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Nucleic acid vaccines

Abstract

The invention relates to compositions and methods for the preparation, manufacture and therapeutic use ribonucleic acid vaccines (NAVs) comprising polynucleotide molecules encoding one or more antigens.

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chemically modified. As used herein, a "structural" modification is one in which two or more linked nucleosides are inserted, deleted, duplicated, inverted or randomized in a polynucleotide without significant chemical modification to the nucleotides themselves. Because chemical bonds will necessarily be broken and reformed to effect a structural modification, structural modifications are of a chemical nature and hence are chemical modifications. However, structural modifications will result in a different se uence of nucleotides. For e ample, the polynucleotide "ATCG" may be chemically modified to "AT-5meC-G". The same polynucleotide may be structurally modified from "ATCG" to "ATCCCG". Here, the dinucleotide "CC" has been inserted, resulting in a structural modification to the polynucleotide.

In one embodiment, the polynucleotides of the present invention, such as IVT polynucleotides or circular polynculeotides, may have a uniform chemical modification of all or any of the same nucleoside type or a population of modifications produced by mere downward titration of the same starting modification in all or any of the same nucleoside type, or a measured percent of a chemical modification of all any of the same nucleoside type but with random incorporation, such as where all uridines are replaced by a uridine analog, e.g., pseudouridine. In another embodiment, the polynucleotides may have a uniform chemical modification of two, three, or four of the same nucleoside type throughout the entire polynucleotide (such as all uridines and all cytosines, etc. are modified in the same way).

When the polynucleotides of the present invention are chemically and/or structurally modified the polynucleotides may be referred to as "modified polynucleotides."

In one embodiment, the polynucleotides of the present invention may include a se uence encoding a self-cleaving peptide. The self-cleaving peptide may be, but is not limited to, a 2A peptide. As a non-limiting e ample, the 2A peptide may have the protein se uence: GSGATNFSLLK AGDVEENPGP (SE ID NO: 963), fragments or variants thereof. In one embodiment, the 2A peptide cleaves between the last glycine and last proline. As another non-limiting e ample, the polynucleotides of the present invention may include a polynucleotide se uence encoding the 2A peptide having the protein se uence GSGATNFSLLK AGDVEENPGP (SE ID NO: 963) fragments or variants thereof.

One such polynucleotide se uence encoding the 2A peptide is GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGA GGAGAACCCTGGACCT (SE ID NO: 964). The polynucleotide se uence of the 2A peptide may be modified or codon optimized by the methods described herein and/or are known in the art.

In one embodiment, this se uence may be used to separate the coding region of two or more polypeptides of interest. As a non-limiting e ample, the se uence encoding the 2A peptide may be between a first coding region A and a second coding region B (A-2Apep-B). The presence of the 2A peptide would result in the cleavage of one long protein into protein A, protein B and the 2A peptide. Protein A and protein B may be the same or different peptides or polypeptides of interest. In another embodiment, the 2A peptide may be used in the polynucleotides of the present invention to produce two, three, four, five, si , seven, eight, nine, ten or more proteins.

Polynucleotide Architecture

Traditionally, the basic components of an mRNA molecule include at least a coding region, a 5 UTR, a 3 UTR, a 5 cap and a poly-A tail. The IVT polynucleotides of the present invention may function as mRNA but are distinguished from wild-type mRNA in their functional and/or structural design features which serve to overcome e isting problems of effective polypeptide production using nucleic-acid based therapeutics. It is to be understood that the antigens of the NAVs of the present invention may be encoded by IVT polynucleotides, as described herein.

FIG. 1 shows a primary construct 100 of an IVT polynucleotide of the present invention. Such polynucleotides are useful in NAV compositions, RNAV compositions or mRNA vaccines. As used herein, "primary construct" refers to a polynucleotide of the present invention which encodes one or more polypeptides of interest and which retains sufficient structural and/or chemical features to allow the polypeptide of interest encoded therein to be translated.

According to FIGS. 1A and 1B, the polynucleotide 100 here contains a first region of linked nucleotides 102 that is flanked by a first flanking region 104 and a second flaking region 106. The polypeptide of interest may comprise at its 5 terminus one or more signal se uences encoded by a signal se uence region 103. The flanking region 104 may comprise a region of linked nucleotides comprising one or more complete or incomplete 5 UTRs se uences which may be completely codon optimized or partially codon optimized. The flanking region 104 may include at least one nucleic acid se uence including, but not limited to, miR se uences, TER AK.TM. se uences and translation control se uences. The flanking region 104 may also comprise a 5 terminal cap 108. The 5 terminal capping region 108 may include a naturally occurring cap, a synthetic cap or an optimized cap. Non-limiting e amples of optimized caps include the caps taught by Rhoads in U.S. Pat. No. 7,074,596 and International Patent Publication No. WO2008157668, WO2009149253 and WO2013103659, the contents of each of which are herein incorporated by reference in its entirety. The second flanking region 106 may comprise a region of linked nucleotides comprising one or more complete or incomplete 3 UTRs. The second flanking region 106 may be completely codon optimized or partially codon optimized. The flanking region 106 may include at least one nucleic acid se uence including, but not limited to, miR se uences and translation control se uences. The flanking region 106 may also comprise a 3 tailing se uence 110. The 3 tailing se uence 110 may include a synthetic tailing region 112 and/or a chain terminating nucleoside 114.

Non-liming e amples of a synthetic tailing region include a polyA se uence, a polyC se uence, and/or a polyA-G uartet. Non-limiting e amples of chain terminating nucleosides include 2 -O methyl, F and locked nucleic acids (LNA).

Bridging the 5 terminus of the first region 102 and the first flanking region 104 is a first operational region 105. Traditionally this operational region comprises a Start codon. The operational region may alternatively comprise any translation initiation se uence or signal including a Start codon.

Bridging the 3 terminus of the first region 102 and the second flanking region 106 is a second operational region 107. Traditionally this operational region comprises a Stop codon. The operational region may alternatively comprise any translation initiation se uence or signal including a Stop codon. Multiple serial stop codons may also be used in the IVT polynucleotide. In one embodiment, the operation region of the present invention may comprise two stop codons. The first stop codon may be "TGA" or "UGA" and the second stop codon may be selected from the group consisting of "TAA," "TGA," "TAG," "UAA," "UGA" or "UAG."

The shortest length of the first region of the primary construct of the IVT polynucleotide of the present invention can be the length of a nucleic acid se uence that is sufficient to encode for a dipeptide, a tripeptide, a tetrapeptide, a pentapeptide, a he apeptide, a heptapeptide, an octapeptide, a nonapeptide, or a decapeptide. In another embodiment, the length may be sufficient to encode a peptide of 2-30 amino acids, e.g. 5-30, 10-30, 2-25, 5-25, 10-25, or 10-20 amino acids. The length may be sufficient to encode for a peptide of at least 11, 12, 13, 14, 15, 17, 20, 25 or 30 amino acids, or a peptide that is no longer than 40 amino acids, e.g. no longer than 35, 30, 25, 20, 17, 15, 14, 13, 12, 11 or 10 amino acids. E amples of dipeptides that the polynucleotide se uences can encode or include, but are not limited to, carnosine and anserine. It is understood that the NAV, RNAV or mRNA vaccines of the invention may be translatable and include such first region of a primary construct.

The length of the first region of the primary construct of the IVT polynucleotide encoding the polypeptide of interest of the present invention is greater than about 30 nucleotides in length (e.g., at least or greater than about 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, and 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000 or up to and including 100,000 nucleotides).

In some embodiments, the IVT polynucleotide includes from about 30 to about 100,000 nucleotides (e.g., from 30 to 50, from 30 to 100, from 30 to 250, from 30 to 500, from 30 to 1,000, from 30 to 1,500, from 30 to 3,000, from 30 to 5,000, from 30 to 7,000, from 30 to 10,000, from 30 to 25,000, from 30 to 50,000, from 30 to 70,000, from 100 to 250, from 100 to 500, from 100 to 1,000, from 100 to 1,500, from 100 to 3,000, from 100 to 5,000, from 100 to 7,000, from 100 to 10,000, from 100 to 25,000, from 100 to 50,000, from 100 to 70,000, from 100 to 100,000, from 500 to 1,000, from 500 to 1,500, from 500 to 2,000, from 500 to 3,000, from 500 to 5,000, from 500 to 7,000, from 500 to 10,000, from 500 to 25,000, from 500 to 50,000, from 500 to 70,000, from 500 to 100,000, from 1,000 to 1,500, from 1,000 to 2,000, from 1,000 to 3,000, from 1,000 to 5,000, from 1,000 to 7,000, from 1,000 to 10,000, from 1,000 to 25,000, from 1,000 to 50,000, from 1,000 to 70,000, from 1,000 to 100,000, from 1,500 to 3,000, from 1,500 to 5,000, from 1,500 to 7,000, from 1,500 to 10,000, from 1,500 to 25,000, from 1,500 to 50,000, from 1,500 to 70,000, from 1,500 to 100,000, from 2,000 to 3,000, from 2,000 to 5,000, from 2,000 to 7,000, from 2,000 to 10,000, from 2,000 to 25,000, from 2,000 to 50,000, from 2,000 to 70,000, and from 2,000 to 100,000).

According to the present invention, the first and second flanking regions of the IVT polynucleotide may range independently from 15-1,000 nucleotides in length (e.g., greater than 30, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, and 900 nucleotides or at least 30, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, and 1,000 nucleotides).

According to the present invention, the tailing se uence of the IVT polynucleotide may range from absent to 500 nucleotides in length (e.g., at least 60, 70, 80, 90, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 nucleotides). Where the tailing region is a polyA tail, the length may be determined in units of or as a function of polyA Binding Protein binding. In this embodiment, the polyA tail is long enough to bind at least 4 monomers of PolyA Binding Protein. PolyA Binding Protein monomers bind to stretches of appro imately 38 nucleotides. As such, it has been observed that polyA tails of about 80 nucleotides and 160 nucleotides are functional.

According to the present invention, the capping region of the IVT polynucleotide may comprise a single cap or a series of nucleotides forming the cap. In this embodiment the capping region may be from 1 to 10, e.g. 2-9, 3-8, 4-7, 1-5, 5-10, or at least 2, or 10 or fewer nucleotides in length. In some embodiments, the cap is absent.

According to the present invention, the first and second operational regions of the IVT polynucleotide may range from 3 to 40, e.g., 5-30, 10-20, 15, or at least 4, or 30 or fewer nucleotides in length and may comprise, in addition to a Start and/or Stop codon, one or more signal and/or restriction se uences.

In one embodiment, the IVT polynucleotides of the present invention may be structurally modified or chemically modified. When the IVT polynucleotides of the present invention are chemically and/or structurally modified the polynucleotides may be referred to as "modified IVT polynucleotides."

In one embodiment, if the IVT polynucleotides of the present invention are chemically modified they may have a uniform chemical modification of all or any of the same nucleoside type or a population of modifications produced by mere downward titration of the same starting modification in all or any of the

same nucleoside type, or a measured percent of a chemical modification of all any of the same nucleoside type but with random incorporation, such as where all uridines are replaced by a uridine analog, e.g., pseudouridine. In another embodiment, the IVT polynucleotides may have a uniform chemical modification of two, three, or four of the same nucleoside type throughout the entire polynucleotide (such as all uridines and all cytosines, etc. are modified in the same way).

In one embodiment, the IVT polynucleotides of the present invention may include a se uence encoding a self-cleaving peptide, described herein, such as but not limited to the 2A peptide. The polynucleotide se uence of the 2A peptide in the IVT polynucleotide may be modified or codon optimized by the methods described herein and/or are known in the art.

In one embodiment, this se uence may be used to separate the coding region of two or more polypeptides of interest in the IVT polynucleotide.

In one embodiment, the IVT polynucleotide of the present invention may be structurally and/or chemically modified. When chemically modified and/or structurally modified the IVT polynucleotide may be referred to as a "modified IVT polynucleotide."

In one embodiment, the IVT polynucleotide may encode at least one peptide or polypeptide of interest. In another embodiment, the IVT polynucleotide may encode two or more peptides or polypeptides of interest. Non-limiting e amples of peptides or polypeptides of intest include heavy and light chains of antibodies, an enzyme and its substrate, a label and its binding molecule, a second messenger and its enzyme or the components of multimeric proteins or comple es.

Chimeric Polynucleotide Architecture

The chimeric polynucleotides or RNA constructs of the present invention maintain a modular organization similar to IVT polynucleotides, but the chimeric polynucleotides comprise one or more structural and/or chemical modifications or alterations which impart useful properties to the polynucleotide. As such, the chimeric polynucleotides which are modified mRNA molecules of the present invention are termed "chimeric modified mRNA" or "chimeric mRNA."

It is to be understood that the antigens of the NAVs of the present invention may be encoded by a chimeric polynucleotide, RNA construct, chimeric modified mRNA or chimeric mRNA.

Chimeric polynucleotides have portions or regions which differ in size and/or chemical modification pattern, chemical modification position, chemical modification percent or chemical modification population and combinations of the foregoing.

E amples of parts or regions, where the chimeric polynucleotide functions as an mRNA and encodes a polypeptide of interest include, but are not limited to, untranslated regions (UTRs, such as the 5 UTR or 3 UTR), coding regions, cap regions, polyA tail regions, start regions, stop regions, signal se uence regions, and combinations thereof. FIG. 2 illustrates certain embodiments of the chimeric polynucleotides of the invention which may be used as mRNA. FIG. 3 illustrates a schematic of a series of chimeric polynucleotides identifying various patterns of positional modifications and showing regions analogous to those regions of an mRNA polynucleotide. Regions or parts that oin or lie between other regions may also be designed to have subregions. These are shown in the figure.

In some embodiments, the chimeric polynucleotides of the invention have a structure comprising Formula I. 5 A.sub.n .sub. -L1- B.sub.o .sub.y-L2- C.sub.p .sub.z-L3 3 Formula I

wherein:

each of A and B independently comprise a region of linked nucleosides;

C is an optional region of linked nucleosides;

at least one of regions A, B, or C is positionally modified, wherein said positionally modified region comprises at least two chemically modified nucleosides of one or more of the same nucleoside type of adenosine, thymidine, guanosine, cytidine, or uridine, and wherein at least two of the chemical modifications of nucleosides of the same type are different chemical modifications;

n, o and p are independenty an integer between 15-1000;

and y are independently 1-20;

z is 0-5;

L1 and L2 are independently optional linker moieties, said linker moieties being either nucleic acid based or non-nucleic acid based; and

L3 is an optional con ugate or an optional linker moiety, said linker moiety being either nucleic acid based or non-nucleic acid based.

In some embodiments the chimeric polynucleotide of Formula I encodes one or more peptides or polypeptides of interest. Such encoded molecules may be encoded across two or more regions.

In another aspect, the invention features a chimeric polynucleotide encoding a polypeptide, wherein the polynucleotide has a se uence including Formula II: A.sub.n -L.sup.1- B.sub.o Formula II

wherein each A and B is independently any nucleoside;

n and o are, independently 15 to 1000; and

L.sup.1 has the structure of Formula III:

STR00001

wherein a, b, c, d, e, and f are each, independently, 0 or 1;

each of R.sup.1, R.sup.3, R.sup.5, and R.sup.7, is, independently, selected from optionally substituted C.sub.1-C.sub.6 alkylene, optionally substituted C.sub.1-C.sub.6 heteroalkylene, O, S, and NR.sup.8;

R.sup.2 and R.sup.6 are each, independently, selected from carbonyl, thiocarbonyl, sulfonyl, or phosphoryl;

R.sup.4 is optionally substituted C.sub.1-10 alkylene, optionally substituted C.sub.2-10 alkenylene, optionally substituted C.sub.2-10 alkynylene, optionally substituted C.sub.2-C.sub.9 heterocyclylene, optionally substituted C.sub.6-C.sub.12 arylene, optionally substituted C.sub.2-C.sub.100 polyethylene glycolene, or optionally substituted C.sub.1-10 heteroalkylene, or a bond linking (R.sup.1).sub.a--(R.sup.2).sub.b--(R.sup.3).sub.c to (R.sup.5).sub.d--(R.sup.6).sub.e--(R.sup.7).sub.f, wherein if c, d, e, f, g, and h are 0, R.sup.4 is not a bond; and