

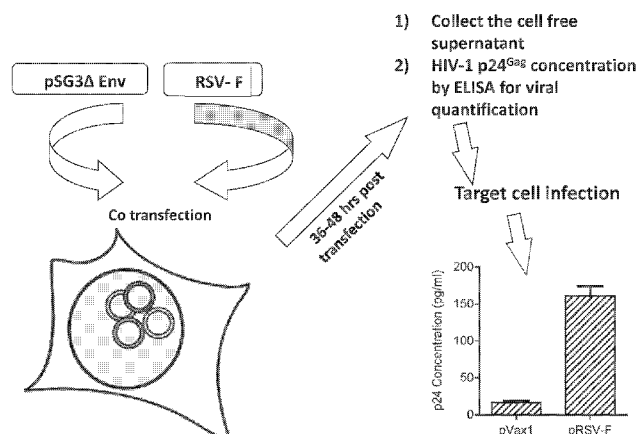


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(54) **Title:** HUMAN RESPIRATORY SYNCYTIAL VIRUS CONSENSUS ANTIGENS, NUCLEIC ACID CONSTRUCTS AND VACCINES MADE THEREFROM, AND METHODS OF USING SAME

Figure 12  
RSV-Fusion Pseudo viral production in 293T cells



(57) **Abstract:** Nucleic acid molecules and compositions comprising one or more nucleic acid sequences that encode an RSV immunogen are disclosed. Nucleic acid are disclosed that comprise the sequences that encodes consensus RSV F protein or immunogenic fragment thereof, sequences that encodes an RSV G(A) protein or immunogenic fragment thereof and sequences that encodes an RSV G(B) protein or immunogenic fragment thereof. Compositions comprising one, combinations of two or all three sequences are disclosed. The coding sequences optionally include operable linked coding sequence that encode a signal peptide. Nucleic acid molecules and compositions comprising the chemokine CC20 and/or a consensus RSV M2-1 protein or immunogenic fragment thereof are also disclosed. Immunomodulatory methods and methods of inducing an immune response against RSV are disclosed. Method of preventing RSV infection and methods of treating individuals infected with RSV are disclosed.



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**HUMAN RESPIRATORY SYNCYTIAL VIRUS CONSENSUS ANTIGENS,  
NUCLEIC ACID CONSTRUCTS AND VACCINES MADE THEREFROM, AND  
METHODS OF USING SAME**

**FIELD OF THE INVENTION**

The present invention relates to consensus antigenic respiratory syncytia virus proteins and nucleic acid molecules which encode the same; improved respiratory syncytia virus vaccines including such proteins and/or nucleic acid molecules; and methods for using the vaccines for inducing immune responses and preventing respiratory syncytia virus infection and/or treating individuals infected with respiratory syncytia virus.

**BACKGROUND OF THE INVENTION**

This application claims priority to U.S. Provisional Application No. 61/622,279, which is incorporated herein, in its entirety, by reference.

Human Respiratory Syncytial Virus (RSV) is the most common cause of upper and lower respiratory tract infections among infants and young children (premature infants especially prone to disease). World-wide, RSV is the leading cause of serious lower respiratory infections in infants (especially among those born prematurely and having chronic lung diseases or congenital heart diseases) and young children worldwide, and is responsible for a variety of illnesses, including 20-25% of pneumonia cases and 45-50% of bronchiolitis cases among hospitalized children. In the U.S., RSV infection results in approximately 120,000 hospitalization and 500+ deaths each year. In the population of <1 year old, RSV is the leading cause of infant viral death and mortality among this population is 10 times greater than mortality due to influenza infection. Global annual morbidity and mortality estimated to be 64 million and 160,000 deaths respectively. RSV-related medical costs estimated to be >\$650 million/year.

While essentially all children experience an infection by two years of age, the peak age for serious RSV infection is at 2-6 months of age. The majority of infections resolve uneventfully. In some children, however, infection may predispose children for asthma and

airway hyper-responsiveness later in life. Natural RSV infection does not confer lifetime immunity, and therefore, individuals may be repeatedly infected.

RSV infection raises significant issue among the elderly and other vulnerable populations. Among the elderly, RSV is the second leading cause of viral death. Transplant recipients and other immunocompromised populations as well as individuals suffering from cystic fibrosis are vulnerable to serious health consequences due to infection.

Respiratory Syncytial Virus (RSV) is an enveloped, negative-sense, single-stranded RNA virus of the family *Paramyxoviridae*, a family which includes common respiratory viruses such as influenza and those causing mumps and measles. In all, RSV has ten genes encoding eleven different proteins. The eleven RSV proteins include: proteins 1) protein “NS1” and 2) protein “NS2”, which inhibit type I interferon activity; 3) protein “N”, the nucleocapsid protein which associates with RNA forming nucleocapsid; 4) protein “P”, which is a cofactor for protein L; 5) protein “M”, the matrix protein which required for viral assembly; 6) protein “SH”, which is expressed on the viral surface forms the viral coat with protein G and protein F; 7) protein “G”, which is highly glycosylated, expressed on the surface, involved in viral attachment and binds glycosaminoglycans (GAGs); 8) protein “F”, which is expressed on the surface, viral-cell membrane fusion and mediating fusion to allow entry of virus into the cell cytoplasm; 9) protein “M2-1”, which is a matrix protein and elongation factor; 10) protein “M2-2”, which is a matrix protein and transcription factor; and, 11) protein “L”, which is RNA polymerase. The M2 gene encodes both protein M2-1 and protein M2-2 in overlapping open reading frames. The primary CD8 T cell epitope is encoded by the M2 gene. There are two major subtypes of human RSV - A and B. The major difference between the two subtypes resides within the G protein.

RSV infection often results both immune mediated pathology and virus mediated pathology. Primary RSV infection often results in acute bronchiolitis that leads to inflammation-induced airway obstruction. RSV F binding has been shown to induce apoptosis resulting in the sloughing of ciliated epithelial cells, compromised pulmonary clearance, and consequent secondary infections.

Unfortunately, despite the immense effort, there are still no effective vaccines available for RSV. In 1966-1967, first RSV vaccine candidate, a formalin-inactivated alum-precipitated



RSV preparation (FI-RSV vaccine) resulted in enhanced disease in vaccinated children upon subsequent natural infection.. Histological analysis of lungs of children who died from enhanced disease caused by infection after vaccination revealed extensive mononuclear cell infiltration including pulmonary eosinophilia. The FI-RSV vaccine generated only binding antibodies without neutralizing activity because of denatured F protein and did not induce CTL activity.

Subsequent experiments have suggested that this enhanced pulmonary disease is associated with an exaggerated Th2-type cytokine response by CD4 T cells, a poor cytolytic response by CD8 T cells, and a weak neutralizing antibody response. RSV infection of FI-RSV vaccinated BALB/c and C57BL/6 mice resulted in enhanced disease observed in FI-RSV vaccinated children. Characteristic of a Th2-mediated immune response suggested immunized children were primed for Th2 immune response by vaccine. Increased levels of Th2-associated cytokines IL-5, IL-4, and IL-13 and chemokine eotaxin with a decrease in Th1-associated cytokine IL-12 were exhibited. Depletion of IL-4, IL-10, or IL-13 resulted in significant decrease in enhanced disease after RSV challenge.

Live attenuated and inactivated whole virus vaccines have also failed to protect. The candidate vaccines were either insufficiently attenuated or demonstrated the potential for enhanced disease. In 1982, a live attenuated RSV vaccine was found to be safe, but not effective for prevention of RSV illness.

In 1983, a Native American infant, "Baby Moose", who was thought to have B streptococcal disease but who actually was infected with RSV, serendipitously improved when he received IGIV. This result prompted study of IGIV for RSV disease. In the mid 1980s through 1990 studies of standard IGIV for treatment and prevention of RSV illness validated the role of antibodies in prevention of RSV disease.

In the early 1990s, RSV vaccine studies were re-initiated using various subunit varieties. These trials failed to show significant protection from disease.

From mid 1990s to early 2002, clinical trials of palivizumab (monoclonal antibody specific for RSV-F) for prevention of serious respiratory tract disease caused by RSV produced positive results. In September 2003, palivizumab was approved for prevention of RSV-associated disease in high-risk children. Prophylactic treatment with palivizumab is effective in

reducing the severity of disease, but is only recommended for high-risk patients due to the high cost involved in the treatment.

Although past studies have failed to yield in effective RSV vaccines, they have convincingly demonstrated the importance of immune responses in providing a thorough protection against RSV infection. Studies have found evidence supporting the importance of humoral responses and antibodies in protection against RSV-mediated disease. The presence of IgG antibodies in the lung directly correlates with reduced viral load and children with less severe RSV disease often have significantly higher anti-RSV antibody titers before infection. Antibody to fusion protein is an important correlate of immunity. Infants who did not become infected with RSV had higher mean titers of IgG than infected infants and were born to mothers who had significantly higher maternal RSV-specific IgG antibody levels than the mothers of infants who became infected. The importance of antibody in mediating protection helps explain why premature infants are at such high risk for serious illness after RSV infection. Maternal IgG is not efficiently transferred to the fetus until the third trimester of pregnancy.

Similarly, the importance of cellular immune responses in providing a thorough protection against RSV infection has also been demonstrated. Children with T cell deficiency have difficulty clearing the virus and are more susceptible to subsequent RSV infection. In animal studies, depletion of CD8 T cells alone in mice does not result in chronic infection, but does result in delayed viral clearance. Clearance requires IFN- $\gamma$ , FasL, TNF- $\alpha$ . Deficiency in any of these results in delayed viral clearance. Further, C57BL/6 mice immunized with VV-G do not develop RSV-associated enhanced disease, but depletion of CD8 T cells before immunization results in disease.

There remains a need for an RSV vaccine, including an immunogenic antigen, that induces long-term protection against RSV. There is also a need for a cost-effective delivery system to enable mass prophylactic vaccination against RSV. There is also a need for additional therapeutic agents to treat individuals infected with RSV.

## SUMMARY OF THE INVENTION

Nucleic acid molecules are provided which comprising one or more nucleic acid sequences that encode one or more consensus RSV immunogens. The RSV immunogens include the consensus RSV F immunogen, the consensus RSV-G(A) immunogen, and the consensus RSV G(B) immunogen.

Compositions are provided which comprise one or more nucleic acid sequences that encode an RSV immunogen. The nucleic acids are selected from the group consisting of: a) a nucleic acid coding sequence that encodes an RSV F immunogen, b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, and c) a nucleic acid coding sequence that encodes an RSV Gb immunogen.

In some embodiments, the nucleic acid coding sequence that encodes an RSV F immunogen encodes an RSV F immunogen selected from the group consisting of: SEQ ID NO:2, a fragment of SEQ ID NO:2, a protein that is at least 98% homologous to SEQ ID NO:2 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:2. In each instance, the nucleic acid coding sequence that encodes an RSV F immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Ga immunogen encodes an RSV Ga immunogen selected from the group consisting of: SEQ ID NO:6, a fragment of SEQ ID NO:6, a protein that is at least 98% homologous to SEQ ID NO:6 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:6. In each instance, the nucleic acid coding sequence that encodes an RSV Ga immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Gb immunogen encodes an RSV Gb immunogen selected from the group consisting of: SEQ ID NO:8, a fragment of SEQ ID NO:8, a protein that is at least 98% homologous to SEQ ID NO:8 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:8. In each instance, the nucleic acid coding sequence that encodes an RSV Gb immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV F immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:1, nucleic acid sequences comprising a fragment of SEQ ID NO:1, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:1, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:1. In each instance, the nucleic acid coding sequence that encodes an RSV F immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:1 preferable encode a protein that is at least 98% homologous to SEQ ID NO:2. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:1 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:2.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Ga immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:5, nucleic acid sequences comprising a fragment of SEQ ID NO:5, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:5, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:5. In each instance, the nucleic acid coding sequence that encodes an RSV Ga immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:5 preferable encode a protein that is at least 98% homologous to SEQ ID NO:6. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:5 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:6.

In some embodiments, the nucleic acid coding sequence that encodes an RSV Gb immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:7, nucleic acid sequences comprising a fragment of SEQ ID NO:7, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:7, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID

NO:7. In each instance, the nucleic acid coding sequence that encodes an RSV Gb immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:7 preferable encode a protein that is at least 98% homologous to SEQ ID NO:8. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:7 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:8.

In some embodiments, the compositions comprises one or more nucleic acid sequences selected from the group consisting of: a nucleic acid sequence that encodes SEQ ID NO:10; a nucleic acid sequence that encodes SEQ ID NO:14; and a nucleic acid sequence that encodes SEQ ID NO:16.

In some embodiments, the compositions comprise one or more nucleic acid sequences selected from the group consisting of: a nucleic acid sequence comprising SEQ ID NO:9; a nucleic acid sequence comprising SEQ ID NO:13; and a nucleic acid sequence comprising SEQ ID NO:15.

In some embodiments, the compositions compriss nucleic acid sequences selected from the group consisting of:

- a nucleic acid coding sequence that encodes an RSV F immunogen,
- a nucleic acid coding sequence that encodes an RSV Ga immunogen,
- a nucleic acid coding sequence that encodes an RSV Gb immunogen,
- a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,
- a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,
- a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,
- a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

The composition may further comprise a nucleic acid coding sequence that encodes CCL20 and/or be formulated for delivery to an individual using electroporation and/or further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

In some embodiments, one or more nucleic acid coding sequences are part of one or more plasmids. In some embodiments, one or more nucleic acid coding sequences are each incorporated into a separate plasmid.

Method are provided for inducing an immune response against RSV comprising administering a composition to an individual in an amount effective to induce an immune response in said individual.

Method are provided for treating an individual who has been diagnosed with RSV comprising administering a therapeutically effective amount of a composition to an individual.

Method are provided for preventing RSV infection an individual comprising administering a prophylactically effective amount of the composition to an individual.

Novel “RSV F immunogen”, “RSV Ga immunogen”, and “RSV Gb immunogen” are provided. In some embodiments, proteins selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:14; and SEQ ID NO:16 are provided.

Methods of modulating immune responses using a nucleic acid coding sequence that encodes the chemokine CCL20 are also provided.

In addition, provided herein is a nucleic acid coding sequence that encodes a consensus RSV M2-1 immunogen. In some embodiments, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen encodes an RSV M2-1 immunogen selected from the group consisting of: SEQ ID NO:4, a fragment of SEQ ID NO:4, a protein that is at least 98% homologous to SEQ ID NO:4 and a fragment of a protein that is at least 98% homologous to SEQ ID NO:4. In each instance, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence.

In some embodiments, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen is selected from the group consisting of: nucleic acid sequences comprising SEQ ID NO:3, nucleic acid sequences comprising a fragment of SEQ ID NO:3, nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:3, and nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:3. In each instance, the nucleic acid coding sequence that encodes an RSV M2-1 immunogen may optionally further comprise coding sequence that encodes a signal peptide operably linked to the sequence. Nucleic acid sequences comprising sequences that is at least 98% homologous to SEQ ID NO:3 preferable encode a protein that is at least 98% homologous to SEQ ID NO:4. Nucleic acid sequences comprising a fragment of a sequence that is at least 98% homologous to SEQ ID NO:3 preferable encode a fragment of a protein that is at least 98% homologous to SEQ ID NO:4.

In some embodiments, compositions are provided which comprises one or more nucleic acid sequences including a nucleic acid sequence that encodes SEQ ID NO:12.

In some embodiments, the compositions are provided which comprise one or more nucleic acid sequences including a nucleic acid sequence comprising SEQ ID NO:11.

In some embodiments, the compositions comprise nucleic acid sequences selected from the group consisting of: a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen, a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic

acid coding sequence that encodes an RSV Gb immunogen, and a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen.

Novel “ “RSV M2-1 immunogen is also provided. In some embodiments, proteins selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:12 are provided.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows the phylogenetic tree of RSV-F Protein, the consensus RSV F protein amino acid sequence is set forth in SEQ ID NO:2.

Figure 2 shows the phylogenetic tree of RSV M2-1 Protein, the consensus RSV M2-1 protein amino acid sequence is set forth in SEQ ID NO:4.

Figure 3 shows results anti-RSV F protein IgG at various time points following immunization with RSV F vaccine.

Figure 4 shows results anti-RSV F protein IgA at various time points following immunization with RSV F vaccine.

Figure 5 shows results from a comparison of IFN- $\gamma$  production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization.

Figure 6 shows results from a comparison of IFN- $\gamma$ + CD8 and CD4 T cells in spleen after RSV-F +/- chemokine immunization in the spleen.

Figure 7 shows results from a comparison of IFN- $\gamma$ + CD8 and CD4 T cells in spleen after RSV-F +/- chemokine immunization in the lung.

Figure 8 shows results from a comparison of IgG Endpoint Titer in Sera and IgG subtypes (IgG1 vs IgG2a) in Sera.

Figure 9 shows a comparison of IgG2a/IgG1 ratio.

Figure 10 depicts Env-Pseudotyped viruses in TZM-BL Cells/ “ON”.

Figure 11 depicts Env-Pseudotyped viruses in TZM-BL Cells/ “OFF”.

Figure 12 depicts RSV-Fusion Pseudo viral production in 293T cells.



Figure 13 shows the immunization protocol used for a RSV-F Neutralization Study using the assay.

Figure 14 shows data from quantification of RSV-F mediated infection in different target cells.

Figure 15 shows data from quantification of RSV-F neutralization activity in TZM-bl HeLa cells.

Figure 16 shows data from quantification of cell death activity by RSV-F pseudo virus in HeLa cells.

Figure 17 shows the immunization schedule for RSV-M2 dosage study that was undertaken.

Figure 18 shows data collected from that study including a comparison of IFN- $\gamma$  production at different dosages of RSV-M2 vaccine 1 week after last immunization.

Figure 19 depicts an immunization schedule for evaluation of the combination of RSV-F vaccine with CCL20.

Figure 20 shows data from the study including data from a comparison of IFN- $\gamma$  production in each of spleen, liver and lung with different dosages of CCL20 at 1 week after last immunization.

Figure 21 shows data of a comparison of IgG subtypes (IgG1 vs IgG2a) in Sera.

Figures 22A-22C show plasmid designs and construction of RSV-F, RSV-G(A), and RSV-G(B) constructs. Figures 22A shows plasmid design and construction of RSV-F construct. Figures 22B shows plasmid design and construction of RSV-G(A) construct. Figures 22C shows plasmid design and construction of RSV-G(B) construct.

Figure 23 shows results confirming in vitro expression of RSV-F, RSV-G(A), and RSV-G(B) constructs.

Figure 24 shows the immunization schedule for an RSV-F Dosage Study.

Figure 25 shows IgG data from RSV-F Dosage Study.

Figure 26 shows IFN- $\gamma$  data from RSV-F Dosage Study.

Figure 27 shows the immunization schedule for a RSV-F PSEUDO Neutralization Study.

Figure 28 shows data from the RSV-F PSEUDO Neutralization Study.

Figure 29 shows data from two comparisons to determine inhibition of apoptotic cell death activity by RSV-F pseudovirus in HeLa cells.

Figure 30 shows the immunization schedule for an RSV-G(A) and RSV-(B) Dosage Study.

Figure 31 shows IgG data from RSV-G(A) from the RSV-G(A) and RSV-(B) Dosage Study.

Figure 32 shows IgG data from RSV-G(A) from the RSV-G(A) and RSV-(B) Dosage Study.

Figure 33 is a chart showing a summary of RSV Preliminary Neutralization results using Mono Vaccines in Mouse Studies.

Figure 34 shows the RSV Rabbit Study Design that was followed.

Figure 35 shows data from the RSV Rabbit Study of measured humoral immunity against RSV-F.

Figure 36 shows data from the RSV Rabbit Study of measured humoral immunity against RSV-G.

Figure 37 shows the RSV Non-Human Primate Study Design that was followed.

Figure 38A-38D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-F. Figure 38A shows data from measured humoral immunity against RSV-F in naïve animals. Figure 38B shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine IM at one site. Figure 38C shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine plus rhMEC construct IM at one site. Figure 38D shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine ID at three sites.

Figure 39A-39D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-G. Figure 39A shows data from measured humoral immunity against RSV-G in naïve animals. Figure 39B shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine IM at one site. Figure 39C shows data from measured humoral immunity against RSV-G in animals who received the cocktail

vaccine plus rhMEC construct IM at one site. Figure 39D shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine ID at three sites.

Figure 40 is a chart showing a summary of RSV Preliminary Neutralization results using cocktail vaccines in Rabbit Non-Human Primate Studies.

#### **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT**

In one aspect of the invention, it is desired that the consensus antigen provides for improved transcription and translation, including having one or more of the following: low GC content leader sequence to increase transcription; mRNA stability and codon optimization; eliminating to the extent possible cis-acting sequence motifs (i.e., internal TATA-boxes).

In some aspects of the invention, it is desired to generate a consensus antigen that generates a broad immune response across multiple strains, including having one or more of the following: incorporate all available full-length sequences; computer generate sequences that utilize the most commonly occurring amino acid at each position; and increase cross-reactivity between strains.

The attached Sequence Listing includes SEQ ID NOs:1-16 which are various RSV sequences and embodiments.

SEQ ID NO:1 shows a nucleotide sequence encoding an RSV F without start codon (ATG) or signal peptide.

SEQ ID NO:2 shows an amino sequence encoding an RSV F without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:3 shows a nucleotide sequence encoding an RSV M2-1 without start codon (ATG) or signal peptide.

SEQ ID NO:4 shows an amino sequence encoding an RSV M2-1 without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:5 shows a nucleotide sequence encoding an RSV Ga without start codon (ATG) or signal peptide.

SEQ ID NO:6 shows an amino sequence encoding RSV Ga without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:7 shows a nucleotide sequence encoding an RSV Gb without start codon (ATG) or signal peptide.

SEQ ID NO:8 shows an amino sequence encoding an RSV Gb without Met encoded by a start codon (ATG) or signal peptide.

SEQ ID NO:9 shows a nucleotide sequence encoding an RSV F including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:10 shows an amino sequence encoding an RSV F with Met encoded by start codon/IgE signal peptide.

SEQ ID NO:11 shows a nucleotide sequence encoding an RSV M2-1 including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:12 shows an amino sequence encoding an RSV M2-1 with Met encoded by start codon/IgE signal peptide.

SEQ ID NO:13 shows a nucleotide sequence encoding an RSV Ga including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:14 shows an amino sequence encoding an RSV Ga with Met encoded by start codon/IgE signal peptide.

SEQ ID NO:15 shows a nucleotide sequence encoding an RSV Gb including start codon (ATG)/sequences encoding IgE signal peptide.

SEQ ID NO:16 shows an amino sequence encoding an RSV Gb with Met encoded by start codon/IgE signal peptide.

The attached Sequence Listing includes SEQ ID NO:17 which is the amino acid sequence of the IgE signal peptide.

SEQ ID NO:17 shows an amino acid sequence of an IgE signal peptide.

The attached Sequence Listing includes SEQ ID NOs:18-21 which are various CCL20 sequences and embodiments.

SEQ ID NO:18 shows a nucleotide sequence encoding an CCL20 without start codon (ATG) or signal peptide

SEQ ID NO:19 CCL20 without Met encoded by a start codon (ATG) or signal peptide

SEQ ID NO:20 shows a nucleotide sequence encoding an CCL20 including start codon (ATG)/sequences encoding IgE signal peptide

SEQ ID NO:21 CCL20 with Met encoded by start codon/IgE signal peptide

The attached Sequence Listing includes SEQ ID NO:22-31 which are amino acid sequences of derived from RSV immunogens.

SEQ ID NO:22 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:23 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:24 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:25 is a T cell epitope of RSV F Immunogen.

SEQ ID NO:26 is a RSV F Immunogen protease cleavage site.

SEQ ID NO:27 is a RSV F Immunogen protease cleavage site.

SEQ ID NO:28 is a RSV F Immunogen fusion peptide.

SEQ ID NO:29 is a RSV F Immunogen binding site for palivizumab.

SEQ ID NO:30 is a RSV M2-1 Immunodominant T cell epitope.

SEQ ID NO:31 is a RSV M2-1 Subdominant T cell epitope.

In some aspects of the invention, RSV F immunogen and sequences that encode the RSV F immunogen are provided. In some aspects of the invention, RSV Ga immunogen and sequences that encode the RSV Ga immunogen are provided. In some aspects of the invention, RSV Gb immunogen and sequences that encode the RSV Gb immunogen are provided. Compositions comprising combinations sequences that encode the RSV F immunogen, sequences that encode the RSV Ga immunogen and sequences that encode the RSV Gb immunogen are provided, including each of sequences that encode the RSV F immunogen, sequences that encode the RSV Ga immunogen and sequences that encode the RSV Gb immunogen.

RSV M2-1 immunogen and sequences that encode the RSV M2-1 immunogen are also provided and may be used alone or in combination with other proteins and/or nucleic acid sequences.

## **1. Definitions.**

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

### **a. Adjuvant**

“Adjuvant” as used herein may mean any molecule added to the DNA plasmid vaccines described herein to enhance antigenicity of the one or more Respiratory Syncytial Virus (RSV.) antigens encoded by the DNA plasmids and encoding nucleic acid sequences described hereinafter.

### **b. Antibody**

“Antibody” may mean an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, fragments or derivatives thereof, including Fab, F(ab')<sub>2</sub>, Fd, and single chain antibodies, diabodies, bispecific antibodies, bifunctional antibodies and derivatives thereof. The antibody may be an antibody isolated from the serum sample of mammal, a polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom.

### **c. Coding Sequence**

“Coding sequence” or “encoding nucleic acid” as used herein may mean refers to the nucleic acid (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein. The coding sequence may further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to whom the nucleic acid is administered.

In some embodiments, the coding sequence for the amino acid sequence of consensus RSV F immunogen, which is set forth in SEQ ID NO:2, is SEQ ID NO:1. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:10 corresponds to SEQ ID NO:2 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:9 encodes SEQ ID NO:10.

In some embodiments, the coding sequence for the amino acid sequence of consensus RSV Ga immunogen, which is set forth in SEQ ID NO:6, is SEQ ID NO:5. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:14 corresponds to SEQ ID NO:6 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:13 encodes SEQ ID NO:14.

In some embodiments, the coding sequence for the amino acid sequence of consensus RSV Gb immunogen, which is set forth in SEQ ID NO:8, is SEQ ID NO:7. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:16 corresponds to SEQ ID NO:8 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:15 encodes SEQ ID NO:16.

Also provides is a coding sequence, SEQ ID NO:3, which encodes the amino acid sequence of consensus RSV M2-1 immunogen, which is set forth in SEQ ID NO:4. Such coding sequence may optionally further comprise a start codon that encodes an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:12 corresponds to SEQ ID NO:4 with an IgE signal peptide (SEQ ID NO:17). SEQ ID NO:11 encodes SEQ ID NO:12.

#### **d. Complement**

“Complement” or “complementary” as used herein may mean a nucleic acid may mean Watson-Crick (*e.g.*, A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

#### **e. Consensus or Consensus Sequence**

“Consensus” or “consensus sequence” as used herein may mean a synthetic nucleic acid sequence, or corresponding polypeptide sequence, constructed based on analysis of an alignment of multiple subtypes of a particular RSV antigen, that can be used to induce broad immunity against multiple subtypes or serotypes of a particular RSV antigen. Consensus RSV antigens

may include consensus amino acid sequences of protein F, protein G and protein M2. Nucleotide sequences that encode the consensus amino acid sequences are also provided. Also, synthetic antigens such as fusion proteins may be manipulated to include consensus sequences (or consensus antigens).

The amino acid sequence of consensus RSV F immunogen is set forth in SEQ ID NO:2. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:10 corresponds to SEQ ID NO:2 with an IgE signal peptide (SEQ ID NO:17).

The amino acid sequence of consensus RSV Ga immunogen is set forth in SEQ ID NO:6. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:14 corresponds to SEQ ID NO:6 with an IgE signal peptide (SEQ ID NO:17).

The amino acid sequence of consensus RSV Gb immunogen is set forth in SEQ ID NO:8. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:16 corresponds to SEQ ID NO:8 with an IgE signal peptide (SEQ ID NO:17).

Also provided is the amino acid sequence of consensus RSV M2-1 immunogen which is set forth in SEQ ID NO:4. Such sequence may optionally comprise an N terminal methionine or a signal peptide such as an IgE or IgG signal peptide. SEQ ID NO:12 corresponds to SEQ ID NO:4 with an IgE signal peptide (SEQ ID NO:17).

#### **f. Constant Current**

“Constant current” as used herein to define a current that is received or experienced by a tissue, or cells defining said tissue, over the duration of an electrical pulse delivered to same tissue. The electrical pulse is delivered from the electroporation devices described herein. This current remains at a constant amperage in said tissue over the life of an electrical pulse because the electroporation device provided herein has a feedback element, preferably having instantaneous feedback. The feedback element can measure the resistance of the tissue (or cells) throughout the duration of the pulse and cause the electroporation device to alter its electrical energy output (e.g., increase voltage) so current in same tissue remains constant throughout the



electrical pulse (on the order of microseconds), and from pulse to pulse. In some embodiments, the feedback element comprises a controller.

**g. Current Feedback or Feedback**

“Current feedback” or “feedback” as used herein may be used interchangeably and may mean the active response of the provided electroporation devices, which comprises measuring the current in tissue between electrodes and altering the energy output delivered by the EP device accordingly in order to maintain the current at a constant level. This constant level is preset by a user prior to initiation of a pulse sequence or electrical treatment. The feedback may be accomplished by the electroporation component, e.g., controller, of the electroporation device, as the electrical circuit therein is able to continuously monitor the current in tissue between electrodes and compare that monitored current (or current within tissue) to a preset current and continuously make energy-output adjustments to maintain the monitored current at preset levels. The feedback loop may be instantaneous as it is an analog closed-loop feedback.

**h. Decentralized Current**

“Decentralized current” as used herein may mean the pattern of electrical currents delivered from the various needle electrode arrays of the electroporation devices described herein, wherein the patterns minimize, or preferably eliminate, the occurrence of electroporation related heat stress on any area of tissue being electroporated.

**i. Electroporation**

“Electroporation,” “electro-permeabilization,” or “electro-kinetic enhancement” (“EP”) as used interchangeably herein may refer to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

**j. Feedback Mechanism**

“Feedback mechanism” as used herein may refer to a process performed by either software or hardware (or firmware), which process receives and compares the impedance of the desired tissue (before, during, and/or after the delivery of pulse of energy) with a present value,

preferably current, and adjusts the pulse of energy delivered to achieve the preset value. A feedback mechanism may be performed by an analog closed loop circuit.

**k. Fragment**

“Fragment” may mean a polypeptide fragment of an RSV Immunogen that is capable of eliciting an immune response in a mammal against RSV by recognizing the particular RSV antigen. An RSV Immunogen is refers to the consensus sequences set forth herein for RSV F Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen, RSV Gb Immunogen, in each case with or without signal peptides and/or a methionine at position 1, proteins 98% or more homologous to the consensus sequences set forth herein, proteins 99% or more homologous to the consensus sequences set forth herein, and proteins 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. A fragment may or may not for example comprise a fragments of an RSV Immunogen linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide.

A fragment of RSV F consensus Immunogen may be a fragment of SEQ ID NO:2, of SEQ ID NO:10, of a protein 98% or more homologous to the RSV F consensus Immunogen sequences set forth herein, of a protein 99% or more homologous to the RSV F consensus Immunogen sequences set forth herein, or of RSV F consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. Such fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV F consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV F consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or

more homologous, or 100% identical to the RSV F consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV F Immunogen may comprise a fragment of a SEQ ID NO:2. The fragment may comprise a fragments of SEQ ID NO:2 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:10 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:2 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:2 . The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV F Immunogen is 575 amino acids. In some embodiments, the fragments thereof may be 115 or more amino acids in length, 163 or more, 230 or more, 288 or more, 345 or more, 403 or more, 460 or more, 515 or more, 520 or more, 525 or more, 530 or more, 535 or more, 540 or more, 545 or more, 550 or more, 555 or more, 560 or more, 565 or more, 570 or more, 574 or more in length. Polypeptide fragments may be fewer than 140 amino acids, fewer than 190, fewer than 250, fewer than 283, fewer than 300, fewer than 365, fewer than 425, fewer than 495, fewer than 518, fewer than 523, fewer than 528, fewer than 533, fewer than 547, fewer than 552, fewer than 563, or fewer than 572 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a

574 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 574 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 574 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 574 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 574 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:2 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:10 comprises SEQ ID NO:2 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:10 comprises a fragment of SEQ ID NO:2, that is 100% homologous to a fragment of SEQ ID NO:2, notwithstanding the signal peptide which is absent in SEQ ID NO:2. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:2 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:2 that are at least 115 amino acids and may optionally be linked to a, for example, a signal peptide. SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25 correspond to T cell epitopes of RSV F immunogen set forth in SEQ ID NO:2. In some embodiments, fragments comprise all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them. SEQ ID NO:26 and SEQ ID NO:27 are RSV F Immunogen protease cleavage sites. SEQ ID NO:28 is an RSV F Immunogen fusion peptide. SEQ ID NO:29 is the RSV F Immunogen binding site for palivizumab.

A fragment of RSV Ga consensus Immunogen may be a fragment of SEQ ID NO:6, of SEQ ID NO:14, of a protein 98% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, of a protein 99% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, or of RSV Ga consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. Such fragments may comprise may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more,

91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Ga consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Ga consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Ga consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV Ga Immunogen may comprise a fragment of SEQ ID NO:6. The fragment may comprise a fragments of SEQ ID NO:6 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:14 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:6 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:6. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV Ga Immunogen is 297 amino acids excluding an N terminal methionine and/or signal peptide. In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more,

200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 290 or more, 295 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 292, or fewer than 296 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a 296 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 296 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 296 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 296 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 296 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:6 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:14 comprises SEQ ID NO:6 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:14 comprises a fragment of SEQ ID NO:6, that is 100% homologous to a fragment of SEQ ID NO:6, notwithstanding the signal peptide which is absent in SEQ ID NO:6. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:6 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:6 that are at least 125 amino acids and may optionally be linked to a, for example, a signal peptide.

A fragment of RSV Gb consensus Immunogen such as that which is disclosed in SEQ ID NO:8, SEQ ID NO:16, proteins 98% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, and RSV Gb consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a

methionine at position 1, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Gb consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Gb consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Gb consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV Gb Immunogen may comprise a fragment of SEQ ID NO:8. The fragment may comprise a fragments of SEQ ID NO:8 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:16 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:8 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:8. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV Gb Immunogen is 291

amino acids excluding an N terminal methionine and/or signal peptide. In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more, 200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 288 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 290 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a 290 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 290 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 290 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 290 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 290 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:6 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:16 comprises SEQ ID NO:8 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:16 comprises a fragment of SEQ ID NO:8, that is 100% homologous to a fragment of SEQ ID NO:8, notwithstanding the signal peptide which is absent in SEQ ID NO:8. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:8 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:8 that are at least 125 amino acids and may optionally be linked to a, for example, a signal peptide.

In addition, a fragment of RSV M2-1 consensus Immunogen may be a fragment of SEQ ID NO:4, of SEQ ID NO:12, of a protein 98% or more homologous to the RSV M2-1 consensus Immunogen sequences set forth herein, of a protein 99% or more homologous to the RSV M2-1



consensus Immunogen sequences set forth herein, or of RSV M2-1 consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. Such fragments may comprise may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV M2-1 consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV M2-1 consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV M2-1 consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment of RSV M2-1 Immunogen may comprise a fragment of a SEQ ID NO:4. The fragment may comprise a fragments of SEQ ID NO:4 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:12 which comprises SEQ ID NO:17. The fragment may comprise fragments of SEQ ID NO:4 linked to an N terminal methionine. Fragments also refer to fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:4. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when

calculating percent homology. The fragment may comprise a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The RSV M2-1 Immunogen is 195 amino acids. In some embodiments, the fragments thereof may be 25 or more amino acids in length, 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more, 110 or more, 120 or more, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 184 or more, 190 or more, 193 or more in length. Polypeptide fragments may be fewer than 35 amino acids, fewer than 45, fewer than 55, fewer than 65, fewer than 75, fewer than 85, fewer than 95, fewer than 105, fewer than 115, fewer than 125, fewer than 135, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 188, fewer than 192, or fewer than 194 in length. Fragments may further comprise an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to a 194 amino acid or smaller fragment thereof. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 98% homologous to the 194 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 194 amino acid sequence. The N terminal methionine and/or signal peptide may be linked to a polypeptide that is 99% homologous to the 194 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 194 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:4 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:12 comprises SEQ ID NO:4 operably linked to a signal peptide SEQ ID NO:17, SEQ ID NO:12 comprises a fragment of SEQ ID NO:4, that is 100% homologous to a fragment of SEQ ID NO:4, notwithstanding the signal peptide which is absent in SEQ ID NO:4. Thus, proteins which comprise fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:4 intended to refer to proteins which fragments of a polypeptide that is at least 98% homologous to a fragment of SEQ ID NO:4 that are at least 25 amino acids and may optionally be linked to a, for example, a signal peptide. SEQ ID NO:30 corresponds to an immunodominant T cell epitope of RSV M2-1

immunogen set forth in SEQ ID NO:4. SEQ ID NO:31 refers to a subdominant T cell epitope corresponds to an immunodominant T cell epitope of RSV M2-1 immunogen set forth in SEQ ID NO:4. In some embodiments, fragments comprise all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them.

“Fragment” may also mean a nucleic acid fragment of that encodes an RSV Immunogen fragment set forth above, i.e. RSV F Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen, RSV Gb Immunogen, in each case with or without signal peptides and/or a methionine at position 1, proteins 98% or more homologous to the consensus sequences set forth herein, proteins 99% or more homologous to the consensus sequences set forth herein, and proteins 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1. A fragment may or may not for example comprise a fragments of nucleic acid sequences that encode an RSV Immunogen linked to coding sequence for an N terminal methionine (i.e. a start codon) or coding sequences encoding a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. A fragment of a coding sequence that encodes an RSV Immunogen may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of coding sequence of the particular full length RSV Immunogen sequence set forth herein, excluding any coding sequence that encodes non-RSV signal peptide added. Fragments also refer to fragments of coding sequences which encode a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Immunogen set forth herein. The fragment may comprise a fragment of a polypeptide that is 98% or more homologous to and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology.

In some embodiments, the fragments are fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO1. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:1. In some embodiments, the

fragments are fragments of SEQ ID NO:1. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments are fragments of SEQ ID NO:1. A fragment of a coding sequence that encodes RSV F consensus Immunogen such as that which is disclosed in SEQ ID NO:2, SEQ ID NO:10, proteins 98% or more homologous to the RSV F consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV F consensus Immunogen sequences set forth herein, and RSV F consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:1 or SEQ ID NO:9, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV F consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV F consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV F consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV F Immunogen may encode a fragment of a SEQ ID NO:2 and comprise a fragment of SEQ ID NO:1. The fragment may encode fragments of SEQ ID NO:2 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:10 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:9. The fragment may comprise fragments of SEQ ID NO:1 linked to start codon. Fragments

also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:2. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:2 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:1. The fragment may be 98% or more homologous to SEQ ID NO:1 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:1 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:1 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:1 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:1, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:1, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:1, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV F immunogen that may be 115 or more amino acids in length, 163 or more, 230 or more, 288 or more, 345 or more, 403 or more, 460 or more, 515 or more, 520 or more, 525 or more, 530 or more, 535 or more, 540 or more, 545 or more, 550 or more, 555 or more, 560 or more, 565 or more, 570 or more, 574 or more in length. Polypeptide fragments may be fewer than 140 amino acids, fewer than 190, fewer than 250, fewer than 283, fewer than 300, fewer than 365, fewer than 425, fewer than 495,

fewer than 518, fewer than 523, fewer than 528, fewer than 533, fewer than 547, fewer than 552, fewer than 563, or fewer than 572 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 574 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 574 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 574 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 574 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 574 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:2 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:7 comprises SEQ ID NO:1 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:7 comprises a fragment of SEQ ID NO:1 that is 100% homologous to a fragment of SEQ ID NO:1, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:1. SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25 correspond to T cell epitopes of RSV F immunogen set forth in SEQ ID NO:2. In some embodiments, fragments comprise coding sequences that encode all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them including fragments of SEQ ID NO:1 or sequences that are 98% or more or 99% or more homologous to SEQ ID NO:1.

In some embodiments, the fragments are fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO:5. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:5. In some embodiments, the fragments are fragments of SEQ ID NO:5. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size

and homology of the peptide fragments are fragments of SEQ ID NO:5. A fragment of a coding sequence that encodes RSV Ga consensus Immunogen such as that which is disclosed in SEQ ID NO:6, SEQ ID NO:14, proteins 98% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV Ga consensus Immunogen sequences set forth herein, and RSV Ga consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:5 or SEQ ID NO:13, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Ga consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Ga consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Ga consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV Ga Immunogen may encode a fragment of a SEQ ID NO:6 and comprise a fragment of SEQ ID NO:5. The fragment may encode fragments of SEQ ID NO:6 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:14 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:13. The fragment may comprise fragments of SEQ ID NO:5 linked to start codon. Fragments also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:6. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The

fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:6 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:5. The fragment may be 98% or more homologous to SEQ ID NO:5 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:5 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:5 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:5 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:5, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:5, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:5, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV Ga immunogen that may be In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more, 200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 290 or more, 295 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 292, or fewer than 296 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an



IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 296 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 296 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 296 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 296 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 296 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:6 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:13 comprises SEQ ID NO:5 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:13 comprises a fragment of SEQ ID NO:5 that is 100% homologous to a fragment of SEQ ID NO:5, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:5.

In some embodiments, the fragments are fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO:7. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:7. In some embodiments, the fragments are fragments of SEQ ID NO:7. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments are fragments of SEQ ID NO:7. A fragment of a coding sequence that encodes RSV Gb consensus Immunogen such as that which is disclosed in SEQ ID NO:8, SEQ ID NO:16, proteins 98% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV Gb consensus Immunogen sequences set forth herein, and RSV Gb consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:7 or SEQ ID NO:15, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or

more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV Gb consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV Gb consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV Gb consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV Gb Immunogen may encode a fragment of a SEQ ID NO:8 and comprise a fragment of SEQ ID NO:7. The fragment may encode fragments of SEQ ID NO:8 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:16 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:15. The fragment may comprise fragments of SEQ ID NO:7 linked to start codon. Fragments also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:8. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:8 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:7. The

fragment may be 98% or more homologous to SEQ ID NO:7 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:7 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:7 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:7 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:7, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:7, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:7, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV Gb immunogen that may be In some embodiments, the fragments thereof may be 125 or more amino acids in length, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 190 or more, 200 or more, 210 or more, 220 or more, 230 or more, 240 or more, 250 or more, 260 or more, 270 or more, 280 or more, 285 or more, 288 or more in length. Polypeptide fragments may be fewer than 135 amino acids, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 195, fewer than 205, fewer than 215, fewer than 225, fewer than 235, fewer than 245, fewer than 255, fewer than 265, fewer than 275, fewer than 285, fewer than 288, fewer than 290 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 290 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 290 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 290 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 290 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 290 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:8 or a fragment thereof, an N terminal methionine

and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:15 comprises SEQ ID NO:7 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:15 comprises a fragment of SEQ ID NO:7 that is 100% homologous to a fragment of SEQ ID NO:7, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:7.

Also provides are sequences related to RSV M2-1 including fragments of a nucleic acid sequence 98% or more homologous to SEQ ID NO:3. In some embodiments, the fragments are fragments of a nucleic acid sequence 99% or more homologous to SEQ ID NO:3. In some embodiments, the fragments are fragments of SEQ ID NO:3. It is intended that nucleic acid sequences encoding the polypeptide fragments described herein are expressly disclosed, i.e. fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments. In some embodiments, fragments of nucleic acid sequences that encode the proteins having the size and homology of the peptide fragments are fragments of SEQ ID NO:3. A fragment of a coding sequence that encodes RSV M2-1 consensus Immunogen such as that which is disclosed in SEQ ID NO:4, SEQ ID NO:12, proteins 98% or more homologous to the RSV M2-1 consensus Immunogen sequences set forth herein, proteins 99% or more homologous to the RSV M2-1 consensus Immunogen sequences set forth herein, and RSV M2-1 consensus Immunogen 100% identical to the consensus sequences set forth herein, in each case with or without signal peptides and/or a methionine at position 1, such as for example SEQ ID NO:3 or SEQ ID NO:11, may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length RSV M2-1 consensus Immunogen sequence set forth herein, excluding any non-RSV signal peptide added. Fragments may encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to the sequences of the RSV M2-1 consensus Immunogen set forth herein and 100% identical to such sequence, in each case with or without signal peptides and/or a methionine at position 1. The fragment may encode a

fragment of a polypeptide that is 98% or more homologous, 99% or more homologous, or 100% identical to the RSV M2-1 consensus Immunogen and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. In some embodiments, a fragment that encodes RSV M2-1 Immunogen may encode a fragment of a SEQ ID NO:4 and comprise a fragment of SEQ ID NO:3. The fragment may encode fragments of SEQ ID NO:4 linked to a signal peptide such as an immunoglobulin signal peptide for example IgE signal peptide (SEQ ID NO:17) or IgG signal peptide. Thus in some embodiments, the fragment is a fragment of SEQ ID NO:12 which comprises SEQ ID NO:17 and may be encoded by SEQ ID NO:11. The fragment may comprise fragments of SEQ ID NO:3 linked to start codon. Fragments also encode fragments of a polypeptide that is 98% or more, or 99% or more homologous to SEQ ID NO:4. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 98% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may encode a fragment of a polypeptide that is 99% or more homologous to SEQ ID NO:4 and additionally comprise an N terminal methionine which is not included when calculating percent homology. Fragments may be 98% or more, or 99% or more homologous to SEQ ID NO:3. The fragment may be 98% or more homologous to SEQ ID NO:3 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:3 and additionally comprise a signal peptide such as an immunoglobulin signal peptide which is not included when calculating percent homology. The fragment may be 98% or more homologous to SEQ ID NO:3 and additionally comprise an N terminal methionine which is not included when calculating percent homology. The fragment may be 99% or more homologous to SEQ ID NO:3 and additionally comprise an N terminal

methionine which is not included when calculating percent homology. Fragments of SEQ ID NO:3, fragments of nucleic acid sequence 98% or more homologous to SEQ ID NO:3, and fragments of nucleic acid sequence 99% or more homologous to SEQ ID NO:3, in each case with or without start codons or coding sequences that encode signal peptides may encode fragments of RSV M2-1 immunogen that may be In some embodiments, the fragments thereof may be 25 or more amino acids in length, 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more, 110 or more, 120 or more, 130 or more, 140 or more, 150 or more, 160 or more, 170 or more, 180 or more, 184 or more, 190 or more, 193 or more in length. Polypeptide fragments may be fewer than 35 amino acids, fewer than 45, fewer than 55, fewer than 65, fewer than 75, fewer than 85, fewer than 95, fewer than 105, fewer than 115, fewer than 125, fewer than 135, fewer than 145, fewer than 155, fewer than 165, fewer than 175, fewer than 185, fewer than 188, fewer than 192, or fewer than 194 in length. Fragments may further encode an N terminal methionine and/or a signal peptide such as an immunoglobulin signal peptide, for example an IgE or IgG signal peptide. The N terminal methionine and/or signal peptide may be linked to sequences that encode a 194 amino acid or smaller fragment thereof. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 98% homologous to the 194 amino acid or to a smaller fragment of a polypeptide that is 98% homologous to the 194 amino acid sequence. The fragment may encode an N terminal methionine and/or signal peptide linked to a polypeptide that is 99% homologous to the 194 amino acid sequence or to a smaller fragment of a polypeptide that is 99% homologous to the 194 amino acid sequence. In calculating degrees of homology a polypeptide has to SEQ ID NO:4 or a fragment thereof, an N terminal methionine and/or any signal peptide is not included in such calculation. The sequences of the signal peptide are not used in determining homology. Thus, for example, although SEQ ID NO:9 comprises SEQ ID NO:3 operably linked to a coding sequences that encode signal peptide SEQ ID NO:17, SEQ ID NO:9 comprises a fragment of SEQ ID NO:3 that is 100% homologous to a fragment of SEQ ID NO:3, notwithstanding the signal peptide coding sequence which is absent in SEQ ID NO:3. SEQ ID NO:30 corresponds to an immunodominant T cell epitope of RSV M2-1 immunogen set forth in SEQ ID NO:4. SEQ ID NO:31 refers to a subdominant T cell epitope corresponds to an

immunodominant T cell epitope of RSV M2-1 immunogen set forth in SEQ ID NO:4. In some embodiments, fragments comprise coding sequences that encode all or part of one or more of these sequences or sequences 98% or more or 99% or more homologous to them including fragments of SEQ ID NO:3 or sequences that are 98% or more or 99% or more homologous to SEQ ID NO:3.

**l. Identical**

"Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

**m. Impedance**

"Impedance" as used herein may be used when discussing the feedback mechanism and can be converted to a current value according to Ohm's law, thus enabling comparisons with the preset current.

**n. Immune Response**

"Immune response" as used herein may mean the activation of a host's immune system, e.g., that of a mammal, in response to the introduction of one or more RSV consensus antigen via the provided DNA plasmid vaccines. The immune response can be in the form of a cellular or humoral response, or both.

**o. Nucleic Acid**

“Nucleic acid” or “oligonucleotide” or “polynucleotide” as used herein may mean at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

**p. Operably Linked**

“Operably linked” as used herein may mean that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene may be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

**q. Promoter**

“Promoter” as used herein may mean a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter may comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter may also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter may be derived from



sources including viral, bacterial, fungal, plants, insects, and animals. A promoter may regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV40 late promoter and the CMV IE promoter.

#### **r. Stringent Hybridization Conditions**

“Stringent hybridization conditions” as used herein may mean conditions under which a first nucleic acid sequence (e.g., probe) will hybridize to a second nucleic acid sequence (e.g., target), such as in a complex mixture of nucleic acids. Stringent conditions are sequence-dependent and will be different in different circumstances. Stringent conditions may be selected to be about 5-10°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength pH. The  $T_m$  may be the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at  $T_m$ , 50% of the probes are occupied at equilibrium). Stringent conditions may be those in which the salt concentration is less than about 1.0 M sodium ion, such as about 0.01-1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., about 10-50 nucleotides) and at least about 60°C for long probes (e.g., greater than about 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal may be at least 2 to 10 times background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

#### **s. Substantially Complementary**

“Substantially complementary” as used herein may mean that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the complement of

a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

**t. Substantially Identical**

“Substantially identical” as used herein may mean that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

**u. Variant**

“Variant” used herein with respect to a nucleic acid may mean (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

“Variant” with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant may also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of  $\pm 2$  are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in

the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference.

Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within  $\pm 2$  of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

#### **v. Vector**

"Vector" used herein may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

## **2. RSV Proteins**

Provided herein are RSV proteins, also referred to herein as "RSV immunogens", are consensus antigens capable of eliciting an immune response against RSV. Three of the consensus RSV immunogens are: RSV F immunogen, and two forms of RSV G immunogen, referred to herein as RSV Ga immunogen and RSV Gb immunogen. Two RSV G immunogen forms are provided due to the diversity among RSV G proteins. In some embodiments, the RSV immunogens may comprise a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide (SEQ ID NO:17) or an IgG signal peptide. RSV immunogen include RSV F immunogen, RSV Ga immunogen and RSV Gb immunogen. Also provided herein is the "RSV immunogens" that is a consensus antigens capable of eliciting an immune response against RSV M2-1, i.e. RSV M2-1 immunogen which may optionally comprise a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide (SEQ ID NO:17) or an IgG signal peptide.

“RSV F immunogens” refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:2. The RSV F immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:2. The RSV F immunogen may comprise immunogenic fragments of SEQ ID NO:2. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV F immunogen that comprises an immunogenic fragment of SEQ ID NO:2 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:10 that comprises SEQ ID NO:17. RSV F immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:2, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:2. The RSV F immunogen may comprise SEQ ID NO:2. In each instance the RSV F immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV F immunogen that comprises SEQ ID NO:2 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:10.

“RSV Ga immunogens” refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:6. The RSV Ga immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:6. The RSV Ga immunogen may comprise immunogenic fragments of SEQ ID NO:6. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Ga immunogen that comprises an immunogenic fragment of SEQ ID NO:6 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:14 that comprises SEQ ID NO:17. RSV Ga immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:6, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:6. The RSV Ga immunogen may comprise SEQ ID NO:6. In each instance the RSV Ga immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Ga immunogen that comprises SEQ ID NO:6 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:14.

“RSV Gb immunogens” refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:8. The RSV Gb immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:8. The

RSV Gb immunogen may comprise immunogenic fragments of SEQ ID NO:8. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Gb immunogen that comprises an immunogenic fragment of SEQ ID NO:8 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:16 that comprises SEQ ID NO:17. RSV Gb immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:8, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:8. The RSV Gb immunogen may comprise SEQ ID NO:8. In each instance the RSV Gb immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV Gb immunogen that comprises SEQ ID NO:8 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:16.

In some embodiments, fusion proteins are provided which comprise a combination of two or more of the RSV proteins set forth herein. For example, fusion proteins may comprise: SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof; or SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof. Fusion proteins may comprise: SEQ ID NO:2 or an immunogenic fragment thereof, SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof. Alternatively one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:2 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:2 or an immunogenic fragment. Likewise, one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:6 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:6 or an immunogenic fragment; and/or one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:8 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:8 or an immunogenic fragment. Fusion proteins may include combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins.

The consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins may be linked directly adjacent to each other or linked with a spacer or one more amino acids in between. In some embodiments, the space may be a proteolytic cleavage site. In some embodiments, the space may be a proteolytic cleavage site recognized by a protease found in cells to which the vaccine is intended to be administered and/or taken up.

“RSV M2-1 immunogens” refer to proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:4. The RSV M2-1 immunogen may comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:4. The RSV M2-1 immunogen may comprise immunogenic fragments of SEQ ID NO:4. In each instance the immunogenic fragments may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV M2-1 immunogen that comprises an immunogenic fragment of SEQ ID NO:4 and signal peptide SEQ ID NO:17 may be an immunogenic fragments of SEQ ID NO:12 that comprises SEQ ID NO:17. RSV M2-1 immunogen may comprise a protein that is at least 98% homologous to SEQ ID NO:4, and in some embodiments may comprise a protein that is at least 99% homologous to SEQ ID NO:4. The RSV M2-1 immunogen may comprise SEQ ID NO:4. In each instance the RSV M2-1 immunogen may optionally further comprise a signal peptide such as SEQ ID NO:17. The RSV M2-1 immunogen that comprises SEQ ID NO:4 and signal peptide SEQ ID NO:17 may comprise SEQ ID NO:12. In some embodiments, fusion proteins are provided which comprise a combination of RSV M2-1 and another RSV immunogen proteins set forth herein. For example, fusion proteins may comprise: SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:4 or an immunogenic fragment thereof; SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof; or SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof. Fusion proteins may comprise: SEQ ID NO:4 or an immunogenic fragment thereof. Alternatively one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:4 or an

immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:4 or an immunogenic fragment.

### **3. Coding sequences encoding RSV Proteins**

Provided herein are nucleic acid sequences that encode the “RSV immunogens” set forth herein. Administration of nucleic acid molecules comprising the nucleic acid sequences when taken up and expressed by cells results in a broad immune response against RSV. Coding sequences for RSV immunogens are provided, i.e. nucleic acid sequences that encode RSV F immunogen, nucleic acid sequences that encode RSV Ga immunogen and nucleic acid sequences that encode RSV Gb immunogen as well as for nucleic acid sequences that encode RSV M2-1 immunogen. In some embodiments, the nucleic acid sequences that encode RSV immunogens may comprise nucleic acid sequences that encode a signal peptide from a different protein such as an immunoglobulin protein, for example an IgE signal peptide (SEQ ID NO:17) or an IgG signal peptide.

Nucleic acid sequences that encode RSV F immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:2. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:2. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:2. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprises an immunogenic fragment of SEQ ID NO:2 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:10 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:2, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:2. Nucleic acid sequences that encode RSV F immunogens may encode proteins that comprise SEQ ID NO:2. In each instance nucleic acid

sequences that encode RSV F immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode a protein that comprises SEQ ID NO:2 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode a protein that comprises SEQ ID NO:10.

Nucleic acid sequences that encode RSV F immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encodes a protein that is at least 98% homologous to SEQ ID NO:2. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:2. In some embodiments, the nucleic acid sequences that encodes the RSV F immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:2, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:2, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:2 such as an immunogenic fragment SEQ ID NO:2. In some such embodiments, the nucleic acid sequences that encodes the RSV F immunogen may comprise fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:1 and in some embodiments, fragments of SEQ ID NO:1. In some embodiments, the nucleic acid sequences that encode RSV F immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may comprise fragments of SEQ ID NO:2 and coding sequence encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may comprise fragments of SEQ ID NO:9 that include coding sequence encoding signal peptide SEQ ID NO:17.

Nucleic acid sequences that encode RSV Ga immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:6. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:6.



Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:6. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprises an immunogenic fragment of SEQ ID NO:6 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:14 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:6, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:6. Nucleic acid sequences that encode RSV Ga immunogens may encode proteins that comprise SEQ ID NO:6. In each instance nucleic acid sequences that encode RSV Ga immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode a protein that comprises SEQ ID NO:6 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may encode a protein that comprises SEQ ID NO:14.

Nucleic acid sequences that encode RSV Ga immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encodes a protein that is at least 98% homologous to SEQ ID NO:6. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:6. In some embodiments, the nucleic acid sequences that encodes the RSV Ga immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:6, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:6, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:6 such as an immunogenic fragment SEQ ID NO:6. In some such embodiments, the nucleic acid sequences that encodes the RSV Ga immunogen may comprise

fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:5 and in some embodiments, fragments of SEQ ID NO:5. In some embodiments, the nucleic acid sequences that encode RSV Ga immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may comprise fragments of SEQ ID NO:6 and coding sequence encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Ga immunogens may comprise fragments of SEQ ID NO:13 that include coding sequence encoding signal peptide SEQ ID NO:17.

Nucleic acid sequences that encode RSV Gb immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:8. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:8. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:8. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprises an immunogenic fragment of SEQ ID NO:8 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:16 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:8, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:8. Nucleic acid sequences that encode RSV Gb immunogens may encode proteins that comprise SEQ ID NO:8. In each instance nucleic acid sequences that encode RSV Gb immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode a protein that comprises SEQ ID NO:8 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may encode a protein that comprises SEQ ID NO:16.

Nucleic acid sequences that encode RSV Gb immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encodes a protein that is at least 98% homologous to SEQ ID NO:8. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:8. In some embodiments, the nucleic acid sequences that encodes the RSV Gb immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:8, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:8, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:8 such as an immunogenic fragment SEQ ID NO:8. In some such embodiments, the nucleic acid sequences that encodes the RSV Gb immunogen may comprise fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:7 and in some embodiments, fragments of SEQ ID NO:7. In some embodiments, the nucleic acid sequences that encode RSV Gb immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may comprise fragments of SEQ ID NO:8 and coding sequence encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV Gb immunogens may comprise fragments of SEQ ID NO:15 that include coding sequence encoding signal peptide SEQ ID NO:17.

Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins comprising immunogenic fragments of proteins that are at least 98% homologous to SEQ ID NO:4. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise immunogenic fragments of proteins that are at least 99% homologous to SEQ ID NO:4. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise immunogenic fragments of SEQ ID NO:4. In each instance the nucleic acid sequences that encode proteins that comprise the immunogenic fragments may optionally further comprise nucleic acid sequences that encode a signal peptide such as nucleic acid sequences that encode SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode

proteins that comprises an immunogenic fragment of SEQ ID NO:4 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV F immunogens may encode proteins that may be an immunogenic fragment of SEQ ID NO:12 that comprises SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise a protein that is at least 98% homologous to SEQ ID NO:4, and in some embodiments may encode a protein that is at least 99% homologous to SEQ ID NO:4. Nucleic acid sequences that encode RSV M2-1 immunogens may encode proteins that comprise SEQ ID NO:4. In each instance nucleic acid sequences that encode RSV M2-1 immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode a protein that comprises SEQ ID NO:4 and signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may encode a protein that comprises SEQ ID NO:12.

Nucleic acid sequences that encode RSV M2-1 immunogens may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3. The nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encodes a protein that is at least 98% homologous to SEQ ID NO:4. The fragment encodes an immunogenic fragment of the protein that is at least 98% homologous to SEQ ID NO:4. In some embodiments, the nucleic acid sequences that encodes the RSV M2-1 immunogen may comprise fragments of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 wherein the nucleic acid sequence encodes a protein that is at least 99% homologous to SEQ ID NO:4, in some embodiments nucleic acid sequence encodes a protein that comprises SEQ ID NO:4, and the fragment of the nucleic acid sequence encodes an immunogenic fragment of the protein that is at least 99% homologous to SEQ ID NO:4 such as an immunogenic fragment SEQ ID NO:4. In some such embodiments, the nucleic acid sequences that encodes the RSV M2-1 immunogen may comprise fragments of a nucleic acid sequence that is at least 99% homologous to SEQ ID NO:3 and in some embodiments, fragments of SEQ ID NO:3. In some embodiments, the nucleic acid sequences that encode RSV M2-1 immunogens may optionally further comprise nucleic acid sequences that encode a signal peptide such as SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may comprise fragments of SEQ ID NO:4 and coding sequence

encoding signal peptide SEQ ID NO:17. Nucleic acid sequences that encode RSV M2-1 immunogens may comprise fragments of SEQ ID NO:11 that include coding sequence encoding signal peptide SEQ ID NO:17.

Coding sequences may encode fusion proteins which comprise a combination of two or more of the RSV proteins set forth herein. Coding sequences may encode fusion proteins that include combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins. The consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins may be linked directly adjacent to each other or linked with a spacer or one more amino acids in between. In some embodiments, the space may be a proteolytic cleavage site. In some embodiments, the space may be a proteolytic cleavage site recognized by a protease found in cells to which the vaccine is intended to be administered and/or taken up.

In some embodiments, coding sequences encode fusion proteins which comprise a combination of two or more of the RSV proteins set forth herein. For example, coding sequences may encode fusion proteins that comprise: SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:4 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, such as SEQ ID NO:3 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof; SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:3 or an

immunogenic fragment-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof; and SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:5 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment thereof-encoding fragment thereof. Coding sequences may encode fusion proteins which comprise a combination of three RSV proteins set forth herein. For example, coding sequences may encode fusion proteins that comprise: SEQ ID NO:2 or an immunogenic fragment thereof, SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:6 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof and SEQ ID NO:5 or an immunogenic fragment-encoding fragment thereof; SEQ ID NO:2 or an immunogenic fragment thereof, SEQ ID NO:4 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment-encoding fragment thereof; and SEQ ID NO:4 or an immunogenic fragment thereof, SEQ ID NO:6 or an immunogenic fragment thereof and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:3 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:5 or an immunogenic fragment thereof-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment-encoding fragment thereof. Coding sequences may encode fusion proteins which comprise a combination of the four RSV proteins set forth herein such as coding sequences that encode SEQ ID NO:2 or an immunogenic fragment thereof, SEQ ID NO:4 or an immunogenic fragment thereof, SEQ ID NO:6 or an immunogenic fragment thereof, and SEQ ID NO:8 or an immunogenic fragment thereof, such as SEQ ID NO:1 or an immunogenic fragment-encoding fragment thereof, SEQ ID NO:3 or an immunogenic fragment thereof-encoding fragment thereof, SEQ ID NO:5 or an immunogenic fragment-encoding fragment thereof and SEQ ID NO:7 or an immunogenic fragment-encoding fragment thereof. Alternatively coding sequence encode one or more of the fusion proteins above may comprise a sequence that encodes a protein that is at least 98% or at least 99% homologous to SEQ ID NO:2 or an immunogenic fragment

thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:2 or an immunogenic fragment. Likewise, coding sequences for one or more of the fusion proteins above comprise coding sequence at least 98% or at least 99% homologous to SEQ ID NO:4 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:4 or an immunogenic fragment; one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:6 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:6 or an immunogenic fragment; and/or one or more of the fusion proteins above may comprise a sequence at least 98% or at least 99% homologous to SEQ ID NO:8 or an immunogenic fragment thereof in place of the portion of the fusion protein described above corresponding to SEQ ID NO:8 or an immunogenic fragment. In some embodiments, coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:1 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:2 in place of the portion of the coding sequence of the fusion protein described above corresponding to portion that encodes SEQ ID NO:2 or an immunogenic fragment. Coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:3 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:4 in place of the portion of the coding sequence of the fusion protein described above corresponding to portion that encodes SEQ ID NO:4 or an immunogenic fragment. Coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:5 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:6 in place of the portion of the coding sequence of the fusion protein described above corresponding to portion that encodes SEQ ID NO:6 or an immunogenic fragment. Coding sequence encode one or more of the fusion proteins above may comprise a sequence that is at least 98% or at least 99% homologous to SEQ ID NO:7 or fragment thereof that is at least 98% or at least 99% homologous to an immunogenic fragment of SEQ ID NO:8 in place of the portion of the coding sequence of the fusion protein described above corresponding

to portion that encodes SEQ ID NO:8 or an immunogenic fragment. Fusion proteins may include combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins. The consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins may be linked directly adjacent to each other or linked with a spacer or one more amino acids in between. In some embodiments, the space may be a proteolytic cleavage site. In some embodiments, the space may be a proteolytic cleavage site recognized by a protease found in cells to which the vaccine is intended to be administered and/or taken up.

#### **4. Plasmid**

Provided herein is a vector that is capable of expressing one or more RSV proteins in the cell of a mammal in a quantity effective to elicit an immune response in the mammal. The vector may comprise heterologous nucleic acid encoding the one or more RSV antigens. The vector may be a plasmid. The plasmid may be useful for transfecting cells with nucleic acid encoding an RSV antigen, which the transformed host cell is cultured and maintained under conditions wherein expression of the RSV antigen takes place.

The plasmid may comprise a nucleic acid sequence that encodes one or more of the various RSV proteins disclosed above including coding sequences that encode synthetic, consensus antigen capable of eliciting an immune response against RSV are provided for each of the following three proteins: F, Ga and Gb, fragments of such proteins, variants of such proteins, fragments of variants or fusion proteins which are made up of combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins. The plasmid may comprise a nucleic acid sequence that encodes RSV M2-1 proteins disclosed above including coding sequences that encode synthetic, consensus antigen capable of eliciting an immune response against RSV M2-1, fragments of such proteins, variants of such proteins, fragments of variants or fusion proteins which are made up of combinations of consensus proteins and/or fragments of consensus protein and/or variants of consensus protein and/or fragments of variants consensus proteins.



A single plasmid may contain coding sequence for a single RSV Immunogen, coding sequence for two RSV Immunogens, or coding sequence for three RSV Immunogens. In some embodiments, a coding sequence for a fourth RSV Immunogen may be provided. A single plasmid may contain coding sequence for RSV F Immunogen. A single plasmid may contain coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen and coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen.

If RSV M2-1 coding sequence is provided, a single plasmid may contain coding sequence for RSV M2-1 Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen and coding sequence for RSV M2-1 Immunogen. A single plasmid may contain coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Ga Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV M2-1 Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV M2-1 Immunogen, coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen. A single plasmid may contain coding sequence for RSV F Immunogen, coding sequence for RSV M2-1 Immunogen, coding sequence for RSV Ga Immunogen and coding sequence for RSV Gb Immunogen.

In some embodiments, a plasmid may comprise coding sequence that encodes CCR20 alone or as part of one these plasmids. Similarly, plasmids may further comprise coding sequences for IL-12, IL-15 and/or IL-28.

The plasmid may further comprise an initiation codon, which may be upstream of the coding sequence, and a stop codon, which may be downstream of the coding sequence. The initiation and termination codon may be in frame with the coding sequence.

The plasmid may also comprise a promoter that is operably linked to the coding sequence. The promoter operably linked to the coding sequence may be a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter may also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, or human metallothionein. The promoter may also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no. US20040175727, the contents of which are incorporated herein in its entirety.

The plasmid may also comprise a polyadenylation signal, which may be downstream of the coding sequence. The polyadenylation signal may be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human  $\beta$ -globin polyadenylation signal. The SV40 polyadenylation signal may be a polyadenylation signal from a pCEP4 plasmid (Invitrogen, San Diego, CA).

The plasmid may also comprise an enhancer upstream of the coding sequence. The enhancer may be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, FMDV, RSV or EBV. Polynucleotide function enhances are described in U.S. Patent Nos. 5,593,972, 5,962,428, and WO94/016737, the contents of each are fully incorporated by reference.

The plasmid may also comprise a mammalian origin of replication in order to maintain the plasmid extrachromosomally and produce multiple copies of the plasmid in a cell. The plasmid may be pVAX1, pCEP4 or pREP4 from Invitrogen (San Diego, CA), which may

comprise the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which may produce high copy episomal replication without integration. The backbone of the plasmid may be pAV0242. The plasmid may be a replication defective adenovirus type 5 (Ad5) plasmid.

The plasmid may also comprise a regulatory sequence, which may be well suited for gene expression in a cell into which the plasmid is administered. The coding sequence may comprise a codon that may allow more efficient transcription of the coding sequence in the host cell.

The coding sequence may also comprise an Ig leader sequence. The leader sequence may be 5' of the coding sequence. The consensus antigens encoded by this sequence may comprise an N-terminal Ig leader followed by a consensus antigen protein. The N-terminal Ig leader may be IgE or IgG.

The plasmid may be pSE420 (Invitrogen, San Diego, Calif.), which may be used for protein production in *Escherichia coli* (E.coli). The plasmid may also be pYES2 (Invitrogen, San Diego, Calif.), which may be used for protein production in *Saccharomyces cerevisiae* strains of yeast. The plasmid may also be of the MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.), which may be used for protein production in insect cells. The plasmid may also be pcDNA I or pcDNA3 (Invitrogen, San Diego, Calif.), which may be used for protein production in mammalian cells such as Chinese hamster ovary (CHO) cells.

## **5. Compositions**

Compositions are provided which comprise nucleic acid molecules. The compositions may comprise a plurality of copies of a single nucleic acid molecule such a single plasmid, a plurality of copies of a two or more different nucleic acid molecules such as two or more different plasmids. For example a compositions may comprise plurality of two, three, four, five, six, seven, eight, nine or ten or more different nucleic acid molecules. Such a compositions may comprise plurality of two, three, four, five, six, seven, eight, nine or ten or more different plasmids.

Compositions may comprise nucleic acid molecules, such as plasmids, that collectively contain coding sequence for a single RSV Immunogen, coding sequence for two RSV

Immunogens, or coding sequence for three RSV Immunogens. In some instances, coding sequence for a fourth RSV Immunogen may be provided.

Compositions comprising coding sequence of two RSV Immunogens may be on a single nucleic acid molecule such as a single plasmid or the compositions may comprise two different nucleic acid molecule such as two different plasmids wherein one nucleic acid molecule comprises the coding sequence one RSV Immunogen and the other nucleic acid molecule comprises the coding sequence different RSV Immunogen. Similarly, compositions comprising coding sequence three RSV Immunogens may comprise a single nucleic acid molecule such as a single plasmid, two different nucleic acid molecules or three different nucleic acid molecules. Likewise, compositions comprising coding sequence four RSV Immunogens may comprise a single nucleic acid molecule such as a single plasmid, two different nucleic acid molecules, three different nucleic acid molecules or four different nucleic acid molecule.

In some embodiments, a composition comprises a plurality single nucleic acid molecule encoding one RSV Immunogen such as RSV F Immunogen, RSV Ga Immunogen or RSV Gb Immunogen. In some embodiments, a composition comprises a plurality single nucleic acid molecule, such a single plasmid encoding two RSV Immunogen such as RSV F Immunogen and RSV Ga Immunogen, RSV F Immunogen and RSV Gb Immunogen, and RSV Ga Immunogen and RSV Gb Immunogen. In some embodiments, a composition comprises a plurality single nucleic acid molecule, such a single plasmid encoding three RSV Immunogen such as RSV Ga Immunogen, RSV F Immunogen and RSV Gb Immunogen.

In some embodiments, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, each different nucleic acid molecule comprising a single different coding sequence for a different RSV Immunogen wherein pairs of different nucleic acid molecule comprise RSV F Immunogen and RSV Ga Immunogen, RSV F Immunogen and RSV Gb Immunogen, and RSV Ga Immunogen and RSV Gb Immunogen.

In some embodiments, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, which collectively comprising coding sequence for three different RSV Immunogen. In some embodiments, one encodes RSV F Immunogen and RSV Ga Immunogen, and the second encodes RSV Gb Immunogen. In some embodiments, one

encodes RSV F Immunogen and RSV Gb Immunogen, and the second encodes RSV Ga Immunogen. In some embodiments, one encodes Ga Immunogen and RSV Gb Immunogen, and the second encodes RSV F Immunogen.

In some embodiments, a composition comprises a plurality single nucleic acid molecule encoding RSV M2-1 Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality single nucleic acid molecules, such a single plasmid encoding two RSV Immunogen such as RSV F Immunogen and RSV M2-1 Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, and RSV M2-1 Immunogen and RSV Gb Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a plurality single nucleic acid molecules, such a single plasmid encoding three RSV Immunogen such as RSV F Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, RSV F Immunogen, RSV M2-1 Immunogen and RSV Gb Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen and RSV Gb Immunogen. In some embodiments, a composition comprises a plurality single nucleic acid molecule, such a single plasmid encoding three RSV Immunogen such as RSV F Immunogen, RSV M2-1 Immunogen, RSV Ga Immunogen and RSV Gb Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, each different nucleic acid molecule comprising a single different coding sequence for a different RSV Immunogen wherein pairs of different nucleic acid molecule comprise RSV F Immunogen and RSV M2-1 Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, and RSV M2-1 Immunogen and RSV Gb Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, which collectively comprising coding sequence for three different RSV Immunogen or which one is RSV M2-1. Embodiments may be selected from the group consisting of: one nucleic acid molecule encodes RSV F Immunogen and RSV M2-1 Immunogen and the second encodes RSV Ga Immunogen; one nucleic acid molecule encodes RSV F Immunogen and RSV M2-1 Immunogen and the second encodes RSV Gb Immunogen; one encodes RSV F Immunogen and

RSV Ga Immunogen, and the second encodes RSV M2-1 Immunogen; one encodes RSV F Immunogen and RSV Gb Immunogen, and the second encodes RSV M2-1 Immunogen; one encodes RSV M2-1 Immunogen and RSV Ga Immunogen, and the second encodes RSV F Immunogen; one encodes RSV M2-1 Immunogen and RSV Ga Immunogen, and the second encodes RSV Gb Immunogen; one encodes RSV M2-1 Immunogen and RSV Gb Immunogen, and the second encodes RSV F Immunogen; one encodes RSV M2-1 Immunogen and RSV Gb Immunogen, and the second encodes RSV Ga Immunogen; and one encodes Ga Immunogen and RSV Gb Immunogen, and the second encodes RSV M2-1 Immunogen.

In some embodiments in which coding sequences for RSV M2-1 are provided, a composition comprises a plurality two different nucleic acid molecules, such as two plasmids, which collectively comprising coding sequence for four different RSV Immunogen. In some embodiments: one nucleic acid molecule encodes RSV F Immunogen and the second encodes RSV M2-1 Immunogen, RSV Ga Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV M2-1 Immunogen and the second encodes RSV F Immunogen, RSV Ga Immunogen and RSV Gb Immunogen; one encodes RSV Ga Immunogen and the second encodes RSV F Immunogen, RSV M2-1 Immunogen and RSV Gb Immunogen, one encodes RSV Gb Immunogen and the second encodes RSV F Immunogen, RSV M2-1 Immunogen and RSV Ga Immunogen, one encodes RSV F and RSV M2-1 Immunogen the second encodes RSV Ga Immunogen and RSV Gb Immunogen, one encodes RSV F and RSV Ga Immunogen the second encodes RSV M2-1 Immunogen and RSV Gb Immunogen, and one encodes RSV F and RSV Gb Immunogen the second encodes RSV M2-1 Immunogen and RSV Ga Immunogen. In some embodiments, a composition comprises a plurality three different nucleic acid molecules, such as three plasmids, which collectively comprising coding sequence for three different RSV Immunogen. In some embodiments: one nucleic acid molecule encodes RSV F Immunogen, one encodes RSV M2-1 Immunogen and the third second encodes RSV Ga Immunogen; one nucleic acid molecule encodes RSV F Immunogen, one encodes RSV M2-1 Immunogen and the third second encodes RSV Gb Immunogen; one nucleic acid molecule encodes RSV F Immunogen, one encodes RSV Ga Immunogen and the third second encodes RSV Gb Immunogen; and one nucleic acid molecule encodes RSV M2-1 Immunogen, one encodes RSV Ga Immunogen and

the third second encodes RSV Gb Immunogen. In some embodiments, a composition comprises a plurality three different nucleic acid molecules, such as three plasmids, which collectively comprising coding sequence for four different RSV Immunogens. In some embodiments: one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV M2-1 Immunogen and the third encodes RSV Ga Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV Ga Immunogen and the third encodes RSV M2-1 Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV Gb Immunogen and the third encodes RSV M2-1 Immunogen and RSV Ga Immunogen; one nucleic acid molecule encodes RSV M2-1 Immunogen, the second encodes RSV Ga Immunogen and the third encodes RSV F Immunogen and RSV Gb Immunogen; one nucleic acid molecule encodes RSV M2-1 Immunogen, the second encodes RSV Gb Immunogen, and the third encodes RSV F Immunogen and RSV Ga Immunogen; and one encodes RSV Ga Immunogen, the second encodes RSV Gb Immunogen, and the third encodes RSV F Immunogen and RSV M2-1. In some embodiments, a composition comprises a plurality four different nucleic acid molecules, such as four plasmids, which collectively comprising coding sequence for four different RSV Immunogens, i.e. one nucleic acid molecule encodes RSV F Immunogen, the second encodes RSV M2-1 Immunogen, the third encodes RSV Ga Immunogen and the fourth encodes RSV Gb Immunogen.

In some embodiments, a composition further comprises coding sequence for chemokine CCL20, IL-12, IL-15 and/or IL-28. Coding sequence for chemokine CCL20, IL-12, IL-15 and/or IL-28 may be included on one or more nucleic acid molecules that comprise .coding sequence for one or more RSV Immunogens. Coding sequence for chemokine CCL20, IL-12, IL-15 and/or IL-28 may be included on a separate nucleic acid molecules such as a separate plasmid.

## **6. Vaccine**

Provided herein is a vaccine capable of generating in a mammal an immune response against RSV. The vaccine may comprise each plasmid as discussed above. The vaccine may comprise a plurality of the plasmids, or combinations thereof. The vaccine may be provided to induce a therapeutic or prophylactic immune response.

In some embodiments, the vaccine is preferably Th1 biased so as not to play into prior Th2 related pathogenesis and it should be able to function in a neutralization sensitive environment. Preferably, it should be non-live.

Vaccines may be used to deliver one or more immunogenic targets selected from the group consisting of: F, Ga and Gb, fragments of such proteins, variants of such proteins, fragments of variants. In the case of delivery of multiple targets, vaccines may include multiple compositions or a single compositions. Plasmids may be used which encode multiple proteins on a single plasmid or compositions which comprise different plasmids that encode different proteins. In some embodiments, vaccines may be used to deliver M2, fragments thereof, variants of M2-1, fragments of variants, alone or in combination with one or more immunogenic targets selected from the group consisting of: F, Ga and Gb, fragments of such proteins, variants of such proteins, fragments of variants.. In the case of delivery of multiple targets, vaccines may include multiple compositions or a single compositions. Plasmids may be used which encode multiple proteins on a single plasmid or compositions which comprise different plasmids that encode different proteins.

The vaccine may further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient may be functional molecules as vehicles, adjuvants, carriers, or diluents. The pharmaceutically acceptable excipient may be a transfection facilitating agent, which may include surface active agents, such as immune-stimulating complexes (ISCOMS), Freund's incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and more preferably, the poly-L-glutamate is present in the vaccine at a concentration less than 6 mg/ml. The transfection facilitating agent may also include surface active agents such as immune-stimulating complexes (ISCOMS), Freund's incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic



acid may also be used administered in conjunction with the genetic construct. In some embodiments, the DNA plasmid vaccines may also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. Preferably, the transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. Concentration of the transfection agent in the vaccine is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than 0.050 mg/ml, or less than 0.010 mg/ml.

The pharmaceutically acceptable excipient may be one or more adjuvants. An adjuvant may be other genes that are expressed from the same or from an alternative plasmid or are delivered as proteins in combination with the plasmid above in the vaccine. The one or more adjuvants may be proteins and/or nucleic acid molecules that encode proteins selected from the group consisting of: CCL20,  $\alpha$ -interferon (IFN- $\alpha$ ),  $\beta$ -interferon (IFN- $\beta$ ),  $\gamma$ -interferon, platelet derived growth factor (PDGF), TNF $\alpha$ , TNF $\beta$ , GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15 including IL-15 having the signal sequence or coding sequence that encodes the signal sequence deleted and optionally including a different signal peptide such as that from IgE or coding sequence that encodes a difference signal peptide such as that from IgE, IL-28, MHC, CD80, CD86, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-1 $\alpha$ , MIP-1 $\beta$ , IL-8, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, I $\kappa$ B, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NF $\kappa$ B, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E,

NKG2F, TAP1, TAP2 and functional fragments thereof. or a combination thereof. In some embodiments adjuvant may be one or more proteins and/or nucleic acid molecules that encode proteins selected from the group consisting of: CCL-20, IL-12, IL-15, IL-28, CTACK, TECK, MEC or RANTES. Examples of IL-12 constructs and sequences are disclosed in PCT application no. PCT/US1997/019502 and corresponding US Application Serial No. 08/956,865, and U.S. Provisional Application Serial No 61/569600 filed December 12, 2011, which are each incorporated herein by reference. Examples of IL-15 constructs and sequences are disclosed in PCT application no. PCT/US04/18962 and corresponding US Application Serial No. 10/560,650, and in PCT application no. PCT/US07/00886 and corresponding U.S. Application Serial No. 12/160,766, and in PCT application no. PCT/US10/048827, which are each incorporated herein by reference. Examples of IL-28 constructs and sequences are disclosed in PCT application no. PCT/US09/039648 and corresponding U.S. Application Serial No. 12/936,192, which are each incorporated herein by reference. Examples of RANTES and other constructs and sequences are disclosed in PCT application no. PCT/US1999/004332 and corresponding U.S. Application Serial No. and 09/622452, which are each incorporated herein by reference. Other examples of RANTES constructs and sequences are disclosed in PCT application no. PCT/US11/024098, which is incorporated herein by reference. Examples of RANTES and other constructs and sequences are disclosed in PCT application no. PCT/US1999/004332 and corresponding U.S. Application Serial No. 09/622452, which are each incorporated herein by reference. Other examples of RANTES constructs and sequences are disclosed in PCT application no. PCT/US11/024098, which is incorporated herein by reference. Examples of chemokines CTACK, TECK and MEC constructs and sequences are disclosed in PCT application no. PCT/US2005/042231 and corresponding U.S. Application Serial No. 11/719,646, which are each incorporated herein by reference. Examples of OX40 and other immunomodulators are disclosed in U.S. Application Serial No. 10/560,653, which is incorporated herein by reference. Examples of DR5 and other immunomodulators are disclosed in U.S. Application Serial No. 09/622452, which is incorporated herein by reference.

The vaccine may further comprise a genetic vaccine facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

The vaccine may comprise the consensus antigens and plasmids at quantities of from about 1 nanogram to 100 milligrams; about 1 microgram to about 10 milligrams; or preferably about 0.1 microgram to about 10 milligrams; or more preferably about 1 milligram to about 2 milligram. In some preferred embodiments, pharmaceutical compositions according to the present invention comprise about 5 nanogram to about 1000 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 10 nanograms to about 800 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 25 to about 250 micrograms, from about 100 to about 200 microgram, from about 1 nanogram to 100 milligrams; from about 1 microgram to about 10 milligrams; from about 0.1 microgram to about 10 milligrams; from about 1 milligram to about 2 milligram, from about 5 nanogram to about 1000 micrograms, from about 10 nanograms to about 800 micrograms, from about 0.1 to about 500 micrograms, from about 1 to about 350 micrograms, from about 25 to about 250 micrograms, from about 100 to about 200 microgram of the consensus antigen or plasmid thereof.

The vaccine may be formulated according to the mode of administration to be used. An injectable vaccine pharmaceutical composition may be sterile, pyrogen free and particulate free. An isotonic formulation or solution may be used. Additives for isotonicity may include sodium chloride, dextrose, mannitol, sorbitol, and lactose. The vaccine may comprise a vasoconstriction agent. The isotonic solutions may include phosphate buffered saline. Vaccine may further comprise stabilizers including gelatin and albumin. The stabilizing may allow the formulation to be stable at room or ambient temperature for extended periods of time such as LGS or polycations or polyanions to the vaccine formulation.

## **7. Methods of Delivery the Vaccine**

Provided herein is a method for delivering the vaccine for providing genetic constructs and proteins of the consensus antigen which comprise epitopes that make them particular effective against immunogens of RSV against which an immune response can be induced. The method of delivering the vaccine or vaccination may be provided to induce a therapeutic and

prophylactic immune response. The vaccination process may generate in the mammal an immune response against RSV. The vaccine may be delivered to an individual to modulate the activity of the mammal's immune system and enhance the immune response. The delivery of the vaccine may be the transfection of the consensus antigen as a nucleic acid molecule that is expressed in the cell and delivered to the surface of the cell upon which the immune system recognized and induces a cellular, humoral, or cellular and humoral response. The delivery of the vaccine may be used to induce or elicit an immune response in mammals against RSV by administering to the mammals the vaccine as discussed above.

Upon delivery of the vaccine and plasmid into the cells of the mammal, the transfected cells will express and secrete consensus antigens for each of the plasmids injected from the vaccine. These proteins will be recognized as foreign by the immune system and antibodies will be made against them. These antibodies will be maintained by the immune system and allow for an effective response to subsequent RSV infections.

The vaccine may be administered to a mammal to elicit an immune response in a mammal. The mammal may be human, primate, non-human primate, cow, cattle, sheep, goat, antelope, bison, water buffalo, bison, bovids, deer, hedgehogs, elephants, llama, alpaca, mice, rats, and chicken.

#### **a. Combination Treatments**

The vaccine may be administered in combination with other proteins and/or genes encoding CCL20,  $\alpha$ -interferon,  $\gamma$ -interferon, platelet derived growth factor (PDGF), TNF $\alpha$ , TNF $\beta$ , GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15 including IL-15 having the signal sequence deleted and optionally including the different signal peptide such as the IgE signal peptide, MHC, CD80, CD86, IL-28, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-1 $\alpha$ , MIP-1 $\beta$ , IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1,

DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, Ikb, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof or combinations thereof. In some embodiments, the vaccine is administered in combination with one or more of the following nucleic acid molecules and/or proteins: nucleic acid molecules selected from the group consisting of nucleic acid molecules comprising coding sequence that encode one or more of CCL20, IL-12, IL-15, IL-28, CTACK, TECK, MEC and RANTES or functional fragments thereof, and proteins selected from the group consisting of: CCL20, IL-12 protein, IL-15 protein, IL-28 protein, CTACK protein, TECK protein, MEC protein or RANTES protein or functional fragments thereof.

The vaccine may be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal. The vaccine may be administered by traditional syringes, needleless injection devices, "microprojectile bombardment guns", or other physical methods such as electroporation ("EP"), "hydrodynamic method", or ultrasound.

The plasmid of the vaccine may be delivered to the mammal by several well known technologies including DNA injection (also referred to as DNA vaccination) with and without in vivo electroporation, liposome mediated, nanoparticle facilitated, recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. The consensus antigen may be delivered via DNA injection and along with in vivo electroporation.

**b. Electroporation**

Administration of the vaccine via electroporation of the plasmids of the vaccine may be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation device may comprise an electroporation component and an electrode assembly or handle assembly. The electroporation component may include and incorporate one or more of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The electroporation may be accomplished using an in vivo electroporation device, for example CELLECTRA EP system (VGX Pharmaceuticals, Blue Bell, PA) or Elgen electroporator (Genetronics, San Diego, CA) to facilitate transfection of cells by the plasmid.

The electroporation component may function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component may function as more than one element of the electroporation devices, which may be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device may not be limited as the elements can function as one device or as separate elements in communication with one another. The electroporation component may be capable of delivering the pulse of energy that produces the constant current in the desired tissue, and includes a feedback mechanism. The electrode assembly may include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism may receive the measured impedance and

can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

A plurality of electrodes may deliver the pulse of energy in a decentralized pattern. The plurality of electrodes may deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed sequence is input by a user to the electroporation component. The programmed sequence may comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

The feedback mechanism may be performed by either hardware or software. The feedback mechanism may be performed by an analog closed-loop circuit. The feedback occurs every 50  $\mu$ s, 20  $\mu$ s, 10  $\mu$ s or 1  $\mu$ s, but is preferably a real-time feedback or instantaneous (i.e., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode may measure the impedance in the desired tissue and communicates the impedance to the feedback mechanism, and the feedback mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism may maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

Examples of electroporation devices and electroporation methods that may facilitate delivery of the DNA vaccines of the present invention, include those described in U.S. Patent No. 7,245,963 by Draghia-Akli, et al., U.S. Patent Pub. 2005/0052630 submitted by Smith, et al., the contents of which are hereby incorporated by reference in their entirety. Other electroporation devices and electroporation methods that may be used for facilitating delivery of the DNA vaccines include those provided in co-pending and co-owned U.S. Patent Application, Serial No. 11/874072, filed October 17, 2007, which claims the benefit under 35 USC 119(e) to U.S. Provisional Applications Ser. Nos. 60/852,149, filed October 17, 2006, and 60/978,982, filed October 10, 2007, all of which are hereby incorporated in their entirety.

U.S. Patent No. 7,245,963 by Draghia-Akli, et al. describes modular electrode systems and their use for facilitating the introduction of a biomolecule into cells of a selected tissue in a body or plant. The modular electrode systems may comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The biomolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the biomolecule into the cell between the plurality of electrodes. The entire content of U.S. Patent No. 7,245,963 is hereby incorporated by reference.

U.S. Patent Pub. 2005/0052630 submitted by Smith, et al. describes an electroporation device which may be used to effectively facilitate the introduction of a biomolecule into cells of a selected tissue in a body or plant. The electroporation device comprises an electro-kinetic device ("EKD device") whose operation is specified by software or firmware. The EKD device produces a series of programmable constant-current pulse patterns between electrodes in an array based on user control and input of the pulse parameters, and allows the storage and acquisition of current waveform data. The electroporation device also comprises a replaceable electrode disk having an array of needle electrodes, a central injection channel for an injection needle, and a removable guide disk. The entire content of U.S. Patent Pub. 2005/0052630 is hereby incorporated by reference.

The electrode arrays and methods described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/0052630 may be adapted for deep penetration into not only tissues such as muscle, but also other tissues or organs. Because of the configuration of the electrode array, the injection needle (to deliver the biomolecule of choice) is also inserted completely into the target organ, and the injection is administered perpendicular to the target issue, in the area that is pre-delineated by the electrodes. The electrodes described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/005263 are preferably 20 mm long and 21 gauge.



Additionally, contemplated in some embodiments that incorporate electroporation devices and uses thereof, there are electroporation devices that are those described in the following patents: US Patent 5,273,525 issued December 28, 1993, US Patents 6,110,161 issued August 29, 2000, 6,261,281 issued July 17, 2001, and 6,958,060 issued October 25, 2005, and US patent 6,939,862 issued September 6, 2005. Furthermore, patents covering subject matter provided in US patent 6,697,669 issued February 24, 2004, which concerns delivery of DNA using any of a variety of devices, and US patent 7,328,064 issued February 5, 2008, drawn to method of injecting DNA are contemplated herein. The above-patents are incorporated by reference in their entirety.

**c. Method of Preparing DNA Plasmids**

Provided herein is methods for preparing the DNA plasmids that comprise the DNA vaccines discussed herein. The DNA plasmids, after the final subcloning step into the mammalian expression plasmid, can be used to inoculate a cell culture in a large scale fermentation tank, using known methods in the art.

The DNA plasmids for use with the EP devices of the present invention can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using an optimized plasmid manufacturing technique that is described in a licensed, co-pending U.S. provisional application U.S. Serial No. 60/939,792, which was filed on May 23, 2007. In some examples, the DNA plasmids used in these studies can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those described in U.S. Serial No. 60/939792, including those described in a licensed patent, US Patent No. 7,238,522, which issued on July 3, 2007. The above-referenced application and patent, US Serial No. 60/939,792 and US Patent No. 7,238,522, respectively, are hereby incorporated in their entirety.

## EXAMPLES

The present invention is further illustrated in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims

### Example 1

With expected continued increases in the number of individuals at high risk for RSV infection in the coming years (*e.g.*, young infants and the elderly), there is a dire need for the development of an efficacious and financially manageable treatment for RSV infection. DNA vaccines, small bacterial plasmids genetically engineered to express an encoded protein of interest upon immunization, offer several significant advantages that may help in circumventing many of the hurdles involved in designing an efficacious RSV vaccine. For instance, to prevent clinically significant illness and re-infection, the vaccine would have to confer greater and longer lasting immunity than that which occurs from natural RSV infection. DNA vaccines not only elicit both humoral (*e.g.*, neutralization antibody) and cellular (*e.g.*, cytotoxic T lymphocytes) immune response, but can also elicit lifelong immunity against many viruses. In addition, the primary targets for RSV vaccination are neonates and young infants. Neonatal immune system is functionally immature and thus, develops only a weak immune response to conventional vaccines (*e.g.*, live attenuated or inactivated virus). DNA vaccines, on the other hand, have been shown to be safe and immunogenic in neonatal animal models. Furthermore, the presence of maternal antibody at the time of immunization has been known to suppress or inhibit immune responses to a variety of conventional vaccines. With DNA vaccine, however, the immunizing protein is not present in the vaccine preparation and therefore, should not be susceptible to direct inactivation by maternal antibodies.

Currently, there are three main RSV vaccines in or near early stages of clinical trials: 1) live attenuated RSV (MedImmune, Philadelphia, PA); 2) Sendai virus expressing the RSV F and/or G proteins (AmVac, Switzerland); and 3) virus-like particles containing the G protein of RSV (Novavax, Rockville, MD) (<http://www.clinicaltrials.gov/ct2/results?term=RSV&pg=1>). Although these approaches have shown some degree of efficacy in the prevention of RSV infection, there are other inherent disadvantages associated with such vaccine designs. For instance, the biggest issues with live attenuated virus are under-attenuation or mutations leading to reversion to virulence and therefore, should not be administered to immunocompromised individuals. A common drawback to all viral vectors is the host immune response as recognition of viral proteins leads to the generation of neutralizing antibodies that may significantly reduce the efficacy of the vaccine upon subsequent immunization. Lastly, the G protein of RSV is highly variable between the two subtypes of RSV and therefore, any vaccine targeting the G protein is not likely to provide cross protection.

In contrast, DNA vaccines have been shown to be highly immunogenic and lack many of the disadvantages associated with the above described vaccine approach. When delivered via electroporation, DNA vaccine immunogenicity further improves. DNA vaccines contain less than a complete set of viral components and like protein subunit vaccines have no risk for virulence reversion or infection of the host making them safe to use in both infants and in immunocompromised individuals.

Using consensus sequences of both subtype A and B of RSV provides the basis to obtain broader protection against both strains of the virus. Such advantages make consensus RSV DNA vaccine much more beneficial and potentially more efficacious than any other approaches suggested in the past

Major RSV proteins - fusion (F), glycoprotein (Ga), and glycoprotein (Gb) were chosen as targets for new RSV vaccines. Also chosen for evaluation was the second matrix protein (M2). As mentioned above, F and G proteins are expressed on the surface and are ideal targets for neutralizing antibodies. M2 protein contains the major immunodominant CD8 T cell epitope. For each of these RSV proteins, a single consensus sequence was generated based on the amino acid sequences provided by PUBMED for both subtype A and B of RSV (Figure 1 shows the

phylogenetic tree of RSV-F Protein, the consensus RSV F protein amino acid sequence is set forth in SEQ ID NO:2; Figure 2 shows the phylogenetic tree of RSV M2-1 Protein, the consensus RSV M2-1 protein amino acid sequence is set forth in SEQ ID NO:4. In a preferred embodiment, codon and RNA optimized DNA sequences that encode the consensus protein sequences are produced and used to make DNA vaccines which may be delivered using electroporation.

### **Example 2**

Successful expression of RSV consensus F protein was confirmed through immunofluorescence microscopy and/or western blot analysis. Constructs were used in immunization studies.

Mice were immunized with varying dosages of the different RSV DNA vaccine constructs. One group received the DNA vaccine backbone plasmid pVAX as a control. One group received 5 µg dose of plasmid RSV-F, one group received 15 µg dose of plasmid RSV-F; one group received 30 µg dose of plasmid RSV-F; and one group received 60 µg dose of plasmid RSV-F.

Mice were immunized 2 weeks apart for a total of 3 immunizations (weeks 0, 2 and 4) intramuscularly with electroporation. Mice were bled at weeks 2, 4, 6 and 12 after each immunization and the total RSV F protein specific IgG and IgA antibody was measured in the sera. Figure 3 shows anti-RSV F protein IgG after immunization 1 (i.e. at week 0), immunization 2 (i.e. week 2) and after immunizations 3 (i.e. week 4). Figure 4 shows anti-RSV F protein IgA after immunization 1 (i.e. at week 0), immunization 2 (i.e. week 2) and after immunizations 3 (i.e. week 4). Mice were sacrificed at week 12. In addition to comparisons of total IgG in sera and of total IgA in sera, a comparison of IFN- $\gamma$  production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization was done.

RSV-F DNA vaccine elicited high levels of both IgG and IgA in sera. Responses were detectable as early as 2 weeks after 2nd immunization for all dosage studied. The antibody responses induced appear to be strongly Th1 biased, thus avoiding prior issues of the side effects of Th2 immune responses to RSV. RSV-F specific CD8 T cells were observed even after 8

weeks after the last immunization. Figure 5 shows results from a comparison of IFN- $\gamma$  production at different dosage of RSV-F vaccine approximately 8 weeks after last immunization.

### **Example 3**

Chemokines are a family of small proteins secreted by cells with ability to induce directed chemotaxis toward a higher concentration gradient in nearby responsive cells. Various groups have investigated the feasibility of using chemokines as immune adjuvants.

CCL28/MEC (mucosa-associated epithelial chemokine) which binds CCR10 is expressed on epithelial cells in the gut, lung, breast, and the salivary glands. Use of mucosal chemokines in previous studies showed their utility as immune adjuvants, inducing better immune response in the mucosal tissues after systemic immunization.

Experiments were performed to test the use of a CCL28 genetic construct as an adjuvant for the consensus RSV F protein DNA vaccine construct. A genetic construct that comprised nucleotide sequence encoding CCL20, also referred to a liver activation regulated chemokine (LARC) or Macrophage Inflammatory Protein-3 (MIP3A), was also tested as a possible adjuvant for the consensus RSV F protein DNA vaccine construct.

The immunization schedule for RSV-F plus chemokine experiments utilized three groups of mice: one receiving RSV-F construct only; one receiving RSV-F construct plus CCL20 construct; and one receiving RSV-F construct plus CCL28 construct. Mice were immunized a total of three times: at week 0, at week 2 and at week 4. Mice were bled at week 2, week 4 and week 5. At week 5, mice underwent vaginal wash to collect vaginal samples and were sacrificed after which lungs and spleens were collected.

Comparisons were made of IFN- $\gamma$ + CD8 and CD4 T cells in spleen after RSV-F +/- chemokine immunization in the spleen (Figure 6) and in lung (Figure 7) after RSV-F +/- chemokine immunization.

IgG Endpoint Titer in Sera was measured and IgG subtypes (IgG1 vs IgG2a) in Sera were compared (Figure 8). A comparison of IgG2a/IgG1 ratio was also done (Figure 9).

CCL20 and CCL28 chemokines did not appear to enhance CD8 and CD4 T cell immune responses after RSV-F DNA vaccination in either the spleen or the lung (based on IFN-  $\gamma$  ICS) although it is unclear if the dosage of chemokine construct was insufficient.

Animals receiving the CCL20 immune adjuvant exhibited a heavy bias towards a Th1 immune response (primarily IgG2a) after RSV-F DNA vaccination. Driving immune response away from Th2 and towards Th1 may be useful in order to avoid vaccine-associated enhanced disease after RSV infection.

#### **Example 4**

As noted above, administration of coding sequences that encode CCL20 led to a preferential Th1 immune response and away from Th2 immune responses. This property of CCL20 administration may be useful in the context of both an immunotherapeutic as well as a vaccine adjuvant for other targets in which a shift increasing Th1 immune responses and decreasing Th2 immune responses is desirable.

For example, CCL20 or administration of nucleic acid molecules that encode CCL20 may be useful to treat individuals who have autoimmune diseases or inflammatory conditions characterized by Th2 immune responses. The ability to decrease Th2 immune responses as shown herein can be used to reduce Th2 immune responses associated with certain autoimmune diseases or inflammatory conditions including for example.

In addition, CCL20 may be used as a vaccine adjuvant as described herein with vaccines targeting other infection diseases as well as cancer. The Th1 increasing/Th2 decreasing properties associated with the use of CCL20 as a vaccine adjuvant may be useful in vaccines. SEQ ID NO:18 and SEQ ID NO:20 contains high expression coding sequences for CCL20 with and without coding sequences encoding the IgE signal peptide. In some embodiments, SEQ ID NO:18 further comprises a start codon. SEQ ID NO:19 and SEQ ID NO:21 contains consensus sequences for CCL20 with and without coding sequences encoding the IgE signal peptide. In some embodiments, SEQ ID NO:19 further comprises an N terminal; methionine

#### **Example 5**

The Luciferase Reporter Gene Assay in TZM-bl Cells is based on Single-Round Infection with Molecularly Cloned Env-Pseudotyped Viruses. TZM-bl (JC53-bl) is a genetically

engineered HeLa cell line that expresses CD4, CXCR4 and CCR5 and contains Tat-inducible Luc and  $\beta$ -Gal reporter genes. The assay provides high success rate in single-round infections, increased assay capacity (2-day assay), increased precision (accurately measure 50% neutralization), improved level of standardization (stable cell line), and an optimized and validated assay platform. Montefiori, D.C. (2004) Current Protocols in Immunology, John Wiley & Sons, 12.11.1-12.11.15, which is incorporated herein by reference, described the assay.

Figure 10 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "ON".

Figure 11 depicts Env-Pseudotyped viruses in TZM-BL Cells/ "OFF".

Figure 12 depicts RSV-Fusion Pseudo viral production in 293T cells.

Figure 13 shows the immunization protocol used for a RSV-F Neutralization Study using the assay.

Figure 14 shows data from quantification of RSV-F mediated infection in different target cells. Infectivity of pseudotypes bearing RSV-F proteins. Pseudotype viruses were pelleted, and the particle amounts were estimated by quantifying HIV p24 antigen. Cells were infected with pseudotype viruses or no envelope bearing a panel of diverse cell lines containing 10ng of particulate HIV p24 antigen. At 72 h postinfection, cells supernatant and assayed for p24 antigen. Values are the means of triplicate wells with the standard deviations.

Figure 15 shows data from quantification of RSV-F neutralization activity in TZM-bl HeLa cells. The data show results of a comparison of neutralizing ability to RSV-F using luciferase assay from RSV-F immunized serum samples from DNA vaccinated animals that can block the HIV-Luc/FMDV pseudovirus from entering and infecting target cells. The titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of virus. The ability of the serum sample from the vaccinated mice to neutralize and block the infectivity of HIV-1 Luc/RSV-F the absence of such blocking activity in normal mouse serum (NMS).

Figure 16 shows data from quantification of cell death activity by RSV-F pseudo virus in HeLa cells. The results show that consensus RSV-F DNA plasmid elicits potent humoral and cellular immune responses in mice after 3 vaccinations with electroporation. A high level of RSV-F specific IgG in sera was observed as early as 1 week post 2<sup>nd</sup> immunization with just 5 ug DNA. Cell-mediated immune response were detectable as far out as 8 weeks post 3<sup>rd</sup>

immunization suggesting the long-term persistence of immune response after RSV-F DNA vaccination. Increase in RSV-F DNA concentration did not result in increased immune response. Antibodies generated after 3 vaccinations with RSV-F DNA plus electroporation were capable of neutralizing infection as measured by pseudo-virus neutralization assay. Results also showed mitigated RSV-F mediated apoptosis of target cells.

#### **Example 6**

Figure 17 shows the immunization schedule for RSV-M2 dosage study that was undertaken. Data collected from that study includes a comparison of IFN-g production at different dosages of RSV-M2 vaccine 1 week after last immunization (Figure 18). The results showed that a consensus-based RSV-M2 DNA plasmid elicited significant cellular immune responses in mice after 3 vaccinations with electroporation. High level of IFN-g<sup>+</sup> T cells specific for either the immunodominant or subdominant epitopes of RSV-M2 in both spleen and in lung were observed. Maximum immune response was observed with 60 ug DNA (however, response not much greater than that observed with 10 ug DNA).

#### **Example 7**

Further study was undertaken to evaluate CCL20 as potential immune adjuvant to enhance mucosal immune response after DNA immunization. CCL20 (liver activation regulated chemokine – LARC; macrophage inflammatory protein 3 – MIP3a) binds to CCR6 and is expressed in lymph nodes, liver, appendix, fetal lung (lower levels in thymus, testis, prostate, and gut).

Figure 19 depicts an immunization schedule for evaluation of the combination of RSV-F vaccine with CCL20.

Figure 20 shows data from the study including data from a comparison of IFN-g production in each of spleen, liver and lung with different dosages of CCL20 at 1 week after last immunization.

Figure 21 shows data of a comparison of IgG subtypes (IgG1 vs IgG2a) in Sera.

Results show that co-immunization with CCL20 does not enhance cell-mediated immune response in both the lung and the liver after RSV-F DNA immunization. CCL20 appeared to bias RSV-F specific IgG antibodies generated after DNA immunization towards a Th1-isotype.



Less IgG1 and more IgG2a were observed in sera after CCL20 + RSV-F co-immunization compared to RSV-F immunization alone.

Thus, provided are improved vaccines, improved methods for inducing immune such responses, including mucosal immune responses, and for prophylactically and/or therapeutically immunizing individuals against immunogens, particularly those associated with pathogens and other agents which enter the host via mucosal tissue. For examples, infectious agents commonly enter the host across a mucosal tissue such as the oral mucosa and other mucosa of the alimentary canal, the respiratory tract including olfactory and conjunctival mucosa, the mammary glands, and the genitourinary tract.

Composition comprising nucleic acid sequences that encodes an immunogen in combination with nucleic acid sequences that encodes CCL20 and functional fragments thereof are provided. The composition may comprise an isolated nucleic acid molecule that encodes both an immunogen and CCL20 and functional fragments thereof and/or compositions comprising an isolated nucleic acid molecule that encodes an immunogen in combination with an isolated nucleic acid molecule that encodes CCL20 and functional fragments thereof. Such compositions may be provided as injectable pharmaceutical.

The compositions may be used in methods of inducing an immune response in an individual against an immunogen, comprising administering to the individual such a composition.

Recombinant vaccines comprising a nucleotide sequence that encodes an immunogen operably linked to regulatory elements and a nucleotide sequences that CCL20 or functional fragments thereof are provided as are methods of inducing an immune response, including methods of inducing a mucosal immune response, in an individual against an immunogen comprising administering such a recombinant vaccine to an individual.

Live attenuated pathogens comprising a nucleotide sequence that CCL20 or functional fragments thereof, and to methods of inducing an immune response, including methods of inducing a mucosal immune response, in an individual against a pathogen comprising administering the live attenuated pathogen to an individual are also provided.

Methods of inducing an immune response in an individual against an immunogen comprising administering to said individual CCL20 protein or a functional fragment thereof in combination with an isolated nucleic acid molecule that encodes an immunogen; and/or a recombinant vaccine that encodes an immunogen and/or a subunit vaccine that comprises an immunogen and/or a live attenuated vaccine and/or a killed vaccine. Compositions comprising CCL20 protein or a functional fragments thereof in combination with one or more of an isolated nucleic acid molecule that encodes an immunogen; a recombinant vaccine comprising a nucleotide sequence that encodes an immunogen operably linked to regulatory elements; a live attenuated pathogen; and a killed pathogen are provided. As used herein, "functional fragment of CCL20" is meant to refer to a fragment of an iCCL20protein that, when delivered in conjunction with an immunogen, provides an modified immune response compared to the immune response that is induced when the immunogen is delivered without the fragment. Fragments are generally 10 or more amino acids in length. In some embodiments, functional fragments of CCL20 may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the length of the particular full length CCL20 protein.

When delivered in combination with an immunogen, CCL20 and functional fragments thereof, and combinations thereof modulates immune responses. Accordingly, a combination of these proteins may be delivered as components of a DNA or protein based vaccine in order to induce a therapeutic or prophylactic immune response or in compositions useful to induce an immune response. In some embodiments, the means to deliver the immunogen is a DNA vaccine, a recombinant vaccine, a protein subunit vaccine, an attenuated vaccine or a killed vaccine. In some embodiments, the means to deliver one or more of CCL20 and functional fragments thereof is by expression of coding sequences included in a DNA vaccine, a recombinant vaccine or an attenuated vaccine.

Immune responses result in the production of antigen specific antibodies and/or antigen specific T- and B-cells. Antigen specific antibodies and/or cells provide the means to protect

against infection, to reduce or to clear existing infection. They can also be isolated from the individual and used in other applications such as passive immunity protocols, immunocolumns or as reagents.

In some embodiments, CCL20 is useful to induce mucosal immune responses, particularly increased Th1 responses characterized by increased IgG2a responses and decreased IgG1 responses, even in protocols where the composition is delivered systemically. Co-immunization with an immunogen such as by DNA vaccine or other means plasmid plus one or more of these chemokines, such as in a DNA vaccine or part of the coding sequence of another type of vaccine, will provide a unique adjuvanting property.

CCL20 protein and nucleotides which encode it can be obtained from many sources, natural and synthetic. In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, isolates CCL20 protein from natural sources using, for example, immuno columns which contain antibodies that specifically bind to the protein. Alternatively, the protein may be separated using electrophoresis, isolated from the electrophoresis matrix and purified by for example dialysis to yield essentially pure protein. Other well known protein purification technologies can be employed to produce isolated, essentially pure protein. In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, inserts DNA molecules that encode any of CCL20 and functional fragments thereof into a commercially available expression vector for use in well known expression systems. In addition to isolating proteins from natural sources or producing proteins by recombinant techniques, automated peptide synthesizers may also be employed to produce isolated, essentially pure protein.

According to some aspects of the present invention, compositions and methods are provided which prophylactically and/or therapeutically immunize an individual against an immunogen such as an allergen, a pathogen or abnormal, disease-related cells. The vaccine may be any type of vaccine such as, a live attenuated vaccine, a cell vaccine, a recombinant vaccine or a nucleic acid or DNA vaccine. By delivering nucleic acid molecules that encode an immunogen and one or more of CCL20 and functional fragments thereof the immune response induced by the vaccine may be modulated.

Compositions for delivering immunogens and CCL20 are provided, particularly those comprising one or more nucleic acid molecules that comprise a nucleotide sequence that encodes one or more of CCL20 and functional fragments thereof operably linked to regulatory elements in combination with a nucleotide sequence that encodes an immunogen operably linked to regulatory elements. The nucleic acid sequences encoding CCL20 and the immunogen may be one the same molecule or on separate molecules.

The nucleic acid molecules may be delivered using any of several well known technologies including DNA injection (also referred to as DNA vaccination), recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. In some embodiments, DNA vaccines are constructed as described herein and delivered using electroporation as described herein. The descriptions for RSV vaccines set forth herein, for example, may be applied to the use of CCL20 with other immunogens. DNA vaccines are described in U.S. Pat. Nos. 5,593,972, 5,739,118, 5,817,637, 5,830,876, 5,962,428, 5,981,505, 5,580,859, 5,703,055, 5,676,594, and the priority applications cited therein, which are each incorporated herein by reference. In addition to the delivery protocols described in those applications, alternative methods of delivering DNA are described in U.S. Pat. Nos. 4,945,050 and 5,036,006, which are both incorporated herein by reference. Routes of administration include, but are not limited to, intramuscular, intranasally, intraperitoneal, intradermal, subcutaneous, intravenous, intraarterially, intraocularly and oral as well as topically, transdermally, by inhalation or suppository or to mucosal tissue such as by lavage to vaginal, rectal, urethral, buccal and sublingual tissue. Preferred routes of administration include intramuscular, intraperitoneal, intradermal and subcutaneous injection. In some embodiments, nucleotide sequences that encode CCL20 or functional fragments thereof may be linked to IgE signal peptide. In some embodiments, the immunogen is a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune diseases. The present invention may be used to immunize an individual against all pathogens such as viruses, prokaryote and pathogenic eukaryotic organisms such as unicellular pathogenic organisms and multicellular parasites. The present invention is particularly useful to immunize an individual against those pathogens which infect cells and which are not encapsulated such as viruses, and prokaryote

such as gonorrhea, listeria and shigella. In addition, the present invention is also useful to immunize an individual against protozoan pathogens that include a stage in the life cycle where they are intracellular pathogens. Examples of pathogens include the following. Viruses in the Picornavirus Family Genera such as rhinoviruses (responsible for - 50% cases of the common cold), Etheroviruses, (include polioviruses, coxsackieviruses, echoviruses, and human enteroviruses such as hepatitis A virus), and Aphthoviruses (Veterinary; target antigens include: VP1, VP2, VP3, VP4, VPG). Viruses in the Calcivirus Family Genera include Norwalk Group of Viruses (causative agent of epidemic gastroenteritis). Viruses of the Togavirus Family Genera include Alphaviruses: (examples include Senilis viruses, RossRiver virus and Eastern & Western, Equine encephalitis). Reovirus include Rubella virus. Flariviridae Family Examples include dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne encephalitis viruses. West Nile virus (Genbank NC001563, AF533540, AF404757, AF404756, AF404755, AF404754, AF404753, AF481864, M12294, AF317203, AF196835, AF260969, AF260968, AF260967, AF206518 and AF202541) Representative Target antigens: E NS5 C. Hepatitis C Virus. Coronavirus Family include infectious bronchitis virus. porcine transmissible gastroenteric virus, Porcine hemagglutinating encephalomyelitis virus, Feline infectious peritonitis virus, Feline enteric coronavirus, Canine coronavirus, SARS associated coronavirus, human respiratory coronaviruses cause ~40 cases of common cold. EX. 224E, OC43. Target antigens: E1 - also called M or matrix protein E2 - also called S or Spike protein E3 - also called BE or hemagglutinin- elterose glycoprotein N – nucleocapsid. Rhabdovirus Family Genera include Vesiliovirus Lyssavirus such as rabies. Target antigen: G protein N protein. Filoviridae Family includes Hemorrhagic fever viruses such as (Medical) Marburg and Ebola virus. Paramyxovirus Genera includes paramyxovirus: Family such as Mumps virus, New Castle disease virus, Morbillivirus such as Measles, canine distemper, Pneumovirus such as Respiratory syncytial virus, Orthomyxovirus. The Influenza virus Family includes Bunyavirus Family Genera such as bunyavirus, California encephalitis, LA Crosse, Phlebovirus such as Rift Valley Fever, Hantavirus such as puremala, a hemahagin fever virus, Nairovirus, unassigned bunyaviruses Arenavirus Family LCM, and Lassa fever virus. Reovirus Family Genera includes Reovirus, Rotavirus, Orbiviruses such as Colorado Tick fever, Lebombo, equine encephalosis, blue tongue.

Retrovirus Family Sub-Family includes oncorivirinal such as leukemia virus, HTLV I and HTLV II, Lentivirinal such as HIV, feline immunodeficiency virus, equine infections, and anemia virus. Spumavirinal Papovavirus Family Sub-Family includes Polyomaviruses such as BKU and JCU viruses, Sub-Family Papillomavirus. Adenovirus such as EX AD7, ARD., O.B., 275. Parvovirus Family includes Feline parvovirus, Feline panleucopeniavirus, Canine parvovirus, Porcine parvovirus. Herpesvirus Family Sub-Family includes alpha-Genera Simplexvirus herpesviridae HSV I (Genbank X14112, NC001806), HSV II (NC001798) Varicellovirus, pseudorabies, varicella zoster. Sub-Family - beta- includes Genera Cytomegalovirus such as herpesviridae HCMV. Muromegalovirus Sub-Family includes Genera: Lymphocryptovirus, Gamma- EBV - (Burkitt's lymphoma), herpesviridae. Rhadinovirus Poxvirus Family Sub-Family Genera Variola includes (Smallpox) Chordopoxviridae Vaccinia (Cowpox) (Parapoxvirus) Aupoxvirus, Capripoxvirus Leporipoxvirus. Suipoxvirus's Sub-Family includes Entomopoxviridae Hepadnavirus. Hepatitis B virus Family Unclassified Hepatitis delta virus. Bacterial pathogens include pathogenic gram-positive cocci such as pneumococcal; staphylococcal; and streptococcal. Pathogenic gram-negative cocci include: meningococcal; and gonococcal. Pathogenic enteric gram-negative bacilli include: enterobacteriaceae; pseudomonas, acinetobacteria and eikenella, melioidosis; salmonella; shigellosis; hemophilus; chancroid; brucellosis; tularemia; yersinia (pasteurella); streptobacillus mortiformis and spirillum; listeria monocytogenes; erysipelas; rickettsial; diphtheria, cholera, anthrax; donovanosis (granuloma inguinale); and bartonellosis. Pathogenic anaerobic bacteria include: tetanus; botulism; other clostridia; tuberculosis; leprosy; and other mycobacteria. Pathogenic spirochetal diseases include: syphilis; - treponematoses: yaws, pinta and endemic syphilis; and leptospirosis. Other infections caused by higher pathogen bacteria and pathogenic fungi include: actinomycosis; nocardiosis; cryptococcosis, blastomycosis, histoplasmosis and coccidioidomycosis; candidiasis, aspergillosis, and mucormycosis; sporotrichosis; paracoccidioidomycosis, petriellidiosis, torulopsosis, mycetoma, and chromomycosis; and dermatophytosis. Rickettsial infections include rickettsial and rickettsioses. Examples of mycoplasma and chlamydial infections include: mycoplasma pneumoniae; lymphogranuloma venereum; psittacosis; and perinatal chlamydial infections. Pathogenic eukaryotes Pathogenic

protozoans and helminths and infections thereby include: amebiasis; malaria; leishmaniasis; trypanosomiasis; toxoplasmosis; pneumocystis carinii; babesiosis; giardiasis; trichinosis; filariasis; schistosomiasis; nematodes; trematodes or flukes; and cestode (tapeworm) infections. Vaccines can be made such as DNA constructs that comprise DNA sequences that encode the peptides that comprise at least an epitope identical or substantially similar to an epitope displayed on a pathogen antigen. In some embodiments, the methods of immunizing an individual against a pathogen are directed against RSV, HIV, HSV, HCV, WNV or HBV.

Methods of conferring a protective immune response against hyperproliferating cells utilize as immunogens proteins that are characteristic in hyperproliferative diseases. These are useful in vaccines and methods of treating individuals suffering from hyperproliferative diseases. Examples of hyperproliferative diseases include all forms of cancer and psoriasis. Examples of target proteins against hyperproliferative diseases include those which are proteins encoded by oncogenes such as myb, myc, fyn, and the translocation gene bcr/abl, ras, src, P53, neu, trk and EGRF. In addition to oncogene products as target antigens, target proteins for anti-cancer treatments and protective regimens include variable regions of antibodies made by B cell lymphomas and variable regions of T cell receptors of T cell lymphomas which, in some embodiments, are also used target antigens for autoimmune disease. Other tumor-associated proteins can be used as target proteins such as proteins that are found at higher levels in tumor cells including the protein recognized by monoclonal antibody 17-IA and folate binding proteins or PSA. Methods of treating individuals suffering from autoimmune diseases and disorders by conferring a broad based protective immune response against targets that are associated with autoimmunity including cell receptors and cells which produce "self"-directed antibodies are also provided. T cell mediated autoimmune diseases include Rheumatoid arthritis (RA), multiple sclerosis (MS), Sjogren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Crohn's disease and ulcerative colitis. Each of these diseases is characterized by T cell receptors that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of the T cells would elicit an immune response including

CTLs to eliminate those T cells. In RA, several specific variable regions of T cell receptors (TCRs) that are involved in the disease have been characterized. These TCRs include V.beta.-3, V.beta.-14, 20 V.beta.-17 and V.alpha.-17. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in RA. See: Howell, M. D., et al., 1991 Proc. Nat. Acad. Sci. USA 88:10921-10925; Piliard, X., et al, 1991 Science 253:325-329; Williams, W. V., et al., 1992 J Clin. Invest. 90:326-333; each of which is incorporated herein by reference. In MS, several specific variable regions of TCRs that are involved in the disease have been characterized. These TCRs include V $\beta$ P and V.alpha.-10. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in MS. See: Wucherpfennig, K. W., et al., 1990 Science 248:1016-1019; Oksenberg, J. R., et al, 1990 Nature 345:344-346; each of which is incorporated herein by reference. In scleroderma, several specific variable regions of TCRs that are involved in the disease have been characterized. These TCRs include V.beta.-6, V.beta.-g, V.beta.-14 and V.alpha.-16, V.alpha.-3C, V.alpha.-7, V.alpha.-14, V.alpha.-15, V.alpha.-16, V.alpha.-28 and V.alpha.-12. Thus, vaccination with a DNA construct that encodes at least one of these proteins will elicit an immune response that will target T cells involved in scleroderma. In order to treat patients suffering from a T cell mediated autoimmune disease, particularly those for which the variable region of the TCR has yet to be characterized, a synovial biopsy can be performed. Samples of the T cells present can be taken and the variable region of those TCRs identified using standard techniques. Genetic vaccines can be prepared using this information. B cell mediated autoimmune diseases include Lupus (SLE), Grave's disease, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, asthma, cryoglobulinemia, primary biliary sclerosis and pernicious anemia. Each of these diseases is characterized by antibodies that bind to endogenous antigens and initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of antibodies would elicit an immune response including CTLs to eliminate those B cells that produce the antibody.

In order to treat patients suffering from a B cell mediated autoimmune disease, the variable region of the antibodies involved in the autoimmune activity must be identified. A biopsy can be performed and samples of the antibodies present at a site of inflammation can be



taken. The variable region of those antibodies can be identified using standard techniques. Genetic vaccines can be prepared using this information. In the case of SLE, one antigen is believed to be DNA. Thus, in patients to be immunized against SLE, their sera can be screened for anti-DNA antibodies and a vaccine can be prepared which includes DNA constructs that encode the variable region of such anti-DNA antibodies found in the sera. Common structural features among the variable regions of both TCRs and antibodies are well known. The DNA sequence encoding a particular TCR or antibody can generally be found following well known methods such as those described in Kabat, et al 1987 Sequence of Proteins of Immunological Interest U.S. Department of Health and Human Services, Bethesda Md., which is incorporated herein by reference. In addition, a general method for cloning functional variable regions from antibodies can be found in Chaudhary, V. K., et al, 1990 Proc. Natl. Acad Sci. USA 87:1066, which is incorporated herein by reference.

In addition to using expressible forms of CCL20 protein coding sequence to improve DNA vaccines, improved attenuated live vaccines, improved killed vaccines and improved vaccines that use recombinant vectors to deliver foreign genes that encode antigens and well as subunit and glycoprotein vaccines are provided which comprise CCL20 protein or a functional fragment thereof and/or coding sequences that encode CCL20 or a functional fragment thereof. Examples of attenuated live vaccines, those using recombinant vectors to deliver foreign antigens, subunit vaccines and glycoprotein vaccines are described in U.S. Pat. Nos. 4,510,245; 4,797,368; 4,722,848; 4,790,987; 4,920,209; 5,017,487; 5,077,044; 5,110,587; 5,112,749; 5,174,993; 5,223,424; 5,225,336; 5,240,703; 5,242,829; 5,294,441; 5,294,548; 5,310,668; 5,387,744; 5,389,368; 5,424,065; 5,451,499; 5,453,364; 5,462,734; 5,470,734; 5,474,935; 5,482,713; 5,591,439; 5,643,579; 5,650,309; 5,698,202; 5,955,088; 6,034,298; 6,042,836; 6,156,319 and 6,589,529, which are each incorporated herein by reference.

### **Example 8**

Using consensus sequences of RSV immunogens F, G(A) and G(B) provides the basis to obtain neutralizing immune responses. Such advantages make consensus RSV DNA vaccine much more beneficial and potentially more efficacious than any other approaches suggested in the past

Major RSV proteins - fusion (F), glycoprotein (Ga), and glycoprotein (Ga) were chosen as targets for new RSV vaccines. As mentioned above, F and G proteins are expressed on the surface and are ideal targets for neutralizing antibodies. A cocktail DNA vaccine was prepared comprising plasmids that have sequence which encode and express consensus RSV-F, plasmids that have sequence which encode and express consensus RSV-G(A) and plasmids that have sequence which encode and express consensus RSV-G(B). The individual plasmids as well as the cocktail were used in studies. The cocktail vaccine was used in studies with rabbits and non-human primates and the results of those studies show that high levels of neutralizing immune response are induced, indicating that the cocktail vaccine can be used in both prophylactic and therapeutic applications with particular value in protecting against RSV infection.

As shown in Figures 22A, Figures 22B and Figures 22C, single insert plasmids for RSV-F, RSV-G(A), and RSV-G(B), respectively, were constructed using the pVAX vector. Consensus sequences for each respective plasmid were cloned into the pVAX vector between the BamHI and XhoI sites. The resulting plasmids were: pRSV-F, which comprises an 1813 base pair insert that encodes consensus RSV-F cloned into pVAX to produce a plasmid having 4812 base pairs; pRSV-G(A), which comprises a 945 base pair insert that encodes consensus RSV-G(A) cloned into pVAX to produce a plasmid having 3944 base pairs; and pRSV-G(B), which comprises a 930 base pair insert that encodes consensus RSV-G(B) cloned into pVAX to produce a plasmid having 3929 base pairs.

To confirm expression of RSV-F, RSV-G(A), and RSV-G(B) constructs, RD cells were transfected with pRSV-F, pRSV-G(A) and pRSV-G(B), respectively. Immunofluorescent evaluation was performed using immunized, pooled rabbit sera. RSV Immunogens were visualized by immunofluorescence by binding anti-RSV immunogen antibodies chemically conjugated with fluorescein isothiocyanate (FITC). DAPI was used as a nuclear counterstain. Results in Figure 23 show RD cells transfected with pVAX showed no immunostaining by FITC labeled anti-RSV immunogen antibodies.

An RSV-F Dosage Study was done using mice. The immunization schedule is shown in Figure 24. Five groups of mice were used. Group 1 were control injected with pVAX. Group 2 were injected with 5 $\mu$ g pRSV-F construct. Group 3 were injected with 15 $\mu$ g pRSV-F construct.

Group 4 were injected with 30µg pRSV-F construct. Group 5 were injected with 60µg pRSV-F construct. Animals were immunized a total of three times using electroporation: first immunization at Week 0, second immunization at Week 2 and third immunization at Week 4. Sera was collected and IgG measured a total of four times: first collection at Week 2, second collection at Week 4, third collection at Week 6 and fourth collection at Week 12. Animals were sacrificed at Week 12, spleens were harvested and IFN-gamma was measured via ELISPOT.

Figure 25 shows data from the RSV-F Dosage comparing total RSV-F IgG in Sera. Data is shown from sera collected after immunization #1, after immunization #2, and after immunization #3. Anti-RSV-F IgG was measured at reciprocal serum dilutions of 10, 100, 1,000 and 10,000. Data show pVAX did not induce antibodies after any immunization. Data show pRSV-F construct did not induce antibodies after first immunization. Data show pRSV-F construct induced antibodies after second and third immunization.

Figure 26 shows data from the RSV-F Dosage comparing IFN- $\gamma$  production induced by RSV-F vaccination approximately eight weeks after last immunization. Data is shown comparing IFN- $\gamma$  production (SFU/106 splenocytes) from animals injected with pVAX and 15 µg average dose pRSV-F construct.

An RSV-F PSEUDO Neutralization Study was done using mice. The immunization schedule is shown in Figure 27. Mice were immunized with 15µg pRSV-F construct. Animals were immunized a total of three times using electroporation: first immunization at Week 0, second immunization at Week 2 and third immunization at Week 4. Sera was collected and neutralization assay performed at Week 5.

Data from the RSV-F PSEUDO Neutralization Study is shown in Figure 28. RSV-F pseudo-type neutralization activity in TZM-bl HeLa cells was quantified. A comparison of neutralizing ability of RSV-F using luciferase assay from RSV-F immunized serum samples from DNA vaccinated animals that can block the HIV-Luc/RSV-F pseudovirus from entering and infecting target cells. The titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of virus. Note the ability of the serum sample from the vaccinated mice to neutralize and block infectivity of HIV-Luc/RSV-F in the absence of such blocking activity in normal mouse serum (NMS).

As shown in Figure 29, two comparison were done using sera from two animals vaccinated with pRSV-F (RSV-F2 and RSV-F5) versus sera from pVAX vaccinated animals to determine inhibition of apoptotic cell death activity by RSV-F pseudovirus in HeLa cells. For each comparison, sera was diluted 1:1, 1:10, 1:100 and 1:1000. The level of cell death expressed as a percentage was determined showing that sera from animals vaccinated with pRSV-F inhibited apoptotic cell death of HeLa cell exposed to RSV-F pseudovirus in the presence of sera.

An RSV-G(A) and RSV-(B) Dosage Study was done using mice. The immunization schedule is shown in Figure 30. Six groups of mice were used. Group 1 were control injected with pVAX. Group 2 were injected with 1µg pRSV-G(A) or pRSV-G(B) construct. Group 3 were injected with 5µg pRSV-G(A) or pRSV-G(B) construct. Group 4 were injected with 10µg pRSV-G(A) or pRSV-G(B) construct. Group 5 were injected with 20µg pRSV-G(A) or pRSV-G(B) construct. Group 6 were injected with 40µg pRSV-G(A) or pRSV-G(B) construct. Animals were immunized a total of three times using electroporation: first immunization at Week 0, second immunization at Week 2 and third immunization at Week 4. Sera was collected and IgG measured a total of two times: first collection at Week 4, and the second collection at Week 6.

Figure 31 shows data for IgG in sera after RSV-G(A) DNA immunization in the RSV(A) or RSV-G(B) Dosage Study. Total anti-RSV-G(A) IgG in Sera was measured from the Dosage Study. Data is shown from sera collected at various doses as set out in the x-axis of the graph in Figure 31. Anti-RSV-G(A) IgG was measured at serum dilution of 1:100.

Figure 32 shows data for IgG in sera after RSV-G(B) DNA immunization in the RSV(A) or RSV-G(B) Dosage Study. Total anti-RSV-G(B) IgG in Sera was measured from the Dosage Study. Data is shown from sera collected at various doses as set out in the x-axis of the graph in Figure 31. Anti-RSV-G(B) IgG was measured at serum dilution of 1:100.

Figure 33 is a chart showing s summary of RSV Preliminary Neutralization results using Mono Vaccines in Mouse Studies. Neutralization titers from sera collected 3 days post immunization were measured. Negative serum was used as a negative control. Positive controls included an anti-RSV monoclonal antibody and anti-RSV polyclonal serum. Neutralization

titers were measured for controls and for sera from two mice vaccinated with pRSV-F, two mice vaccinated with pRSV-G(A) and one mouse vaccinated with pRSV-G(B).

An RSV Rabbit Study was designed and undertaken. The RSV Rabbit Study Design is shown in Figure 34. Four female New Zealand white (NZW) rabbits were immunized with a cocktail containing pRSV-F, pRSV-G(A), and pRSV-G(B). Each animal received 200 µg/plasmid in a final 100 µl volume. Animals were immunized a total of four times using intramuscular/electroporation immunization. The first immunization was done at Week 0, the second immunization at Week 3, the third immunization at Week 6 and the fourth at Week 9. Blood was collected from animals six times: Week -2 (Pre-bleed - 2 weeks prior to first immunization), Week 0 (Pre-bleed prior to first immunization), Week 2, Week 5, Week 8 and Week 13. Blood was analyzed using ELISA and virus neutralization assays.

Data from the Rabbit Study is shown in Figure 35. Humoral immunity against RSV-F was measured. Anti-RSV-F IgG from pre-bleed samples taken at Weeks -2 and 0 and from samples taken at Week 2, at Week 5, at Week 8 and at Week 13. At Week 2, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 5, animals from which samples were collected had been immunized two times and the sample was collected 2 weeks after the second immunization. At Week 8, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 13, animals from which samples were collected had been immunized four times and the sample was collected 4 weeks after the fourth immunization.

Data from the Rabbit Study is shown in Figure 36. Humoral immunity against RSV-G was measured. Anti-RSV-G IgG from pre-bleed samples taken at Weeks -2 and 0 and from samples taken at Week 2, at Week 5, at Week 8 and at Week 13. At Week 2, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 5, animals from which samples were collected had been immunized two times and the sample was collected 2 weeks after the second immunization. At Week 8, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 13, animals from which

samples were collected had been immunized four times and the sample was collected 4 weeks after the fourth immunization.

An RSV Non-human Primate Study was undertaken. The purpose of the study was to evaluate the humoral immunogenicity of the single-insert pDNA RSV vaccines administered as a cocktail in the non-human primate (NHP). Also, the study was used to evaluate and determine whether factors such as multiple site delivery of the vaccine, electroporation method, and delivery with an adjuvant would increase the breadth of the humoral responses. The experiments were performed to determine if the synthetic DNA vaccines against RSV-F, RSV-G(A), and RSV-G(B) stimulate high titer of anti-RSV antibodies, to analyze the specific immune responses in animals vaccinated with the RSV cocktail and to evaluate in the effect of adjuvant and/or multiple site delivery on the immune responses generated. The RSV Non-Human Primate Study Design is shown in Figure 37. The non-human primate used were Rhesus macaques. In the study, 15 Rhesus macaques were divided into 4 groups: three groups contained four animals each and one group contained three animals. The groups in which  $n = 4$  were treated groups, i.e. they were vaccinated. The group in which  $n = 3$  was the untreated group which served as a control. The groups were treated as follows.

Group 1 ( $n=4$ ): each animal was administered a cocktail of 1 mg RSV-F, 1 mg RSV-G(A), 1 mg RSV-G(B), mixed and delivered IM in 1 site.

Group 2 ( $n=4$ ): each animal was administered a cocktail of 1 mg RSV-F, 1 mg RSV-G(A), 1 mg RSV-G(B), 1 mg rhMEC, mixed and delivered IM in 1 site.

Group 3 ( $n=4$ ): each animal was administered a cocktail of 1 mg RSV-F, 1 mg RSV-G(A), 1 mg RSV-G(B), 1 mixed and delivered ID in 3 sites.

Group 4 ( $n=3$ ): each animal was untreated.

Animals were immunized a total of three times using electroporation. The first immunization was done at Week 1, the second immunization at Week 5, and the third immunization at Week 13. Blood was collected from animals six times: Week 0 (Pre-bleed prior to first immunization),

Week 3, Week 7, Week 15, Week 17 and Week 18. Blood was analyzed using ELISA and virus neutralization assays.

Figure 38A-38D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-F. Figure 38A shows data from measured humoral immunity against RSV-F in naïve animals. Figure 38B shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine IM at one site. Figure 38C shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine plus rhMEC construct IM at one site. Figure 38D shows data from measured humoral immunity against RSV-F in animals who received the cocktail vaccine ID at three sites. Anti-RSV-F IgG from pre-bleed samples taken at Week 0 and from samples taken at Week 3, at Week 15 and at Week 18. At Week 3, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 15, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 18, animals from which samples were collected had been immunized three times and the sample was collected 5 weeks after the third immunization.

Figure 39A-39D show data from the RSV Non-Human Primate Study of measured humoral immunity against RSV-G. Figure 39A shows data from measured humoral immunity against RSV-G in naïve animals. Figure 39B shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine IM at one site. Figure 39C shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine plus rhMEC construct IM at one site. Figure 39D shows data from measured humoral immunity against RSV-G in animals who received the cocktail vaccine ID at three sites. Anti-RSV-G IgG from pre-bleed samples taken at Week 0 and from samples taken at Week 3, at Week 15 and at Week 18. At Week 3, animals from which samples were collected had been immunized one time and the sample was collected 2 weeks post immunization. At Week 15, animals from which samples were collected had been immunized three times and the sample was collected 2 weeks after the third immunization. At Week 18, animals from which samples were

collected had been immunized three times and the sample was collected 5 weeks after the third immunization.

Figure 40 is a chart showing a summary of RSV Preliminary Neutralization results using a cocktail vaccines in Rabbit Non-Human Primate Studies. Neutralization titers from sera collected 3 days post immunization were measured. Negative serum was used as a negative control. Positive controls included an anti-RSV monoclonal antibody and anti-RSV polyclonal serum. Neutralization titers were measured for controls and for sera from two rabbits vaccinated with the cocktail vaccine and two non-human primates vaccinated with the cocktail vaccine. Data from pre-bleeds shows that neutralization titers in the rabbits and the non-human primates were generated post vaccination.



## CLAIMS

1. A composition comprising one or more nucleic acid sequences that encode an RSV immunogen selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, wherein the nucleic acid coding sequence that encodes the RSV F immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:2 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:2;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:2 that is at least 115 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:2;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:2 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:2; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:2 that is at least 115 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, wherein the nucleic acid coding sequence that encodes the RSV Ga immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:6 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:6;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:6 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:6;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:6 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:6; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:6 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:6;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, wherein the nucleic acid coding sequence that encodes the RSV Gb immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:8 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:8;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:8 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:8;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:8 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:8; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:8 that is at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:8.

2. The composition of claim 1 comprising one or more nucleic acid sequences that encode an RSV immunogen selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, wherein the nucleic acid coding sequence that encodes the RSV F immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:1 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1;

a nucleic acid sequence comprising a fragment of SEQ ID NO:1 encoding at least 115 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:1 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:1; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encoding at least 115 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, wherein the nucleic acid coding sequence that encodes the RSV Ga immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:5 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5;

a nucleic acid sequence comprising a fragment of SEQ ID NO:5 encoding at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:5 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:5; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encoding at least 125 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, wherein the nucleic acid coding sequence that encodes the RSV Gb immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:7 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7;

a nucleic acid sequence comprising a fragment of SEQ ID NO:7 encoding at least 125 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:7 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:7; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encoding at least 125 or more amino acids and optionally further comprises coding

sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7.

3. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising SEQ ID NO:2 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen comprising SEQ ID NO:6 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:6;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising SEQ ID NO:8 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:8.

4. The composition of claim 3 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, the coding sequence comprising SEQ ID NO:1 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, the coding sequence comprising SEQ ID NO:5 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, the coding sequence comprising SEQ ID NO:7 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7.

5. The composition of claim 3 comprising one or more nucleic acid sequences selected from the group consisting of:

- a) a nucleic acid sequence that encodes SEQ ID NO:10;
- b) a nucleic acid sequence that encodes SEQ ID NO:14;

and

- c) a nucleic acid sequence that encodes SEQ ID NO:16;

6. The composition of claim 3 comprising one or more nucleic acid sequences selected from the group consisting of:

- a) a nucleic acid sequence comprising SEQ ID NO:9;
- b) a nucleic acid sequence comprising SEQ ID NO:13;

and

- c) a nucleic acid sequence comprising SEQ ID NO:15.

7. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a fragment of SEQ ID NO:2 having at least 345 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:2, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:2 having at least 345 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen comprising a fragment of SEQ ID NO:6 having at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:6, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:6 having at least 200 or more amino acids and optionally further comprises coding sequence that encodes a signal

peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:6;  
and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:8 having at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:8, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:8 having at least 200 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:8.

8. The composition of claim 7 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a nucleic acid sequence comprising a fragment of SEQ ID NO:1 encoding at least 345 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encoding at least 345 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, comprising a fragment of SEQ ID NO:5 encoding at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encoding at least 125 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5;  
and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:7 encoding at least 200 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encoding at least 200 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7.

9. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a fragment of SEQ ID NO:2 having at least 550 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:2, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:2 having at least 550 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen comprising a fragment of SEQ ID NO:6 having at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:6, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:6 having at least 270 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:6;  
and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:8 having at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:8, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:8 having at least 270 or



more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:8.

10. The composition of claim 9 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen comprising a nucleic acid sequence comprising a fragment of SEQ ID NO:1 encoding at least 550 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:1, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1 encoding at least 550 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, comprising a fragment of SEQ ID NO:5 encoding at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:5, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5 encoding at least 270 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:5;

and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen comprising a fragment of SEQ ID NO:7 encoding at least 270 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:7, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7 encoding at least 270 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:7.

11. The composition of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen at least 99% homologous to SEQ ID NO:2 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:2;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen at least 99% homologous to SEQ ID NO:6 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:6;  
and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen at least 99% homologous to SEQ ID NO:8 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:8.

12. The composition of claim 11 comprising one or more nucleic acid sequences selected from the group consisting of:

a) a nucleic acid coding sequence that encodes an RSV F immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:1 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:1;

b) a nucleic acid coding sequence that encodes an RSV Ga immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:5 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:5;  
and

c) a nucleic acid coding sequence that encodes an RSV Gb immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:7 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:7.

13. A composition of claim 1 comprising nucleic acid sequences selected from the group consisting of:

- a nucleic acid coding sequence that encodes an RSV F immunogen,
- a nucleic acid coding sequence that encodes an RSV Ga immunogen,
- a nucleic acid coding sequence that encodes an RSV Gb immunogen,
- a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,
- a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,
- a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, and
- a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen.

14. The composition of any of claims 1-13 further comprising a nucleic acid coding sequence that encodes CCL20.

15. The composition of any of claims 1-13 formulated for delivery to an individual using electroporation.

16. The composition of any of claims 1-13 further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

17. The composition of any of claims 1-13 wherein said one or more nucleic acid coding sequences are part of one or more plasmids.

18. The composition of any of claims 1-13 wherein each of the one or more nucleic acid coding sequences are part of incorporated into a separate plasmid.

19. A method of inducing an immune response against RSV comprising administering the composition of any of claims 1-13 to an individual in an amount effective to induce an immune response in said individual.

20. A method of treating an individual who has been diagnosed with RSV comprising administering a therapeutically effective amount of the composition of any of claims 1-13 to an individual.

21. A method of preventing RSV infection an individual comprising administering a prophylactically effective amount of the composition of any of claims 1-13 to an individual.

22. A protein selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:14; and SEQ ID NO:16.

23. A composition comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, wherein the nucleic acid coding sequence that encodes the RSV M2-1 immunogen is selected from the group consisting of:

a nucleic acid sequence encoding SEQ ID NO:4 and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes SEQ ID NO:4;

a nucleic acid sequence that encodes a fragment of SEQ ID NO:4 that is at least 25 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of SEQ ID NO:4;

a nucleic acid sequence that encodes a protein that is at least 98% homologous to SEQ ID NO:4 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence that encodes that protein that is at least 98% homologous to SEQ ID NO:4; and

a nucleic acid sequence that encodes a fragment of a protein that is at least 98% homologous to SEQ ID NO:4 that is at least 25 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to the sequence that encodes the fragment of a protein that is at least 98% homologous to SEQ ID NO:4.

24. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, wherein the nucleic acid coding sequence that encodes the RSV M2-1 immunogen is selected from the group consisting of:

a nucleic acid sequence comprising SEQ ID NO:3 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3;

a nucleic acid sequence comprising a fragment of SEQ ID NO:3 encoding at least 25 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3;

a nucleic acid sequence at least 98% homologous to SEQ ID NO:3 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 98% homologous to SEQ ID NO:3; and

a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encoding at least 25 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3.

25. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen comprising SEQ ID NO:4 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:4.

26. The composition of claim 25 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, the coding sequence comprising SEQ ID NO:3 and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3.

27. The composition of claim 25 comprising a nucleic acid sequence that encodes SEQ ID NO:12.

28. The composition of claim 25 comprising a nucleic acid sequence comprising SEQ ID NO:11.

29. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen comprising a fragment of SEQ ID NO:4 having at least 130 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:4, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:4 having at least 130 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:4.

30. The composition of claim 29 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, comprising a fragment of SEQ ID NO:3 encoding at least 130 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encoding at least 130 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3.

31. The composition of claim 23 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen comprising a fragment of SEQ ID NO:4 having at least 165 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide

operably linked to SEQ ID NO:4, or a fragment of a protein that is at least 98% homologous to SEQ ID NO:4 having at least 165 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that encodes a protein least 98% homologous to SEQ ID NO:4.

32. The composition of claim 31 comprising a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, comprising a fragment of SEQ ID NO:3 encoding at least 165 or more amino acids and optionally further comprising coding sequence that encodes a signal peptide operably linked to SEQ ID NO:3, or a fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3 encoding at least 165 or more amino acids and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence of the fragment of a nucleic acid sequence that is at least 98% homologous to SEQ ID NO:3.

33. The composition of claim 23 comprising a nucleic acid coding sequence that encodes the RSV M2-1 immunogen at least 99% homologous to SEQ ID NO:4 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:4.

34. The composition of claim 33 comprising nucleic acid coding sequence that encodes the RSV M2-1 immunogen, the coding sequence being at least 99% homologous to SEQ ID NO:3 and optionally further comprises coding sequence that encodes a signal peptide operably linked to coding sequence at least 99% homologous to SEQ ID NO:3.

35. A composition of claim 23 comprising nucleic acid sequences selected from the group consisting of:

- a nucleic acid coding sequence that encodes an RSV M2-1 immunogen,
- a nucleic acid coding sequence that encodes an RSV F immunogen and a nucleic acid coding sequence that encodes an RSV M2-1 immunogen,

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Ga immunogen,

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen,

a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen, and

a nucleic acid coding sequence that encodes an RSV F immunogen, a nucleic acid coding sequence that encodes an RSV M2-1 immunogen, a nucleic acid coding sequence that encodes an RSV Ga immunogen and a nucleic acid coding sequence that encodes an RSV Gb immunogen.

36. The composition of any of claims 23-35 further comprising a nucleic acid coding sequence that encodes CCL20.

37. The composition of any of claims 23-35 formulated for delivery to an individual using electroporation.

38. The composition of any of claims 23-35 further comprising nucleic acid sequences that encode one or more proteins selected from the group consisting of: IL-12, IL-15 and IL-28.

39. The composition of any of claims 23-35 wherein said one or more nucleic acid coding sequences are part of one or more plasmids.



40. The composition of any of claims 23-35 wherein each of the one or more nucleic acid coding sequences are part of incorporated into a separate plasmid.
41. A method of inducing an immune response against RSV comprising administering the composition of any of claims 23-35 to an individual in an amount effective to induce an immune response in said individual.
42. A method of treating an individual who has been diagnosed with RSV comprising administering a therapeutically effective amount of the composition of any of claims 23-35 to an individual.
43. A method of preventing RSV infection an individual comprising administering a prophylactically effective amount of the composition of any of claims 23-35 to an individual.
44. A protein selected from the group consisting of: SEQ ID NO:4 and SEQ ID NO:12.

Figure 1  
Phylogenetic Tree of RSV-F Protein

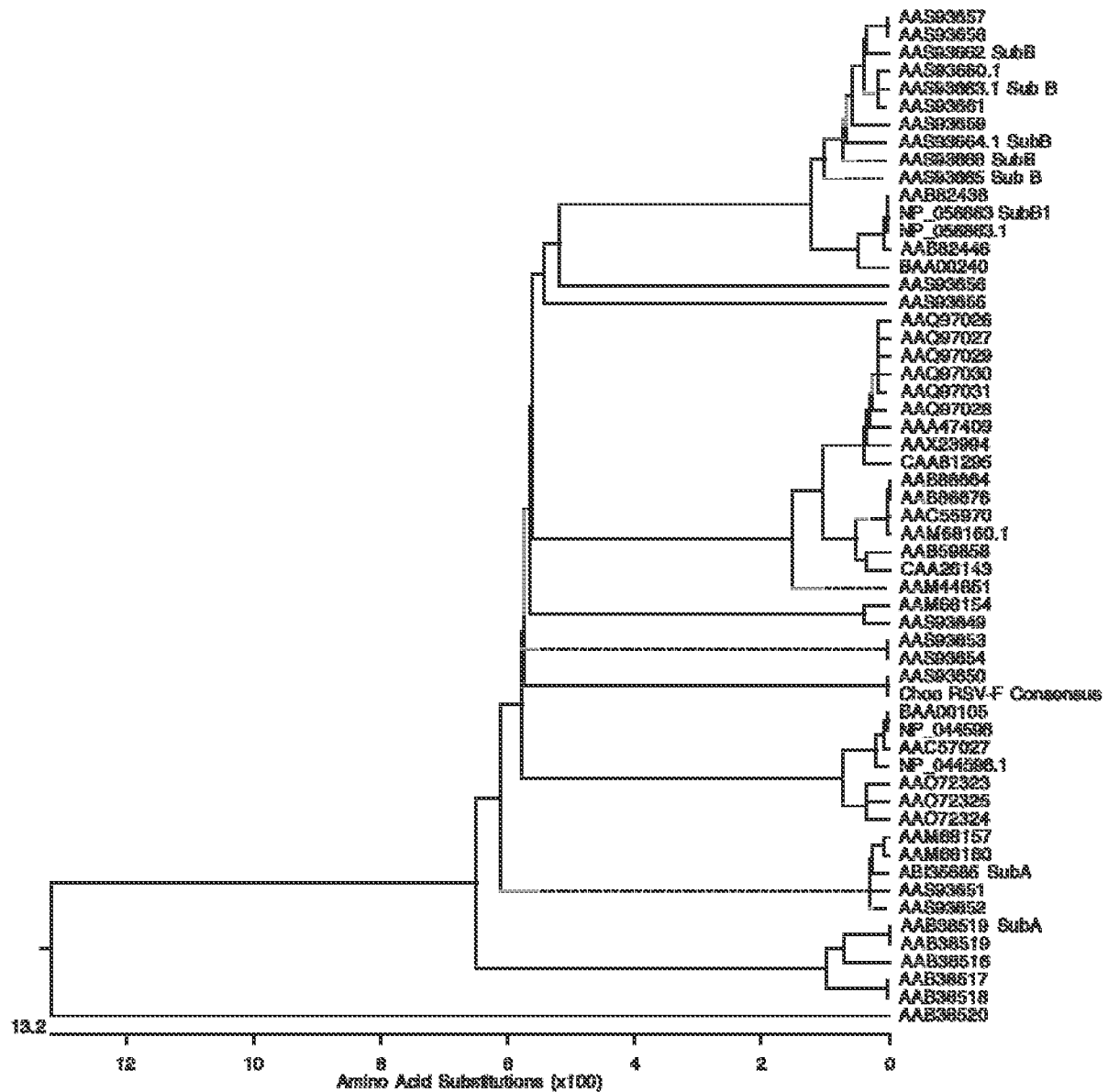


Figure 2

# Phylogenetic Tree of RSV-M2 Protein

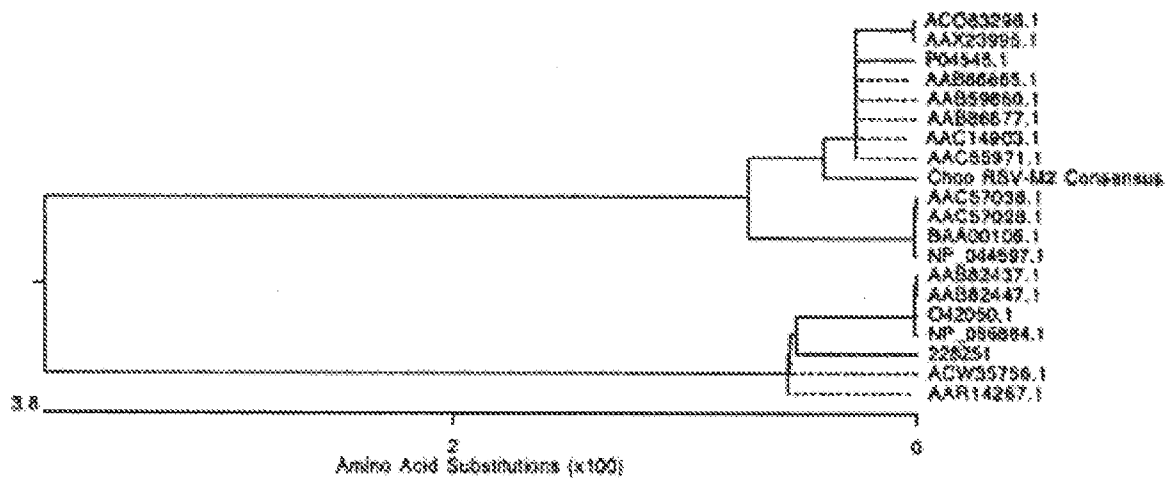


Figure 3  
Comparison of total anti-RSV-F Protein IgG in Sera

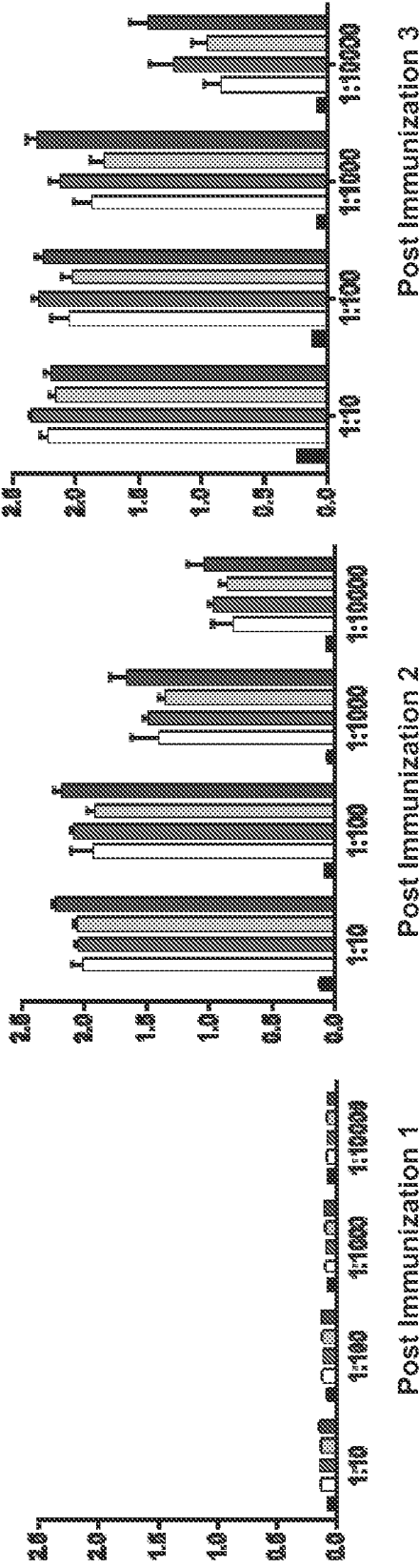
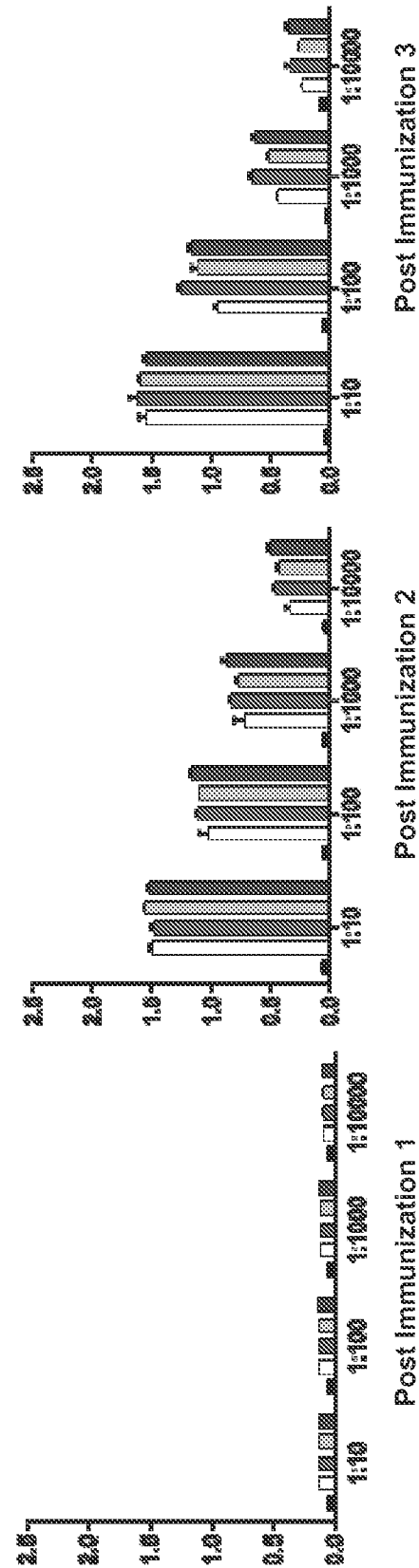
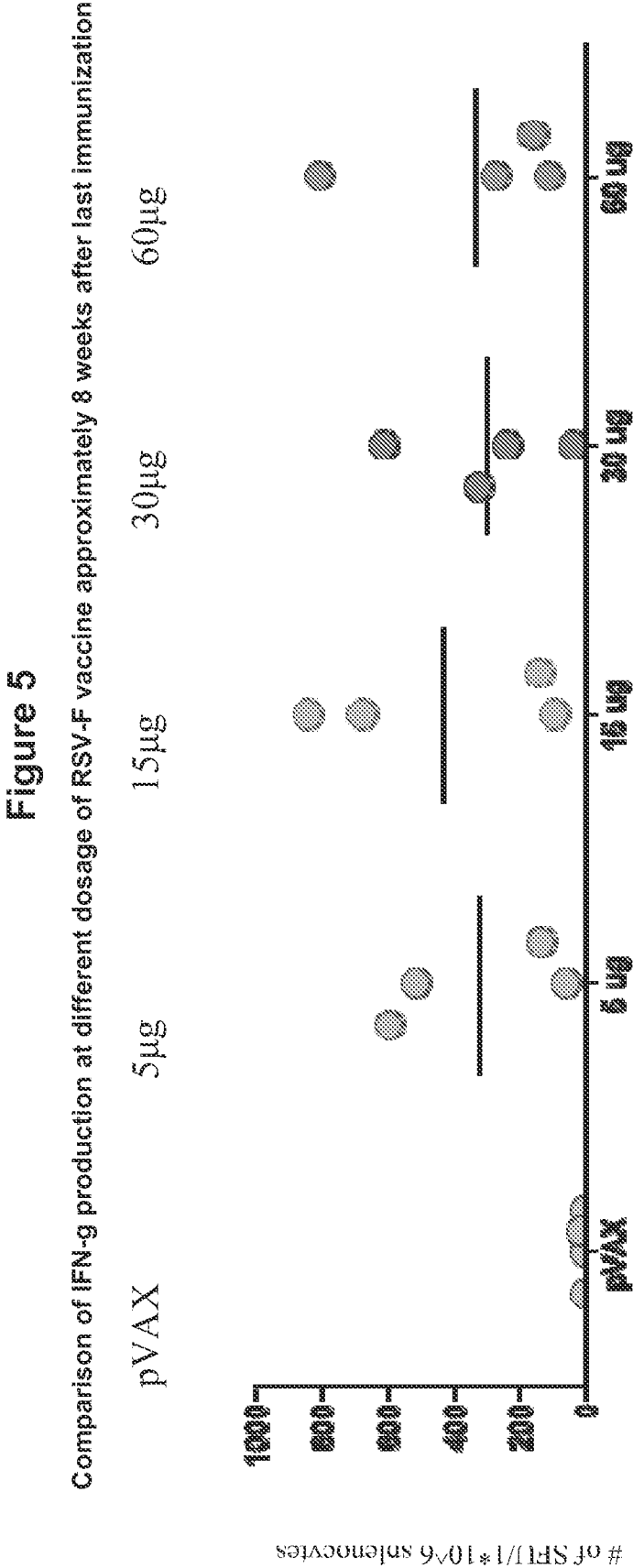


Figure 4  
Comparison of total anti-RSV-F Protein IgA in Sera





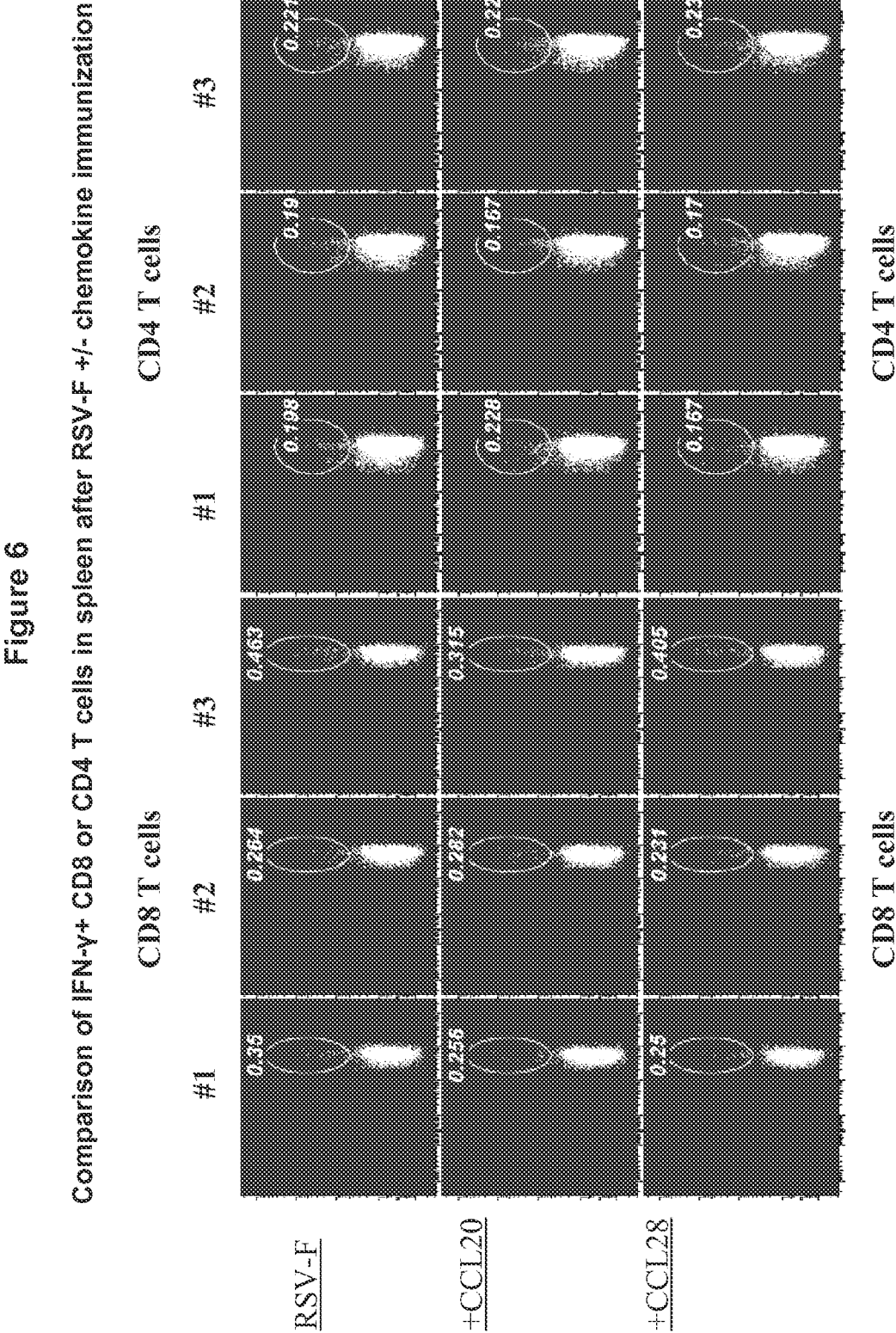
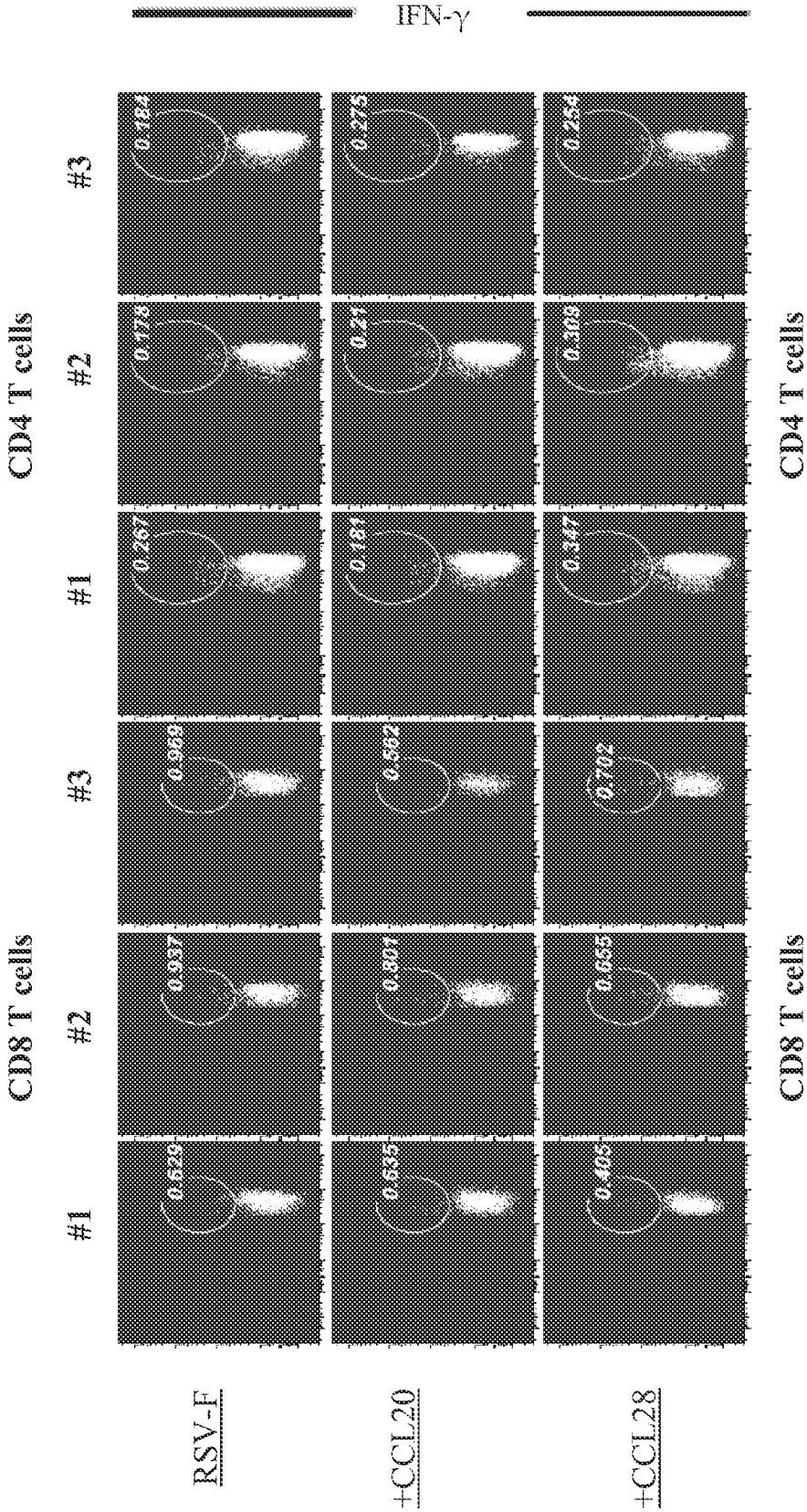
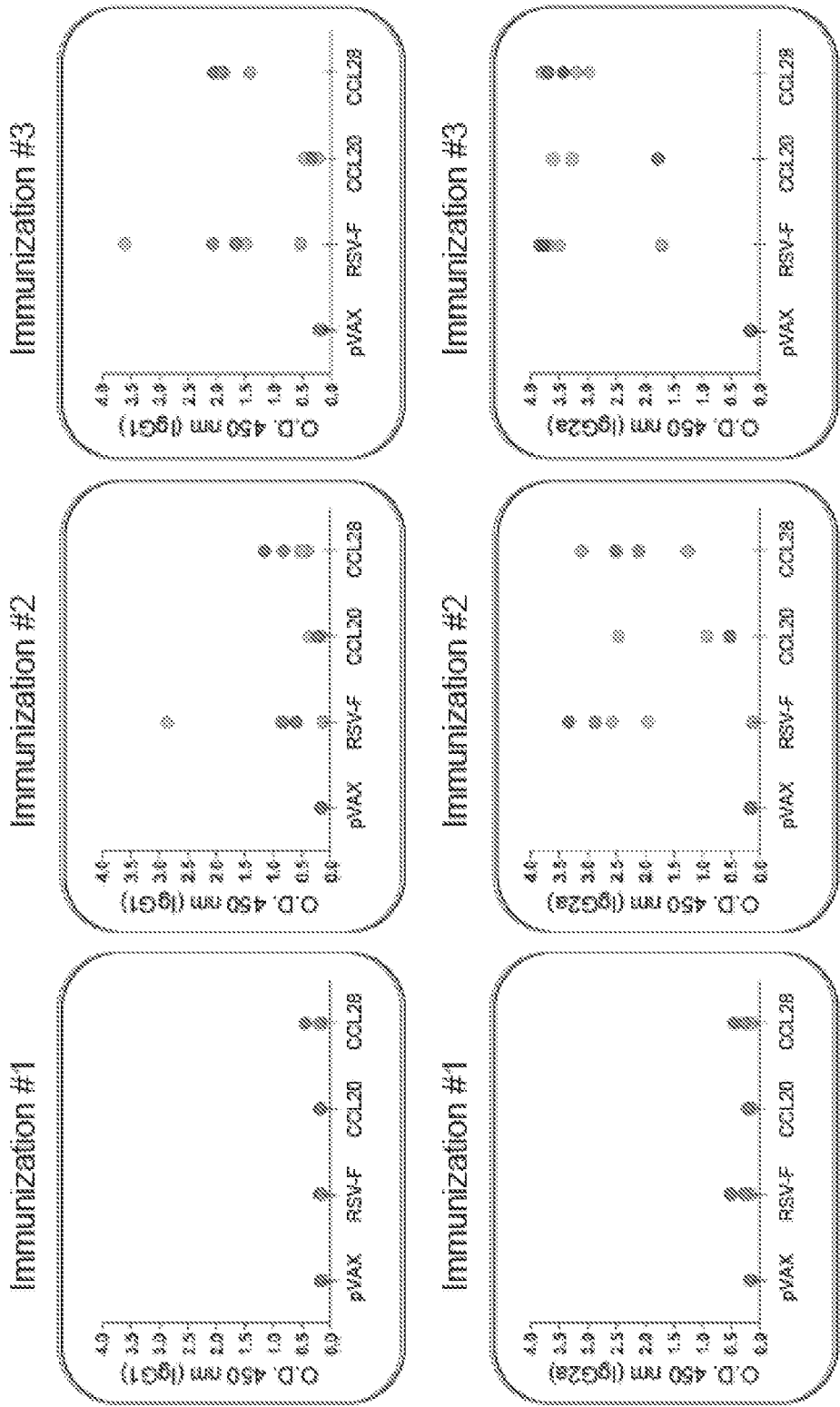


Figure 7  
Comparison of IFN- $\gamma$ + CD8 or CD4 T cells in lung after RSV-F +/- chemokine immunization





**Figure 8**  
Comparison of IgG subtypes (IgG1 vs IgG2a) in Sera



**Figure 9**  
Comparison of IgG2a/IgG Ratio

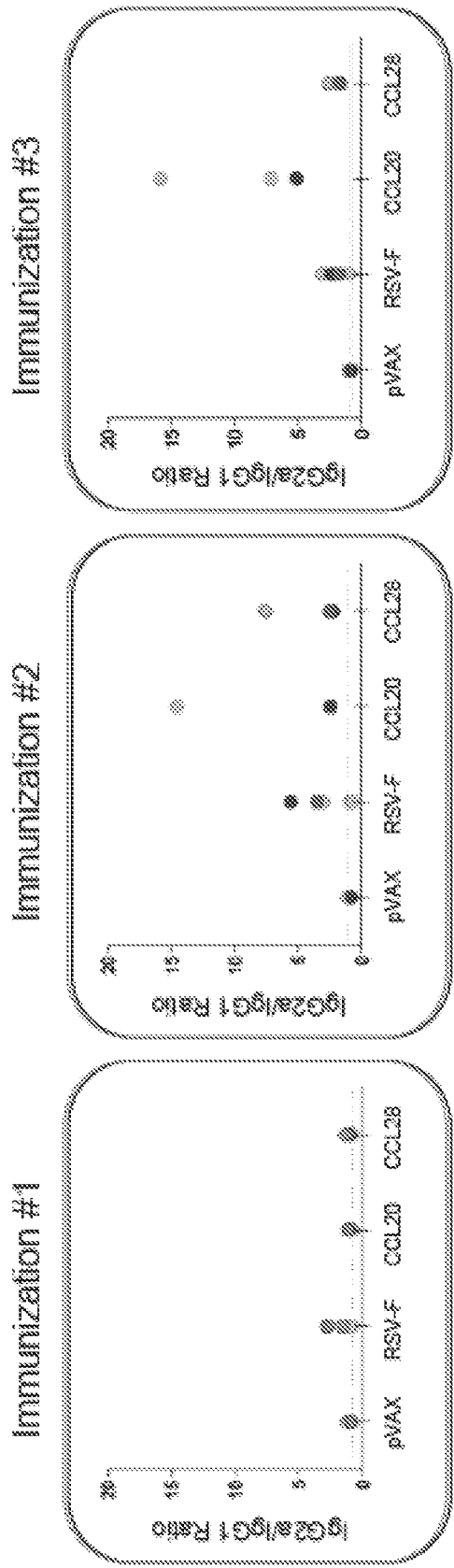


Figure 10  
Env-Pseudotyped viruses in TzM-BL Cells/ "ON"

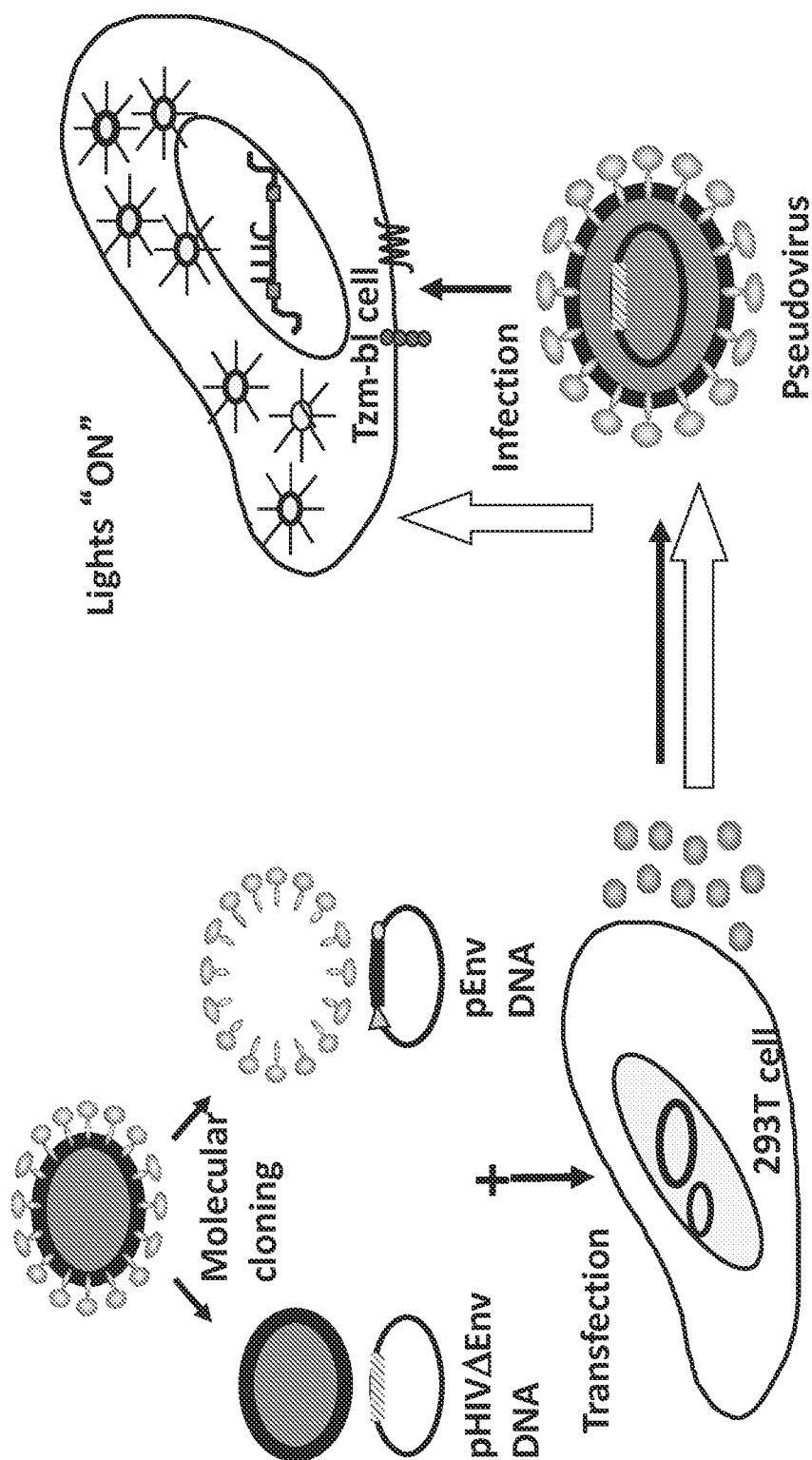
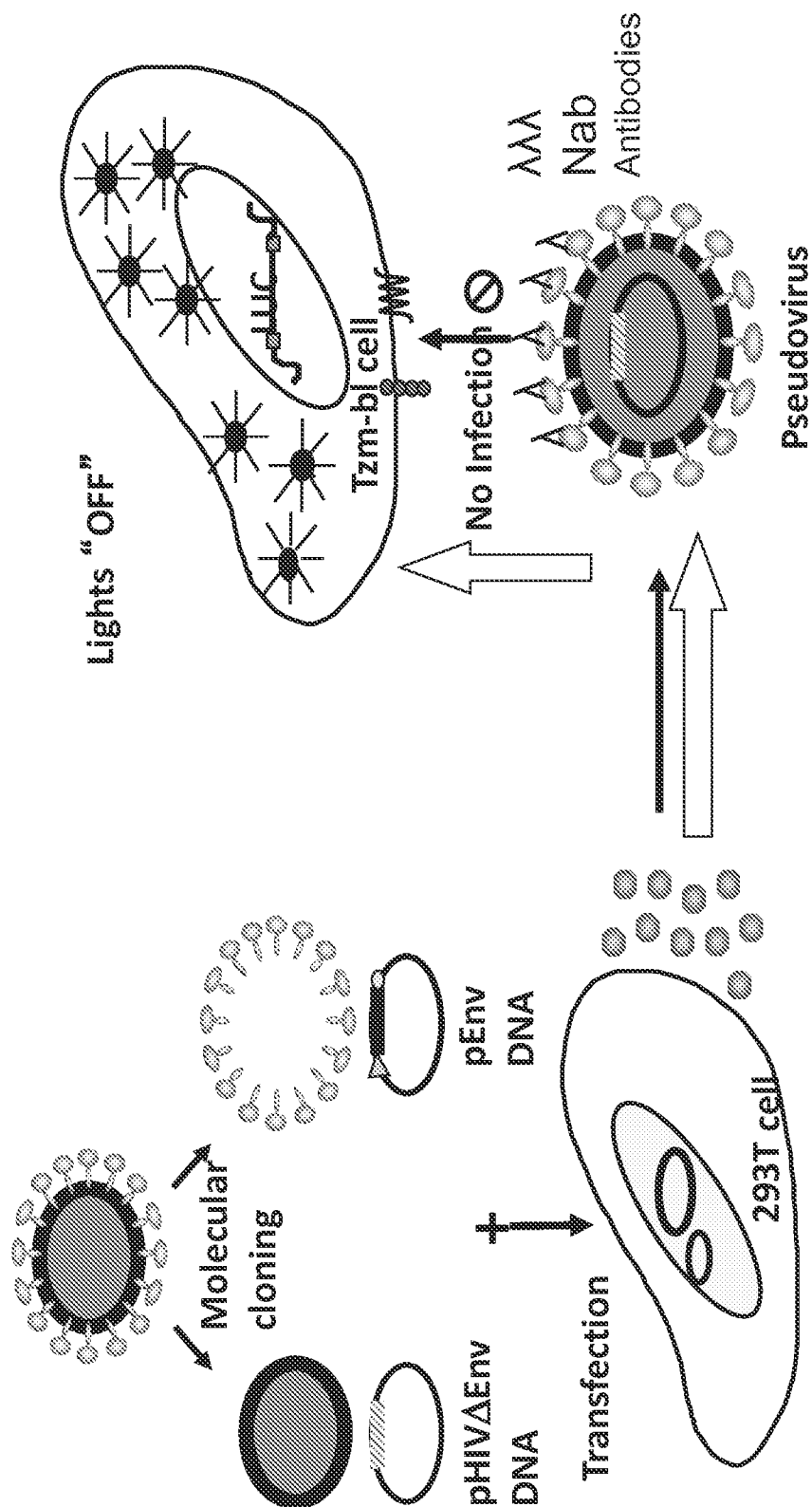
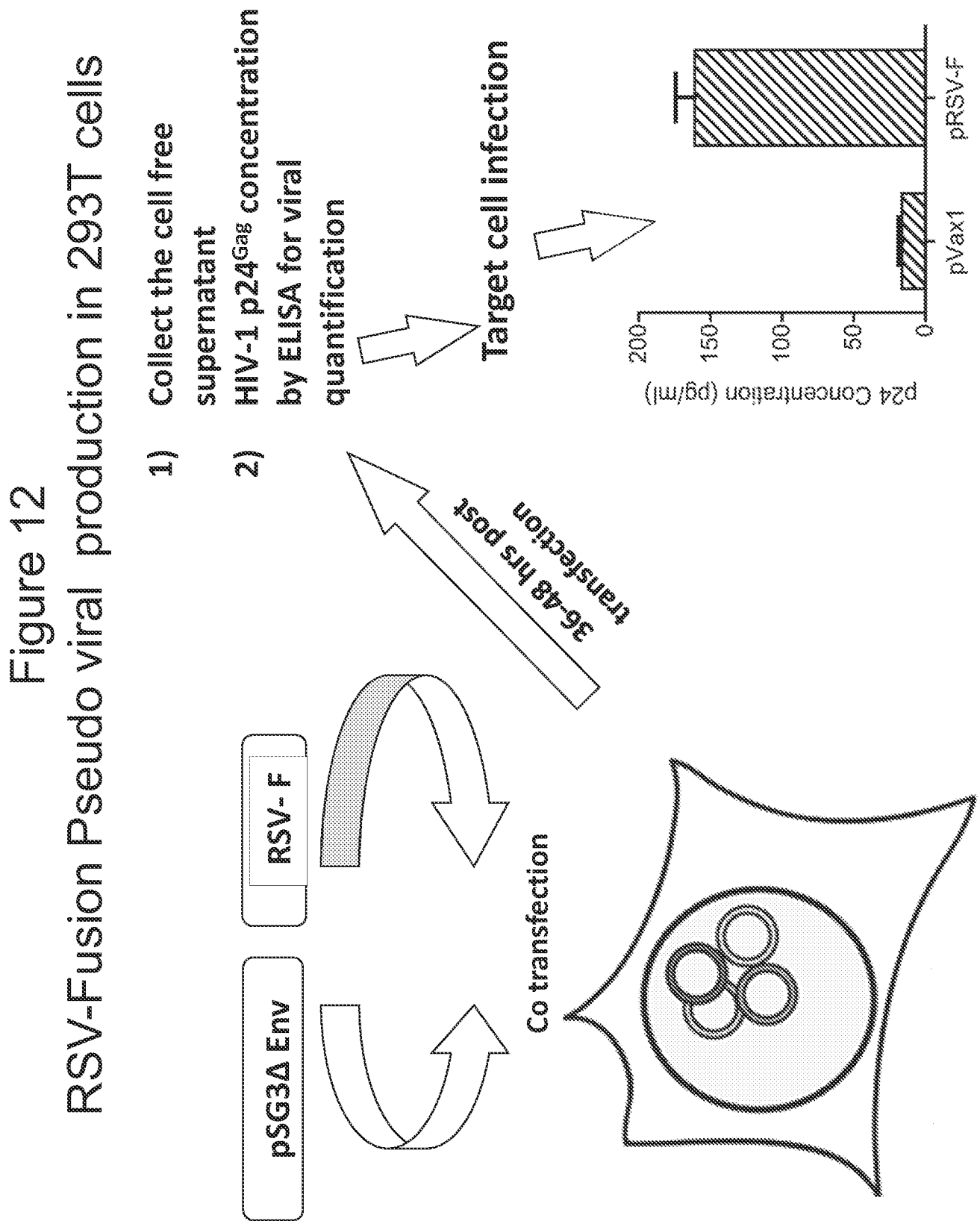


Figure 11  
Env-Pseudotyped viruses in TZM-BL Cells/ "OFF"





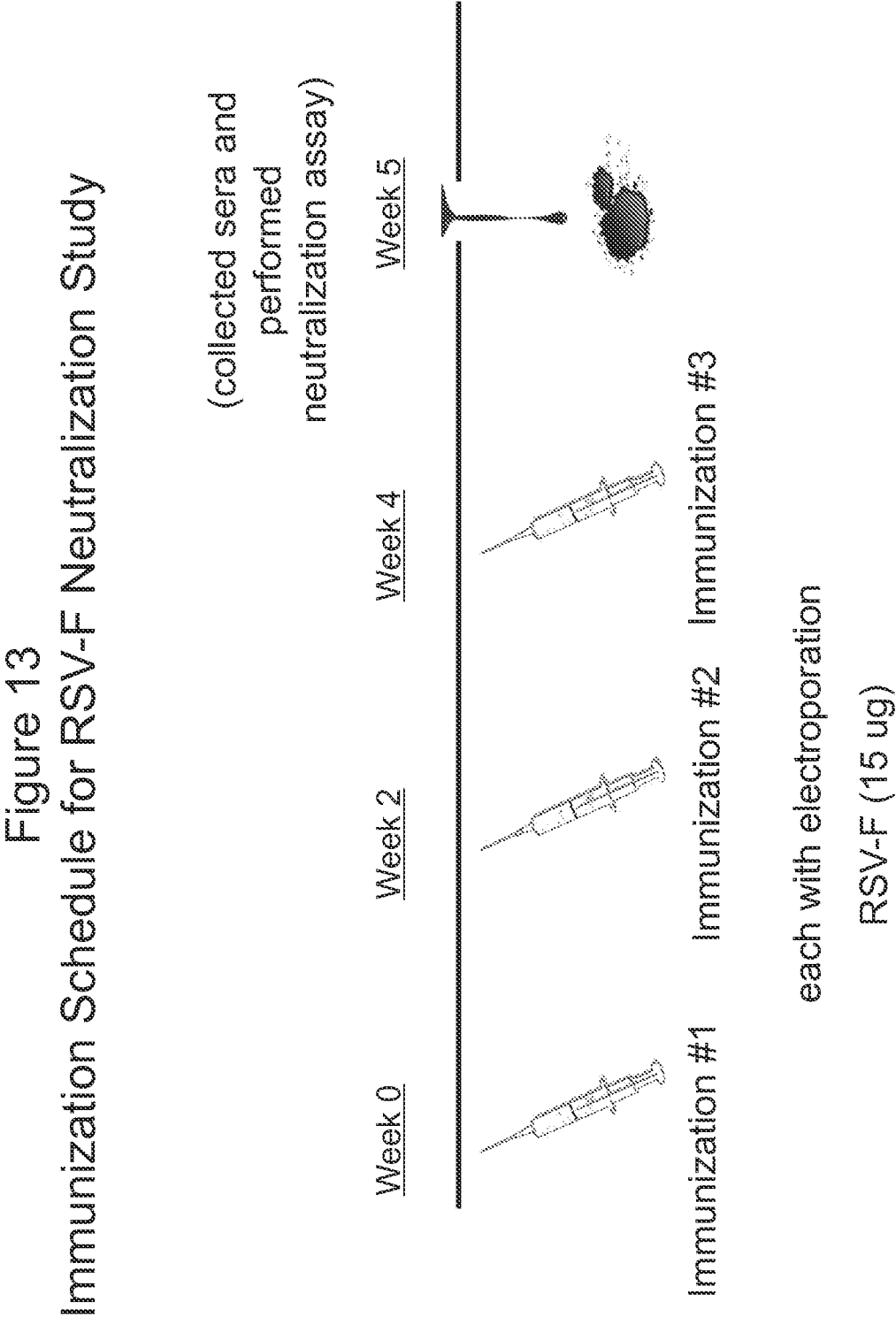


Figure 14

Quantification of RSV-F mediated infection  
in different target cells

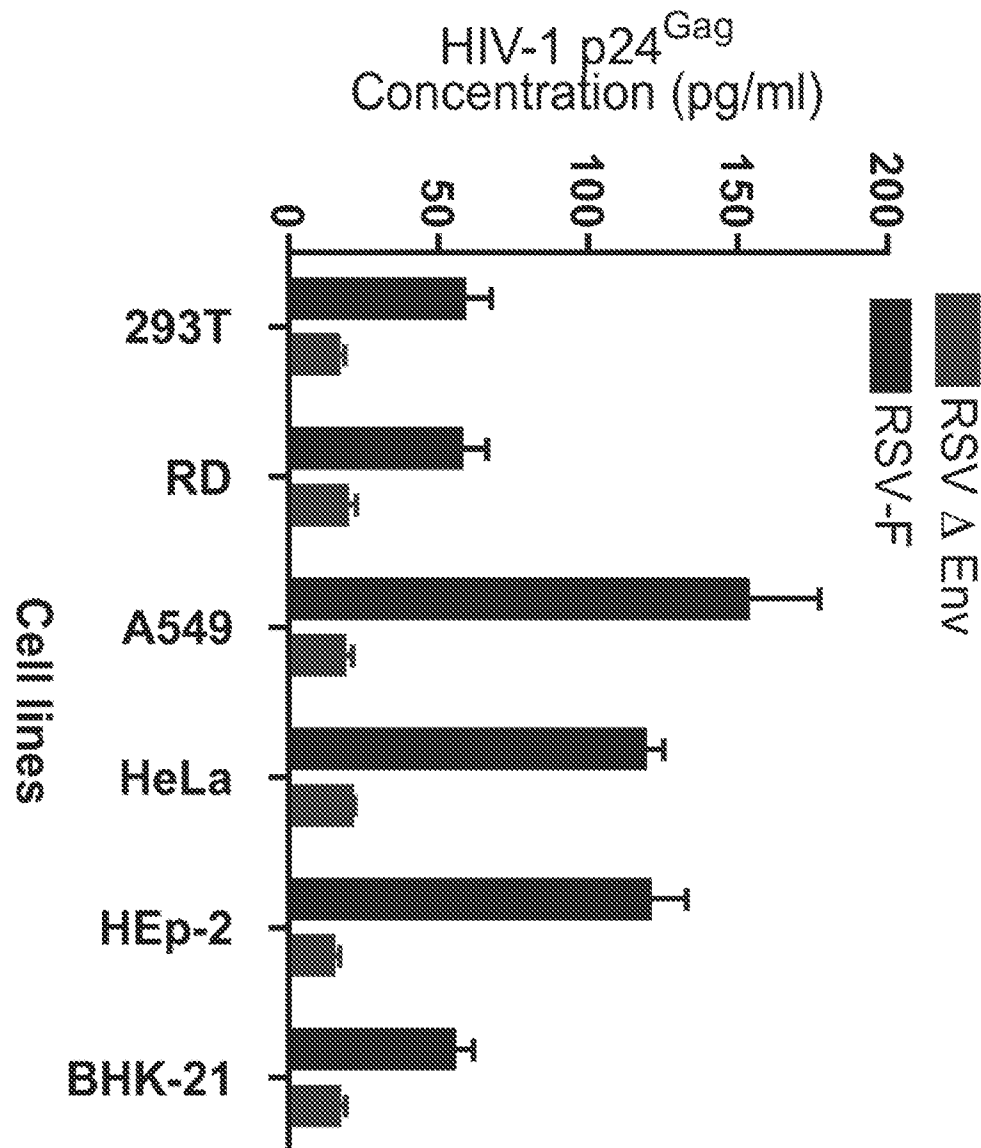


Figure 15  
Quantification of RSV-F neutralization activity in TZM-bl HeLa cells

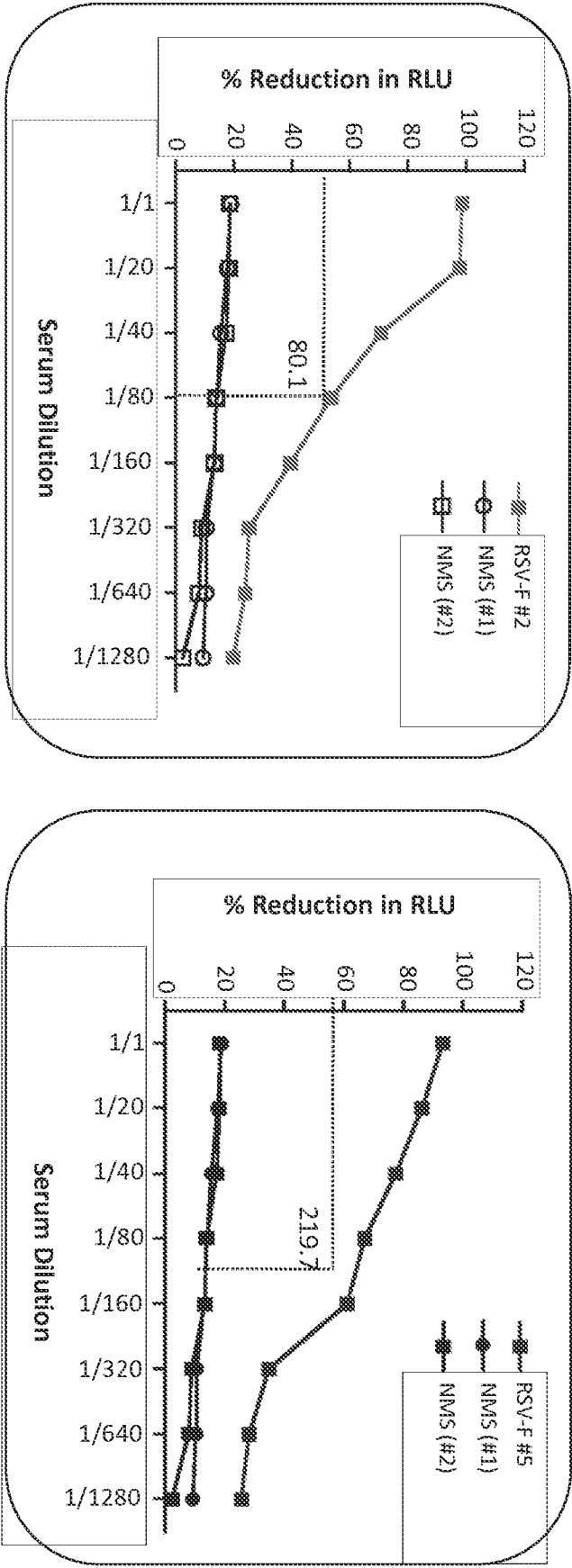




Figure 16  
Quantification of cell death activity by RSV-F pseudo virus in HeLa cells

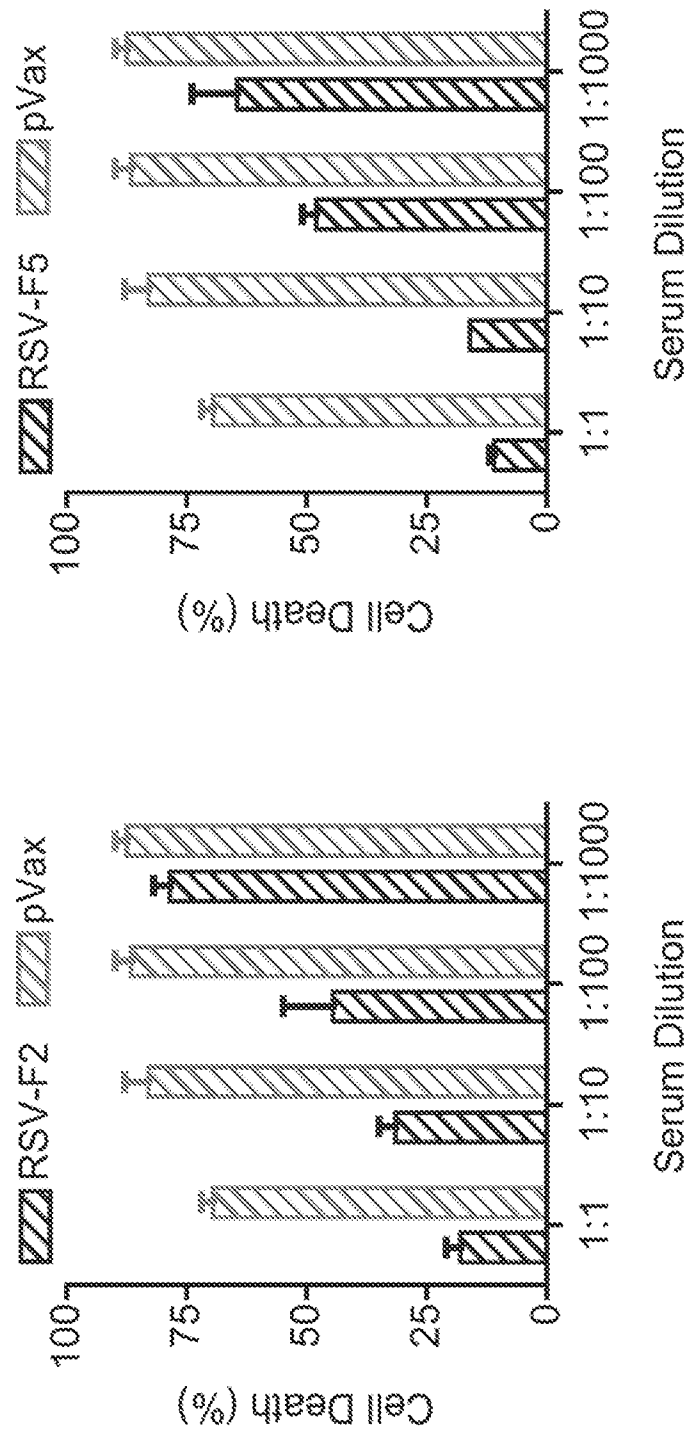


Figure 17  
Immunization Schedule for RSV-M2 Dosage Study

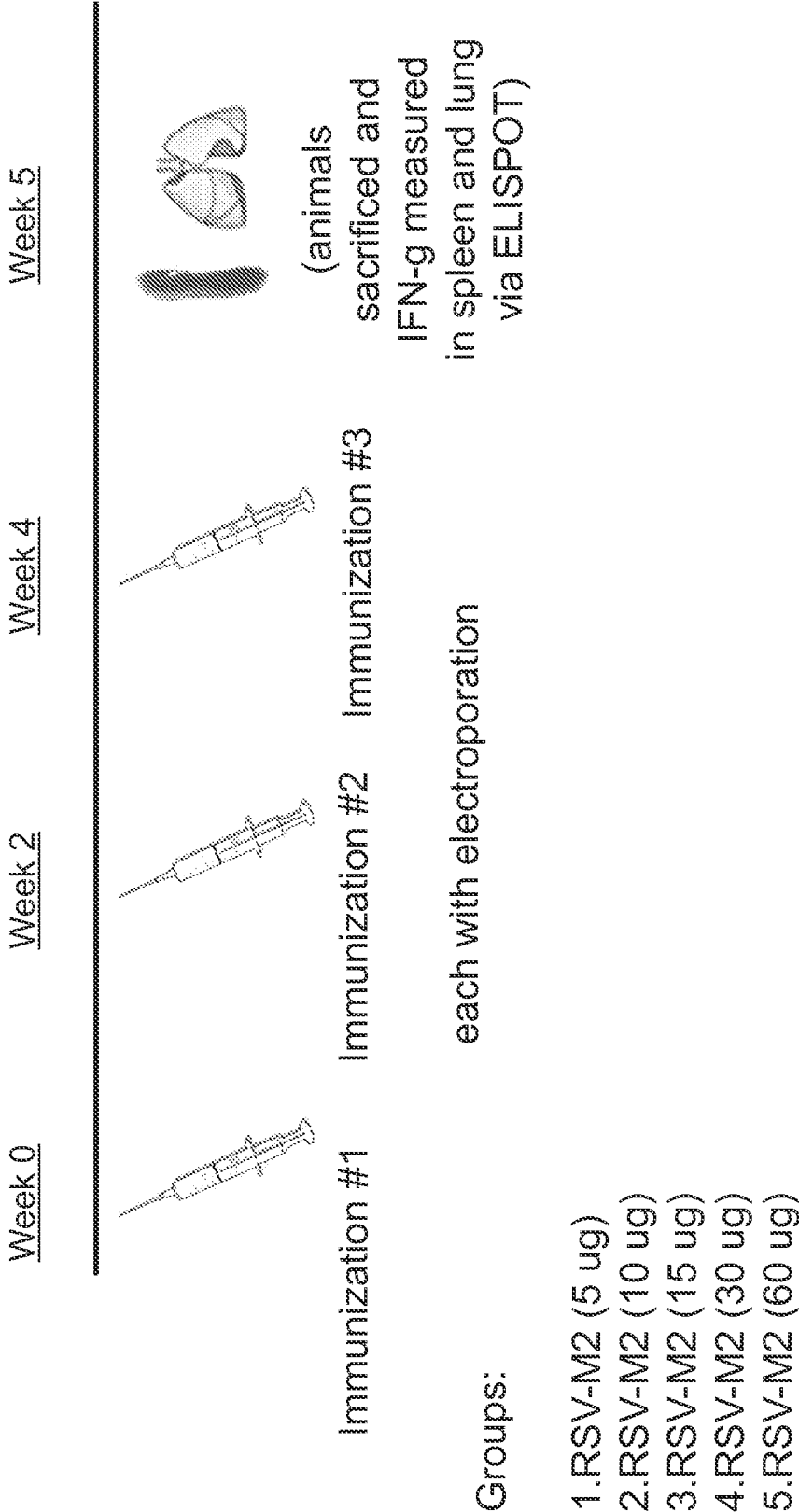


Figure 18  
Comparison of IFN-g production at different dosages of RSV-M2 vaccine 1  
week after last immunization

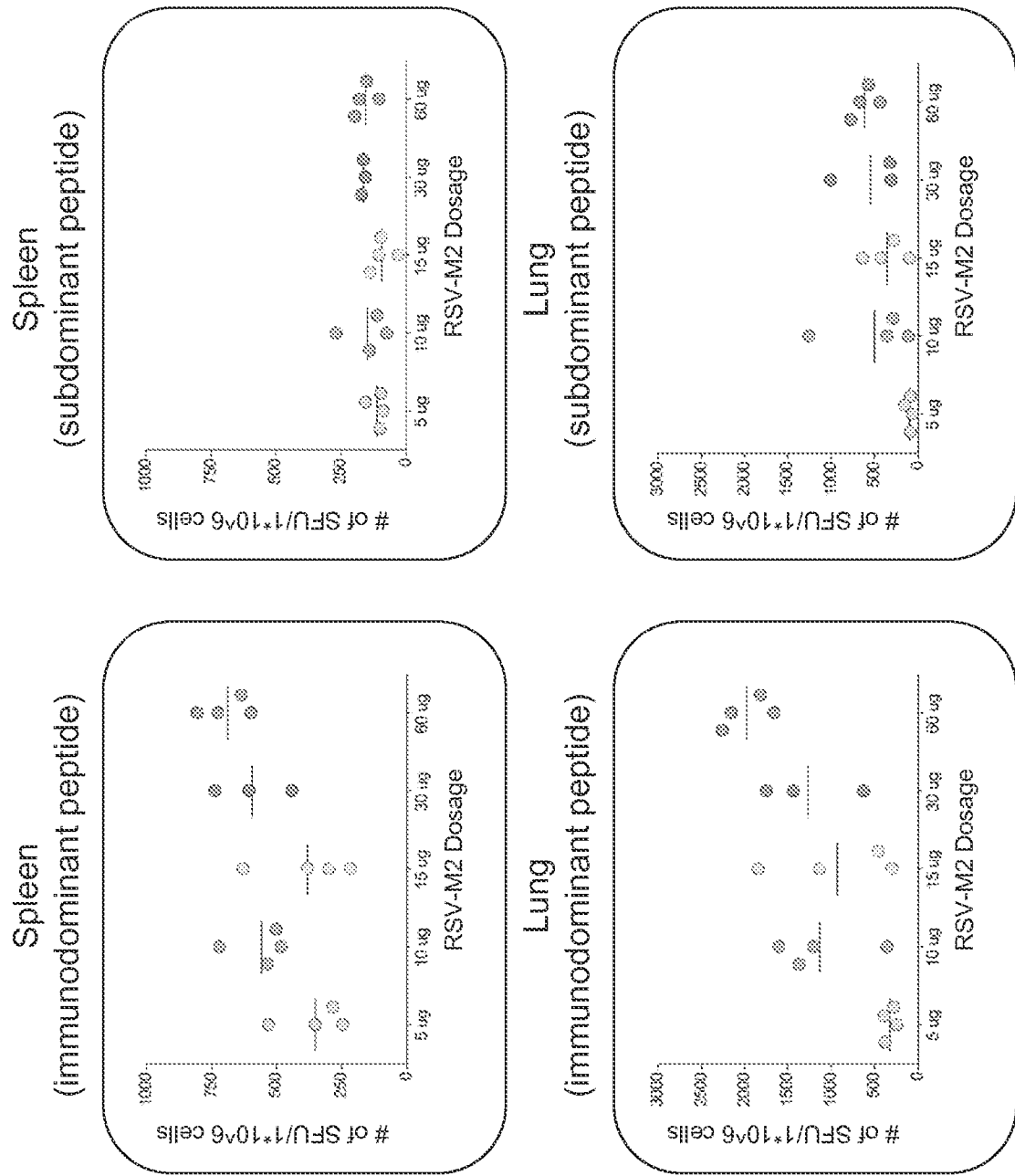


Figure 19  
Immunization Schedule for RSV-F + CCL20 Study

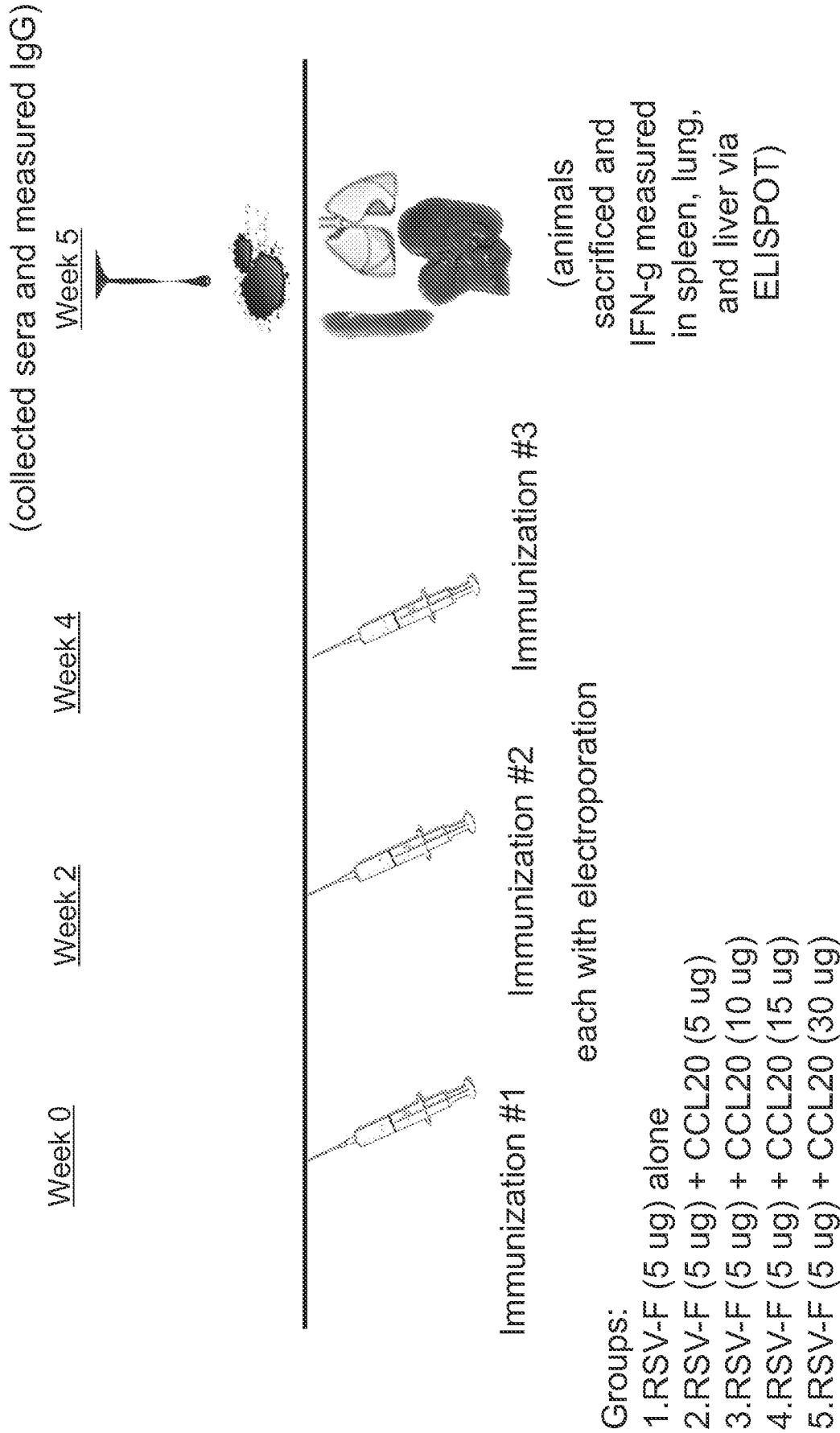


Figure 20  
Comparison of IFN-g production with different dosages of CCL20 at 1 week after last immunization

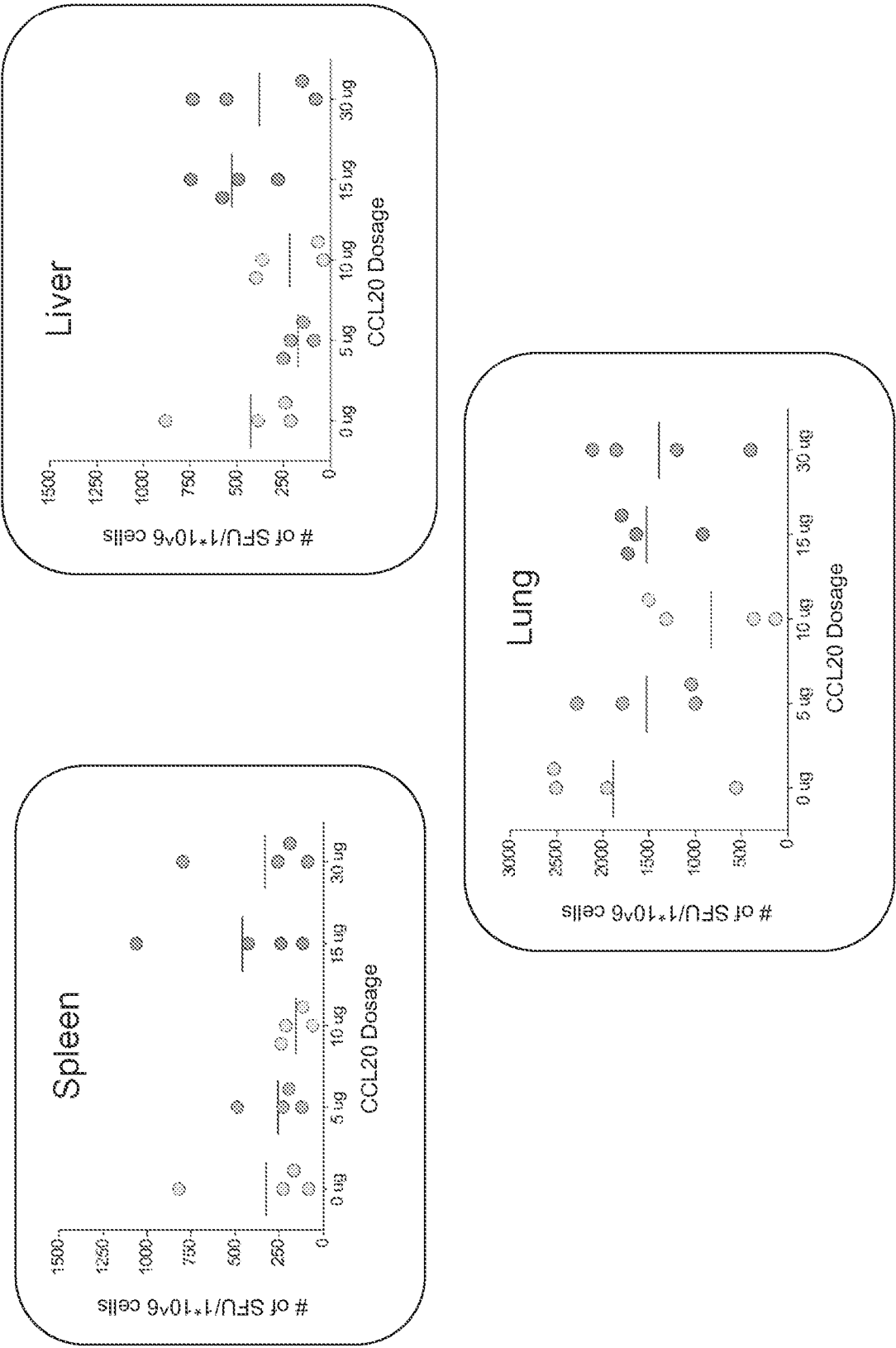


Figure 21  
Comparison of IgG subtypes (IgG1 vs IgG2a) in Sera

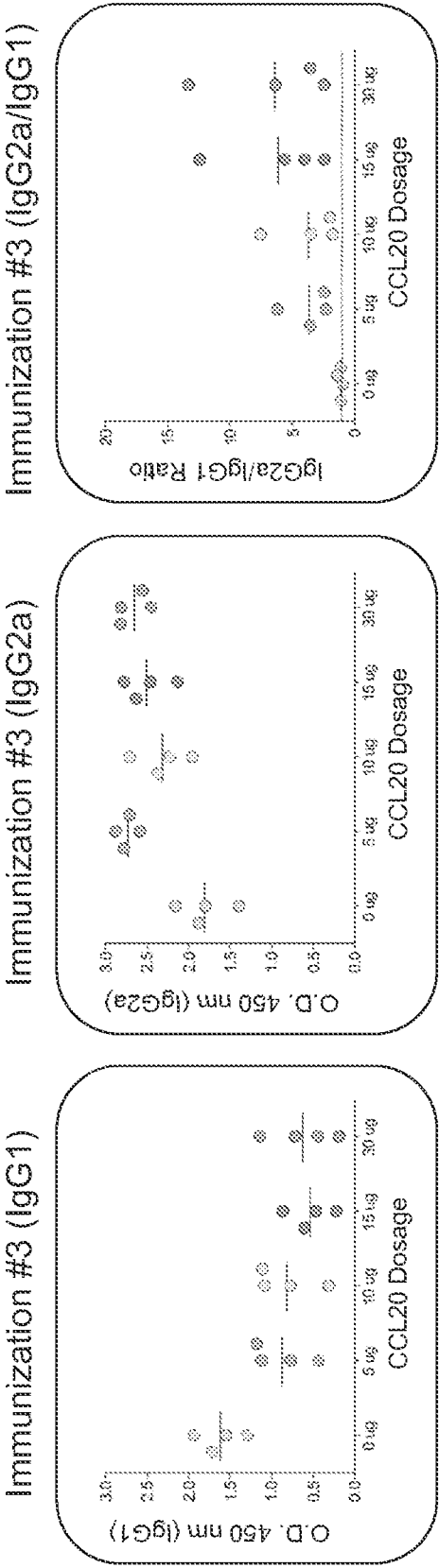


Figure 22A

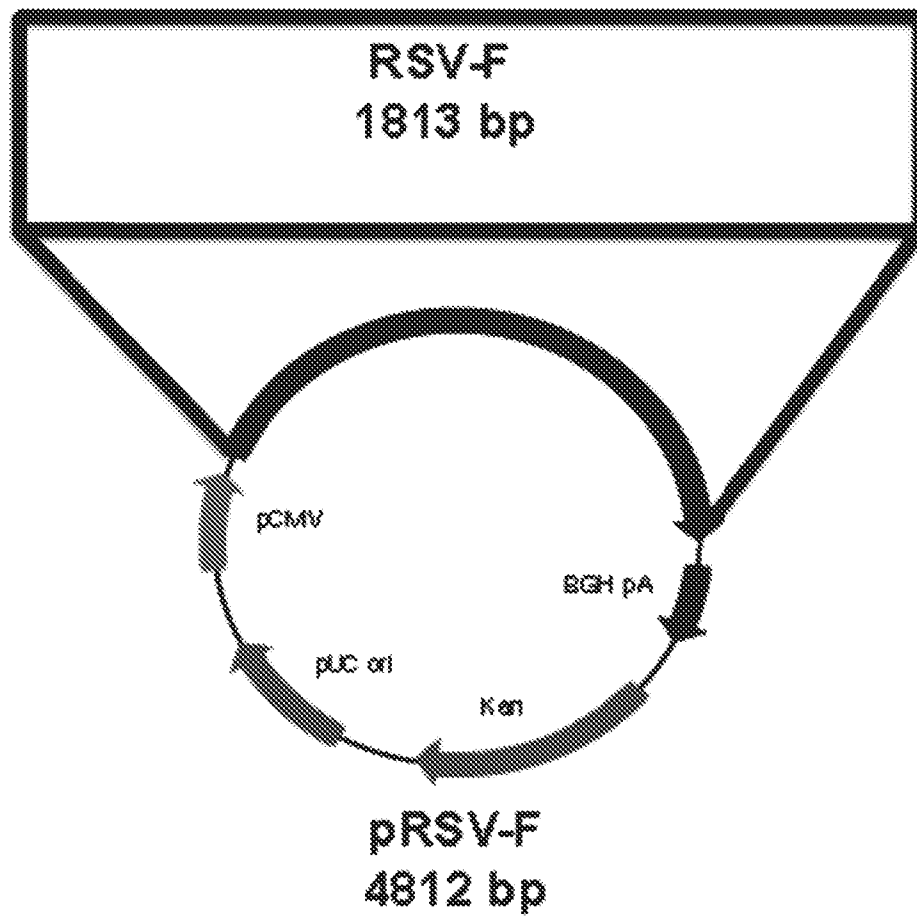


Figure 22B

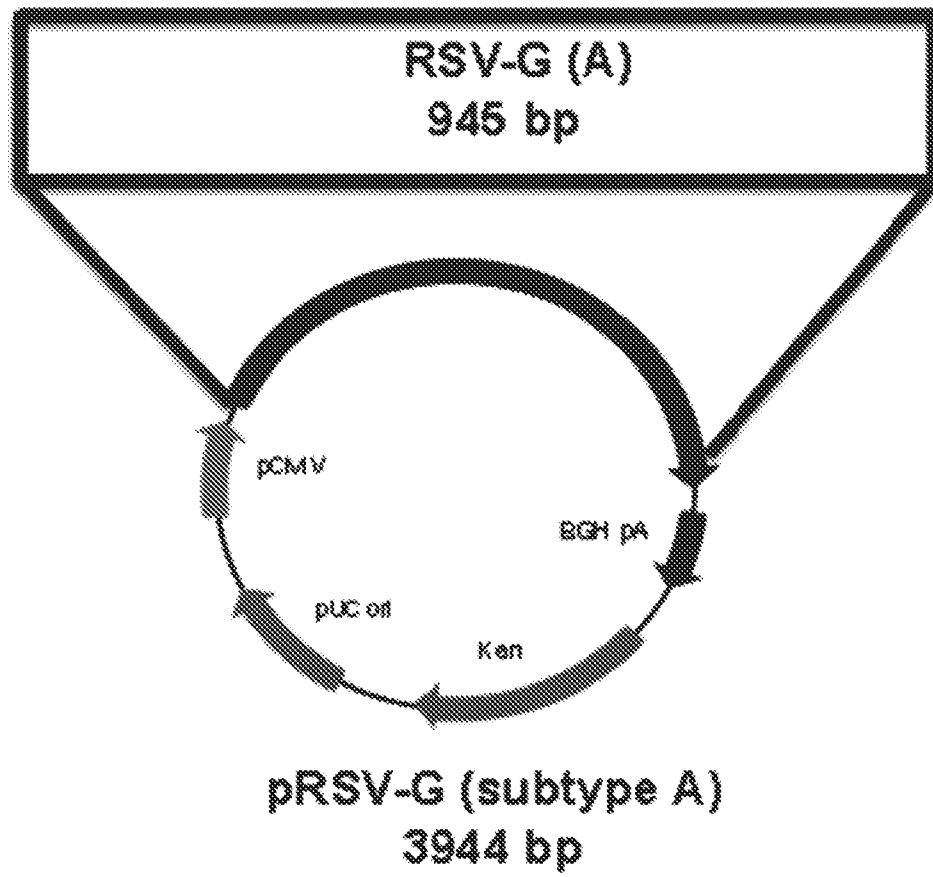




Figure 22C

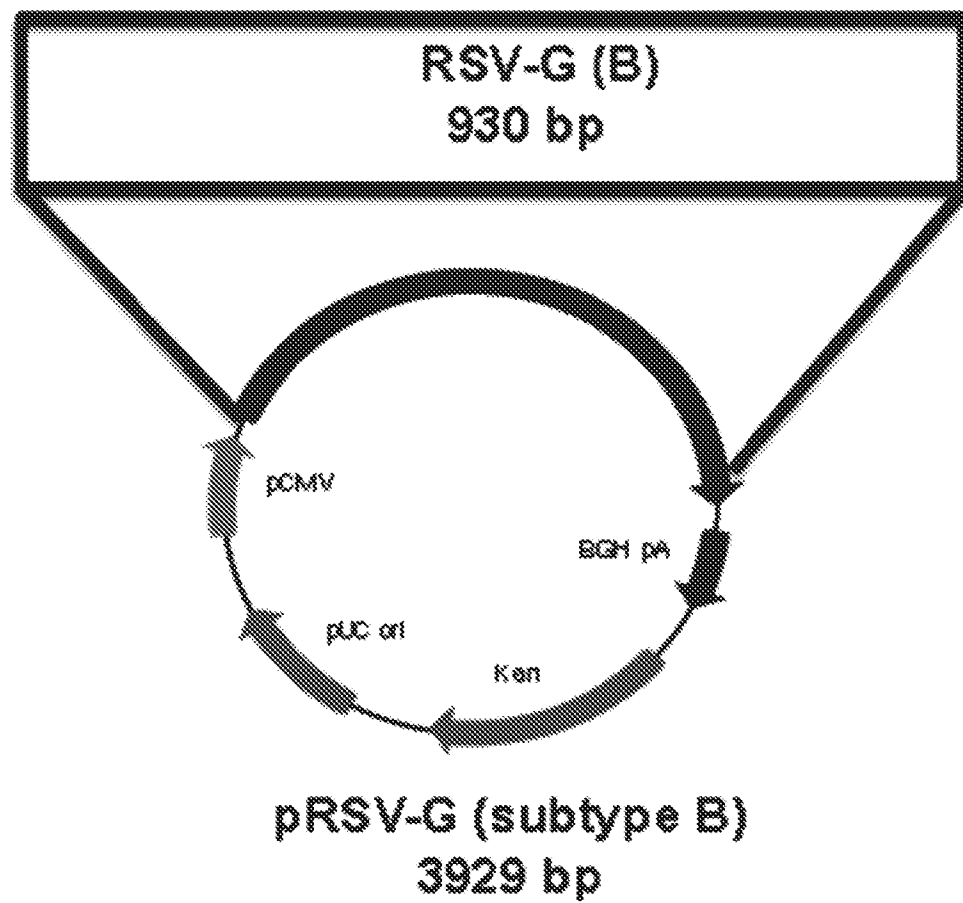


Figure 23

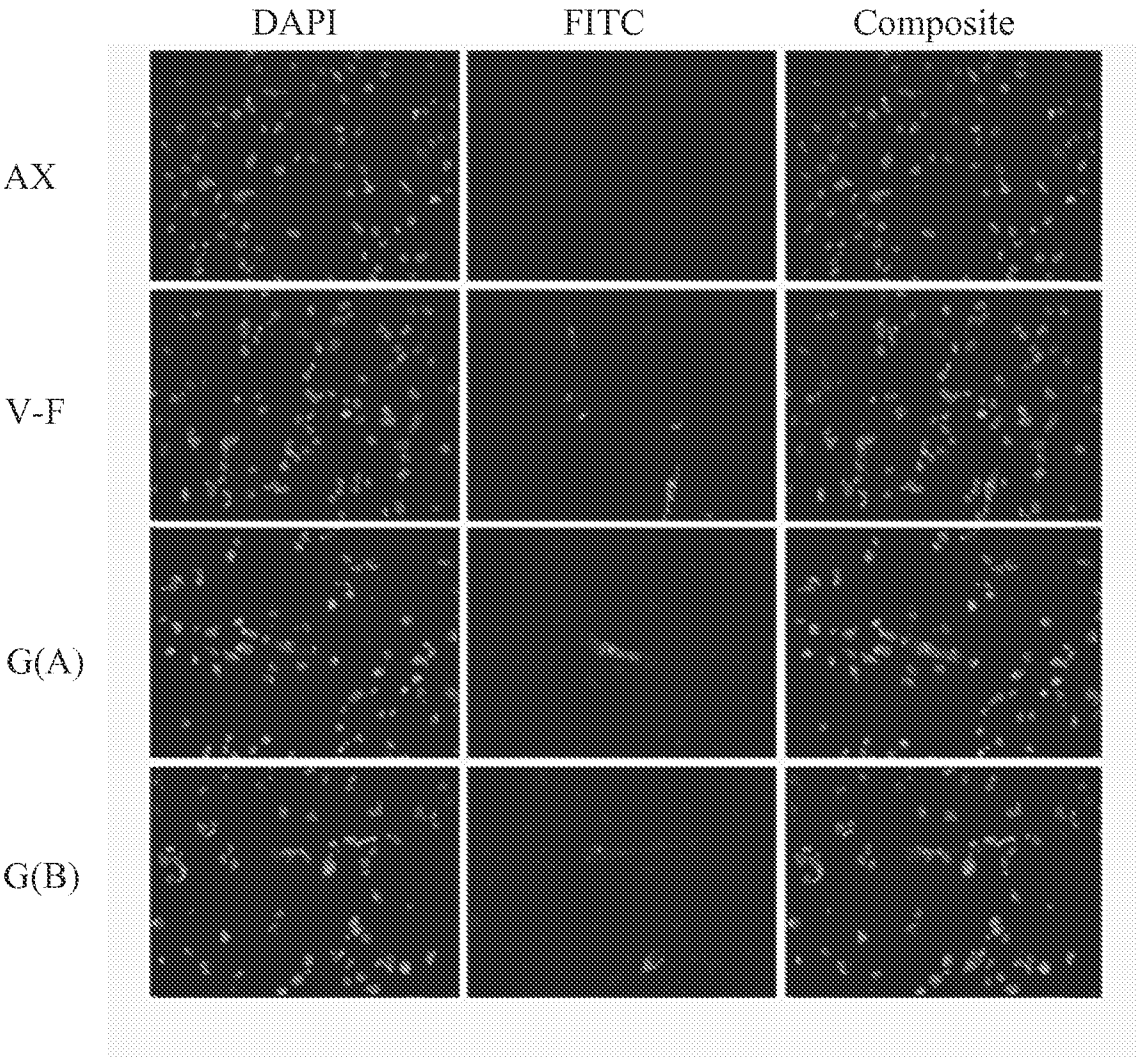


Figure 24  
Immunization Schedule for RSV-F Dosage Study

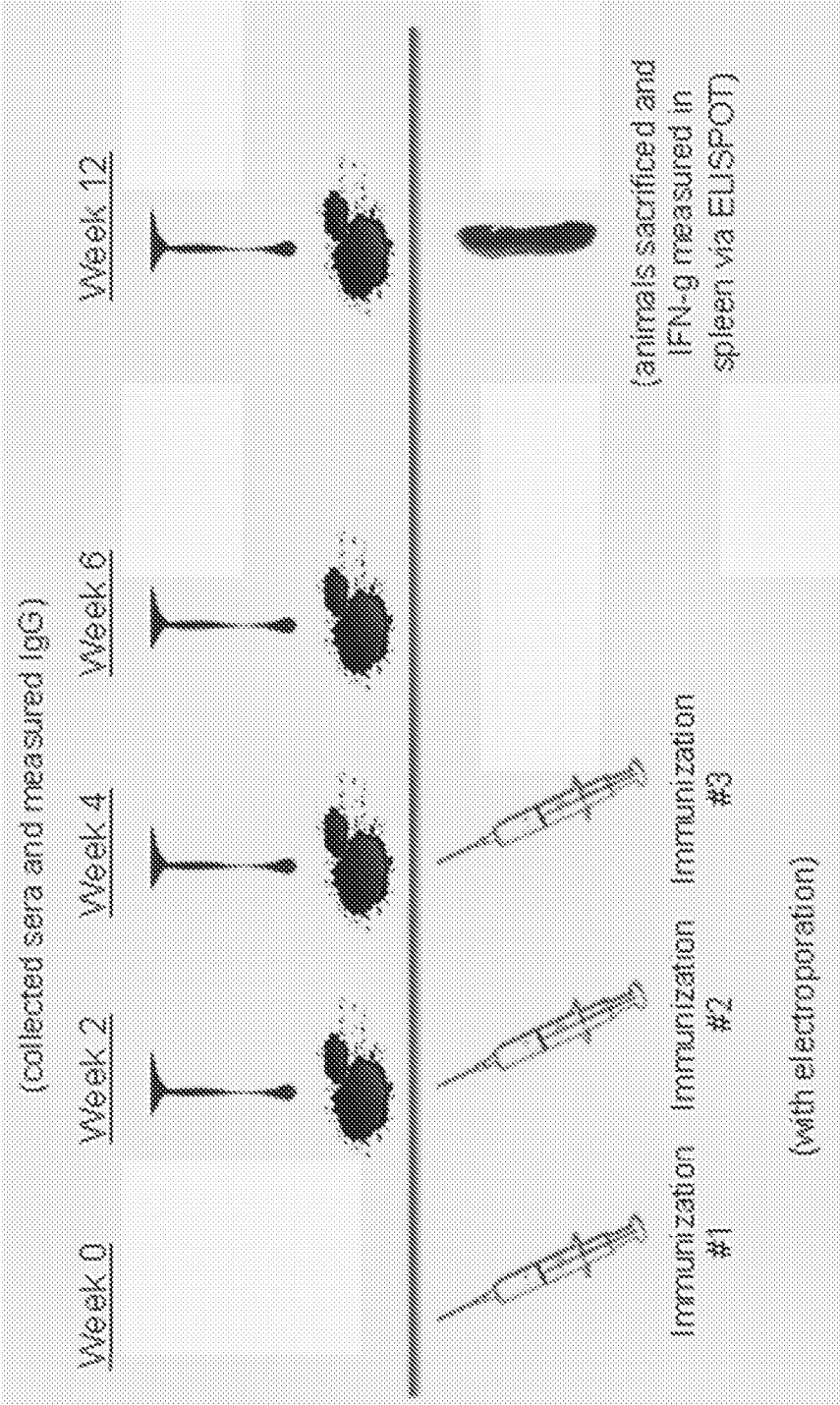


Figure 25

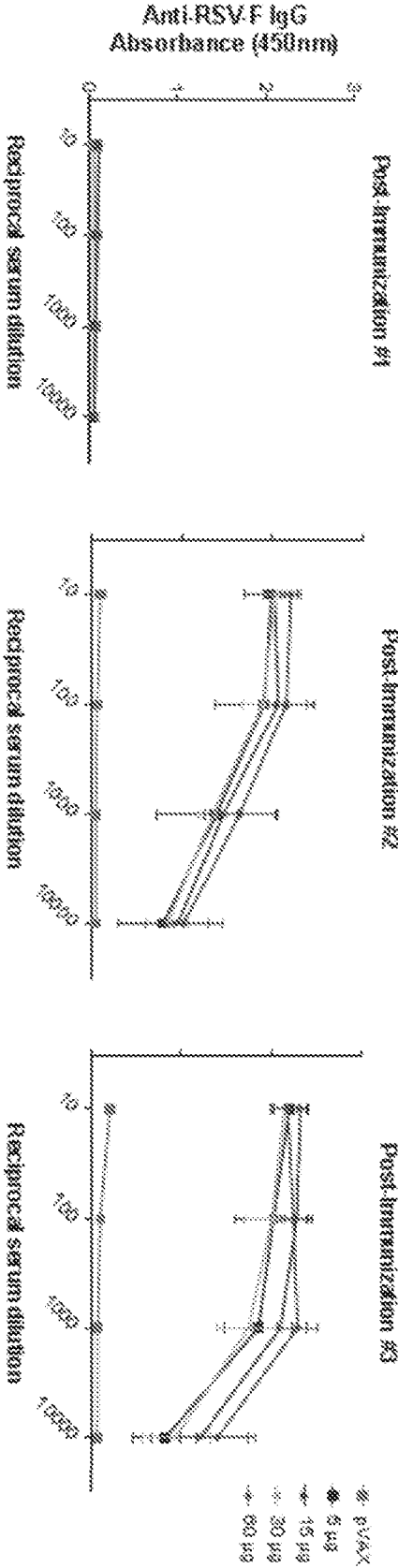


Figure 26

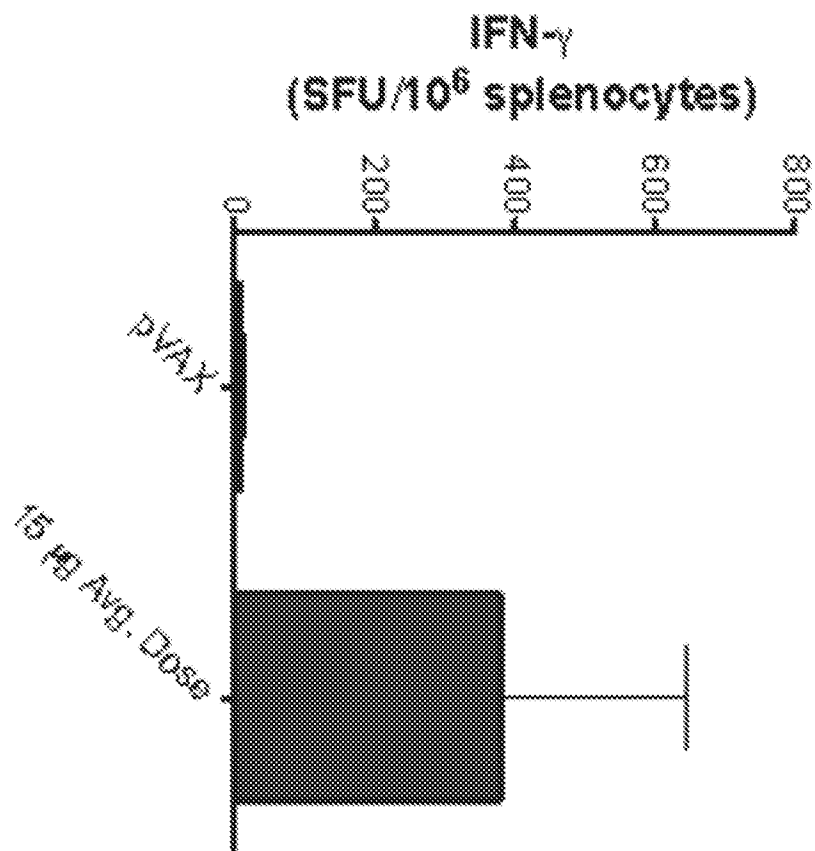


Figure 27

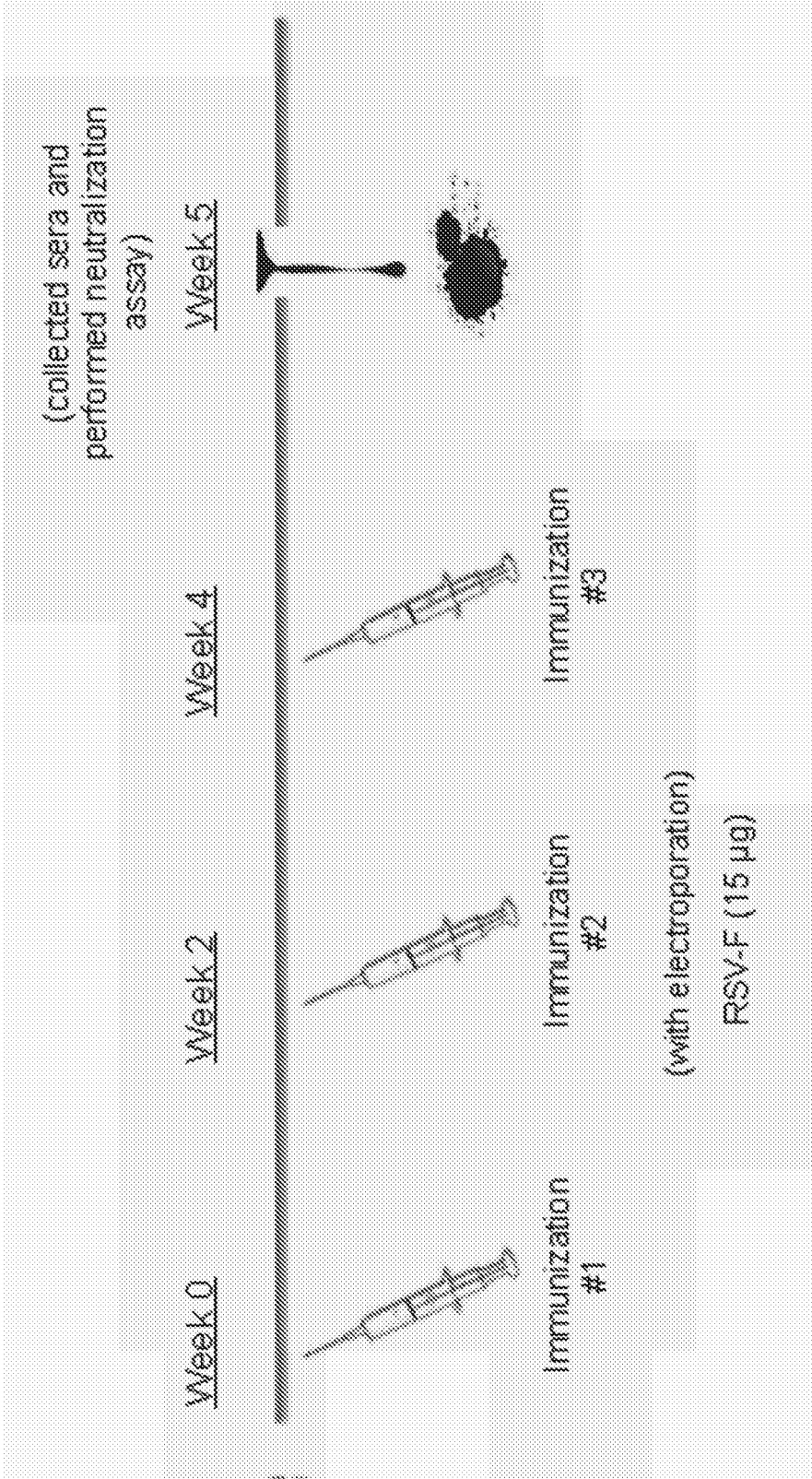
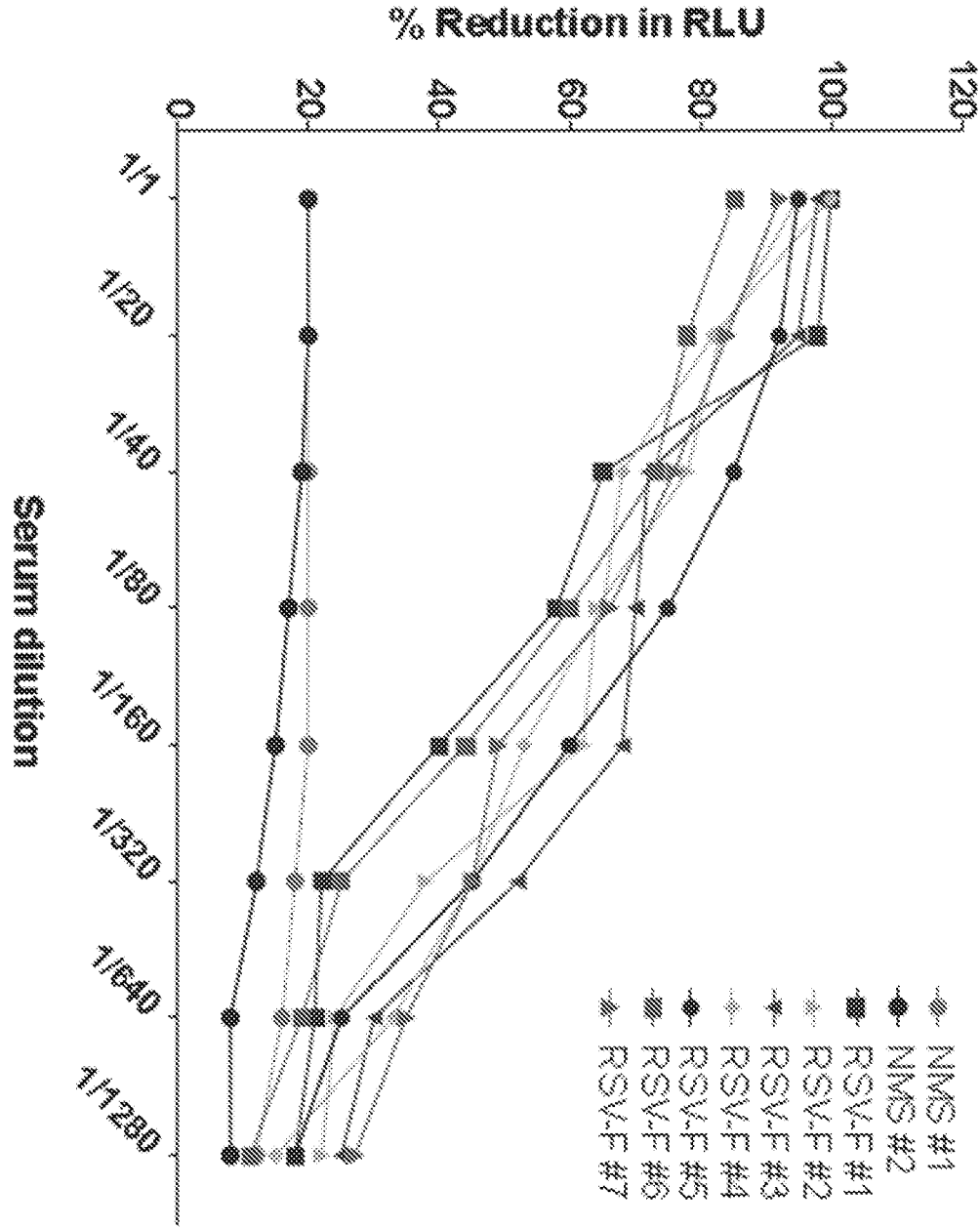


Figure 28



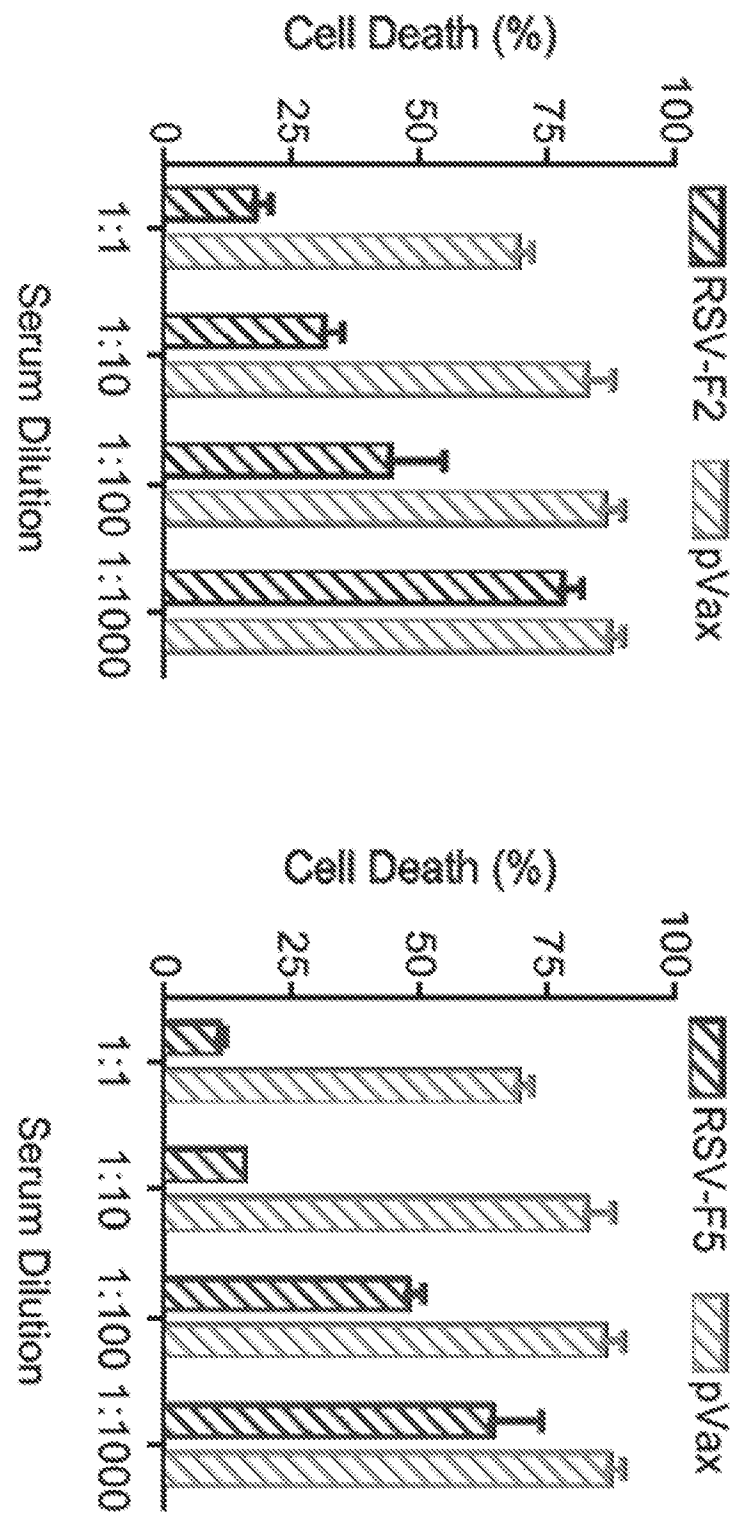


Figure 29



Figure 30

Immunization Schedule for RSV-G(A) and RSV-(B) Dosage Study

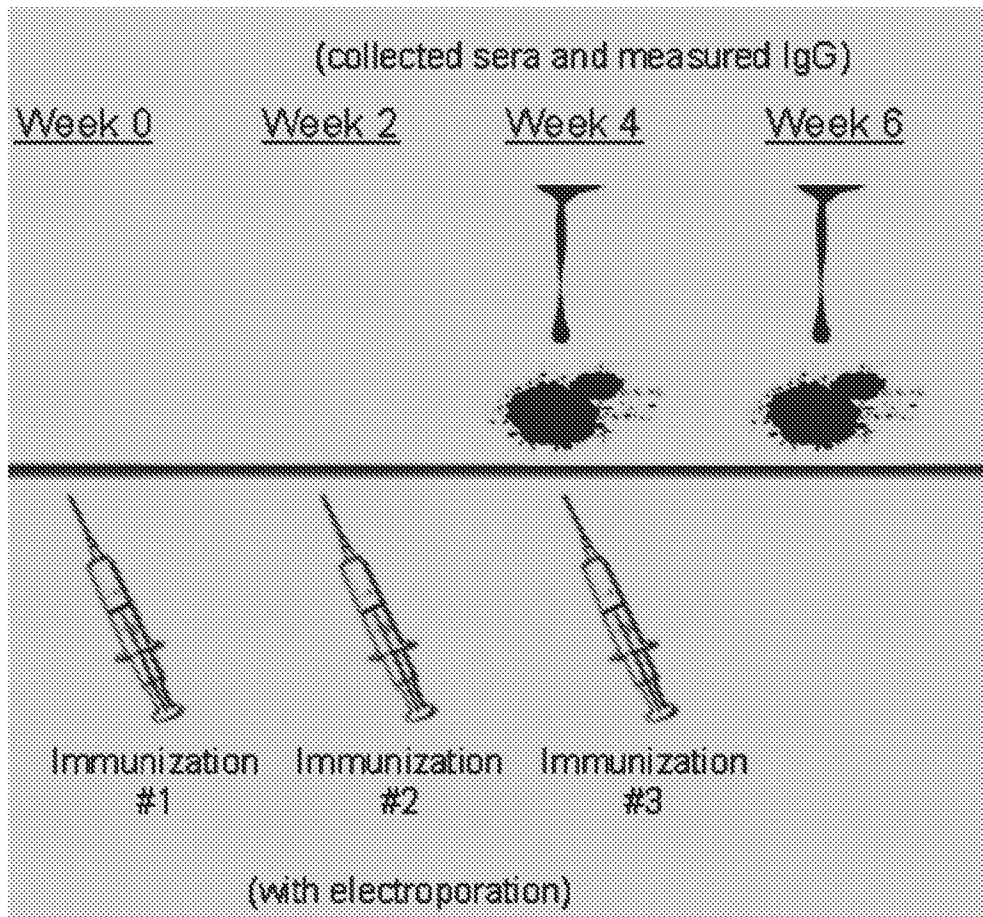


Figure 31

Total IgG in Sera after RSV-G(A) DNA immunization

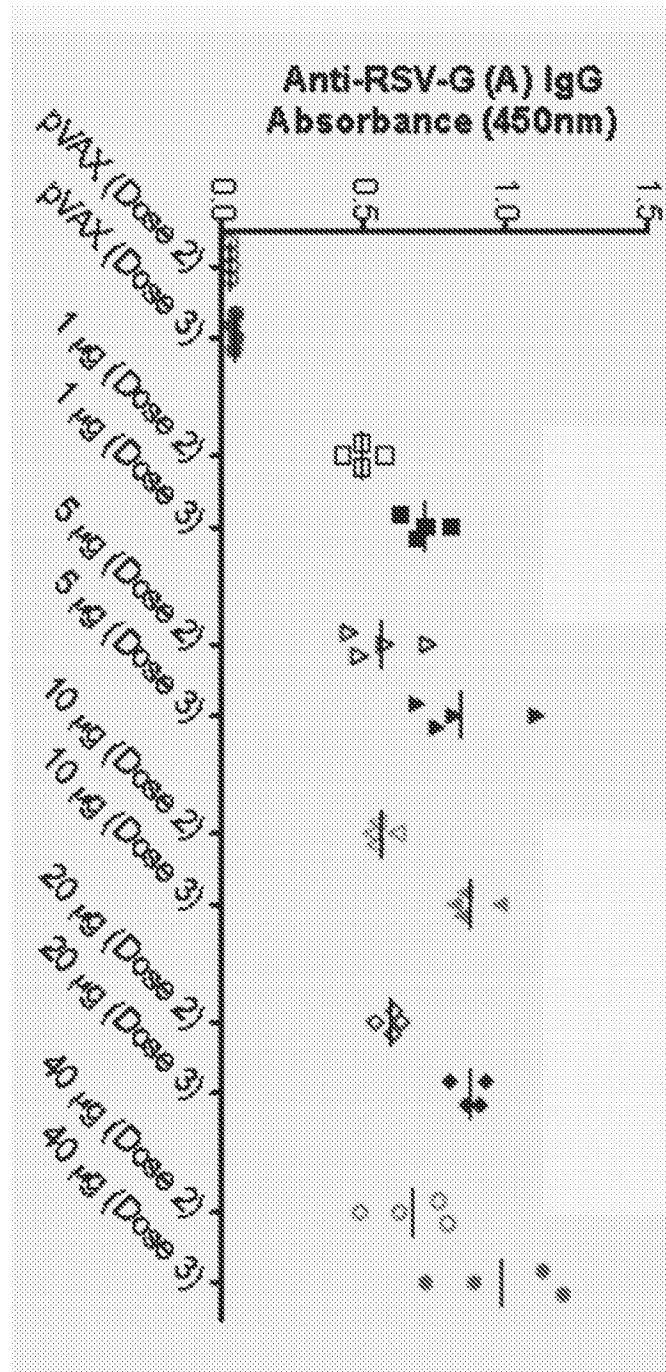


Figure 32

Total IgG in Sera after RSV-G(B) DNA immunization

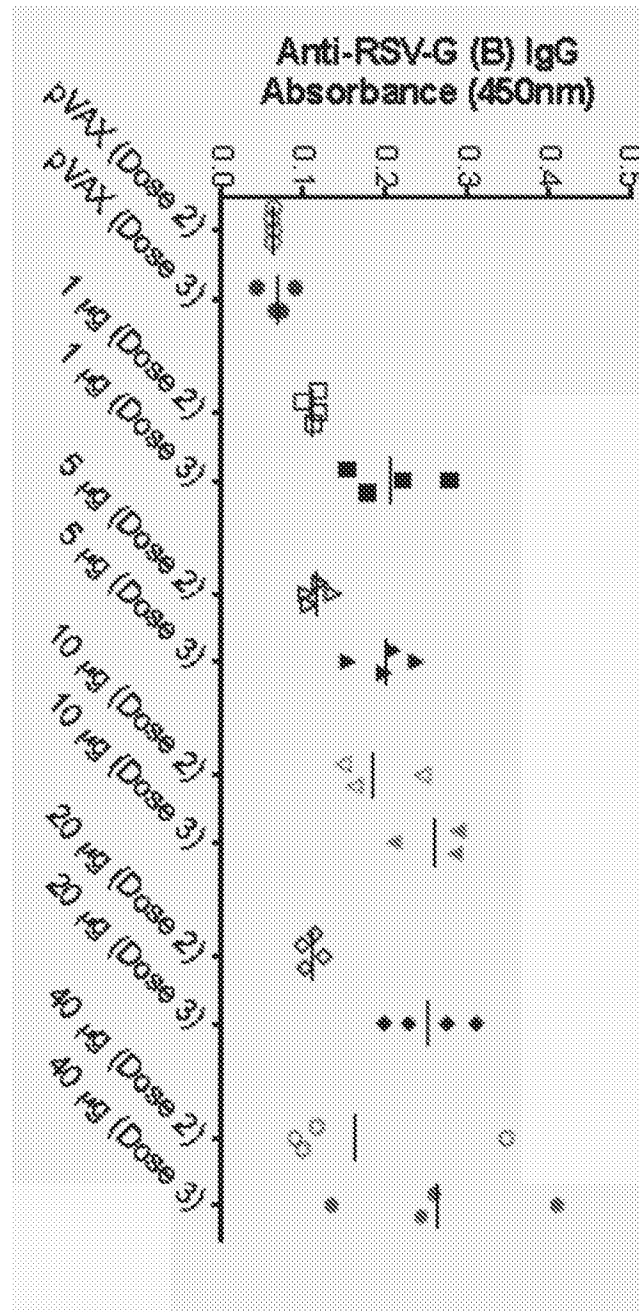


Figure 33  
Preliminary Neutralization Results from Mono Vaccine Mouse Studies

Sample Tested	Neut Titer Post 3d Imm
Negative serum	0
Mab anti RSV	40
Polyclonal Serum	160
Mouse 1-F Ag	320
Mouse 2-F Ag	160
Mouse 3- GA	80
Mouse 4 - GA	320
Mouse 14 -GB	160

Figure 34

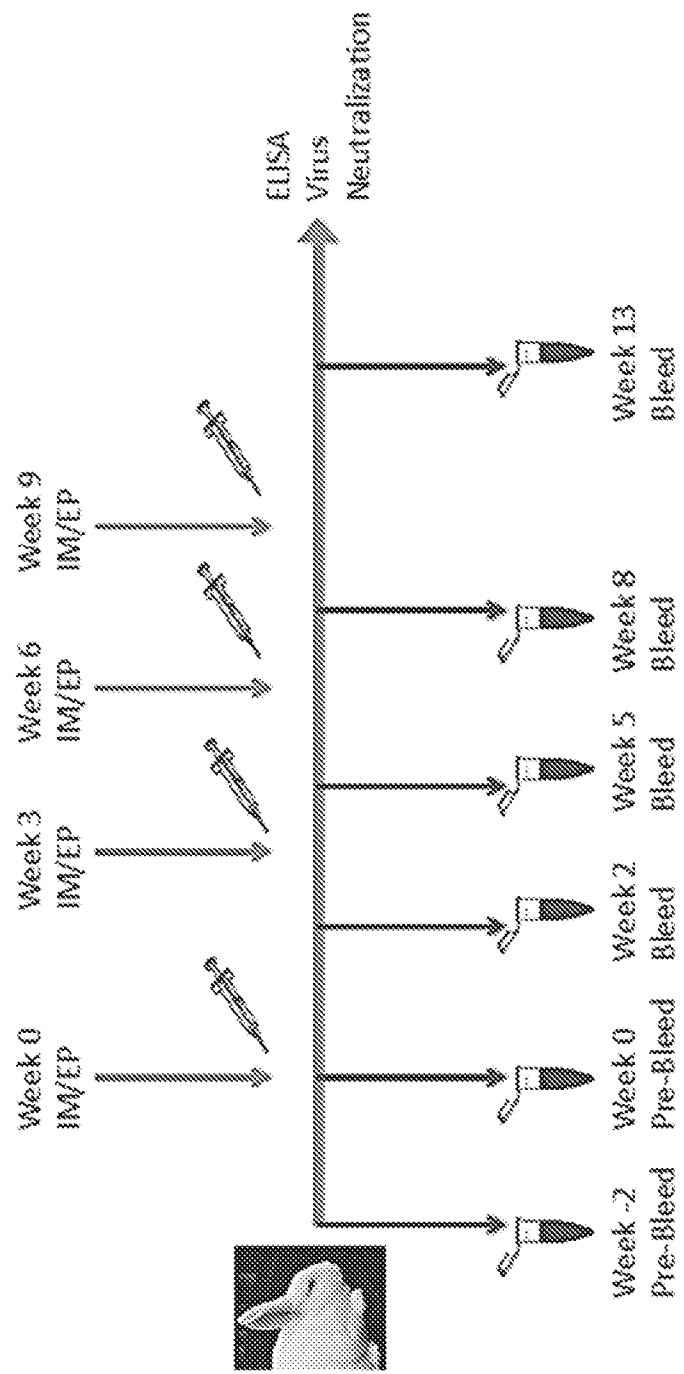


Figure 35

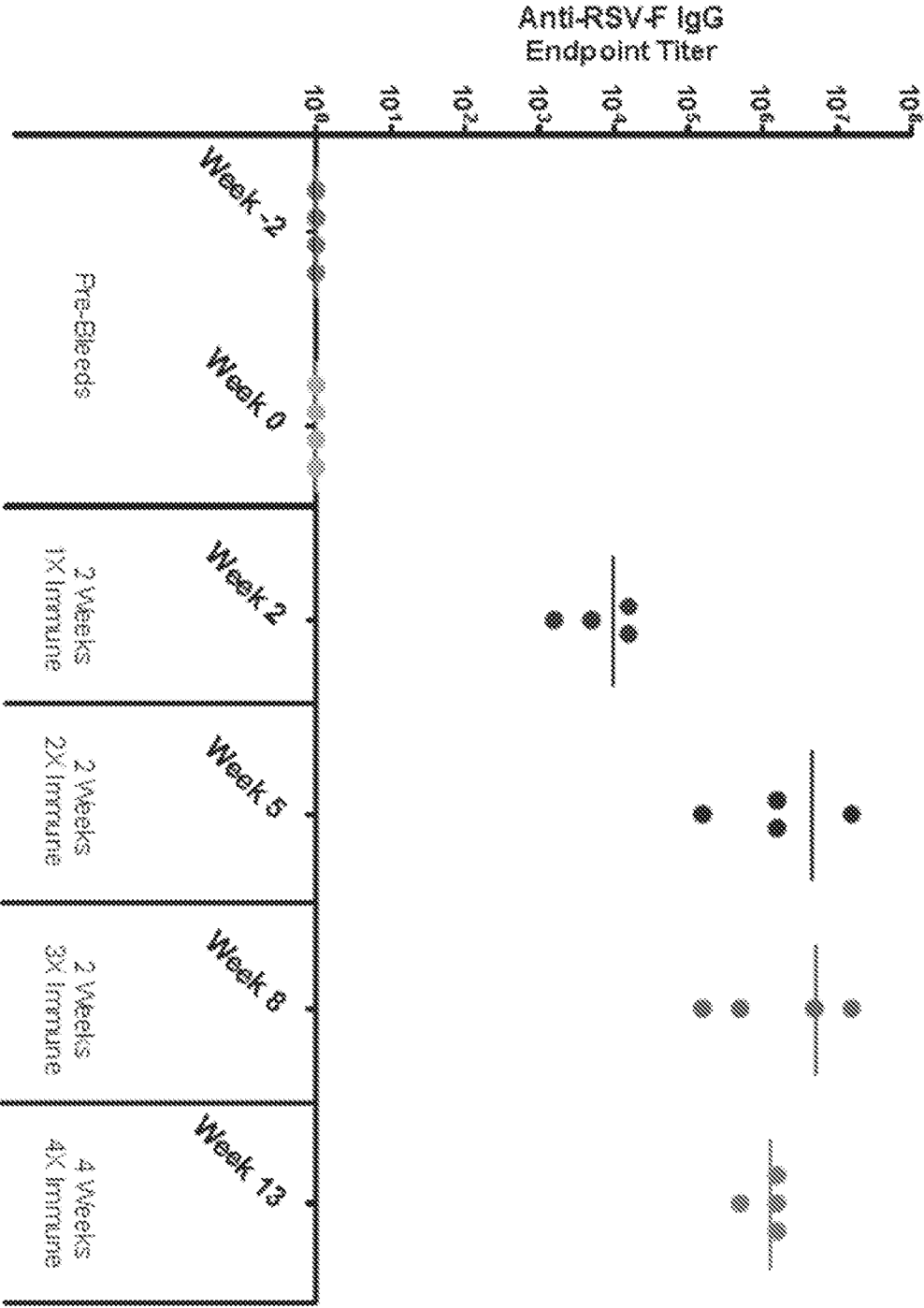


Figure 36

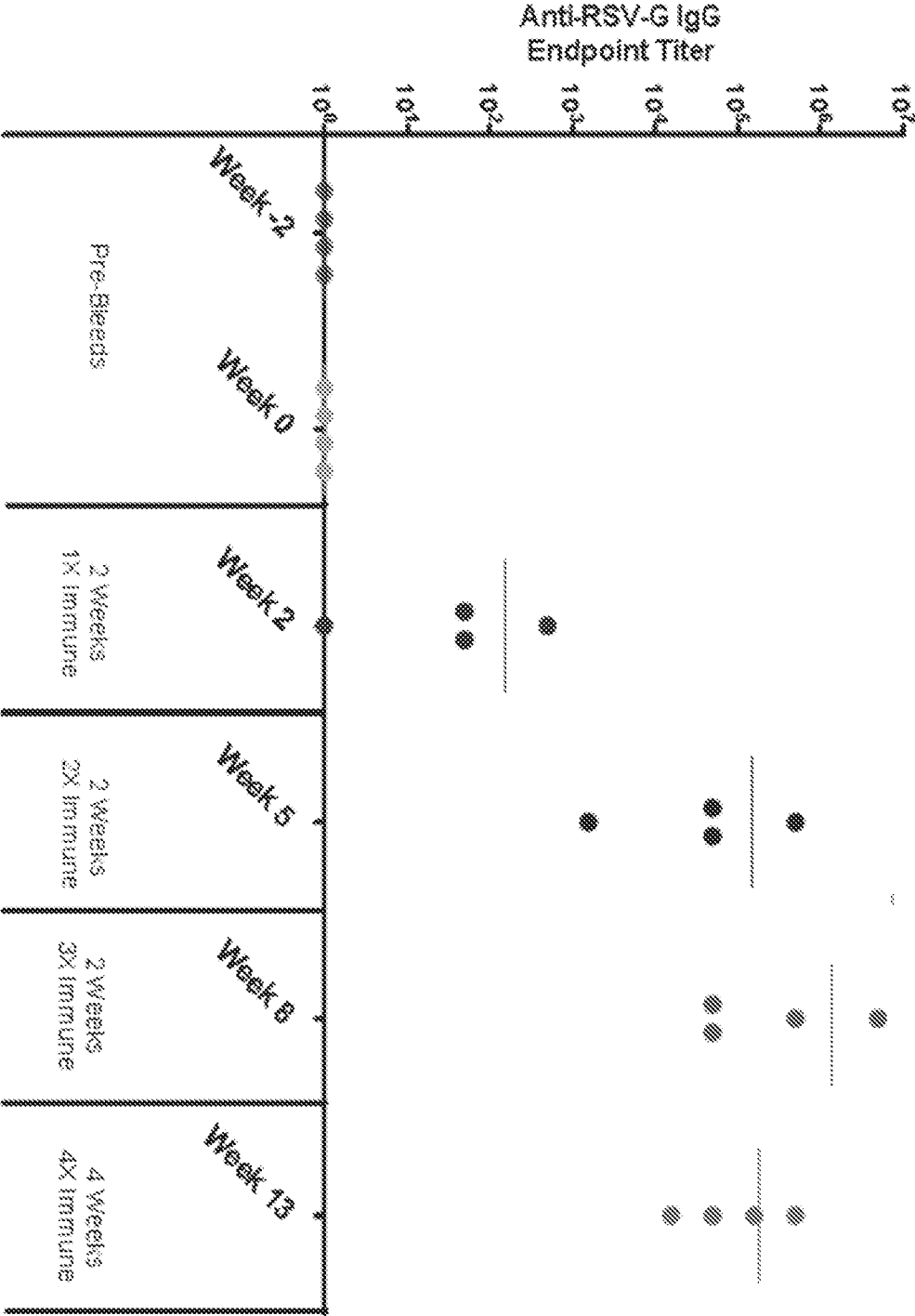


Figure 37

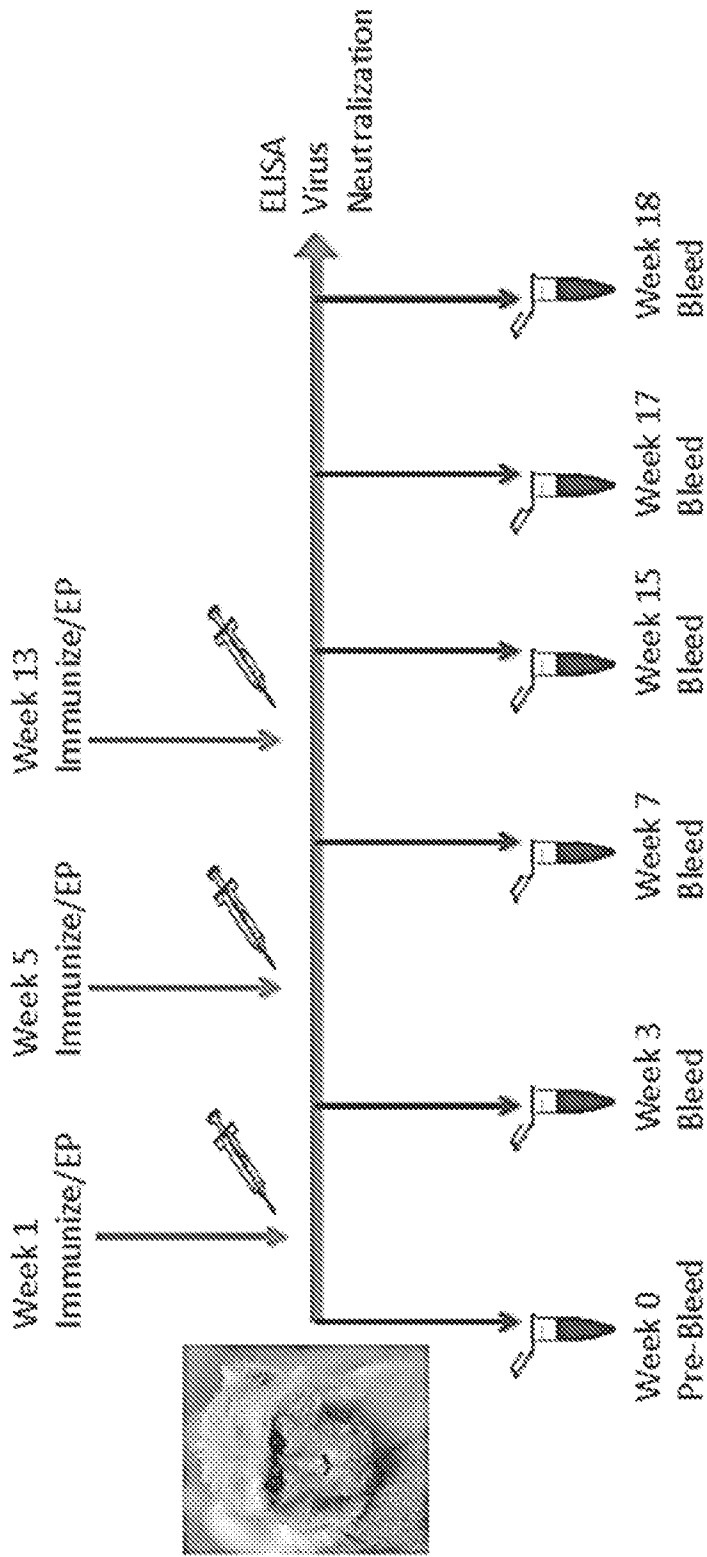




Figure 38A

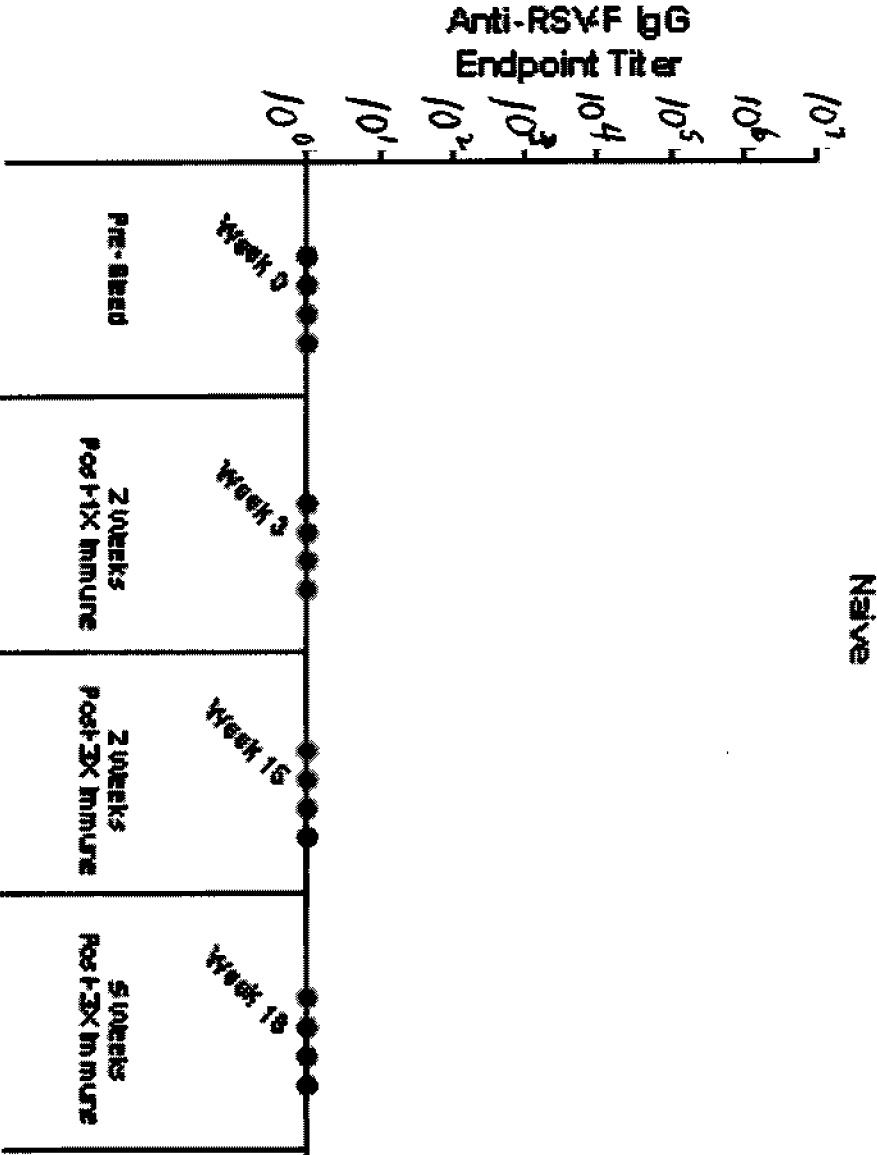


Figure 38B

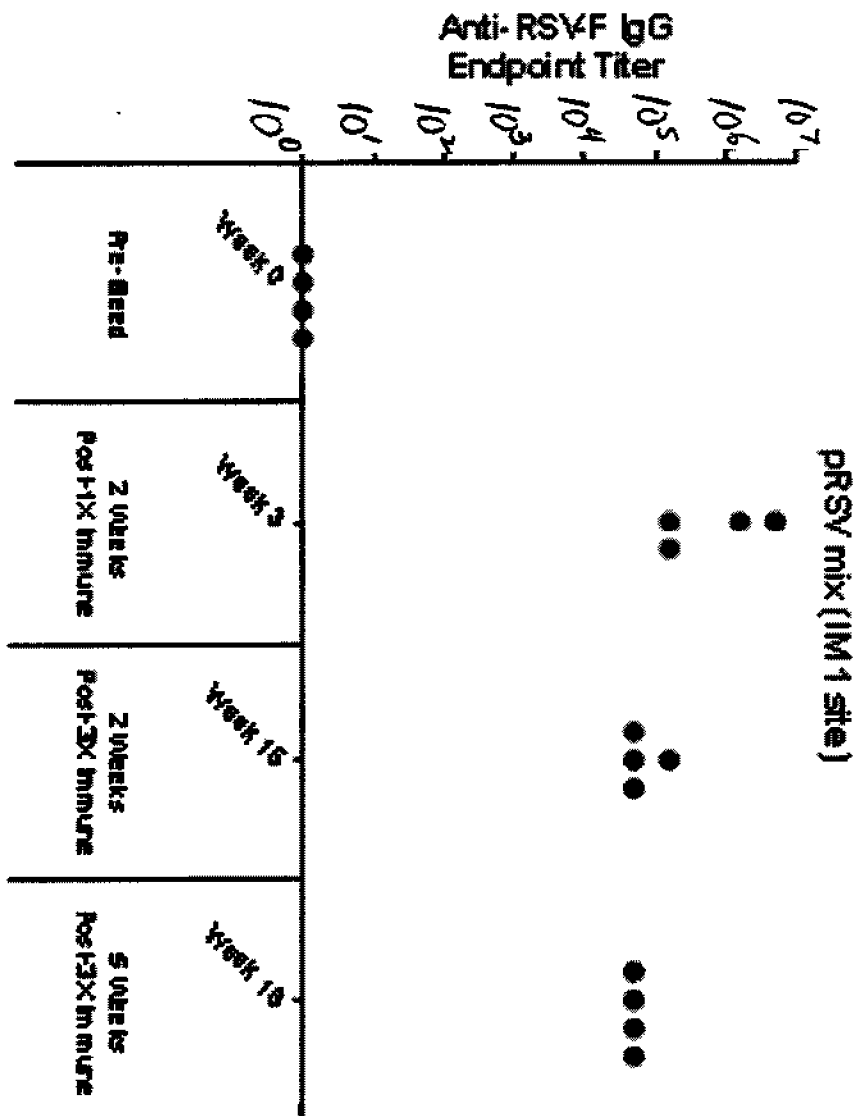




Figure 38D

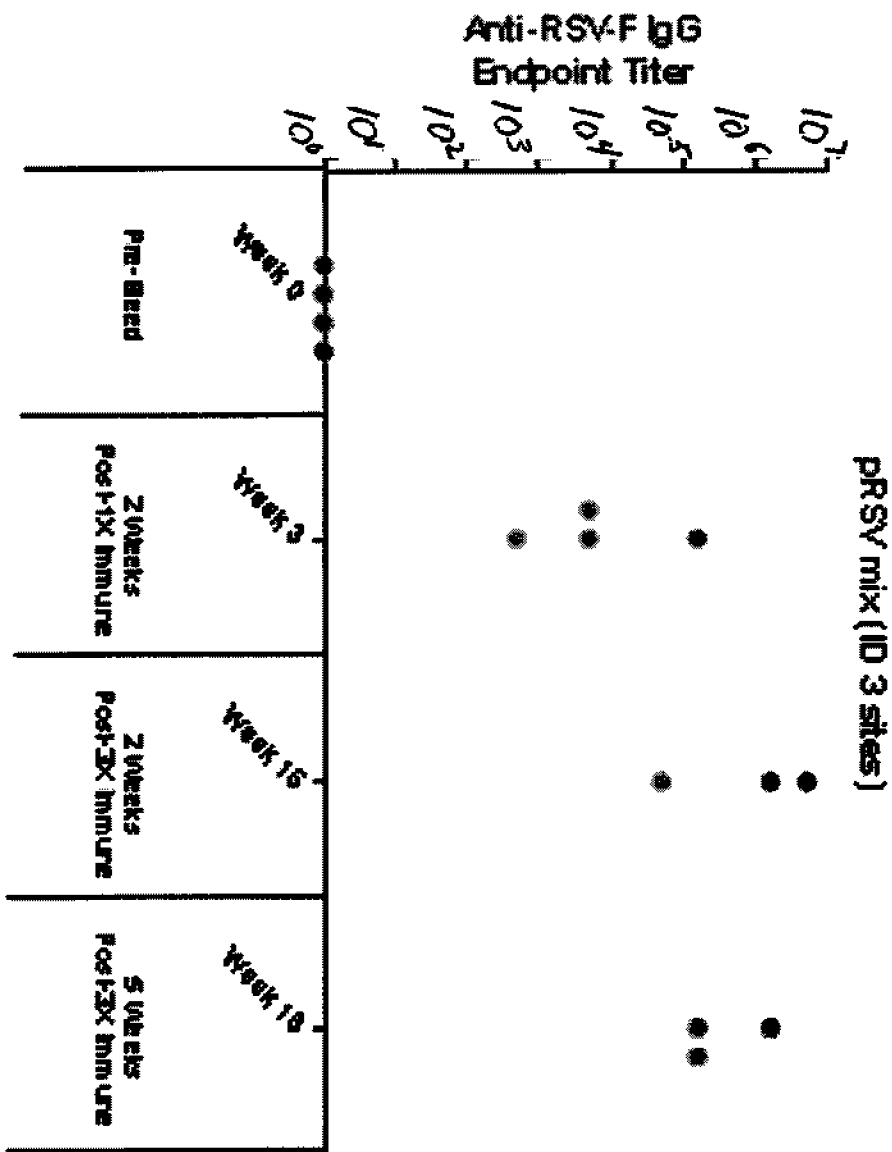


Figure 39A

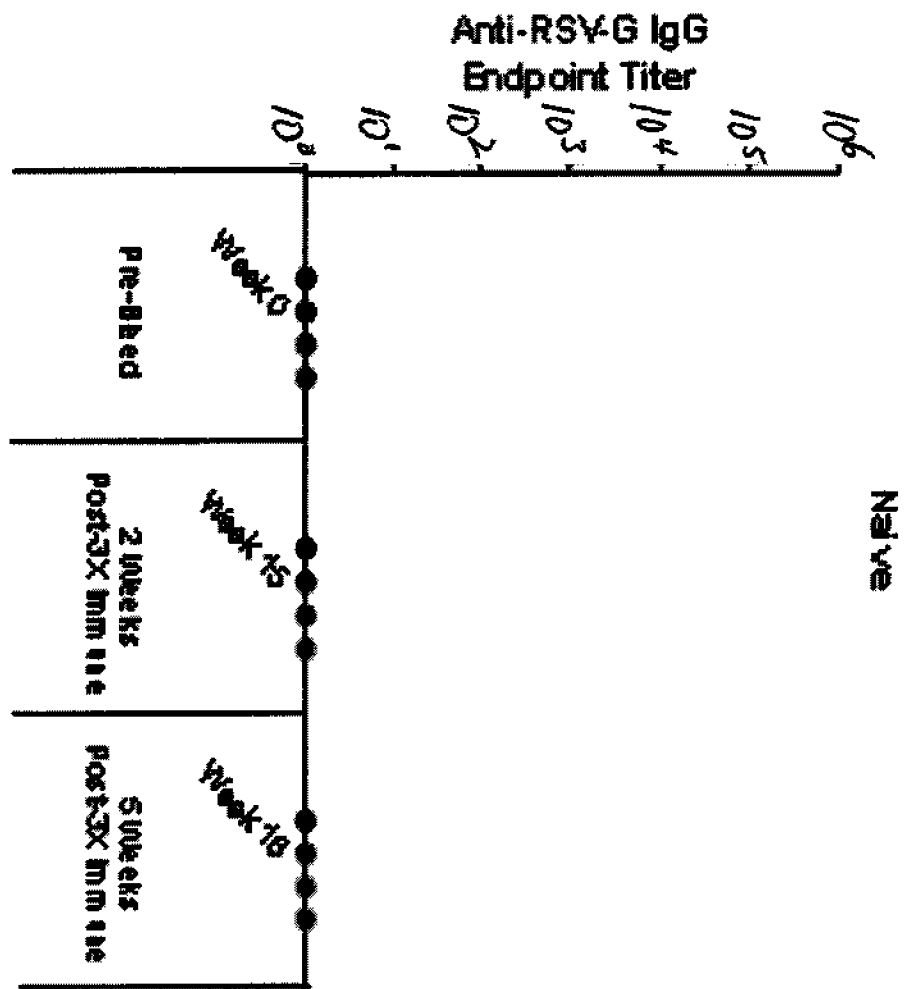




Figure 39C

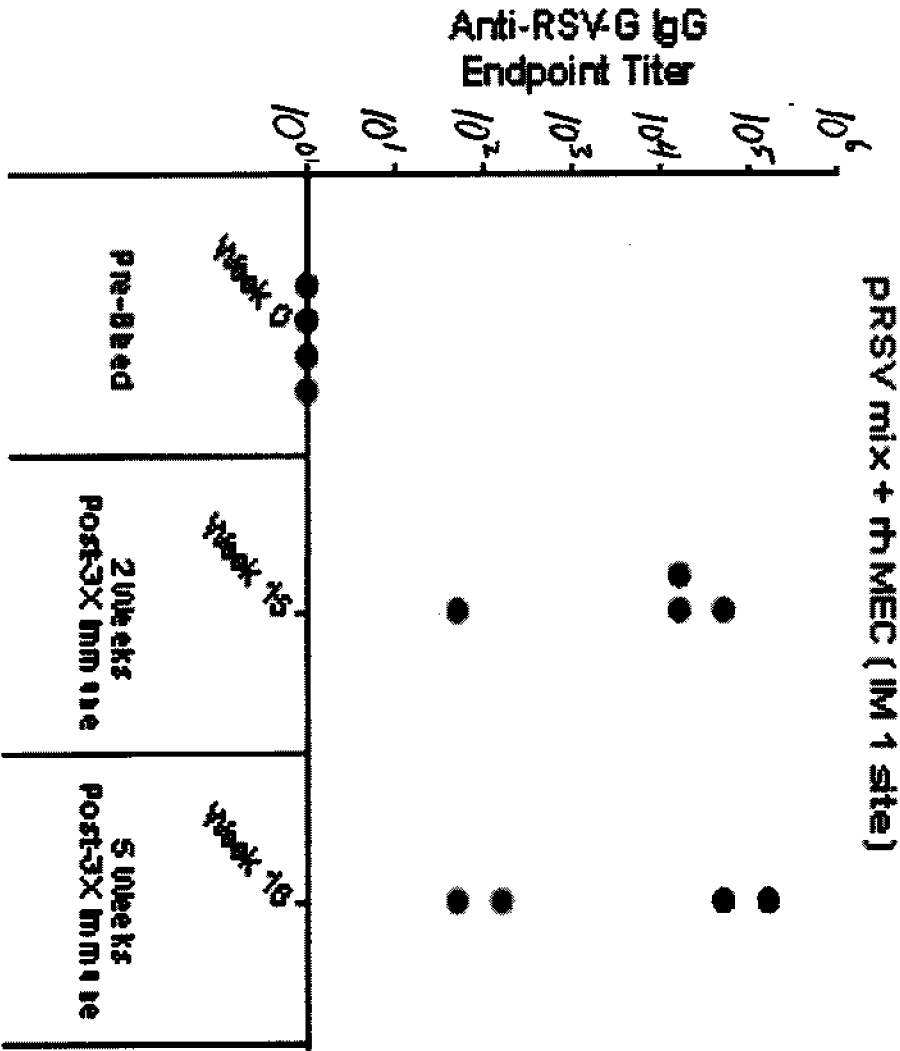


Figure 39D

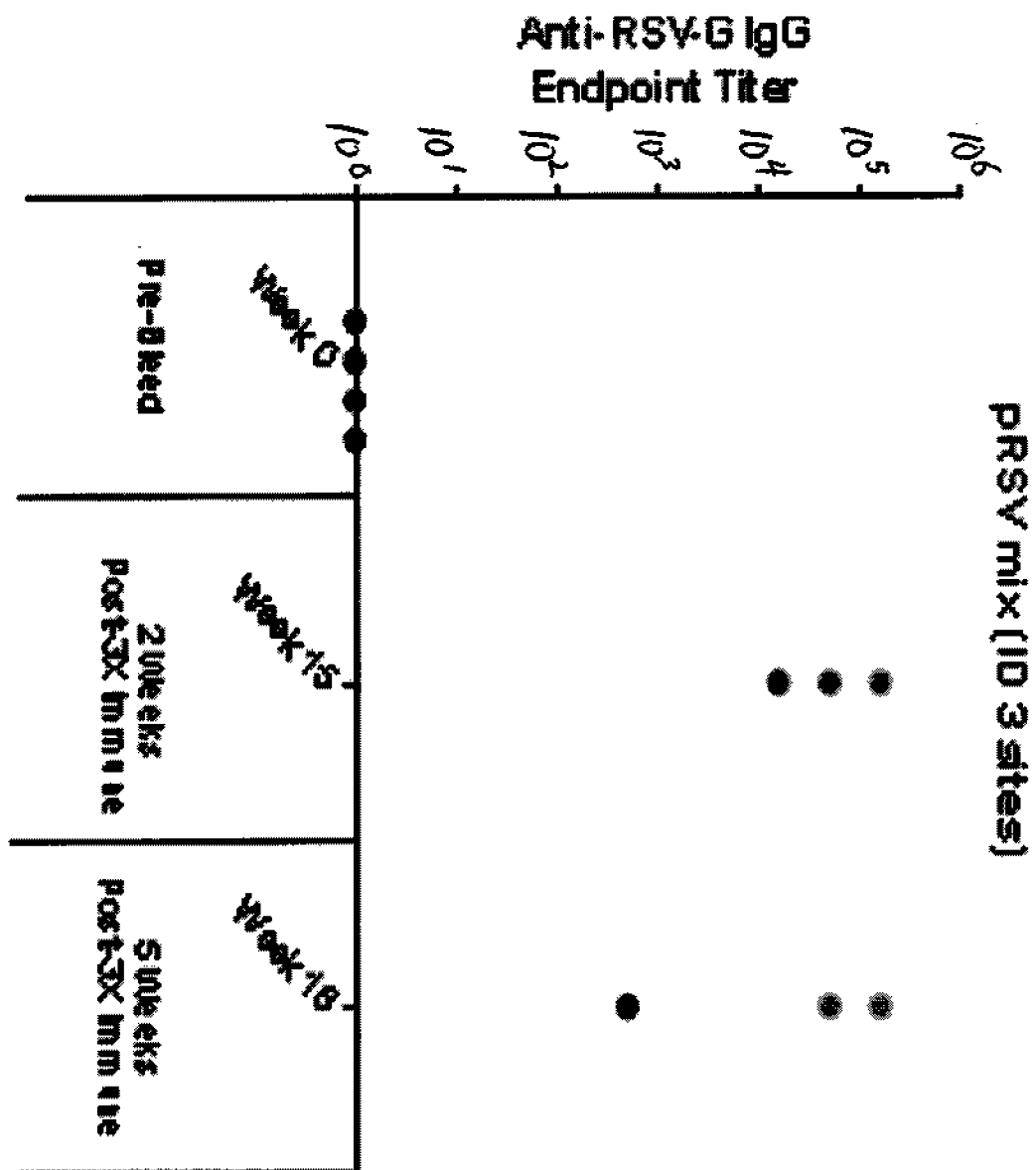




Figure 40

Preliminary Neutralization Results from Cocktail Vaccine  
in Rabbit and Non-Human Primate Studies

Sample Tested	PreBleeds	Neut Titer Post 3d Imm
Negative serum		0
Mab anti RSV		40
Polyclonal Serum		160
Rabbit 71	0	640
Rabbit 72	0	320
NHP IM-1	0	640
NHP IM-2	0	320

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2013/036008

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/135 (2013.01)

USPC - 424/211.1

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)  
IPC(8) - A61K 39/12, 39/155; A61P 31/14, 37/04; C07K 14/115, 14/135 (2013.01)  
USPC - 424/186.1, 199.1, 211.1; 435/69.1; 530/350

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

CPC: A61K 39/00, 39/155, 2039/5256; C07K 14/005, 14/135, 16/1027; C12N 2760/18522, 2760/18534

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

Patbase, Google Patents, Google, Pubmed

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/114149 A2 (BLAIS et al) 25 September 2008 (25.09.2008) entire document	1-22
A	US 2011/0014220 A1 (CHOW et al) 20 January 2011 (20.01.2011) entire document	1-22
A	WO 2009/039178 A1 (KALERGIS et al) 26 March 2009 (26.03.2009) entire document	1-22
A	WO 2004/010935 A2 (YOUNG et al) 05 February 2004 (05.02.2004) entire document This document can be viewed by entering the doc number at the following url: <a href="http://worldwide.espacenet.com/numberSearch?locale=en_EP">http://worldwide.espacenet.com/numberSearch?locale=en_EP</a>	1-22
P, Y	US 2012/0276142 A1 (WEINER et al) 01 November 2012 (01.11.2012) entire document	1-22

☐ Further documents are listed in the continuation of Box C. ☐

\* Special categories of cited documents:

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

"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

13 September 2013

Date of mailing of the international search report

20 SEP 2013

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
P.O. Box 1450, Alexandria, Virginia 22313-1450  
Facsimile No. 571-273-3201

Authorized officer:

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300  
PCT OSP: 571-272-7774

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/036008

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☒

in the international application as filed

☐

together with the international application in electronic form

☐

subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Specifically, SEQ ID NOs:1, 2, 9, and 10 were searched.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/036008

**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.:  
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:

See Extra Sheet

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.:  
1-22, limited to a composition comprising an RSV F immunogen, wherein the RSV F immunogen is selected to be SEQ ID NO:2, further comprising nucleic acid sequence SEQ ID NO:1 that encodes for SEQ ID NO:2; the composition further comprising the RSV F immunogen of SEQ ID NO:2 operably linked to a signal peptide, wherein the RSV F immunogen and signal peptide fusion protein is selected to be SEQ ID NO:10, encoded by nucleic acid sequence SEQ ID NO:9.

**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.