TABLE 49-continued

MN Antibody Titer Results

| Cohort | Treatment | Geomean† Day 0 | Day 7 | Day 21 | Day 49 |
|---|---|---|---|---|---|
| 3 | H7N9/N1-methyl pseudouridine/ C1/MC3 50 μg Vaccine | 13.16 | N/A | N/A | 89.80 |
| 3 | H7N9/N1-methyl pseudouridine/ C1/MC3 10 μg Vaccine | 13.12 | N/A | N/A | 77.51 |
| 3 | PBS | 12.70 | N/A | N/A | 10.68 |
| 3 | H7N9/MC3 (−14 kDa Cap) 200 μg | 10.33 | N/A | N/A | 46.21 |
| 4 | H7N9/N1-methyl pseudouridine/ C1/MC3 200 μg Vaccine | 10.33 | N/A | 63.44 | 697.88 |
| 4 | H7N9/N1-methyl pseudouridine/ C1/MC3 50 μg Vaccine | 10.68 | N/A | 29.99 | 273.04 |
| 4 | H7N9/N1-methyl pseudouridine/ C1/MC3 10 μg Vaccine | 10.68 | N/A | 31.97 | 232.65 |
| 4 | PBS | 12.01 | N/A | 11.03 | 10.68 |
| 4 | H7N9/MC3 (−14 kDa Cap) 200 μg | 11.78 | N/A | 16.47 | 69.06 |

†20 is the lower limit of detection of the MN assay. All <20 were assigned a value of 10 for producing the geomean values.
‡N/A = Not applicable.

Quite surprisingly, the vaccine constructs of the invention reduced viral titers in the lungs when exposed to virus just 7 days following vaccination. Statistically significant increases in antibody titer as measured by HAI and MN were detected as early as 7 days following vaccination. A second vaccination (i.e., booster) did increase antibody titers, but did not statistically reduce the viral titer, as a single vaccination eliminated all virus in most animals. The −14 kDa cap vaccine at 200 μg/animal provided less protection than 10-μg full vaccine, but did reduce viral burden in the lung and increased antibody titers, both relative to PBS control.

While the present invention has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the invention.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit of the invention in its broader aspects.

## SEQUENCE LISTING

The patent contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US10709779B2). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

The invention claimed is:

**1**. A method of vaccinating a subject comprising administering to the subject a nucleic acid vaccine comprising one or more messenger ribonucleic acid (mRNA) polynucleotides comprising an open reading frame encoding an antigenic polypeptide that is derived from an infectious agent, wherein the mRNA polynucleotide is not self-replicating RNA, wherein the mRNA polynucleotides are formulated within a cationic lipid nanoparticle having a molar ratio of about 20-60% ionizable cationic lipid: about 5-25% non-cationic lipid: about 25-55% sterol; and about 0.5-15% PEG-modified lipid, wherein the nucleic acid vaccine elicits an immune response having a longer lasting antibody titer than an antibody titer elicited by a reference nucleic acid vaccine comprising the one or more mRNA polynucleotides not formulated within a cationic lipid nanoparticle having a molar ratio of about 20-60% ionizable cationic lipid: about 5-25% non-cationic lipid: about 25-55% sterol; and about 0.5-15% PEG-modified lipid.

**2**. The method of claim **1**, wherein the method comprises administering to the subject a single dosage of between 0.001 mg/kg and 0.005 mg/kg of the nucleic acid vaccine in an effective amount to vaccinate the subject.

**3**. The method of claim **1**, wherein the open reading frame is codon-optimized.

**4**. The method of claim **1**, wherein the ionizable cationic lipid nanoparticle has a polydispersity value of less than 0.4.

**5**. The method of claim **1**, wherein the polynucleotide has a poly-A tail of 80-250 nucleotides in length.

**6**. The method of claim **1**, wherein a second dose of the nucleic acid vaccine is administered to the subject.

7. The method of claim 1, wherein the mRNA polynucleotide includes a chemical modification selected from the group consisting of pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine, and 2'-O-methyl uridine.

8. The method of claim 1, wherein the mRNA polynucleotide includes from about 500 to about 2,000 nucleotides.

9. The method of claim 1, wherein a single dosage of between 25 ug/kg and 400 ug/kg of the nucleic acid vaccine is administered to the subject in an effective amount to vaccinate the subject.

10. The method of claim 1, wherein the vaccine is administered to the subject via a route selected from the group consisting of intramuscular administration, intradermal administration and subcutaneous administration.

11. The method of claim 1, wherein the method of producing an antigen specific immune response involves a single administration of the vaccine.

* * * * *