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 12^{th} amino acid residue; and

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

a phenylalanine (F) aligned to the 19^{th} 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 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 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 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 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 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 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 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 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 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 examples, the encoded ras derived peptide comprises the following mutations: G12D, G13D, L19F, A59T, G60D, Q61H, K117N, and A146T. In some 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 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 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 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 examples, the vector is a non-viral vector, such as a plasmid, a liposome, or a micelle. In further examples, the vector is pUC57, or pSFV1, or pcDNA3, or pTK126. In yet further 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 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 example, the cell is a prokaryotic cell. In another 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 example, provided is a method of producing an RNA of this disclosure. In some 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 example, the cell comprises the DNA encoding the RNA of this disclosure. In some 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 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 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 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 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 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 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 examples, the cancer comprises a mutated ras gene encoding a neoantigen as disclosed herein. In further 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 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 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.

2. The isolated RNA of claim 1, wherein the ras derived peptide comprises the polypeptide as set forth in 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,

D aligned to the 13th amino acid residue;

F aligned to the 19th amino acid residue;

T aligned to the 59th amino acid residue;

D aligned to the 60th amino acid residue;

H aligned to the 61th amino acid residue;

N aligned to the 117th amino acid residue; or

T aligned to the 146th amino acid residue.

3. The RNA of claim 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.

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

5. The RNA of any one of claims 1 to 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.

6. The RNA of claim 4 or 5, wherein the 3'-UTR comprises a stop codon and a polyA tail, optionally wherein the 3'-UTR comprises GCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUUCCCUAAGUCCA

ACUACUAAACUGGGGGAUAUUAUGAAGGGCCUUGAGCAUCUGGAUUCUGCCUAA
UAAAAAACAUUUAUUUUCAUUGCCAAUAGGCCGAAAUCGGCAAGCGCGAUCGC
(SEQ ID NO: 90) or an equivalent thereof.

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

8. The RNA of any one of claims 1 to 6, 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.

9. The RNA of any one of claim 1 or 8, wherein the GC content of the full-length RNA is 35 % to 70 % of the total RNA content.

10. The RNA of any one of claim 1 or 9, 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 50% to 100% of the uridine residues in the RNA are N1-methyl pseudouridine or pseudouridine.

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

12. The polynucleotide of claim 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.

13. A vector comprising the polynucleotide of claim 11 or 12.

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

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

16. A cell comprising one or more of: the RNA of any one of claims 1 to 10, the polynucleotide of claim 11 or 12, or the vector of any one of claims 13 to 15.

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, the polynucleotide of claim 11 or 12, the vector of any one of claims 13 to 15, or the cell of claim 16.

18. A method of producing an RNA, comprising culturing the cell of claim 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.

19. An immunogenic composition comprising an effective amount of the RNA of any one of claims 1 to 10 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.

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

21. A method of producing a composition, comprising contacting the RNA of any one of claims 1 to 8 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.

22. Use of the RNA of any one of claims 1 to 10, the polynucleotide of claim 11 or 12, the vector of any one of claims 13 to 15, the cell of claim 16, or the composition according to claim 17, 19 or 20 in the manufacture of a medicament for treating a subject having a cancer.

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

24. The use of claim 22 or 23, further comprising that an additional anti-cancer therapy is to be administered to the subject.

25. A kit comprising instructions for use and one or more of: the RNA of any of claims 1 to 10, the polynucleotide of claim 11 or 12, the vector of any one of claims 13 to 15, the cell of claim 16, or the composition according to claim 17, 19 or 20.