or a human.

7323. The method use of claim 22any one of claims 70 to 72, wherein the cancer comprises a mutation of any one or more of: a mutation of SEQ ID NOs: 1 to 69.

~~74~~24. The ~~method~~ use of claim 22 or 23~~any one of claims 70 to 73~~, further comprising ~~administering to the subject~~ that an additional anti-cancer therapy is to be administered to the subject.

~~75~~25. A kit ~~for use in the method of any one of claims 70 to 74~~, comprising instructions for use and one or more of: the RNA of any of claims 1 to 10 ~~claim 1 or 2 any one of claims 1 to 21 and 39~~, the polynucleotide of claim 11 or 12~~22 or 23~~, the vector of any one of claims 13 to 15~~24 to 31~~, the cell of claim 16~~any one of claims 32 to 34~~, ot the composition according to claim 17, 19 or 20 ~~any one of claims 35 or 40 to 59, or an anti-cancer therapy~~.