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(71) Applicant: **THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY** [US/US];
P.O. Box 20386, Office of the General Counsel, Building 170, 3rd Floor, Main Quad, Stanford, California 94305-2038 (US).

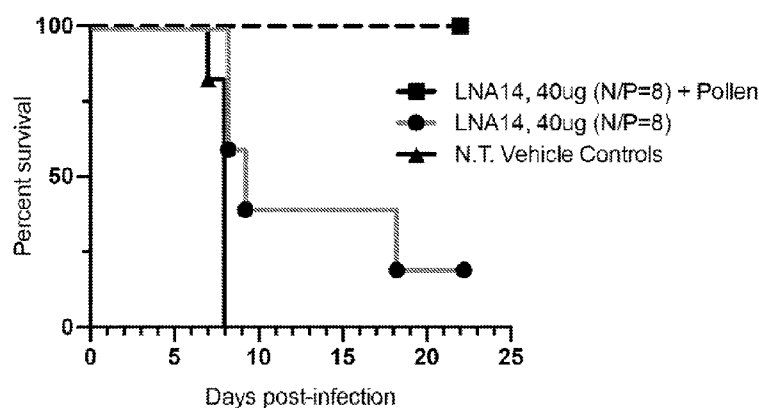
(72) Inventors: **GLENN, Jeffrey S.**; 2061 Webster Street, Palo Alto, California 94301 (US). **ELAZAR, Menashe**; 965 Clark Way, Palo Alto, California 94304 (US). **PHAM, Edward A.**; 3358 Bryant Street, Palo Alto, California 94306 (US). **SALUTI, Rachel**; 269 Campus Drive, CCSR Bldg. Rm. 3115, Stanford, California 94305 (US).

(74) Agent: **FIELD, Bret E.**; 201 Redwood Shores Parkway, Suite 200, Redwood City, California 94065 (US).

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(54) Title: PULMONARY AGENT DELIVERY METHODS AND COMPOSITIONS FOR PRACTICING THE SAME

FIG. 1A



(57) Abstract: Methods of delivering a pulmonary agent to a cell are provided. Aspects of embodiments of the methods include contacting a cell with a pulmonary agent delivery composition, where the composition includes a pulmonary agent and a deproteinized pollen shell component. In some instances, the pulmonary agent delivery composition further includes a transfection agent. Also provided are pulmonary agent delivery compositions, as well as delivery devices, systems and kits, e.g., that find use in practicing the methods. The methods and compositions find use in a variety of different applications, including the treatment or prevention of lung conditions.



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PULMONARY AGENT DELIVERY METHODS AND COMPOSITIONS FOR PRACTICING THE SAME

CROSS-REFERENCE TO RELATED APPLICATION

5 Pursuant to 35 U.S.C. § 119 (e), this application claims priority to the filing date of United States Provisional Patent Application Serial No. 62/988,847 filed March 12, 2020, the disclosure of which application is incorporated herein by reference in its entirety.

GOVERNMENT RIGHTS

10 This invention was made with Government support under contract AI109662 awarded by the National Institutes of Health. The Government has certain rights in the invention.

INTRODUCTION

Pulmonary or nasal delivery is becoming increasingly favored administration
15 route in a number of contexts. Pulmonary or nasal delivery methods are relatively non-invasive, and frequently do not require special medical professional assistance. Pulmonary or nasal delivery is of interest in both local and system administration protocols.

Drugs which are conventionally administered in this way include compounds
20 such as bronchodilators used in the treatment of conditions such as asthma, chronic obstructive pulmonary disease (COPD) or cystic fibrosis, anti-inflammatories for use in the treatment of allergies such as hay fever. However, antibiotics for use in the treatment of pulmonary infections and lung surfactants for treatment of infant respiratory distress syndrome are also being evaluated.

25 Pulmonary delivery also finds use in the systemic delivery of a variety of different types of active agents, including small molecules, as well as biologic agents, such as peptides/proteins and nucleic acids. Pulmonary delivery is a desirable systemic delivery method because of the large surface area of the 300+million alveoli that present a thin barrier (e.g., a 0.2-micron thick epithelial lining) to the blood, as well as a low
30 concentration of proteolytic enzymes.

Nucleic acid-based therapeutics represent a powerful means of addressing a wide range of respiratory conditions, including diseases involving the lung parenchyma. Delivery of such therapeutics, however, remains a challenge, with most means of nucleic acid delivery leading to accumulation in liver and kidney, as opposed to the lung. jetPEI™ (Polyplus Transfection, Illkirch, France) is a polyethylenimine-based transfection reagent that can deliver significant amounts of nucleic acid therapeutics to the lung. Unfortunately, transfection reagents such as jetPEI™ are associated with significant toxicity, which can limit the delivery of optimal ratios of nucleic acid therapeutics, such as locked nucleic acids (LNAs), to jetPEI™ that can be administered *in vivo*.

SUMMARY

Methods of delivering a pulmonary agent to a cell are provided. Aspects of embodiments of the methods include contacting a cell with a pulmonary agent delivery composition, where the composition includes a pulmonary agent and a deproteinized pollen shell component. In some instances, the pulmonary agent delivery composition further includes a transfection agent. Also provided are pulmonary agent delivery compositions, as well as delivery devices, systems and kits, e.g., that find use in practicing the methods. The methods and compositions find use in a variety of different applications, including the treatment or prevention of lung conditions.

BRIEF DESCRIPTION OF THE FIGURES

The skilled artisan will understand that the drawings, described below, are for illustration purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

FIGS. 1A-1B depict percent survival (FIG. 1A), and clinical score (FIG. 1B) of mice treated intranasally with 40 µg of the oligonucleotide LNA14 in combination with in vivo JetPEI, compared to the same combination with the addition of a deproteinized pollen shell component 14 days prior to inoculation with a lethal dose of influenza virus. Mice treated with vehicle (5% Glucose with pollen) served as controls.

FIG. 1C depicts percent survival of mice treated intravenously with 40 µg of the oligonucleotide LNA14 in combination with in vivo JetPEI, compared to the same combination with the addition of a deproteinized pollen shell component 3 days post infection with a lethal dose of influenza virus.

5 FIG1D depicts the percent survival of mice treated with LNA alone ("naked" LNA) without a delivery reagent or carrier, such as in vivo JetPEI or pollen. Mice were intranasally pretreated with various doses of naked LNA14 ranging from 30ug to 1000ug one week prior to lethal infection with PR8 virus.

10 FIG 2 depicts percent survival of mice administered a 40 µg dose of LNA14 mixed with deproteinized empty pollen shells alone and delivered intranasally (IN) 3 days prior to inoculation with a lethal dose of influenza virus. Vehicle alone was administered as a control.

15 FIGS. 3A-3C depict percent survival (FIG. 3A), body weight (FIG. 3B) and clinical score (FIG. 3C) of mice intranasally treated with 20 µg, 40 µg, or 60 µg of the oligonucleotide LNA14 with a deproteinized pollen shell component 3 days before inoculation with lethal dose of influenza virus. Mice treated with 80 µg Scramble LNA (SCR) or Phosphate-buffered saline (PBS) served as controls.

20 FIG. 4 depicts percent survival of mice administered 40 µg of the oligonucleotide LNA14 formulated with the transfection agent jetPEI™, either fresh or lyophilized and then reconstituted with water intranasally, three days prior to the inoculation with a lethal dose of influenza virus. Mice treated with vehicle alone served as controls.

DEFINITIONS

25 Before describing exemplary embodiments in greater detail, the following definitions are set forth to illustrate and define the meaning and scope of the terms used in the description.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton, et al., DICTIONARY OF MICROBIOLOGY AND
30 MOLECULAR BIOLOGY, 2D ED., John Wiley and Sons, New York (1994), and Hale & Markham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial,

N.Y. (1991) provide one of skill with the general meaning of many of the terms used herein. Still, certain terms are defined below for the sake of clarity and ease of reference.

As used herein, the term "effective amount" refers to that amount of a substance (e.g., an agent of interest) that produces some desired local or systemic effect. Effective amounts of pulmonary agents of interest vary depending on a variety of factors including, but not limited to, the weight and age of the subject, the condition being treated, the severity of the condition, the manner of administration and the like, and can readily be determined, e.g., determined empirically using data such as that data provided in the experimental section below.

The term "sample" as used herein relates to a material or mixture of materials, typically, although not necessarily, in fluid, i.e., aqueous, form, containing one or more components of interest. Samples may be derived from a variety of sources such as from a biological sample or solid, such as tissue or fluid isolated from an individual, including but not limited to, for example, plasma, serum, spinal fluid, semen, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs, and also samples of in vitro cell culture constituents (including but not limited to conditioned medium resulting from the growth of cells in cell culture medium, putatively virally infected cells, recombinant cells, and cell components). Components in a sample are termed "analytes" herein. In many embodiments, the sample is a complex sample containing at least about 10^2 , 5×10^2 , 10^3 , 5×10^3 , 10^4 , 5×10^4 , 10^5 , 5×10^5 , 10^6 , 5×10^6 , 10^7 , 5×10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} or more species of analyte.

"Antibody fragments" comprise a portion of an intact antibody, for example, the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 (1995)); single-chain antibody molecules; and multi-specific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation

reflecting the ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

The terms “polypeptide” and “protein”, used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term “fusion protein” or grammatical equivalents thereof is meant a protein composed of a plurality of polypeptide components, that while typically un-joined in their native state, typically are joined by their respective amino and carboxyl termini through a peptide linkage to form a single continuous polypeptide. Fusion proteins may be a combination of two, three or even four or more different proteins. The term polypeptide includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; fusion proteins with detectable fusion partners, e.g., fusion proteins including as a fusion partner a fluorescent protein, β-galactosidase, luciferase, etc.; and the like. In addition, the term protein can further encompass the post-translational modification including, but not limited to, glycosylation, phosphorylation, methylation, acetylation.

In general, polypeptides may be of any length, e.g., greater than 2 amino acids, greater than 4 amino acids, greater than about 10 amino acids, greater than about 20 amino acids, greater than about 50 amino acids, greater than about 100 amino acids, greater than about 300 amino acids, usually up to about 500 or 1000 or more amino acids. “Peptides” are generally greater than 2 amino acids, greater than 4 amino acids, greater than about 10 amino acids, greater than about 20 amino acids, usually up to about 50 amino acids. In some embodiments, peptides are between 5 and 30 amino acids in length.

The term “specific binding” refers to the ability of an agent to preferentially bind to a particular target that is present in a homogeneous mixture of different analytes. In some cases, a specific binding interaction will discriminate between desirable and undesirable analytes in a sample, typically more than about 10 to 100-fold or more (e.g.,

more than about 1000-fold). Specific binding can include hybridization, polypeptide-nucleic acid interactions or small molecule-nucleic acid interactions.

“Oligonucleotide” refers to ribose and/or deoxyribose nucleoside subunit polymers having between about 2 and about 200 contiguous subunits. The nucleoside subunits can be joined by a variety of intersubunit linkages, including, but not limited to, phosphodiester, phosphotriester, an alkylphosphonate, e.g., methylphosphonate, P3'→N5' phosphoramidate, N3'→P5' phosphoramidate, N3'→P5' thiophosphoramidate, phosphorodiamidate, and phosphorothioate linkages. In certain cases, intersubunit linkage has a chiral atom. Representative chiral intersubunit linkages include, but are not limited to, alkylphosphonates, phosphorodiamidates and phosphorothioates. Further, “oligonucleotides” includes modifications, such as those known to one skilled in the art, e.g., to the sugar (e.g., 2' substitutions), the base (see the definition of “nucleoside” below), and/or the 3' and 5' termini. In embodiments where the oligonucleotide moiety includes a plurality of intersubunit linkages, each linkage may be formed using the same chemistry or a mixture of linkage chemistries may be used. In embodiments where the oligonucleotide moiety includes a plurality of intersubunit linkages, one or more of the linkages may be chiral. Linkages having a chiral atom can be prepared as racemic mixtures, or as separate enantiomers. The terms “oligonucleotide”, “nucleic acid,” “nucleic acid molecule,” “nucleic acid fragment,” “nucleic acid sequence or segment,” or “polynucleotide” are used interchangeably and may also be used interchangeably with gene, cDNA, DNA and RNA encoded by a gene.

A “bicyclic nucleic acid” or a “bridged nucleic acid” (BNA) refers to a modified RNA nucleotide where the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon, thereby forming a bicyclic ring system. BNA monomers can contain a five-membered, six-membered or a seven-membered bridge structure with a fixed 3'-endo conformation. Bridged nucleic acids include without limitation, locked nucleic acids (LNA), ethylene-bridged nucleic acids (ENA) and constrained ethyl (cEt).

A “bridge” refers to a chain of atoms or a valence bond connecting two bridgeheads, where a “bridgehead” is any skeletal atom of a ring system (e.g., the ribose ring system) which is bonded to three or more skeletal atoms (excluding hydrogen). In some embodiments, the bridge in a BNA has 7-12 ring members and 1-4

heteroatoms independently selected from nitrogen, oxygen, or sulfur. Unless otherwise specified, a BNA is optionally substituted with one or more substituents, e.g., including, but not limited to alkyl, substituted alkyl, alkoxy, substituted alkoxy, hydroxy, amino and halogen.

5 A “Locked nucleic acid” (LNA) is a modified RNA nucleotide where the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon, thereby forming a bicyclic ring system. The bridge “locks” the ribose in the 3'-endo conformation, which is often found in the A-form duplexes. Locked nucleic acids are also encompassed by the term “bicyclic nucleic acids” or “bridged nucleic acids” (BNA). LNA
10 nucleotides can be mixed with any convenient nucleotides or nucleotide analogs, such as DNA or RNA residues in an oligonucleotide whenever desired. LNA's hybridize with DNA or RNA according to Watson-Crick base-pairing rules. Such oligomers can be synthesized chemically. In general, the locked ribose conformation enhances base stacking and backbone pre-organization to increase the hybridization properties
15 (melting temperature) of the oligonucleotide.

 An “ethylene-bridged nucleic acid” (ENA) refers to an LNA modified RNA nucleotide where the ribose moiety is modified with an extra bridge containing two carbon atoms between the 2' oxygen and the 4' carbon (see, e.g., Morita et al., Bioorganic Medicinal Chemistry, 2003, 11(10), 2211-2226). Ethylene-bridged nucleic
20 acids are also encompassed by the term “bicyclic nucleic acids” or “bridged nucleic acids” (BNA).

 A “constrained ethyl (cEt)” refers to an LNA modified RNA nucleotide where the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon, wherein the carbon atom of the bridge includes a methyl group. In some cases, the cEt
25 is (S)-constrained ethyl. In other cases, the cEt is (R)-constrained ethyl (see, e.g., Pallan et al., Chem. Commun. (Camb)., 2012, 48(66), 8195-8197). Constrained ethyl nucleic acids are also encompassed by the term “bicyclic nucleic acids” or “bridged nucleic acids” (BNA).

 As used herein, the term “2'-modified” or “2'-substituted” means a sugar
30 comprising a substituent at the 2'-position other than H or OH. 2'-modified nucleotides, include moieties with 2' substituents selected from alkyl, allyl, amino, azido, fluoro, thio,

O-alkyl, e.g., O-methyl, O-allyl, OCF_3 , $\text{O}-(\text{CH}_2)_2\text{-O-CH}_3$ (e.g., 2'-O-methoxyethyl (MOE)), $\text{O}-(\text{CH}_2)_2\text{SCH}_3$, $\text{-(CH}_2)_2\text{-ONR}_2$, and $\text{O-CH}_2\text{C(O)-NR}_2$, where each R is independently selected from H, alkyl, and substituted alkyl.

The disclosure encompasses isolated or substantially purified nucleic acid
5 molecules and compositions containing those molecules. In the context of the present disclosure, an "isolated" or "purified" DNA molecule or RNA molecule is a DNA molecule or RNA molecule that exists apart from its native environment and is therefore not a product of nature. An isolated DNA molecule or RNA molecule may exist in a purified form or may exist in a non-native environment such as, for example, a transgenic host
10 cell. For example, an "isolated" or "purified" nucleic acid molecule or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In one embodiment, an "isolated" nucleic acid is free of sequences that naturally flank the nucleic acid (i.e., sequences
15 located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Fragments and
20 variants of the disclosed nucleotide sequences are also encompassed by the present disclosure. By "fragment" or "portion" is meant a full length or less than full length of the nucleotide sequence. The siRNAs of the present disclosure can be generated by any method known to the art, for example, by in vitro transcription, recombinantly, or by synthetic means. In one example, the siRNAs can be generated in vitro by using a
25 recombinant enzyme, such as T7 RNA polymerase, and DNA oligonucleotide templates.

A "small interfering" or "short interfering RNA" or siRNA is an RNA duplex of nucleotides that is targeted to a gene interest. An "RNA duplex" refers to the structure formed by the complementary pairing between two regions of an RNA molecule. siRNA
30 is "targeted" to a gene in that the nucleotide sequence of the duplex portion of the siRNA is complementary to a nucleotide sequence of the targeted gene. In some

embodiments, the length of the duplex of siRNAs is less than 30 nucleotides. In some embodiments, the duplex can be 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 nucleotides in length. In some embodiments, the length of the duplex is 19 - 25 nucleotides in length. The RNA duplex portion of the siRNA can be
5 part of a hairpin structure. In addition to the duplex portion, the hairpin structure may contain a loop portion positioned between the two sequences that form the duplex. The loop can vary in length. In some embodiments the loop is 5, 6, 7, 8, 9, 10, 11, 12 or 13 nucleotides in length. The hairpin structure can also contain 3' or 5' overhang portions. In some embodiments, the overhang is a 3' or a 5' overhang 0, 1, 2, 3, 4 or 5
10 nucleotides in length.

The term "lipid" is used broadly herein to encompass substances that are soluble in organic solvents, but sparingly soluble, if at all, in water. The term lipid includes, but is not limited to, hydrocarbons, oils, fats (such as fatty acids, glycerides), sterols, steroids and derivative forms of these compounds. Preferred lipids are fatty acids and their
15 derivatives, hydrocarbons and their derivatives, and sterols, such as cholesterol. As used herein, the term lipid also includes amphipathic compounds which contain both lipid and hydrophilic moieties. Fatty acids usually contain even numbers of carbon atoms in a straight chain (commonly 12-24 carbons) and may be saturated or unsaturated, and can contain, or be modified to contain, a variety of substituent groups.
20 For simplicity, the term "fatty acid" also encompasses fatty acid derivatives, such as fatty amides produced by the conjugation reactions, e.g., with a modified terminal of an oligonucleotide.

The term 'pulmonary delivery' here is meant to encompass the respiratory tract or components thereof including but not limited to the nose, nasopharynx, oropharynx,
25 laryngopharynx, trachea, and lungs including bronchi, bronchioles, alveoli, interstitium and cells lining or contained within all the above.

Other definitions of terms may appear throughout the specification.

DETAILED DESCRIPTION

30 Methods of delivering a pulmonary agent to a cell are provided. Aspects of embodiments of the methods include contacting a cell with a pulmonary agent delivery

composition, where the composition includes a pulmonary agent and a deproteinized pollen shell component. In some instances, the pulmonary agent delivery composition further includes a transfection agent. Also provided are pulmonary agent delivery compositions, as well as delivery devices, systems and kits, e.g., that find use in practicing the methods. The methods and compositions find use in a variety of different applications, including the treatment or prevention of lung conditions.

Before the present invention is described in greater detail, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Certain ranges are presented herein with numerical values being preceded by the term "about." The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, representative illustrative methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

It is noted that, as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

While the apparatus and method has or will be described for the sake of grammatical fluidity with functional explanations, it is to be expressly understood that the claims, unless expressly formulated under 35 U.S.C. §112, are not to be construed as necessarily limited in any way by the construction of "means" or "steps" limitations, but are to be accorded the full scope of the meaning and equivalents of the definition provided by the claims under the judicial doctrine of equivalents, and in the case where the claims are expressly formulated under 35 U.S.C. §112 are to be accorded full statutory equivalents under 35 U.S.C. §112.

METHODS

As summarized above, methods of delivering a pulmonary agent to a cell are provided. By delivering a pulmonary agent to a cell is meant providing the agent to a cell so that the agent is associated with the cell in some manner, e.g., introduced inside of the cell, contacted with a surface molecule of the cell, etc. Accordingly, embodiments of the methods may result in the association of the pulmonary agent with the cell in a variety of different ways, e.g., by binding to a surface molecule of the cell, by entering the cell and/or an organelle thereof, etc.

Pulmonary Agent Delivery Compositions

Aspects of embodiments of the methods include contacting a cell with a pulmonary agent delivery composition in a manner sufficient to deliver the pulmonary agent to the cell. Pulmonary agent delivery compositions employed in embodiments of the invention may vary. In some instances, the pulmonary agent delivery composition includes a pulmonary agent and a deproteinized shell component. The term “pulmonary agent” is used broadly herein to encompass active agents that can treat or prevent a condition that affects the respiratory tract including the naso-oropharyngeal passage. Non-limiting examples of pulmonary agents include, but are not limited to, nucleic acids, peptides, polypeptides, carbohydrate antigens, antibodies or fragments thereof and small molecule compounds. In certain embodiments, the pulmonary agent is not an antigen.

In some instances, the pulmonary agent is a nucleic acid pulmonary agent. According to some embodiments, the nucleic acid is a deoxyribonucleic acid (DNA). In certain embodiments, the nucleic acid is a ribonucleic acid (RNA). According to some embodiments, the nucleic acid is an oligonucleotide.

Where the nucleic acid pulmonary agent is an oligonucleotide, the oligonucleotide may be of any suitable length. In certain embodiments, the oligonucleotide ranges from 5 nucleotides to 30 nucleotides in length, such as from 5 to 10, from 10 to 15, from 15 to 20, from 20 to 25 or from 25 to 30 nucleotides in length. According to some embodiments, the oligonucleotide comprises a locked nucleic acid (LNA) nucleotide. The oligonucleotide sequences can include any convenient number of DNA, RNA and LNA nucleotides. In some instances of the oligonucleotide sequences

described herein, the sequence is a mixed RNA/DNA sequence. In some instances of the oligonucleotide sequences described herein, the sequence is a mixed LNA/DNA sequence. In some instances of the oligonucleotide sequences described herein, the sequence is a mixed LNA/RNA sequence. In some instances of the oligonucleotide sequences described herein, the sequence includes only LNA nucleotides. In some instances of the oligonucleotide sequences described herein, the sequence includes only DNA nucleotides. In some instances of the oligonucleotide sequences described herein, the sequence includes only RNA nucleotides.

In certain cases, the linkages of the oligonucleotides are modified phosphate groups, e.g., where one or more oxygens of phosphate has been replaced with a different substituent. Without being bound to any particular theory, such modification can increase resistance of the oligonucleotide to nucleolytic breakdown. Examples of modified phosphate groups include, but are not limited to, phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoramidates, alkyl or aryl phosphonates and phosphotriesters. In some cases, the linkages of the oligonucleotides are modified phosphate groups where one or more of the non-bridging phosphonate oxygen atoms in the linkage has been replaced by a group selected from, S, Se, BR^a₃, alkyl, substituted alkyl, aryl, substituted aryl, H, NR^a₂, or OR^b, where R^a is H, alkyl, substituted alkyl, aryl, substituted aryl, and R^b is H, alkyl, substituted alkyl, aryl or substituted aryl. In certain cases, one or more of the linkages of the oligonucleotide the phosphorous atom is chiral, e.g., a stereogenic center. The stereogenic phosphorus atom can possess either the "R" configuration (referred to herein as Rp), or the "S" configuration (referred to herein as Sp). In certain embodiments, the linkages of the oligonucleotide include one or more stereogenic phosphorus atoms with at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more enantiomeric excess of the Sp configuration. In certain other instances, the linkages of the oligonucleotide include one or more stereogenic phosphorus atoms with at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more enantiomeric excess of the Rp configuration.

In certain embodiments, one or more of the linkages of the oligonucleotide are selected from methylphosphonate, P3'→N5' phosphoramidate, N3'→P5'

phosphoramidate, N3'→P5' thiophosphoramidate, phosphorodithioate and phosphorothioate linkages. In certain cases, one or more linkages of the oligonucleotide is a phosphorothioate linkage. In certain cases, the phosphorus atom in one or more of the phosphorothioate linkages is chiral. In some cases, the chiral phosphorus atom in the one or more phosphorothioate linkages has Rp configuration. In some cases, the chiral phosphorus atom in the one or more phosphorothioate linkages has Sp configuration. In certain embodiments, the linkages of the oligonucleotide include one or more phosphorothioate linkages with at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more enantiomeric excess of the Sp configuration. In certain other embodiments, the linkages of the oligonucleotide includes one or more phosphorothioates with at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more enantiomeric excess of the Rp configuration.

In certain instances, the oligonucleotide sequence is a bridged nucleic acid (e.g., as described herein). In certain instances, the oligonucleotide sequence includes one or more bridged nucleic acid nucleotides, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or even more.

In certain instances, the oligonucleotide sequence is a locked nucleic acid. In certain instances, the oligonucleotide sequence includes one or more locked nucleic acid nucleotides, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or even more.

In certain instances, the oligonucleotide sequence is an ethylene-bridged nucleic acid (ENA). In certain instances, the oligonucleotide sequence includes one or more ENA nucleotides, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or even more.

In certain instances, the oligonucleotide sequence is constrained ethyl (cEt) nucleic acid. In certain instances, the oligonucleotide sequence includes one or more cEt nucleotides, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or even more. In certain instances, the oligonucleotide sequence includes one or more (S)-constrained ethyl nucleic acids, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or even more. In certain instances, the oligonucleotide sequence

includes one or more (R)-constrained ethyl nucleic acids, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or even more. In certain instances, the oligonucleotide sequence includes one or more ribose modifications. In some cases, the oligonucleotide sequence includes one or more 2'-modified ribose sugars (also referred to herein as 2'-modified nucleotides). In certain instances, the oligonucleotide sequence includes one or more 2'-modified nucleotides, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or even more. 2'-modified nucleotides, include, but are not limited to moieties with 2' substituents selected from alkyl, allyl, amino, azido, fluoro, thio, O-alkyl, e.g., O-methyl, O-allyl, OCF₃, O-(CH₂)₂-O-CH₃ (e.g., 2'-O-methoxyethyl (MOE)), O-(CH₂)₂SCH₃, -(CH₂)₂-ONR₂, and O-CH₂C(O)-NR₂, where each R is independently selected from H, alkyl, and substituted alkyl. In certain instances, the oligonucleotide sequence includes one or more 2'-O-methoxyethyl (MOE) modifications, such as 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or even more.

In some embodiments, the agent is an oligonucleotide that comprises at least 5 deoxyribonucleotide units (e.g., least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20) and is capable of recruiting an RNase. In some case, the oligonucleotide recruits an RNase to catalyze the degradation of the target vRNA into smaller components. Any convenient methods and moieties for recruiting an RNase can be incorporated into the subject agents (e.g., oligonucleotides). In some instances, the oligonucleotide agent further includes a sequence that recruits an RNase of interest. It is understood that unless indicated otherwise, an oligonucleotide sequence as depicted herein is meant to include DNA sequences, RNA sequences (e.g., where U can optionally replace T), mixed RNA/DNA sequences, and analogs thereof, including analogs where one or more nucleotides of the sequence are modified nucleotides, such as BNA analogs, LNA analogs, ENA analogs, cEt analogs, 2'-modified analogs, and/or analogs where one or more internucleoside linkages are replaced, e.g., with a non-naturally occurring linkage such as a phosphorothioate, phosphorodithioate, phosphoramidate or thiophosphoramidate linkage. It will be understood that in embodiments where one or more of the linkages of

the oligonucleotide include a chiral phosphorous atom, e.g., a stereogenic center, the stereogenic phosphorus atom can possess either the “R” configuration (referred to herein as Rp), or the “S” configuration (referred to herein as Sp).

Oligonucleotides may be chemically synthesized by methods known in the art (see Wagner *et al.* (1993), *supra*, and Milligan *et al.*, *supra*.) Oligonucleotides may be chemically modified from the native phosphodiester structure, in order to increase their intracellular stability and binding affinity. A number of such modifications have been described in the literature, which alter the chemistry of the backbone, sugars or heterocyclic bases.

Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur; phosphoroamidites; alkyl phosphotriesters and boranophosphates. Achiral phosphate derivatives include 3'-O'-5'-S-phosphorothioate, 3'-S-5'-O-phosphorothioate, 3'-CH₂-5'-O-phosphonate, 3'-NH-5'-O-phosphoroamidate, and thiophosphoramidates. Peptide nucleic acids replace the entire ribose phosphodiester backbone with a peptide linkage. Sugar modifications are also used to enhance stability and affinity. The α -anomer of deoxyribose may be used, where the base is inverted with respect to the natural β -anomer. In certain cases, the 2'-OH of the ribose sugar may be altered, e.g., as described herein. The 2'-OH of the ribose sugar may be altered to form 2'-O-methyl or 2'-O-allyl sugars, which provides resistance to degradation without comprising affinity. In certain cases, modification of the 2'-OH of the ribose sugar can improve toxicity. Modification of the heterocyclic bases must maintain proper base pairing. Some useful substitutions include deoxyuridine for deoxythymidine; 5-methyl-2'-deoxycytidine and 5-bromo-2'-deoxycytidine for deoxycytidine. 5-propynyl-2'-deoxyuridine and 5-propynyl-2'-deoxycytidine have been shown to increase affinity and biological activity when substituted for deoxythymidine and deoxycytidine, respectively.

The oligonucleotide agents may be derivatized with any convenient modifying agent, e.g., by conjugation of the modifying agent to the 5'- and/or 3'terminal of the oligonucleotide sequence. In some cases, the modifying agent is a moiety that enhances cellular uptake (e.g., a lipid). Any convenient lipids may be conjugated to the subject oligonucleotides. In some instances, the modifying agent is a fatty acid,

connected to the 5' or 3' terminal via an optional linker. The lipid group can be an aliphatic hydrocarbon or fatty acid, including but not limited to, derivatives of hydrocarbons and fatty acids, with examples being saturated straight chain compounds having 14-20 carbons, such as myristic (tetradecanoic) acid, palmitic (hexadecanoic) acid, and stearic (octadecanoic) acid, and their corresponding aliphatic hydrocarbon forms, tetradecane, hexadecane and octadecane. Examples of other suitable lipid groups that may be employed are sterols, such as cholesterol, and substituted fatty acids and hydrocarbons, particularly polyfluorinated forms of these groups. The scope of the lipid group includes derivatives such as amine, amide, ester and carbamate derivatives.

In some cases, the modifying agent is a further nucleic acid sequence having a desirable activity (e.g., recruitment of an RNase, as described herein). In certain instances, the modifying agent has a specific binding activity that provides for delivery of the oligonucleotide to a particular target, such as a cell-specific protein. In some cases, the modifying agent is an antibody of interest that specifically binds a cell-specific target of interest. In certain instances, the antibody modifying agent is specifically binds a hemagglutinin (HA) target.

The oligonucleotide active agent can be utilized in any convenient form. In some instances, the oligonucleotide active agent is single stranded. In some instances, the oligonucleotide active agent is double stranded. In some instances, the oligonucleotide active agent is an siRNA. In some instances, the oligonucleotide active agent is an shRNA. In some instances, the oligonucleotide active agent is a ssRNA. In some instances, one or more nucleotides of the ssRNA can be replaced with LNA nucleotides. In some instances, the oligonucleotide active agent is a ssDNA.

In some instances, one or more nucleotides of the ssDNA can be replaced with LNA nucleotides. Non-limiting examples of nucleic acid pulmonary agents are provided in U.S. Patent Application No. 16/081,818 published as US20190136242; PCT Application Serial No. PCT/US2021/018025; and U.S. Patent Nos. 6,211,162, 8,808,747, 8,153,602, 9,840,705, 7,034,007, 7,585,968, 10,119,135, 9,487,778, 9,752,143; the disclosures of which are herein incorporated by reference. In some embodiments, the pulmonary agent is a nucleic acid that is not an antigen.

The term “antimicrobial oligonucleotide” is used broadly herein to encompass oligonucleotides that can treat or prevent a lung condition that is caused by a microbe in a subject. Non-limiting examples of antimicrobial oligonucleotides include antibacterial oligonucleotides, antifungal oligonucleotides and antiviral oligonucleotides. Non-limiting
5 examples of antibacterial oligonucleotides are provided in U.S. Patent Nos. 9,499,583, 7,790,694, 9,534,220 and 7,049,431; the disclosures of which oligonucleotides are herein incorporated by reference. Non-limiting examples of antiviral oligonucleotides are provided in U.S. Patent Application No. 16/081,818 published as US20190136242; PCT Application Serial No. PCT/ US2021/018025; and U.S. Patent Nos. 5,637,573,
10 6,495,675, 5,194,428, 10,378,014, 5,580,767 and 8,697,858; the disclosures of which antiviral oligonucleotides are herein incorporated by reference.

Nucleic acid pulmonary agents of interest that may be delivered to a cell in accordance with embodiments of the invention further include expression vectors, which expression vectors may vary. In some embodiments, such expression vectors include at
15 least a first nucleic acid segment that encodes a therapeutic peptide, protein, or polypeptide. In other embodiments, such expression vectors include at least a first nucleic acid segment that encodes an antisense molecule. In other embodiments, such expression vectors include a sequence region that encodes a functional mRNA, a tRNA, a ribozyme or an antisense RNA. When the use of such vectors is contemplated for
20 introduction of one or more exogenous proteins, polypeptides, peptides, ribozymes, and/or antisense oligonucleotides, to a particular cell transfected with the vector, one may employ at least a first exogenous polynucleotide operably positioned downstream and under the control of at least a first heterologous promoter that expresses the polynucleotide in a cell comprising the vector to produce the encoded peptide, protein,
25 polypeptide, ribozyme, siRNA, RNAi or antisense oligonucleotide. Such constructs may employ heterologous promoters that are constitutive, inducible, or even cell-specific promoters. Exemplary such promoters include, but are not limited to, viral, mammalian, and avian promoters, including for example a CMV promoter, a β -actin promoter, a hybrid CMV promoter, a hybrid β -actin promoter, an EF1 promoter, a U1a promoter, a
30 U1b promoter, a Tet-inducible promoter, a VP16-LexA promoter, and such like. The vectors or expression systems may also further include one or more enhancers,

regulatory elements, transcriptional elements, to alter or effect transcription of the heterologous gene cloned in the rAAV vectors. For example, the rAAV vectors of the present invention may further comprise at least a first CMV enhancer, a synthetic enhancer, or a cell- or tissue-specific enhancer. The exogenous polynucleotide may also further comprise one or more intron sequences. Various vectors (e.g., viral vectors, bacterial vectors, or vectors capable of replication in eukaryotic hosts) can be used in accordance with the present invention. Numerous vectors which can replicate in eukaryotic hosts are known in the art and are commercially available. In some instances, such vectors used in accordance with the invention are composed of a bacterial origin of replication and a eukaryotic promoter operably linked to the coding sequence of interest. Viral vectors used in accordance with the invention may be composed of a viral particle derived from a naturally-occurring virus which has been genetically altered to render the virus replication-defective and to express a recombinant gene of interest in accordance with the invention. Once the virus delivers its genetic material to a cell, it does not generate additional infectious virus but does introduce exogenous recombinant genes into the cell, and in some instances into the genome of the cell. Numerous viral vectors are known in the art, including, for example, retrovirus, adenovirus, helper-dependent adenovirus, adeno-associated virus (AAV), herpes simplex virus (HSV), cytomegalovirus (CMV), vaccinia and poliovirus vectors, lentivirus, poxvirus, hemagglutinin virus of Japan-liposome (HVJ) complex, Moloney murine leukemia virus, and HIV-based virus. In some instances, the vector that is employed is a non-integrating vector.

Pulmonary agents that may be delivered in accordance with embodiments of the methods also include peptides and proteins. As reviewed above, the terms “polypeptide” and “protein”, used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term “fusion protein” or grammatical equivalents thereof is meant a protein composed of a plurality of polypeptide components, that while typically un-joined in their native state, typically are joined by their respective amino and carboxyl termini through a peptide linkage to form a single

continuous polypeptide. Fusion proteins may be a combination of two, three or even four or more different proteins. The term polypeptide includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; fusion proteins with detectable fusion partners, e.g., fusion proteins including as a fusion partner a fluorescent protein, β -galactosidase, luciferase, etc.; and the like. In general, polypeptides may be of any length, e.g., greater than 2 amino acids, greater than 4 amino acids, greater than about 10 amino acids, greater than about 20 amino acids, greater than about 50 amino acids, greater than about 100 amino acids, greater than about 300 amino acids, usually up to about 500 or 1000 or more amino acids. "Peptides" are generally greater than 2 amino acids, greater than 4 amino acids, greater than about 10 amino acids, greater than about 20 amino acids, usually up to about 50 amino acids. In some embodiments, peptides are between 5 and 30 amino acids in length. Non-limiting examples of pulmonary peptide and polypeptide agents are provided in U.S. Patent Nos. 9,180,161, 9,655,973, 8,828,926, 8,153,599, 8,183,203, 5,952,303, 6,743,429, 5,744,445, 8,193,149, 5,164,369, 5,874,406, 6,013,619, 8,444,932, 7,863,241 and 8,338,380, the disclosures of which peptide/protein pulmonary agents is herein incorporated by reference.

In some instances, the pulmonary agent is a pulmonary antigen agent. By pulmonary antigen agent is meant a toxin or other foreign substance which induces an immune response in the body, especially the production of antibodies. Non-limiting examples of pulmonary antigen agents are provided in U.S. Patent Application No. 15/627,489 published as US20170281545 and U.S. Patent Nos. 9,561,271, 10,159,644, 10,391,167, 9,526,778, 8,741,313, 10,010,595, 9,492,525, 7,052,701 and 7,368,537, the disclosures of which antigen agents in herein incorporated by reference. However, as mentioned above, in some embodiments the pulmonary agent is not an antigen agent.

In some instances, the pulmonary agent is an antibody. Antibodies that may be used as agents in connection with the present disclosure can encompass, but are not limited to, monoclonal antibodies, polyclonal antibodies, bispecific antibodies, Fab

antibody fragments, F(ab)₂ antibody fragments, Fv antibody fragments (e.g., V_H or V_L), single chain Fv antibody fragments and dsFv antibody fragments. Furthermore, the antibody molecules may be fully human antibodies, humanized antibodies, or chimeric antibodies. The antibodies that may be used in connection with the present disclosure

5 can include any antibody variable region, mature or unprocessed, linked to any immunoglobulin constant region. Minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are encompassed by the present disclosure, providing that the variations in the amino acid sequence maintain 75% or more, e.g., 80% or more, 90% or more, 95% or more, or 99% or more of the sequence. In

10 particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether an amino acid change results in a functional peptide can be determined by assaying the specific activity of the polypeptide derivative. In some embodiments, the agent is an antibody fragment (e.g., as described herein). Non-

15 limiting examples of pulmonary antibody agents are provided in U.S. Patent Nos. 5,580,740, 7,754,207, 5,081,032, 10,479,836, 9,212,227, 8,574,572, 8,795,668, 9,803,024, 8,642,357, 10,125,191 and 10,376,581; the disclosures of which antibody agents are herein incorporated by reference.

In some embodiments, the pulmonary agent is a small molecule. Small

20 molecules of interest include, but are not limited to, small organic or inorganic compounds having a molecular weight (MW) of more than 50 and less than about 2,500 daltons (Da), such as more than 50 and less than about 1000 Da, or more than 50 and less than about 500 Da. "Small molecules" encompasses numerous biological and chemical classes, including synthetic, semi-synthetic, or naturally-occurring inorganic or

25 organic molecules, including synthetic, recombinant or naturally-occurring polypeptides and nucleic acids. Small molecules of interest can comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and can include at least an amine, carbonyl, hydroxyl or carboxyl group, and can contain at least two of the functional chemical groups. The small molecules can comprise cyclical

30 carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Small molecules are also found among

biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Non-limiting examples of pulmonary small molecule agents are provided in U.S. Patent Nos. 9,663,499, 10,344,003, 10,336,735, 9,233,089, 10,420,759, 9,200,035, 10,149,838 and 10,278,942; the disclosures of which small molecule agents are herein incorporated by reference.

As summarized above, in addition to the pulmonary agent, the pulmonary agent delivery compositions of embodiments of the invention further include a deproteinized pollen shell component. The term “deproteinized pollen shell component” refers to deproteinized empty pollen shells that have been treated to remove their potential allergy-inducing protein components. Accordingly, the deproteinized pollen shell component of this disclosure is inert and non-toxic. The deproteinized pollen shell component may include pollen from any suitable source. In certain embodiments, the deproteinized pollen shell component pollen includes deproteinized echinate pollen shells. In some embodiments, the deproteinized pollen shell component is obtained from at least one of: *Lycopodium clavatum*, paper mulberry (*Broussonetia papyrifera*), corn (*Zea mays*), Cocklebur (*Xanthium commune*), Goldenrod (*Solidago* spp.), Poverty weed (*Iva axillaris*), Desert Ragweed (*Ambrosia dumos*), False Ragweed (*Ambrosia acanthicarpa*), Giant Ragweed (*Ambrosia trifida*), Short Ragweed (*Ambrosia artemisifolia*), Slender Ragweed (*Ambrosia tenuifolia*), Southern Ragweed (*Ambrosia bidentata*), Western Ragweed (*Ambrosia psilostachya*), Prairie Sage (*Artemisia ludoviciana*), Common Sagebrush (*Artemisia tridentate*), Annual Wormwood (*artemisia annua*), Marsh Elder, Sunflower, Black Alder (*Alnus glutinosa*), Lamb’s Quarters (*Chenopodium album*) and High-Water Shrub. The deproteinized pollen shell component may be produced using any convenient protocol, where suitable methods of producing deproteinized pollen shells are known in the art. Non-limiting examples of methods of preparing deproteinized pollen shell components are provided in U.S. Patent Application No. 15/725,099 published as US20180092852 and U.S. Patent Nos. 7,846,654, 5,013,552 and 5,275,819, the disclosures of which methods as well as the deproteinized shell components produced thereby are herein incorporated by reference. The amount of deproteinized pollen shell component present in the pulmonary agent

delivery composition may vary as desired. Where the composition is a liquid, e.g., aqueous composition, the amount, in some instances, ranges from 0.5 µg/ml to 25 mg/ml, such as .1 µg/ml to 20 mg/ml

Pulmonary agent delivery compositions employed in embodiments of the methods may include one or more additional components. In some instances, the pulmonary agent delivery compositions may include a transfection agent. The term “transfection agent” is used broadly herein to encompass agents that facilitate delivery of nucleic acids, peptides, and/or polypeptides to cells. Transfection agents include polymers, lipids and polypeptide vesicles. Suitable transfection agents include polyethylenimine (PEI; ExGen500 (MBI Fermentas)), LipofectAMINE 2000 (Invitrogen) or derivatives thereof, or similar cationic polymers, including polypropyleneimine or polyethylenimine copolymers (PECs) and derivatives, synthetic amphiphils (SAINT-18), Lipofectin, DOTAP and/or viral capsid proteins that are capable of self-assembly into particles that can deliver each constituent as defined herein to a cell, etc. In certain embodiments, the transfection agents are cationic lipids, where examples of such transfection agents include, but are not limited to: Oligofectamin, TRANSIT-TKO, LipofectAmine2000, SiGuide, RNAiFect, and jetSi. In some embodiments, the transfection agents are cationic polymers, where examples of such transfection agents include, but are not limited to Superfect, jetPEI, and X-TREMGene. In some instances, the transfection agent is jetPEI (cationic polymer, linear polyethylenimine derivative, for nucleic acid transfection) from Polyplus-Transfection (France), e.g., as described in United States Patent Publication No. 20140343125 the disclosure of which is herein incorporated by reference. Other transfection agents of interest that may be employed in delivery compositions of the invention include, but are not limited to: Xfect, CRISPRMAX™. When present, the transfection agent is present in an amount ranging from N/P ratio of 4 to N/P ratio of 10. In those embodiments where the pulmonary agent is a nucleic acid pulmonary agent and the transfection agent is a cationic polymer, e.g., jetPEI, the N/P ratio may vary, and in some instances ranges from 4 to 10, such as 6 to 8.

In certain embodiments, the pulmonary agent delivery compositions may further include one or more additional components. Any convenient excipients, carriers, or

other components, etc. can be utilized in the compositions. Pharmaceutically acceptable carriers that find use in the compositions may include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable
5 organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and
10 the like. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like. Examples of carriers which may be used include, but are not limited to, alum, microparticles, liposomes, and nanoparticles. Any convenient additives can be included in the subject compositions to enhance the delivery of the subject pulmonary agent. Additives of
15 interest include, cellular uptake enhancers, carrier proteins, lipids, dendrimer carriers, carbohydrates, and the like. When present, these one or more additional components, collectively referred to as the vehicle, may make up any desired amount of the delivery composition.

The composition may be present in any convenient format, such as in liquid
20 format, dry format, etc. In some instances, the composition is a lyophilized composition. Lyophilization, also known as freeze-drying or cryodesiccation, is a low temperature dehydration process that involves freezing the product, lowering pressure, then removing the ice by sublimation. This process is in contrast to dehydration by most conventional methods that evaporate water using heat. Where the composition is a
25 lyophilized composition, the composition may be reconstituted, e.g. by combination with a suitable amount of a liquid, such as described above, e.g., an aqueous liquid, prior to use, e.g., contact with the cell. With respect to freeze dried compositions, embodiments of the invention include freeze dried compositions that include a pulmonary agent and a transfection agent, but not a deproteinized pollen shell component. As reported in the
30 experimental section below, the inventors have discovered that that co-formulated LNA/jetPEI mixtures can be lyophilized for future use, and that upon reconstitution the

mixture retains *in vivo* efficacy. As such, embodiments of the invention include freeze-dried pulmonary agents, such as described above, and a transfection agent. In some such embodiments, the transfection agents are cationic polymers, where examples of such transfection agents include, but are not limited to Superfect, jetPEI, and X-

5 TREMGene. In some instances, the transfection agent is jetPEI (cationic polymer, linear polyethylenimine derivative, for nucleic acid transfection) from Polyplus-Transfection (France), e.g., as described in United States Patent Publication No. 20140343125 the disclosure of which is herein incorporated by reference.

In some embodiments of the subject methods, the composition is an inhalable
10 composition. In certain embodiments of the subject methods, the composition is aerosolized. In some embodiments, the aerosol comprises particles having an average particle size of 1 to 100 micrometers in diameter. According to certain embodiments, the aerosol comprises particles having an average particle size of 1 to 1.5, 1.5 to 2, 2 to 2.5, 2.5 to 3, 3 to 3.5, 3.5 to 4, 4 to 4.5, 4.5 to 5, 5 to 5.5, or 5.5 to 6 micrometers in
15 diameter.

The pulmonary agent delivery compositions can be made using any convenient protocol and aspects of the invention further include methods of making the pulmonary agent delivery compositions disclosed herein. The fabrication methods may include combining the pulmonary agent and the deproteinized pollen shell component to
20 produce the pulmonary agent delivery composition. In some embodiments, the methods include combining the pulmonary agent, the deproteinized pollen shell component and the transfection agent. In certain embodiments, the methods include combining the pulmonary agent and the transfection agent. The methods may further include aerosolizing the composition. In some embodiments, the methods include
25 lyophilizing the compositions. In certain embodiments, the methods include lyophilizing and reconstituting the compositions.

Delivery of Pulmonary Agent to a Cell

Aspects of embodiments of the methods include contacting a cell with a
30 pulmonary agent delivery composition in a manner sufficient to deliver the pulmonary agent to the cell. The cell being contacted with a pulmonary agent can be any suitable

cell, where cells of interest include cells of the respiratory system. According to some embodiments, the cell is an epithelial cell, where in some instances the cell is a pulmonary cell. In some embodiments, the cell is a mammalian cell. In certain embodiments, the mammalian cell is a human cell. According to some embodiments, the cell is *in vitro*. In some embodiments, the cell is *in vivo*. Contact of the delivery composition with the cell may be achieved using any convenient protocol, where the particular protocol employed may depend on the environment of the target cell. For example, where the cell is *in vitro*, contact may be achieved by introducing the composition into the media of the cell, by introducing the cell into the composition, etc.

Where the cell is *in vivo*, contact may be achieved by administering the composition to a subject harboring the cell, where the administration protocol may be local or systemic, as desired.

Administration of a Pulmonary Agent Delivery Composition to a Subject

Aspects of embodiments of the methods include methods of treating or preventing a lung condition in a subject by administering to a subject in need thereof an effective amount of a pulmonary agent delivery composition as described herein. By “an effective amount” is meant the concentration of an agent that is sufficient to elicit the desired biological effect (e.g., treatment or prevention of the lung condition). By “treatment” is meant that at least an amelioration of the symptoms associated with the condition afflicting the host is achieved, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g., symptom, associated with the condition being treated. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, e.g., prevented from happening, or stopped, e.g., terminated, such that the host no longer suffers from the condition, or at least the symptoms that characterize the condition. Thus treatment includes: (i) prevention, that is, reducing the risk of development of clinical symptoms, including causing the clinical symptoms not to develop, e.g., preventing disease progression to a harmful state; (ii) inhibition, that is, arresting the development or further development of clinical symptoms, e.g., mitigating or completely inhibiting an active disease (e.g., infection); and/or (iii) relief, that is,

causing the regression of clinical symptoms. In the context of influenza A virus infection, the term “treating” includes any or all of: reducing the number of viral-infected cells in patient samples, inhibiting viral replication in the cells, and ameliorating one or more symptoms associated with an infection. By “prevention” is meant that the subject at risk of acquiring a respiratory condition is not infected despite exposure to the microorganism under conditions that would normally lead to the lung condition. In some cases, the administering of the subject pulmonary agent (e.g., oligonucleotide) protects the subject against infection instantaneously, for 1 day or more, 3 days or more, 1 week or more, 2 weeks or more, 3 weeks or more, 1 month or more, 2 months or more, 3 months or more, etc.

The term “lung condition” is used broadly herein to encompass conditions that may affect any part of the respiratory system. Non-limiting examples of lung conditions include, but are not limited to: influenza, asbestosis, asthma, bronchiectasis, bronchitis, chronic cough, chronic obstructive pulmonary disease (COPD), common cold, croup, cystic fibrosis, hantavirus, idiopathic pulmonary fibrosis, lung cancer, pandemic flu, pertussis, pleurisy, pneumonia, pulmonary embolism, pulmonary hypertension, respiratory syncytial virus (RSV), coxsackievirus, Epstein-Barr virus, cytomegalocirus, herpes simplex virus, varicella-zoster virus, hantavirus, human rhinovirus, enterovirus, adenovirus, bocavirus, human metapneumovirus, parainfluenza virus, coronavirus, sarcoidosis, sleep apnea, spirometry, sudden infant death syndrome (SIDS), bacterial pathogens such as *S. pneumoniae*, *H. influenzae*, *S. pyogenes*, *M. catarrhalis*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *M. pneumoniae*, *Legionella spp*, *anaerobic bacteria*, *Mycoplasma spp*, *C. burnelli*, *Chlamydia spp.*, and *S. aureus*, tuberculosis and work-related asthma, fungal infections such as by *C. albicans*, *H. capsulatum*, *B. dermatidis*, *P. brasiliensis*, *C. immitis*, *Aspergillus spp*, *Pneumocystis carinii* etc. In some embodiments, the lung condition is a lung infection. In some embodiments, the lung infection is a bacterial lung infection. In certain embodiments, the lung infection is a fungal lung infection. In some embodiments, the lung infection is a viral lung infection. In certain embodiments, the viral lung infection is an influenza infection. According to some embodiments, the influenza infection is an influenza A virus infection. According to certain embodiments, the influenza infection is an influenza B virus infection. In

certain embodiments, the cell is in vivo. In some embodiments, contacting the cell with the composition results in at least 1 log₁₀ titer deficits of the virus, such as at least 2.5, at least 3, at least 3.5, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 log₁₀ titer deficits of the virus.

5 In some embodiments, the compositions can be broad spectrum compositions. As used herein, the term “broad spectrum” refers to the anti-viral activity of a single moiety that is active against two or more different viruses, such as three or more, four or more, five or more, six or more, eight or more, 10 or more different viruses. The two or more different viruses may be selected from different virus sub-groups (e.g., Influenza A
10 group 1 or Influenza A group 2), or may be selected from within the same group (e.g., two or more of H1, H2, H5, H6, H8 and H9 group 1 influenza A viruses, or two or more of H3, H4, H7 and H10 Group 2 Influenza A viruses).

In some embodiments, the pulmonary agent is an antimicrobial oligonucleotide. In certain embodiments, the antimicrobial oligonucleotide is an antibacterial
15 oligonucleotide. In some embodiments, the antimicrobial oligonucleotide is an antifungal oligonucleotide. According to some embodiments, the antimicrobial oligonucleotide is an antiviral oligonucleotide. In some embodiments, the antiviral oligonucleotide comprises a sequence complementary to an influenza virus RNA.

In certain embodiments, the antiviral oligonucleotide includes a sequence
20 complementary to a PB2 vRNA region of an influenza A virus. According to some embodiments, the antiviral oligonucleotide comprises a sequence complementary to a PB2 vRNA region comprising the nucleotides 34-87 in the (-)-sense notation of the 5' terminal coding region of the PB2 vRNA, or a salt thereof. According to certain
25 embodiments, the antiviral oligonucleotide comprises a sequence comprising at least 8 nucleotide subunits complementary to a region of the Packaging Stem-Loop 2 (PSL2) motif of the region of PB2 vRNA. In certain embodiments, the antiviral oligonucleotide comprises a sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotide subunits complementary to a region of the Packaging Stem-Loop 2 (PSL2) motif of the region of PB2 vRNA. Further details regarding antiviral
30 oligonucleotides that include a sequence complementary to a PB2 vRNA region of an influenza A virus are found in United States Patent Application Serial No. 16/081,818

published as US 20190136242 and its continuation-in-part United States Patent Application Serial No. 16/792,103 filed on February 14, 2020, as well as PCT Application Serial No. PCT/US2021/018025; the disclosures of such oligonucleotides are herein incorporated by reference.

5 In some embodiments, the composition used in the methods described herein further includes one or more additional pulmonary agents. Additional pulmonary agents may include any convenient antimicrobial compounds or drugs of interest, including but not limited to Amantadine, Rimantadine, Zanamivir, Oseltamivir, Peramivir and the like.

Any convenient protocol for administering the agent to a subject may be
10 employed. The particular protocol that is employed may vary, e.g., depending on the site of administration and whether the agents are e.g., oligonucleotides, antibodies, proteins, peptides, antigens or small molecules. For *in vivo* protocols, any convenient administration protocol may be employed. Depending upon the identity and binding affinity of the agent, the response desired, the manner of administration, e.g., locally or
15 systemic, intraocular, periocular, retrobulbar, intramuscular, intravenous, intraperitoneal, subcutaneous, subconjunctival, by inhalation, e.g., intranasal, topical, eye drops, i.v. s.c., i.p., oral, and the like, the half-life, various protocols may be employed. In some embodiments, the composition is administered intravenously. In certain embodiments of the subject methods, the composition is administered by inhalation, e.g., intranasally.

20 Any suitable means of intranasal delivery can be used. In certain embodiments, the composition administered intranasally in an aerosol. The composition can be administered intranasally using any device disclosed herein, including but not limited to, an inhaler, an atomizer, a nebulizer or a ventilating device. Ventilating devices include, but are not limited to, a non-invasive positive pressure ventilating device and a
25 mechanical ventilating device. Non-limiting examples of non-invasive positive pressure ventilating device include a CPAP (Continuous Airway Pressure) machine and a BPAP (Bilevel Positive Airway Pressure) machine.

The amount of the subject composition administered can be determined using any convenient methods to be an amount sufficient to produce the desired effect in
30 association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the unit dosage forms of the present disclosure will depend on the

particular composition employed and the effect to be achieved, and the pharmacodynamics associated with each composition in the host. In some embodiments, an effective dosage is an effective volume with concentration ranges from about 50 ng/ml to about 50 µg/ml (e.g., from about 50 ng/ml to about 40 µg/ml, from about 30 ng/ml to about 20 µg/ml, from about 50 ng/ml to about 10 µg/ml, from about 50 ng/ml to about 1 µg/ml, from about 50 ng/ml to about 800 ng/ml, from about 50 ng/ml to about 700 ng/ml, from about 50 ng/ml to about 600 ng/ml, from about 50 ng/ml to about 500 ng/ml, from about 50 ng/ml to about 400 ng/ml, from about 60 ng/ml to about 400 ng/ml, from about 70 ng/ml to about 300 ng/ml, from about 60 ng/ml to about 100 ng/ml, from about 65 ng/ml to about 85 ng/ml, from about 70 ng/ml to about 90 ng/ml, from about 200 ng/ml to about 900 ng/ml, from about 200 ng/ml to about 800 ng/ml, from about 200 ng/ml to about 700 ng/ml, from about 200 ng/ml to about 600 ng/ml, from about 200 ng/ml to about 500 ng/ml, from about 200 ng/ml to about 400 ng/ml, or from about 200 ng/ml to about 300 ng/ml).

In some embodiments, an effective amount of a subject composition is an amount that ranges from about 10 pg to about 100 mg, e.g., from about 10 pg to about 50 pg, from about 50 pg to about 150 pg, from about 150 pg to about 250 pg, from about 250 pg to about 500 pg, from about 500 pg to about 750 pg, from about 750 pg to about 1 ng, from about 1 ng to about 10 ng, from about 10 ng to about 50 ng, from about 50 ng to about 150 ng, from about 150 ng to about 250 ng, from about 250 ng to about 500 ng, from about 500 ng to about 750 ng, from about 750 ng to about 1 µg, from about 1 µg to about 10 µg, from about 10 µg to about 50 µg, from about 50 µg to about 150 µg, from about 150 µg to about 250 µg, from about 250 µg to about 500 µg, from about 500 µg to about 750 µg, from about 750 µg to about 1 mg, from about 1 mg to about 50 mg, from about 1 mg to about 100 mg, or from about 50 mg to about 100 mg. The amount can be a single dose amount or can be a total daily amount. The total daily amount can range from 10 pg to 100 mg, or can range from 100 mg to about 500 mg, or can range from 500 mg to about 1000 mg.

Single or multiple doses of the subject compositions can be administered according to the subject methods to provide for protection of the subject from infection for an extended period of time. In some embodiments, a single dose of the subject

composition is administered. In other embodiments, multiple doses of the subject composition are administered. In certain embodiments the subject is administered at least one, two, three, four, five, six, seven, eight, nine, or ten doses of the compositions disclosed herein. The timing and dosage amounts can be readily determined using conventional methods. The subject methods may comprises administering according to a dosing schedule. Where multiple doses are administered over a period of time, the subject compound can be administered twice daily (qid), daily (qd), every other day (qod), every third day, three times per week (tiw), or twice per week (biw) over a period of time. For example, a composition can be administered qid, qd, qod, tiw, or biw over a period of from one day to about 2 years or more. For example, a composition can be administered at any of the aforementioned frequencies for one week, two weeks, one month, two months, six months, one year, or two years, or more, depending on various factors. Single or multiple doses of the subject compositions can be administered according to the subject methods at any suitable period of time before or after exposure to the microbe causing the lung condition. In certain embodiments, the subject compositions are administered one day or more, two days or more, three days or more, four days or more, five days or more, six days or more, a week or more, two weeks or more, three weeks or more, a month or more, two months or more, three months or more, four months or more, five months or more, six months or more, a year or more or two years or more prior to the exposure to the microbe causing the lung condition. According to some embodiments, the subject compositions are administered one day or more, two days or more, three days or more, four days or more, five days or more, six days or more, a week or more, two weeks or more, three weeks or more, a month or more, two months or more, three months or more, four months or more, five months or more, six months or more, a year or more or two years or more after the exposure to the microbe causing the lung condition.

In some embodiments, the subject methods include a step of determining or diagnosing whether the subject has a lung condition. The determining step can be performed using any convenient methods. In some cases, the determining step includes obtaining a biological sample from the subject and assaying the sample for the presence of microorganisms or cells infected with the microorganisms such as viral

cells. The sample can be a cellular sample. The determining step can include identification of viral cells including a particular mutation.

Any of a variety of methods can be used to determine whether a treatment method is effective. For example, a biological sample obtained from an individual who has been treated with a subject method can be assayed for the presence and/or level of cells infected with the microorganisms such as viral cells. Assessment of the effectiveness of the methods of treatment on the subject can include assessment of the subject before, during and/or after treatment, using any convenient methods. Aspects of the subject methods further include a step of assessing the therapeutic response of the subject to the treatment.

In some embodiments, the method includes assessing the condition of the subject, including diagnosing or assessing one or more symptoms of the subject which are associated with the disease or condition of interest being treated (e.g., as described herein). In some embodiments, the method includes obtaining a biological sample from the subject and assaying the sample, e.g., for the presence of viral cells or components thereof that are associated with the disease or condition of interest (e.g., as described herein). The sample can be a cellular sample. The assessment step(s) of the subject method can be performed at one or more times before, during and/or after administration of the subject compounds, using any convenient methods. In certain cases, the assessment step includes identification and/or quantitation of viral cells. In certain instances, assessing the subject include diagnosing whether the subject has a lung condition or symptoms thereof.

A variety of subjects may be amenable to treatment using the subject methods and compositions disclosed herein. As used herein, the terms “subject” and “host” are used interchangeably. Generally, such subjects are “mammals”, with humans being of interest. Other subjects can include domestic pets (e.g., dogs and cats), livestock (e.g., cows, pigs, goats, horses, and the like), rodents (e.g., mice, guinea pigs, and rats, e.g., as in animal models of disease), as well as non-human primates (e.g., chimpanzees, and monkeys). According to some embodiments, the subject is a human. As such, in some cases, the subject is one who has a lung condition. In certain cases, the subject is one who is at risk of having or is suspected of having a lung condition.

SYSTEMS, DEVICES AND KITS

Aspects of the invention further systems, devices and kits for practicing embodiments of the methods, e.g., as described above. Systems are collections of
5 disparate components brought together for the purpose of practicing methods of invention. For example, a systems of the invention include disparate pulmonary agent delivery composition components, e.g., pulmonary agent, deproteinized pollen shell component, transfection agent, etc., brought together, e.g., prior to combination, by a user in order to produce a pulmonary agent delivery composition for use in a method of
10 invention.

Also provided are delivery devices loaded with a pulmonary agent delivery composition that are configured to administer the composition to a subject, e.g., as described above. Devices of invention may vary as desired, e.g., depending on the particular route of administration. For intravenous delivery, the device may be a
15 container, e.g., syringe, bag, etc., that includes an amount of the composition. In some embodiments, the device is a pulmonary delivery device. Pulmonary devices disclosed herein, include but are not limited to, inhalers, atomizers, nebulizers ventilating devices, etc. Ventilating devices include, but are not limited to, non-invasive positive pressure ventilating devices and mechanical ventilating devices. Non-limiting examples of non-
20 invasive positive pressure ventilating devices include CPAP (Continuous Airway Pressure) machines and BiPAP (Bilevel Positive Airway Pressure) machines.

Aspects of the invention also include kits. Embodiments of the kits include one or more of the pulmonary agent delivery compositions, e.g., as described above, or components, thereof. Components of the kits may be present in separate containers, or
25 multiple components may be present in a single container. In addition to the above-mentioned components, a subject kit may further include instructions for using the components of the kit, e.g., to practice the subject methods. The instructions are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may
30 be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or sub-packaging) etc. In other

embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, Hard Disk Drive (HDD), portable flash drive, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

I. Deproteinized Pollen/jetPEI™ Formulations

In vivo JetPEI™ is the best available commercial transfection agent for lung-targeting nucleic acid therapeutics. [Lee, J., Park, E. B., Min, J., Sung, S. E., Jang, Y., Shin, J. S., Chun, D., Kim, K. H., Hwang, J., Lee, M. K., Go, Y. Y., Kwon, D., Kim, M., Kang, S. J., Choi, B. S. (2018). Systematic editing of synthetic RIG-I ligands to produce effective antiviral and anti-tumor RNA immunotherapies., *Nucleic Acids Res* 46, 1635-1647; O'Neil, R. T., Saha, S., Veach, R. A., Welch, R. C., Woodard, L. E., Rooney, C. M., Wilson, M. H. (2018). Transposon-modified antigen-specific T lymphocytes for sustained therapeutic protein delivery *in vivo*., *Nat Commun* 9, 1325; Cao, W., Mishina, M., Amoah, S., Mboko, W. P., Bohannon, C., McCoy, J., Mittal, S. K., Gangappa, S., Sambhara, S. (2018). Nasal delivery of H5N1 avian influenza vaccine formulated with GenJet or *in vivo*-jetPEI((R)) induces enhanced serological, cellular and protective immune responses., *Drug Deliv* 25, 773-779 However, a major limitation with jetPEI™ is its toxicity, which in turn limits the amount of therapeutic nucleic acid that can be delivered using jetPEI™. [Hobel S, Aigner A. Polyethylenimines for siRNA and miRNA delivery *in vivo*. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2013 Sep-Oct;5(5):484-501.; McLean, J. W. *et al*. Organ-specific endothelial cell uptake of cationic

liposome-DNA complexes in mice. *Am J Physiol* 273, H387–404 (1997); Chollet, P., Favrot, M. C., Hurbin, A. & Coll, J. L. Side-effects of a systemic injection of linear polyethylenimine-DNA complexes. *J Gene Med* 4, 84–91 (2002).

As shown below, use of deproteinized pollen shells dramatically improves the therapeutic efficacy, as well as decreases the *in vivo* toxicity, of nucleic acid therapeutics co-formulated with transfection agents such as jetPEI™. Employing deproteinized pollen shells with jetPEI™ provides for impressive *in vivo* efficacy results against a lethal inoculum on influenza virus. Moreover, employing deproteinized pollen shells with jetPEI™ provides improves the therapeutic index whether the deproteinized pollen component-pulmonary agent- jetPEI™ formulation is delivered by inhalation or intravenous routes.

A. Employing deproteinized pollen shells with jetPEI™ provides for greater efficacy and reduced toxicity of LNA pulmonary agents whether delivered by inhalation or intravenously

Applicants have found that a co-formulation of LNA with deproteinized empty pollen shells resulted in greater *in vivo* efficacy of lung-targeting LNA therapeutics, as well as significantly reduced toxicity. FIGS. 1A-1B depict percent survival (FIG. 1A), and clinical score (FIG. 1B) of mice treated intranasally with 40 µg of the oligonucleotide LNA14 (5'CGACcaaaagaATTC3' described in United States Patent Application Serial No. 16/081,818 published as US20190136242) in combination with *in vivo* JetPEI, compared to the same combination with the addition of a deproteinized pollen shell component 14 days prior to inoculation with a lethal dose of influenza virus. Mice treated with vehicle (5% Glucose with pollen) served as controls. The percent survival and clinical score were monitored as a function of time. 100% of mice receiving a combination of 40 µg LNA with JetPEI at an N/P ratio of 8 mixed with deproteinized pollen survived and exhibited a significantly lower clinical score, while 25% of the mice treated with a combination of 40 µg LNA with JetPEI at an N/P ratio of 8 survived and 0% of mice treated with Vehicle control (5% glucose + pollen) survived. FIG. 1C depicts percent survival of mice treated intravenously with 40 µg of the oligonucleotide LNA14 in combination with *in vivo* JetPEI, compared to the same combination with the addition

of a deproteinized pollen shell component 3 days post-infection with a lethal dose of influenza virus. Vehicle treatment was used as control. 100% of the mice treated with 40 µg of the oligonucleotide LNA14 and in vivo JetPEI in combination with deproteinized pollen survived while 25% of mice treated with LNA14 and JetPEI only survived and 0% of vehicle control survived. FIG1D depicts the percent survival of mice treated with LNA alone ("naked" LNA) without a delivery reagent or carrier, such as in vivo JetPEI or pollen. Mice were intranasally pretreated with an increasing dose of naked LNA14 ranging from 30ug to 1000ug one week prior to lethal infection with PR8 virus. Percent survival of mice in each of these groups did not exceed 30%.

II. Deproteinized Pollen Formulations

Applicants found that co-formulation of LNA with deproteinized empty pollen shells alone, i.e., without jetPEITM (such as described above) resulted in significant *in vivo* efficacy of lung-targeting LNA therapeutics. In FIG. 2, one dose of LNA14 was mixed with deproteinized empty pollen shells alone and delivered intranasally (IN) 3 days prior to inoculation with a lethal dose of influenza virus. Vehicle alone was administered as a control. The percent survival was monitored as a function of time. 60% of mice receiving LNA with deproteinized empty pollen shells alone survived, versus 0% of the vehicle controls. These data demonstrate the toxicity associated with jetPEITM can be eliminated when LNAs are directly co-formulated with deproteinized empty pollen shells alone.

Applicants extended these findings to show that a co-formulation of LNA with deproteinized empty pollen shells alone resulted in a dose response in vivo efficacy of lung-targeting LNA therapeutics. FIGS. 3A-3C depict percent survival (FIG. 3A), body weight (FIG. 3B) and clinical score (FIG. 3C) of mice intranasally treated with 20 µg, 40 µg, or 60 µg of the oligonucleotide LNA14 (5'CGACcaaaagaATTC 3' described in United States Patent Application Serial No. 16/081,818 published as US20190136242) with a deproteinized pollen shell component 3 days before inoculation with lethal dose of influenza virus. Mice treated with 80 µg Scramble LNA (SCR) or Phosphate-buffered saline (PBS) served as controls. The percent survival, body weight and clinical score were monitored as a function of time. 100% of mice receiving 60 µg LNA14 co-

formulated with pollen survived and exhibited a significantly lower clinical score, while 0% of the mice treated with 80 µg SCR or PBS survived.

III. Lyophilized jetPEI™ Formulations

5 Applicants have also found that co-formulated LNA/jetPEI™ mixtures can be lyophilized for future use, and that upon reconstitution retain their *in vivo* efficacy while reduce the toxic effect exerted by a high N/P ratio of *in vivo* JetPEI. In FIG. 4, one dose of such fresh formulation or lyophilized and reconstituted LNA14 formulation was delivered intranasally (IN) 3 days prior to inoculation with a lethal dose of influenza
10 virus. Vehicle alone was administered as a control. The percent survival was monitored as a function of time. 100% of mice receiving lyophilized and reconstituted LNA14 co-formulated with jetPEI™ survived, versus 25% of the freshly formulated LNA14 with *in vivo* JetPEI and 0% of vehicle controls. These data demonstrate the surprisingly retained *in vivo* efficacy and lower toxicity of lung-targeting LNA therapeutics prepared
15 by co-formulation with jetPEI™, followed by lyophilization and reconstitution.

Notwithstanding the appended claims, the disclosure is also defined by the following clauses:

1. A method of delivering a pulmonary agent to a cell, the method comprising:
20 contacting the cell with a pulmonary agent delivery composition comprising a pulmonary agent and a deproteinized pollen shell component.
2. The method of clause 1, wherein the deproteinized pollen shell component comprises deproteinized echinate pollen shells.
3. The method of clause 1 or 2, wherein the deproteinized pollen shell component is
25 obtained from at least one of: *Lycopodium clavatum*, paper mulberry (*Broussonetia papyrifera*), corn (*Zea mays*), Cocklebur (*Xanthium commune*), Goldenrod (*Solidago* spp.), Poverty weed (*Iva axillaris*), Desert Ragweed (*Ambrosia dumos*), False Ragweed (*Ambrosia acanthicarpa*), Giant Ragweed (*Ambrosia trifida*), Short Ragweed (*Ambrosia artemisifolia*), Slender Ragweed (*Ambrosia tenuifolia*), Southern Ragweed
30 (*Ambrosia bidentata*), Western Ragweed (*Ambrosia psilostachya*), Prairie Sage (*Artemisia ludoviciana*), Common Sagebrush (*Artemisia tridentate*), Annual Wormwood

(*artemisia annua*), Marsh Elder, Sunflower, Black Alder (*Alnus glutinosa*), Lamb's Quarters (*Chenopodium album*) and High-Water Shrub.

4. The method of any one of clauses 1 to 3, wherein the pulmonary agent is selected from: a nucleic acid, a peptide, a polypeptide, an antigen and a small molecule compound.
5. The method of clause 4, wherein the pulmonary agent is a nucleic acid.
6. The method of clause 5, wherein the nucleic acid is a deoxyribonucleic acid (DNA).
7. The method of clause 5, wherein the nucleic acid is a ribonucleic acid (RNA).
8. The method of clause 5, wherein the nucleic acid is an oligonucleotide.
9. The method of clause 8, wherein the oligonucleotide is from 5 nucleotides to 30 nucleotides in length.
10. The method of clause 8 or 9, wherein the oligonucleotide comprises an internucleoside linkage selected from: phosphorothioate, phosphorodithioate, phosphoramidate and thiophosphoramidate linkages.
11. The method of clause 10, wherein the oligonucleotide comprises one or more chiral internucleoside linkages.
12. The method of any one of clauses 8 to 11, wherein the oligonucleotide comprises a bridged nucleic acid (BNA) nucleotide.
13. The method of clause 12, wherein the BNA nucleotide is selected from the group consisting of, locked nucleic acid (LNA) nucleotides, ethylene-bridged nucleic acid (ENA) nucleotides, and constrained ethyl (cEt) nucleotides.
14. The method of clause 13, wherein the oligonucleotide comprises a locked nucleic acid (LNA) nucleotide.
15. The method of any one of clauses 8 to 14, wherein the oligonucleotide comprises one or more 2'-modified nucleotides.
16. The method of any one of the preceding clauses, wherein the composition further comprises a transfection agent.
17. The method of clause 16, wherein the transfection agent is a cationic transfection agent.
18. The method of clause 16 or 17, wherein the transfection agent comprises a lipid.

19. The method of clause 16 or 17, wherein the transfection agent comprises a polymer.
20. The method of clause 19, wherein the transfection agent is a linear polyethylenimine polymer.
- 5 21. The method of any one of the preceding clauses, wherein the cell is a mammalian cell.
22. The method of clause 21, wherein the mammalian cell is a human cell.
23. The method of clause 21 or 22, wherein the cell is an epithelial cell.
24. The method of any one of the preceding clauses, wherein the cell is *in vitro*.
- 10 25. The method of any one of clauses 1 to 23, wherein the cell is *in vivo*.
26. The method of clause 25, wherein the method is a method of treating or preventing a lung condition in a subject by administering to a subject in need thereof an effective amount of the composition.
27. The method of clause 26, wherein the lung condition is a lung infection.
- 15 28. The method of clause 27, wherein the lung infection is an influenza infection.
29. The method of clause 28, wherein the influenza infection is an influenza A virus infection.
30. The method of any one of clauses 26 to 29, wherein the pulmonary agent is an antimicrobial oligonucleotide.
- 20 31. The method of clause 30, wherein the antimicrobial oligonucleotide is an antibacterial oligonucleotide, antifungal oligonucleotide or antiviral oligonucleotide.
32. The method of clause 31, wherein the antimicrobial oligonucleotide is an antiviral oligonucleotide.
33. The method of clause 32, wherein the antiviral oligonucleotide comprises a
25 sequence complementary to an influenza virus RNA.
34. The method of clause 33, wherein the antiviral oligonucleotide comprises a sequence complementary to a PB2 vRNA region of an influenza A virus.
35. The method of clause 34, wherein the antiviral oligonucleotide comprises a sequence complementary to a PB2 vRNA region comprising the nucleotides 34-87 in
30 the (-)-sense notation of the 5' terminal coding region of the PB2 vRNA, or a salt thereof.

36. The method of clause 34 or 35, wherein the antiviral oligonucleotide comprises a sequence comprising at least 8 nucleotide subunits complementary to a region of the Packaging Stem-Loop 2 (PSL2) motif of the region of PB2 vRNA.
37. The method of any one of clauses 26 to 36, wherein the composition is
5 administered by inhalation.
38. The method of any one of clauses 26 to 36, wherein the composition is administered intravenously.
39. The method of any one of clauses 26 to 38, wherein the method comprises administering according to a dosing schedule.
- 10 40. The method of clause 39, wherein the composition is administered once.
41. The method of clause 39, wherein the composition is administered once daily, twice daily, or thrice daily
42. The method of clause 39, wherein the composition is administered once weekly, twice weekly, or thrice weekly.
- 15 43. The method of clause 39, wherein the composition is administered once monthly, twice monthly, or thrice monthly.
44. The method of clause 39, wherein the composition is administered once every 3 months, twice every 3 month, or thrice every 3 month.
45. The method of clause 39, wherein the composition is administered once yearly,
20 twice yearly, or thrice yearly.
46. The method of any one of clauses 26 to 42, wherein the subject is a human.
47. The method of any one of the preceding clauses, wherein the composition further comprises a pharmaceutically acceptable carrier.
48. The method of any of the preceding clauses, wherein the composition is an
25 inhalable composition.
49. The method of clause 48, wherein the composition is aerosolized.
50. The method of clause 49, wherein the aerosol comprises particles having an average particle size of 1 to 40 micrometers in diameter.
51. A pulmonary agent delivery composition, the composition comprising:
30 a pulmonary agent and a deproteinized pollen shell component.

52. The composition of clause 51, wherein the deproteinized pollen shell component pollen comprises deproteinized echinate pollen shells.

53. The composition of clause 51 or 52, wherein the deproteinized pollen shell component is obtained from at least one of: *Lycopodium clavatum*, paper mulberry

5 (*Broussonetia papyrifera*), corn (*Zea mays*), Cocklebur (*Xanthium commune*),

Goldenrod (*Solidago* spp.), Poverty weed (*Iva axillaris*), Desert Ragweed (*Ambrosia*

dumos), False Ragweed (*Ambrosia acanthicarpa*), Giant Ragweed (*Ambrosia trifida*),

Short Ragweed (*Ambrosia artemisifolia*), Slender Ragweed (*Ambrosia tenuifolia*),

Southern Ragweed (*Ambrosia bidentata*), Western Ragweed (*Ambrosia psilostachya*),

10 Prairie Sage (*Artemisia ludoviciana*), Common Sagebrush (*Artemisia tridentate*), Annual

Wormwood (*artemisia annua*), Marsh Elder, Sunflower, Black Alder (*Alnus glutinosa*),

Lamb's Quarters (*Chenopodium album*) and High-Water Shrub.

54. The composition of any one of clauses 51 to 53, wherein the pulmonary agent is selected from: a nucleic acid, a peptide, a polypeptide, an antigen and a small molecule
15 compound.

55. The composition of clause 54, wherein the pulmonary agent is a nucleic acid.

56. The composition of clause 55, wherein the nucleic acid is a deoxyribonucleic acid (DNA).

57. The composition of clause 55, wherein the nucleic acid is a ribonucleic acid
20 (RNA).

58. The composition of clause 55, wherein the nucleic acid is an oligonucleotide.

59. The composition of clause 58, wherein the oligonucleotide is from 5 nucleotides to 30 nucleotides in length.

60. The composition of clause 58 or 59, wherein the oligonucleotide comprises an
25 internucleoside linkage selected from: phosphorothioate, phosphorodithioate, phosphoramidate and thiophosphoramidate linkages.

61. The composition of clause 60, wherein the oligonucleotide comprises one or more chiral internucleoside linkages.
62. The composition of any one of clauses 58 to 61, wherein the oligonucleotide comprises a bridged nucleic acid (BNA) nucleotide.
- 5 63. The composition of clause 62, wherein the BNA nucleotide is selected from the group consisting of, locked nucleic acid (LNA) nucleotides, ethylene-bridged nucleic acid (ENA) nucleotides, and constrained ethyl (cEt) nucleotides.
64. The composition of clause 63, wherein the oligonucleotide comprises a locked nucleic acid (LNA) nucleotide.
- 10 65. The composition of clauses any one of clauses 58 to 64, wherein the oligonucleotide comprises one or more 2'-modified nucleotides.
66. The composition of any one of clauses 58 to 65, wherein the oligonucleotide is an antimicrobial oligonucleotide.
67. The composition of clause 66, wherein the antimicrobial oligonucleotide is an
15 antibacterial oligonucleotide, and antifungal oligonucleotide or an antiviral oligonucleotide.
68. The composition of clause 67, wherein the antimicrobial oligonucleotide is an antiviral oligonucleotide.
69. The composition of clause 68, wherein the antiviral oligonucleotide comprises a
20 sequence complementary to an influenza virus RNA.
70. The composition of clause 69, wherein the antiviral oligonucleotide comprises a sequence complementary to a PB2 vRNA region of an influenza A virus.
71. The composition of clause 70, wherein the antiviral oligonucleotide comprises a
25 sequence complementary to a PB2 vRNA region comprising the nucleotides 34-87 in the (-)-sense notation of the 5' terminal coding region of the PB2 vRNA.
72. The composition of clause 70 or 71, wherein the antiviral oligonucleotide comprises a sequence comprising at least 8 nucleotide subunits complementary to a region of the Packaging Stem-Loop 2 (PSL2) motif of the region of PB2 vRNA.
73. The composition of any one of clauses 51 to 72, wherein the composition further
30 comprises a transfection agent.

74. The composition of clause 73, wherein the transfection agent is a cationic transfection agent.
75. The composition of clause 73 or 74, wherein the transfection agent comprises a lipid.
- 5 76. The composition of clause 73 or 74, wherein the transfection agent comprises a polymer.
77. The composition of clause 76, wherein the transfection agent is a linear polyethylenimine polymer.
78. The composition of any one of clauses 51 to 77, wherein the composition further
10 comprises a pharmaceutically acceptable carrier.
79. The composition of any one of clauses 51 to 78, wherein the composition is an inhalable composition.
80. The composition of any one of clauses 51 to 79, wherein the composition is aerosolized.
- 15 81. The composition of clause 80, wherein the aerosol comprises particles having an average particle size of 1 to 6 micrometers in diameter.
82. A device comprising the composition of any one of clauses 51 to 81.
83. The device of clause 82, wherein the device is a pulmonary delivery device.
84. The device of clause 83, wherein the pulmonary delivery device is an inhaler.
- 20 85. The device of clause 84, wherein the pulmonary delivery device is an atomizer.
86. The device of clause 85, wherein the pulmonary delivery device is a nebulizer.
87. A method of making a pulmonary agent delivery composition, the method comprising:
combining a pulmonary agent and a deproteinized pollen shell component to
25 produce the pulmonary agent delivery composition.
88. The method of clause 87, further comprising combining the pulmonary agent and a deproteinized pollen shell component and a transfection agent.
89. The method of clause 87 or 88, further comprising aerosolizing the composition.
90. A kit comprising:
30 two or more unit doses of pulmonary agent delivery composition according to any one of clauses 51 to 81.

91. The kit of clause 90, further comprising instructions to carry out the methods of any one of clauses 1 to 47.
92. A lyophilized pulmonary agent delivery composition, the composition comprising: a pulmonary agent and a transfection agent.
- 5 93. The composition of clause 92, wherein the pulmonary agent is selected from: a nucleic acid, a peptide, a polypeptide, an antigen and a small molecule compound.
94. The composition of clause 93, wherein the pulmonary agent is a nucleic acid.
95. The composition of clause 94, wherein the nucleic acid is a deoxyribonucleic acid (DNA).
- 10 96. The composition of clause 94, wherein the nucleic acid is a ribonucleic acid (RNA).
97. The composition of clause 96, wherein the nucleic acid is an oligonucleotide.
98. The composition of clause 97, wherein the oligonucleotide is from 5 nucleotides to 30 nucleotides in length.
- 15 99. The composition of clause 97 or 98, wherein the oligonucleotide comprises an internucleoside linkage selected from: phosphorothioate, phosphorodithioate, phosphoramidate and thiophosphoramidate linkages.
100. The composition of clause 99, wherein the oligonucleotide comprises one or more chiral internucleoside linkages.
- 20 101. The composition of any one of clauses 97 to 100, wherein the oligonucleotide comprises a bridged nucleic acid (BNA) nucleotide.
102. The composition of clause 101, wherein the BNA nucleotide is selected from the group consisting of, locked nucleic acid (LNA) nucleotides, ethylene-bridged nucleic acid (ENA) nucleotides, and constrained ethyl (cEt) nucleotides.
- 25 103. The composition of clause 102, wherein the oligonucleotide comprises a locked nucleic acid (LNA) nucleotide.
104. The composition of clauses any one of clauses 97 to 103, wherein the oligonucleotide comprises one or more 2'-modified nucleotides.
105. The composition of any one of clauses 97 to 104, wherein the oligonucleotide is
- 30 an antimicrobial oligonucleotide.

106. The composition of clause 105, wherein the antimicrobial oligonucleotide is an antibacterial oligonucleotide, and antifungal oligonucleotide or an antiviral oligonucleotide.

107. The composition of clause 106, wherein the antimicrobial oligonucleotide is an antiviral oligonucleotide.

108. The composition of clause 107, wherein the antiviral oligonucleotide comprises a sequence complementary to an influenza virus RNA.

109. The composition of clause 108, wherein the antiviral oligonucleotide comprises a sequence complementary to a PB2 vRNA region of an influenza A virus.

110. The composition of clause 109, wherein the antiviral oligonucleotide comprises a sequence complementary to a PB2 vRNA region comprising the nucleotides 34-87 in the (-)-sense notation of the 5' terminal coding region of the PB2 vRNA.

111. The composition of clause 109 or 110, wherein the antiviral oligonucleotide comprises a sequence comprising at least 8 nucleotide subunits complementary to a region of the Packaging Stem-Loop 2 (PSL2) motif of the region of PB2 vRNA.

112. The composition of any one of clauses 92 to 111, wherein the transfection agent is a cationic transfection agent.

113. The composition of clause 112, wherein the transfection agent comprises a lipid.

114. The composition of clause 112, wherein the transfection agent comprises a polymer.

115. The composition of clause 114, wherein the transfection agent is a linear polyethylenimine polymer.

116. The composition of any one of clauses 92 to 115, wherein the composition further comprises a pharmaceutically acceptable carrier.

117. A method comprising reconstituting a composition according to any one of clauses 92 to 116.

118. A method comprising administering a reconstituted composition producing according to clause 117 to a subject.

In at least some of the previously described embodiments, one or more elements used in an embodiment can interchangeably be used in another embodiment unless

such a replacement is not technically feasible. It will be appreciated by those skilled in the art that various other omissions, additions and modifications may be made to the methods and structures described above without departing from the scope of the claimed subject matter. All such modifications and changes are intended to fall within
5 the scope of the subject matter, as defined by the appended claims.

It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (*e.g.*, bodies of the appended claims) are generally intended as “open” terms (*e.g.*, the term “including” should be interpreted as “including but not limited to,” the term “having” should be interpreted as “having at
10 least,” the term “includes” should be interpreted as “includes but is not limited to,” etc.).

It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the
15 introductory phrases “at least one” and “one or more” to introduce claim recitations.

However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles “a” or “an” limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases “one or more” or
20 “at least one” and indefinite articles such as “a” or “an” (*e.g.*, “a” and/or “an” should be interpreted to mean “at least one” or “one or more”); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number

(*e.g.*, the bare recitation of “two recitations,” without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to “at least one of A, B, and C, etc.” is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, “a system having at least one of A, B, and C” would include but not be
25 limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where
30 limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where

a convention analogous to “at least one of A, B, or C, etc.” is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (*e.g.*, “a system having at least one of A, B, or C” would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase “A or B” will be understood to include the possibilities of “A” or “B” or “A and B.”

In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as “up to,” “at least,” “greater than,” “less than,” and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain

changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Accordingly, the preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. Moreover, nothing disclosed herein is intended to be dedicated to the public regardless of whether such disclosure is explicitly recited in the claims.

The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims. In the claims, 35 U.S.C. §112(f) or 35 U.S.C. §112(6) is expressly defined as being invoked for a limitation in the claim only when the exact phrase "means for" or the exact phrase "step for" is recited at the beginning of such limitation in the claim; if such exact phrase is not used in a limitation in the claim, then 35 U.S.C. § 112 (f) or 35 U.S.C. §112(6) is not invoked.

WHAT IS CLAIMED IS:

1. A method of delivering a pulmonary agent to a cell, the method comprising:
contacting the cell with a pulmonary agent delivery composition comprising a pulmonary agent and a deproteinized pollen shell component.
2. The method of claim 1, wherein the deproteinized pollen shell component comprises deproteinized echinate pollen shells.
3. The method of claim 1 or 2, wherein the deproteinized pollen shell component is obtained from at least one of: *Lycopodium clavatum*, paper mulberry (*Broussonetia papyrifera*), corn (*Zea mays*), Cocklebur (*Xanthium commune*), Goldenrod (*Solidago* spp.), Poverty weed (*Iva axillaris*), Desert Ragweed (*Ambrosia dumos*), False Ragweed (*Ambrosia acanthicarpa*), Giant Ragweed (*Ambrosia trifida*), Short Ragweed (*Ambrosia artemisifolia*), Slender Ragweed (*Ambrosia tenuifolia*), Southern Ragweed (*Ambrosia bidentata*), Western Ragweed (*Ambrosia psilostachya*), Prairie Sage (*Artemisia ludoviciana*), Common Sagebrush (*Artemisia tridentate*), Annual Wormwood (*artemisia annua*), Marsh Elder, Sunflower, Black Alder (*Alnus glutinosa*), Lamb's Quarters (*Chenopodium album*) and High-Water Shrub.
4. The method of any one of claims 1 to 3, wherein the pulmonary agent is selected from: a nucleic acid, a peptide, a polypeptide, an antigen and a small molecule compound.
5. The method of claim 4, wherein the pulmonary agent is a nucleic acid.
6. The method of claim 5, wherein the nucleic acid is a deoxyribonucleic acid (DNA).
7. The method of claim 5, wherein the nucleic acid is a ribonucleic acid (RNA).

8. The method of claim 5, wherein the nucleic acid is an oligonucleotide.
9. The method of claim 8, wherein the oligonucleotide comprises an internucleoside linkage selected from: phosphorothioate, phosphorodithioate, phosphoramidate and thiophosphoramidate linkages.
10. The method of any one of claims 8 to 9, wherein the oligonucleotide comprises a bridged nucleic acid (BNA) nucleotide.
11. The method of claim 10, wherein the BNA nucleotide is selected from the group consisting of, locked nucleic acid (LNA) nucleotides, ethylene-bridged nucleic acid (ENA) nucleotides, and constrained ethyl (cEt) nucleotides.
12. The method of any one of the preceding claims, wherein the composition further comprises a transfection agent.
13. The method of any one of the preceding claims, wherein the composition further comprises a pharmaceutically acceptable carrier.
14. The method of any of the preceding claims, wherein the method is a method of treating or preventing a lung condition in a subject by administering to a subject in need thereof an effective amount of the composition.
15. A pulmonary agent delivery composition as recited in any of the preceding claims.

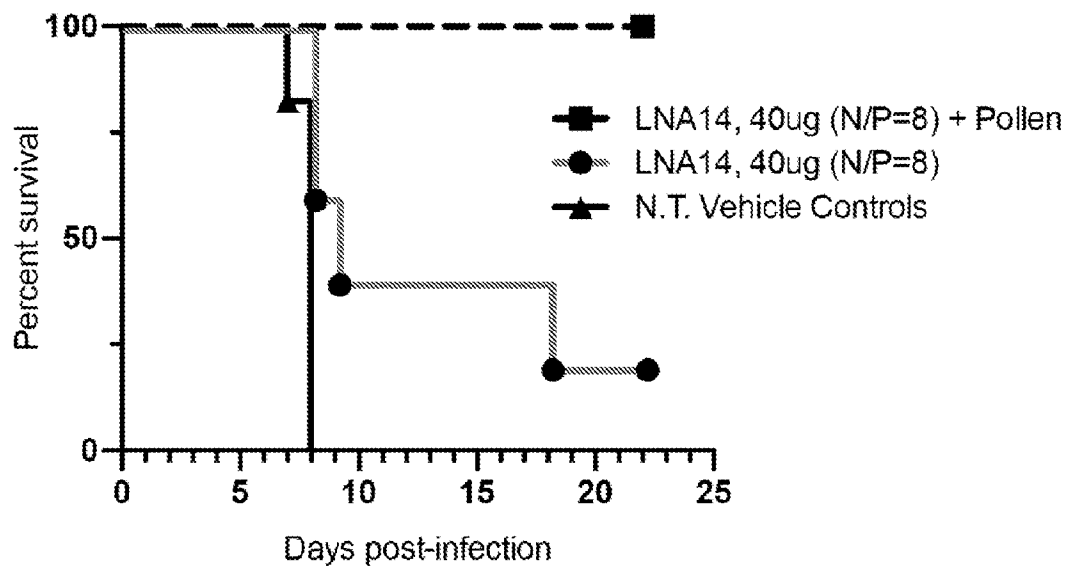
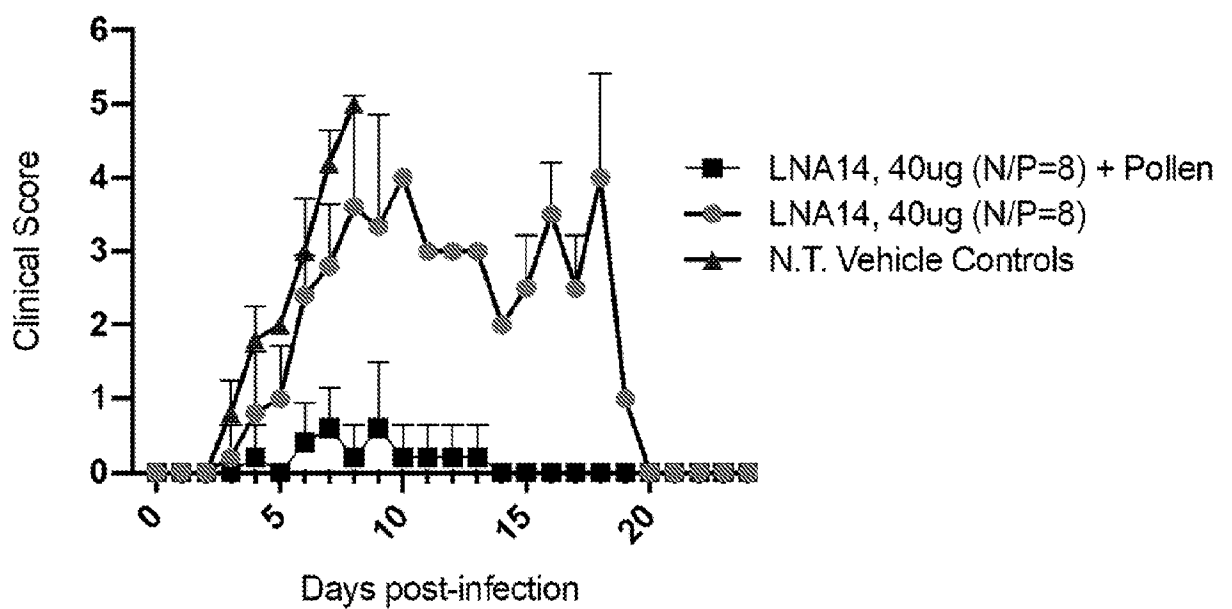
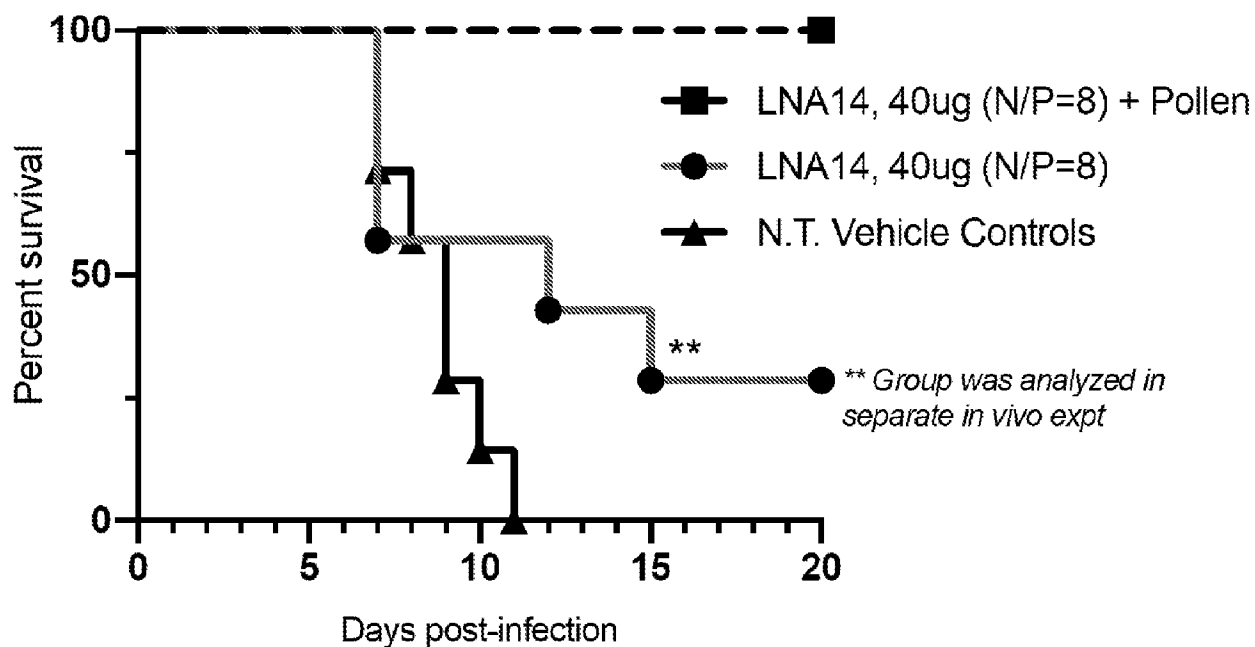
FIGS. 1A-1B**FIG. 1A****FIG. 1B**

FIG. 1C**FIG. 1D**

**Prophylaxis: 1week Pre-RX with
LNA-alone (aka "Naked" delivery)**

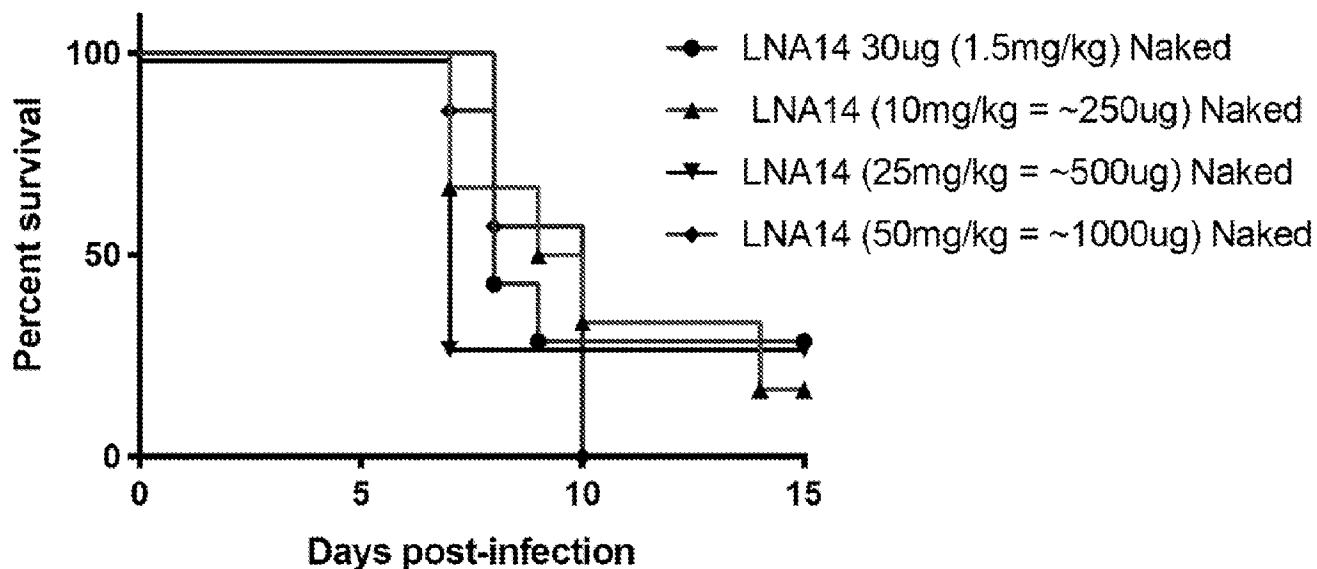
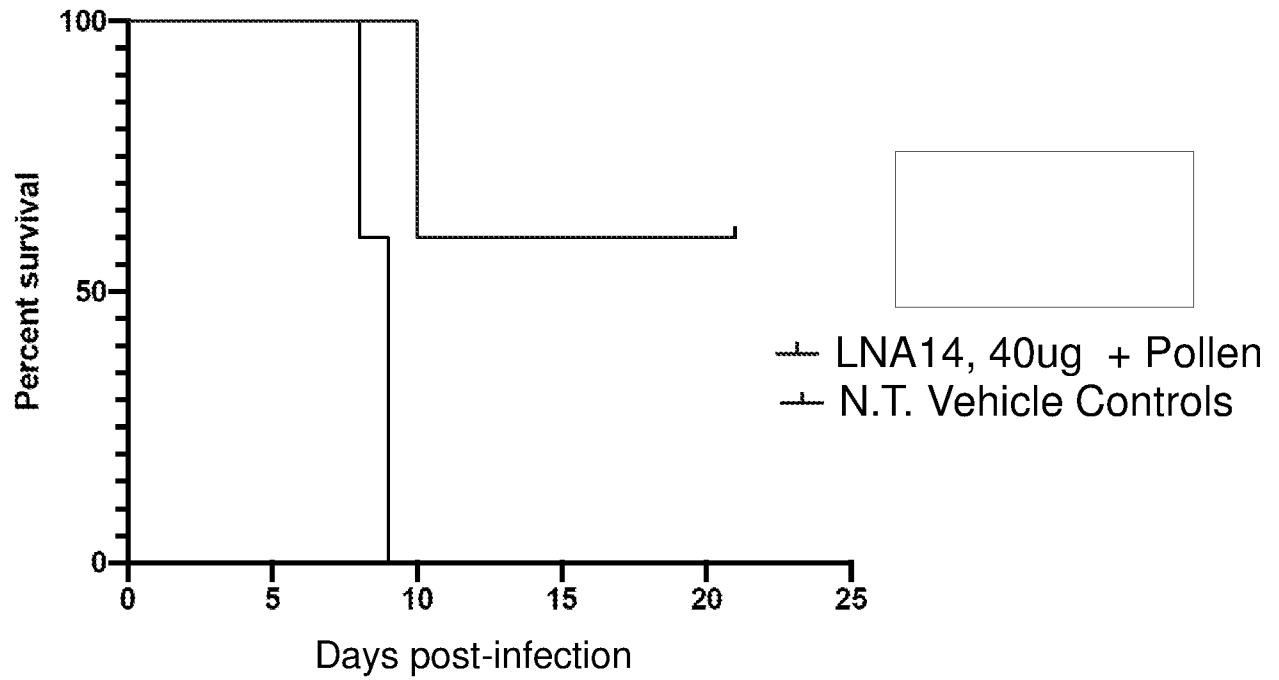


FIG. 2

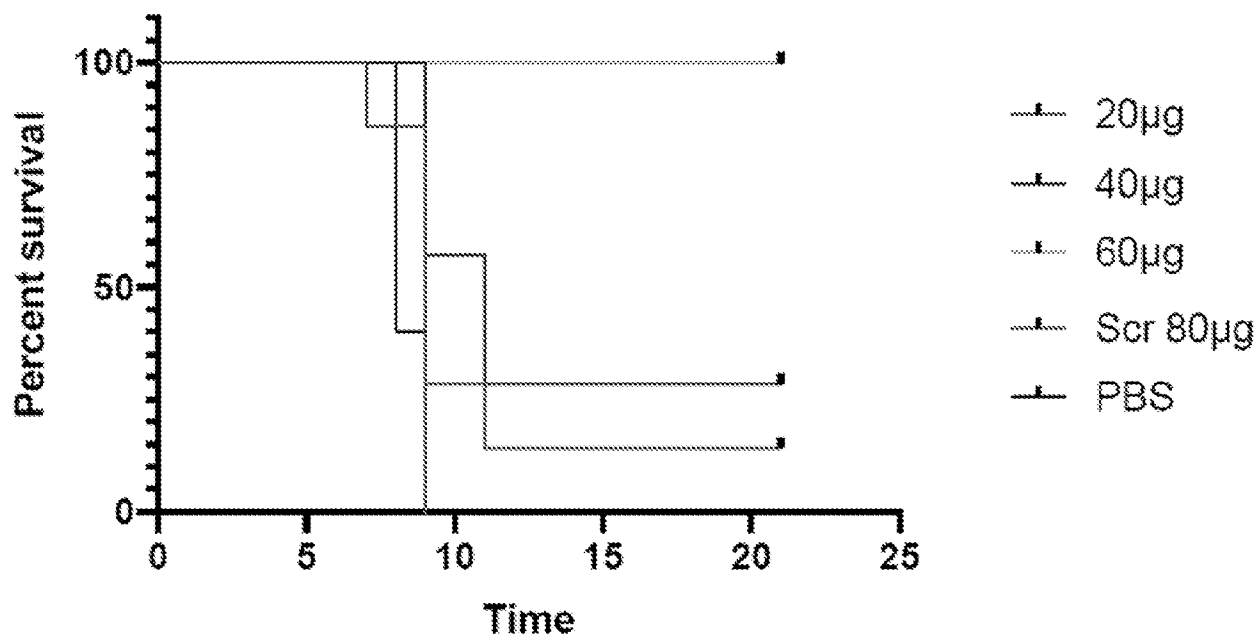
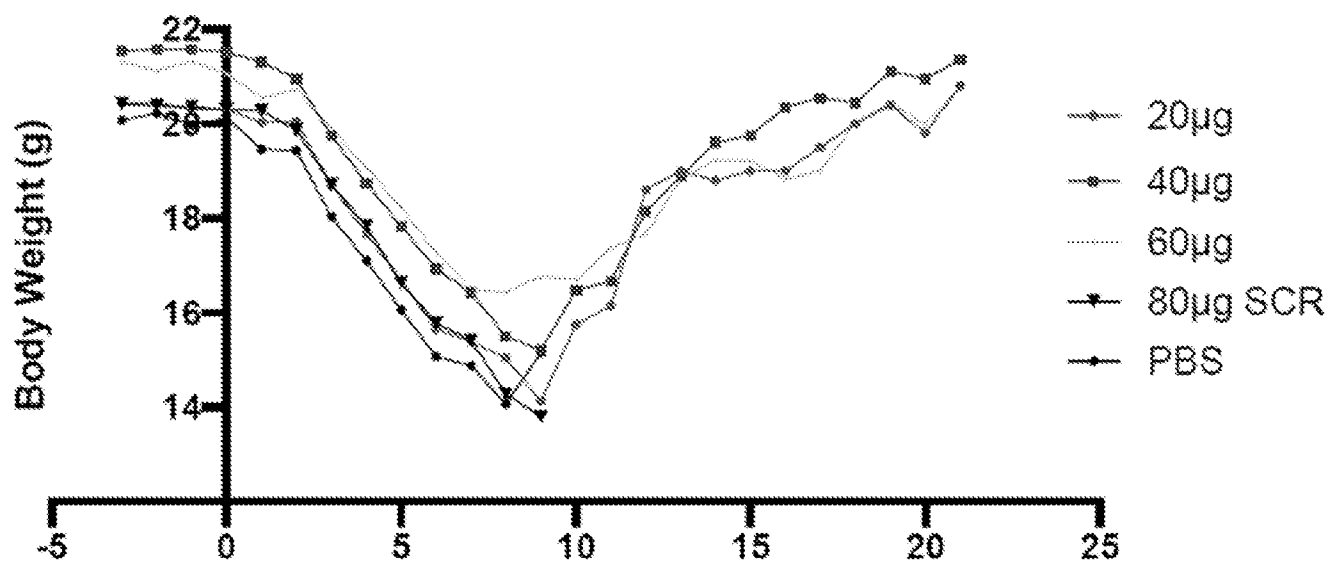
FIGS. 3A-3C**FIG. 3A****FIG. 3B**

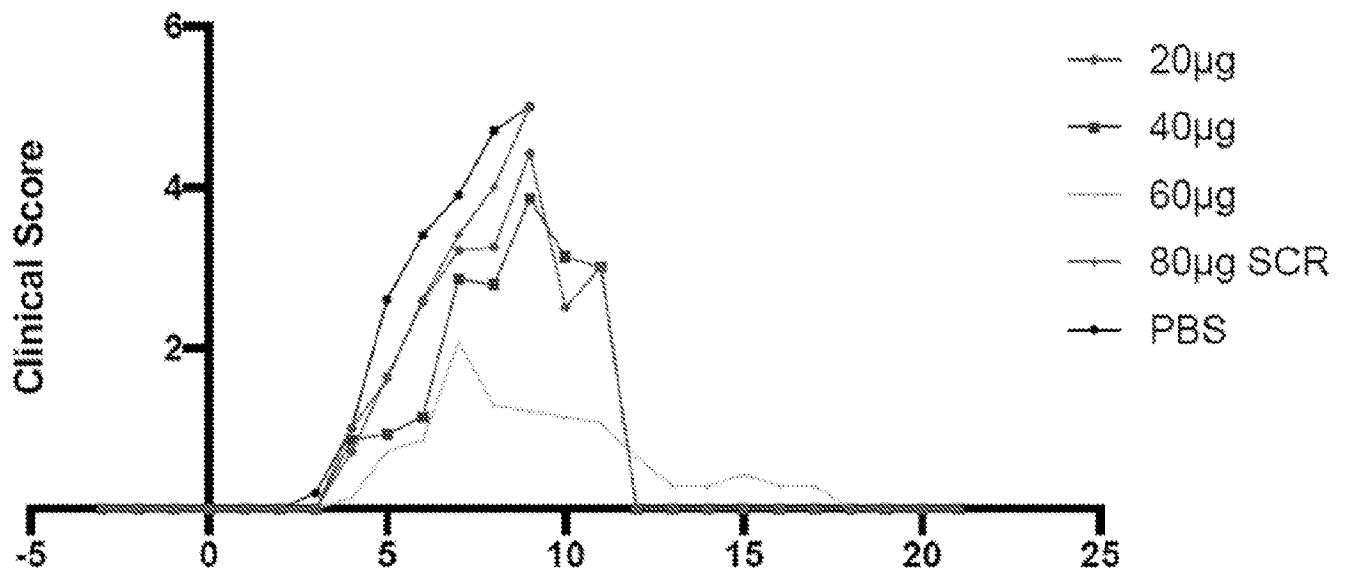
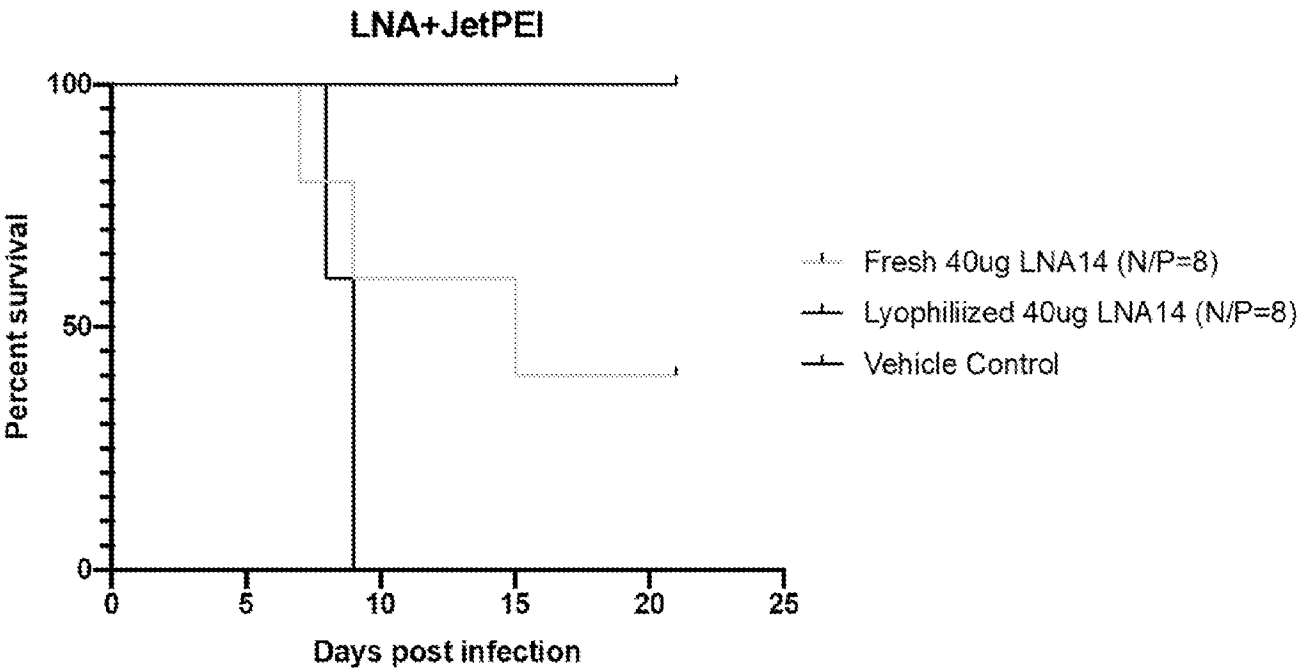
FIG. 3C

FIG. 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/21855

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 9/16; A61P 11/02; A61P 11/12; A01N 25/34; A61P 11/06; A61K 9/50 (2021.01)

CPC - A61K 9/16; A61P 11/02; A61P 11/06; A61P 11/12; A61K 9/50; A01N 25/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2017/0281545 A1 (TEXAS TECH UNIVERSITY SYSTEM) 5 October 2017; abstract; figures 1A, 1D, 2; paragraphs [0016], [0048]; claims 14, 31	1-3
X	US 5,648,101 A (TAWASHI, R) 15 July 1997; column 10, lines 55-68; column 11, lines 1-10	1
A	US 5,013,552 A (AMER, MS et al.) 7 May 1991; entire publication	1

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15 May 2021 (15.05.2021)

Date of mailing of the international search report

MAY 26 2021

Name and mailing address of the ISA/US

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Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/21855

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-15
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.